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Aerobic characteristics of red kangaroo skeletal muscles: is a high aerobic capacity matched by muscle mitochondrial and capillary morphology as in placental mammals?

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Summary

Marsupials and placentals together comprise the Theria, the advanced mammals, but they have had long independent evolutionary histories, with the last common ancestor occurring more than 125 million years ago. Although in the past the marsupials were considered to be metabolically 'primitive', the red kangaroo *Macropus rufus* has been reported to have an aerobic capacity ($\dot{V}_{O_2max}$) comparable to that of the most 'athletic' of placentals such as dogs. However, kangaroos travel at moderate speeds with lower relative cost than quadrupedal placentals. Given the long independent evolution of the two therian groups, and their unusual locomotor energetics, do kangaroos achieve their high aerobic capacity using the same structural and functional mechanisms used by (athletic) placentals?

Red kangaroo skeletal muscle morphometry matched closely the general aerobic characteristics of placental mammals. The relationship between total mitochondrial volume in skeletal muscle and $\dot{V}_{O_2max}$ during exercise was identical to that in quadrupedal placentals, and differed from that in bipedal humans. As for placentals generally, red kangaroo mitochondrial oxygen consumption at $\dot{V}_{O_2max}$ was 4.7 ml O_2 min^{-1} ml^{-1} of mitochondria. Also, the inner mitochondrial membrane densities were 35.8±0.7 m^2 ml^{-1} of mitochondria, which is the same as for placental mammals, and the same pattern of similarity was seen for capillary densities and volumes.

The overall data for kangaroos was equivalent to that seen in athletic placentals such as dogs and pronghorns. Total skeletal muscle mass was high, being around 50% of body mass, and was concentrated around the pelvis and lower back. The majority of the muscles sampled had relatively high mitochondrial volume densities, in the range 8.8–10.6% in the major locomotor muscles. Again, capillary densities and capillary blood volumes followed the pattern seen for mitochondria. Our results indicate that the red kangaroo, despite its locomotion and extreme body form, shows fundamental aerobic/muscular relationships that appear common to both marsupials and placentals. The evolution of such metabolic relationships apparently predates the divergence of the therian groups in the early Cretaceous, and perhaps evolved in the mammal-like reptiles during the Triassic (220 million years ago) before the actual evolution of the mammals.

Key words: kangaroo, marsupial, muscle, mitochondria, capillary, aerobic capacity.

Introduction

The marsupials (Metatheria) and placentals (Eutheria) together comprise the advanced mammals, the Theria. They have had, however, long independent evolutionary histories, with the last common ancestor occurring in the Early Cretaceous, more than 125 million years ago (Ji et al., 2002). Apart from reproduction, therians have many features in common, but there are other differences such as in their energetics. Marsupials have a basal metabolism (BMR) some 70% of that seen in many placentals (Dawson and Hulbert, 1970; Withers et al., 2000) and for many years they were considered to be metabolically 'primitive' with respect to the placentals (see Dawson, 1989). However, now there are indications that the maximum aerobic capacities of the two groups are at least equivalent, i.e. marsupials have a greater aerobic scope (Dawson and Dawson, 1982; Hinds et al., 1993). Dawson et al. (2003) have further suggested that marsupials, despite their relatively low BMR, generally have higher aerobic capacities than placentals.

The red kangaroo *Macropus rufus* has the highest aerobic capacity ($\dot{V}_{O_2max}$) yet measured for a marsupial (Kram and Dawson, 1998). It has a $\dot{V}_{O_2max}$ comparable to that of the most 'athletic' of placentals such as dogs (Kram and Dawson, 1998; Dawson et al., 2003). Given the long independent evolution of the two therian groups, do kangaroos achieve their high aerobic

capacity using the same structural and functional mechanisms as (athletic) placentals? Of note, kangaroos and their relatives have unusual locomotory energetics; they travel above moderate speeds with lower relative cost than quadrupedal placentals (Baudinette et al., 1992; Dawson and Taylor, 1973; Kram and Dawson, 1998; Webster and Dawson, 2003).

The links between the aerobic capability of an animal and the supporting structural elements in the oxygen cascade from the lungs to the muscles have been a matter of conjecture (see discussion in Hoppeler et al., 1981a). Studies initiated by Taylor and Weibel (1981) examined the design of the mammalian (placental) respiratory system. Body size dependent, or allometric, variations in aerobic capacities ($\dot{V}_{O_2max}$) were correlated with variation in structural and functional aspects of the cardiorespiratory system such as pulmonary diffusing capacity and capillary density, and with the densities of mitochondria utilizing oxygen in selected muscles (Gehr et al., 1981; Hoppeler et al., 1981b; Mathieu et al., 1981; Taylor et al., 1981).

Phylogenetic variation in aerobic capacity across vertebrates generally appears to follow a similar pattern. Differences in the aerobic potentials of reptiles, mammals and birds are primarily associated with the amount of mitochondria in organs and skeletal muscle, although some differences in packing density of the inner mitochondrial cristae membranes occur (Else and Hulbert, 1981, 1983, 1985a; Suarez, 1996). However, within the mammals no large differences in total mitochondrial capacity were noted between placental, marsupial and monotreme species (Else and Hulbert, 1985b).

Further studies of placental mammals have shown that differences in metabolic capacity may be associated with induced and adaptive variation (Weibel et al., 1987). Induced variation of $\dot{V}_{O_2max}$ is due to training, as seen in man (Hoppeler and Lindstedt, 1985). Adaptive variation results from different evolutionary pressures; ‘athletic’ species may have a two- to threefold greater oxidative capacity than more ‘sedentary’ species of similar body mass (Taylor et al., 1981). When athletic species such as dogs and horses are compared with more sedentary species like goats and cattle, the athletic species have larger hearts and a bigger mass of muscle, together with higher muscle mitochondrial densities and capillary volumes (Weibel et al., 1991; Hoppeler and Weibel, 1998). This quantitative overall match between design and functional parameters, such as in the pathway for oxygen from the lung to the mitochondria in the muscle cells, has been referred to as ‘symmorphosis’ (Taylor and Weibel, 1981; Weibel et al., 1991; Weibel, 2000).

Aspects of the cardiorespiratory system of marsupials are not the same as those of placentals. They have resting heart rates less than half of those of placentals (Kinnear and Brown, 1967; Dawson and Needham, 1981), but hearts that are generally bigger (Dawson et al., 2003). A similar pattern is also seen with breathing, marsupials having very low resting respiratory rates but large tidal volumes (Dawson and Needham, 1981; Cooper and Withers, 2003). Despite these differences, the mechanisms by which the kangaroos achieve high aerobic capacity may be similar to that seen in athletic placentals. The large heart of the red kangaroo has the same proportionality to $\dot{V}_{O_2max}$ as seen in the athletic dog, and the same is true for the volume of cardiac muscle mitochondria (Dawson et al., 2003). The principal aim of this study was to determine whether similar relationships are seen in skeletal muscles.

Our study was also concerned with the potential for muscular energy output throughout the body of the kangaroo. Hopping is associated with a distinctive body shape, including a concentration of muscle mass in the hind limbs and a large tail. Studies of the functional properties of kangaroo musculature have generally concentrated on the lower leg, specifically the Achilles tendon and the gastrocnemius muscle, because of considerable interest in the role of energy conservation by elastic recoil (e.g. Dennington and Baldwin, 1988; Bennett and Taylor, 1995). However, we know little about the actual sites of power generation and the role of the large tail. An associated question is whether bipedal locomotion and the upright stance at times adopted by kangaroos leads to similarities with humans, who diverge from the usual (placental) relationship between body mass and $\dot{V}_{O_2max}$ (Hoppeler, 1990).

Materials and methods

Animals

Red kangaroos *Macropus rufus* Desmarest 1842 are common in inland Australia (Dawson, 1995); those used were obtained from the wild at the University of New South Wales Arid Zone Field Station, Fowlers Gap, in far western New South Wales. Mature females of medium size were chosen, maturity being confirmed by dental and pouch examination. Red kangaroos of this age/size class predominate in wild populations (Dawson, 1995). Red kangaroos are largely nocturnal feeders so they were taken in the early morning to ensure that gut fill was consistently high. The kangaroos were killed by a shot to the head by an experienced, licensed shooter and transported rapidly (10–20 min) to the field laboratory. When muscle samples were taken for mitochondrial and capillary analysis this procedure was completed within 2 h of death, as recommended by Hoppeler et al. (1981a). Muscle identification generally followed that of Hopwood (1974, 1976) and Hopwood and Butterfield (1976, 1990) for the eastern grey kangaroo *Macropus giganteus* Shaw 1790. We dissected the musculature of the red kangaroo to compare with the eastern grey kangaroo and to familiarize ourselves with functional aspects; we noted minor differences between the species.

Muscle sample collection and preparation

To assess the mitochondrial and capillary characteristics of the skeletal muscle of the whole body we followed a sampling procedure comparable to that of Hoppeler et al. (1984). The kangaroo musculature was divided into seven functional units, head and neck, foreleg, trunk, back, upper hindleg, lower

hindleg and tail (Fig. 1). After being shot the kangaroos were weighed to ±0.05 kg and then eviscerated, with the mass of the large forestomach and its contents determined by weighing on appropriate calibrated electronic balances. The mass of the intestines, including caecum and contents, were similarly determined. Empty body mass was then calculated. The skin was removed and muscle was dissected from one half of the body and weighed. The dissection was carried out in an air conditioned room and evaporation from the muscles was contained by towels dampened with physiological saline. The separation of regions was as follows: head and neck was separated anterior to the first thoracic vertebrae; foreleg included all muscles attached to foreleg; trunk included the m. erector spinae plus rib and abdominal muscles and the diaphragm; back musculature comprised the m. multifidi lumborum and m. sacrocaudalis dorsalis, which run between the spinous and mamillary processes; upper hindleg included all muscles attached; lower hindleg included the m. gastrocnemius and associated muscles; tail was separated immediately behind the pelvis. Diagrams of the principal muscles of the hindleg and back of the red kangaroo are provided in Fig. 2A,B.

The mitochondrial and capillary characteristics of the skeletal muscle of the regions and hence the whole body was determined by sampling from muscles from each region. For four animals, one muscle in each body region was selected by the throw of dice, except in the upper hindleg region, where three muscles were selected (by throw of dice) because of the large mass of this region. Overall, the muscles sampled represented 31.5% of the total musculature. Data was also collected from the diaphragm, so a total of ten muscles were sampled. A concurrent study of the heart was also undertaken (Dawson et al., 2003).

Each muscle was removed and weighed to ±0.1 g. Three sample blocks per muscle (per animal) were cut from random locations (superficial or deep, and proximal, central or distal; again selected by throw of dice). Samples were placed immediately into a fixative solution of 6.25% glutaraldehyde in 0.1 mol l^{-1} sodium cacodylate buffer (pH 7.4), then kept refrigerated at 4°C prior to preparation for electron microscopy.

Preparation for electron microscopy

In total, 30 muscle sample blocks (10 muscles × 3 blocks) per animal were prepared for electron microscopy; three blocks each from the head/neck, foreleg, back, lower hindleg and tail regions, six blocks from the trunk and nine blocks from the upper hindleg. Where required, blocks were trimmed into smaller pieces. Blocks were rinsed in 0.1 mol l^{-1} sodium cacodylate buffer and left overnight in fresh buffer. Blocks were post-fixed in a buffered solution of osmium tetroxide for 4 h, rinsed in 2% sodium acetate solution and placed in 2% uranyl acetate for 1 h. The samples were dehydrated in a series of 15 min ethanol washes, using solutions from 50% to 100% ethanol. After a final 30 min wash in 100% ethanol, the samples were washed twice for 15 min in 100% dry acetone. Spurr's low-viscosity epoxy resin (slow cure) was used to embed the samples. The blocks were placed in a 1:1 acetone:resin mixture for 1 h, then refrigerated for 3–4 days in a 1:9 mixture. This long infiltration period was selected to reduce tearing of the muscle tissue during sectioning. The samples were placed in 100% resin for 30 min at 60°C, transferred into embedding moulds filled with fresh resin, and cured at 60°C for 48 h.

A Reichart-Jung Ultracut ultramicrotome (Vienna, Austria) was used to section the embedded samples. 5–10 ultra-thin sections showing silver (60–90 nm) or gold (90–150 nm) interference colours were cut from each block and mounted on 200 mesh copper grids. Orientation of the sections was generally transverse or oblique to the muscle fibre axis. Grid-mounted sections were stained with 2% uranyl acetate in 50% ethanol for 10 min and then rinsed in distilled water.

Mitochondrial volume

Grids were viewed using a Hitachi 7000 (Tokyo, Japan) transmission electron microscope (TEM) at a magnification of 12 000×. For each sample block, ten grid squares were selected using a systematic random sampling method (Howard and Reed, 1998, pp. 25-29) and a digital image was obtained of the top left corner of each of the grid squares, using an Olympus SQ (Tokyo, Japan) digital camera attached to the TEM and AnalySIS software. For each animal, 30 images were obtained per muscle (10 images × 3 blocks); these were imported into the image analysis software Adobe Photoshop. A human operator selected all mitochondria in an image using a selection tool, coloured the mitochondria black and filtered the background to plain white. The percentage area covered by mitochondria ('mitochondrial area fraction') was thus represented by the area of black in each image, which was calculated with reference to the total image area (51.2 μm^2 for images obtained at 12 000×) using a suite of Photoshop plug-ins (Image Processing Toolkit, Reindeer Graphics, Asheville NC, USA). The mitochondrial area fraction is equivalent to the mitochondrial volume fraction or volume density, V_V(mt,f). The total mitochondrial volume V(mt,m) for each muscle (in cm^3) was calculated from:

$$V(\mathrm{mt,m}) = M_\mathrm{m} \times V_V(\mathrm{mt,f}) \times V_V(\mathrm{f,m}) \times d^{-1}\,, \qquad (1)$$

where M_m is muscle mass, V_V(f,m) is the volume fraction of muscle occupied by muscle fibres, and d is the density of the muscle. A muscle density of 1.06 g ml^{-1} was used (Mendez and Keys, 1960) and it was assumed that V_V(f,m) was equal to 1 (Hoppeler et al., 1987).

Surface density of the inner mitochondrial membranes

The inner mitochondrial membrane surface density (S(im,m)) was estimated in the m. multifidi lumborum, one of the larger skeletal muscles. Grid squares were selected as for mitochondrial density measurements. A mitochondrion at the centre of the field of view (at magnification 12 000×) was then viewed at a magnification of 30 000× or 40 000× so that the inner membranes could be seen clearly. Twenty mitochondria

from each animal were examined. A cycloidal line grid (grid C1; Howard and Reed, 1998, p. 210) was used to estimate the surface density of inner mitochondrial membranes per unit volume of mitochondria, S_V(im,mt) in $m^2\ cm^{-3}$ using equation 6.4 of Howard and Reed (1998). An overall estimation of the total surface area of inner membranes in each multifidi lumborum muscle was given by:

$$S(\text{im,mt}) = V(\text{mt,m}) \times S_V(\text{im,mt}) . \quad (2)$$

Capillary length and volume

Both transverse and longitudinal sections were sliced from muscles and were used to estimate the tortuosity factor c(K,0) of the capillary network, using the shortcut estimation method of Mathieu et al. (1983). The tortuosity factor was determined for the diaphragm, m. multifidi lumborum, m. gastrocnenius, m. vastus lateralis and m. semitendinosus; the mean was c(K,0)=1.37±0.07 (N=5). Grids were viewed using the TEM at a magnification of 1500×. Grid squares were selected using a systematic random sampling method. Ten digital images (of area approximately equal to one grid square) of transverse sections were taken and used to estimate the number of capillaries per unit area (numerical capillary density, N_A(c,f), in mm^{-2}). Capillary length density J_V(c,f) was calculated from numerical capillary densities according to:

$$J_V(\text{c,f}) = c(\text{K,0}) \times N_A(\text{c,f}) . \quad (3)$$

The total capillary length J(c) in km per muscle was then calculated from:

$$J(\text{c}) = J_V(\text{c,f}) \times M_m \times V_V(\text{f,m}) \times d^{-1} . \quad (4)$$

The cross-sectional areas A(c) of capillary profiles in transverse sections were estimated using the same procedure as for mitochondrial area densities, except that the absolute area covered by capillaries was obtained (rather than a percentage). As in previous studies (Conley et al., 1987), we assumed that capillary profiles were approximately circular and estimated the mean capillary radius r_c from A(c):

$$r_c = [A(\text{c})/\pi]^{1/2} . \quad (5)$$

The capillary blood volume V(c) in each muscle was calculated from:

$$V(\text{c}) = \pi r_c^2 \times J(\text{c}) . \quad (6)$$

In this study we examine the relationship between muscle mitochondrial volume and $\dot{V}_{O_2max}$ in *M. rufus*. To do this requires certain assumptions, which have been accepted in the extensive studies of placental mammals undertaken by Hoppeler, Weibel, Taylor and coworkers (for a discussion of these assumptions, see Hoppeler, 1990). We have accepted the same assumptions for comparative purposes, but also because the resultant errors are likely to be small. For example, assuming that the measured muscle volume consists entirely of muscle fibres results in an overestimation of the muscle fibre volume of less than 10% (Hoppeler et al., 1987). Furthermore, some assumptions lead to opposite errors and cancel each other out (Hoppeler, 1990); assuming all oxygen is consumed by muscle mitochondria at $\dot{V}_{O_2max}$ overestimates mitochondrial activity (again of the order of 10%), so that estimates of the maximum oxygen consumption rate per volume of muscle mitochondria will be little affected.

Statistical methods

Comparisons between muscles were analysed using one-way analyses of variance (ANOVAs). A Student–Newman–Keuls (SNK) multiple-range test was applied when significant differences were indicated by the ANOVA (using Statistica/Mac software). Values are given as means ± standard error (S.E.M.). Regression analyses were carried out using Microsoft Excel.

Results

Skeletal muscle comprises 46.8±1.7% of body mass of red kangaroo females, when the gut is relatively full (Table 1). The percentage of skeletal muscle when the gut is empty, empty body mass (57.2±1.6%) is also provided because red kangaroos are herbivores with a large fermentative forestomach and fill can be variable. Table 2 lists the mitochondrial and capillary characteristics of representative muscles from the various regions of the red kangaroo (Fig. 1). These base data were used for the determination of mitochondrial and capillary patterns throughout the total skeletal muscle of the body (Tables 3, 4), in the manner of Hoppeler et al. (1984), Hoppeler (1990) and Hoppeler and Weibel (2000). The principal muscles of the hindleg and lumbar region, including most of those muscles sampled for mitochondria and capillary measurements, are shown in Fig. 2.

Mitochondrial volume density, V_V(mt,f), varies between muscles (Table 2). Those from the back and trunk, including the diaphragm, together with the large m. gluteus medius of the upper hindleg, have V_V(mt,f) in the range 8.8–10.8%, which is significantly higher than in the other muscles measured. On the other hand, muscles in the foreleg and neck have V_V(mt,f) in the range 3.7–3.8%, significantly lower than in other muscles. The area of the inner mitochondrial surface,

Table 1. *Proportion of muscle mass in the body of red kangaroos* Macropus rufus

	Mass (kg)
Body mass	28.48±0.48
Total skeletal muscle	13.33±0.27
Forestomach	4.37±0.38
Forestomach contents	3.85±0.37
Intestines, including caecum	1.94±0.09
Intestine contents	1.31±0.06
Empty body mass	23.32±0.24
Total skeletal muscle / body mass (%)	46.8±1.7
Total skeletal muscle / empty body mass (%)	57.2±1.6

Values are means ± S.E.M., N=4.

Table 2. *Mitochondrial and capillary characteristics of muscles from regions of the body of red kangaroos*

Body section	Muscle	M_m (g)	M_m/M_b (g kg^{-1})	V_V(mt,f) (%)	J_V(c,f) (mm^{-2})	J(c) (km)	V(c)/gM_m (μl g^{-1})	V(c)/V(mt,m) (ml ml^{-1})
Head and neck	Trapezius	103±3^{f}	4.1±0.5^{e}	3.8±0.2^{c}	1102±51^{b,c}	107±6^{d}	16.6±0.8^{b,c}	0.47±0.05^{a}
Foreleg	Triceps	91±12^{f}	3.6±0.4^{e}	3.7±1.0^{c}	790±47^{c}	68±8^{d}	11.9±0.7^{c}	0.35±0.04^{b}
Trunk	Diaphragm	98±13^{f}	3.8±0.2^{e}	10.8±0.9^{a}	1320±112^{b}	123±17^{d}	19.3±2.1^{b}	0.19±0.02^{c}
Trunk	Erector spinae	725±48^{a}	28.6±4.3^{a}	8.8±1.6^{a,b}	1715±169^{a}	1171±122^{a}	25.9±2.8^{a}	0.32±0.04^{b}
Back	Multifidi lumborum	627±81^{b}	24.6±3.0^{a,b}	10.6±1.9^{a}	1103±63^{b,c}	648±35^{c}	16.9±1.3^{b,c}	0.17±0.02^{c}
Upper hindleg	Vastus lateralis	427±48^{d}	16.8±2.0^{c}	6.7±0.9^{b}	785±70^{c}	321±46^{d}	11.8±1.0^{c}	0.19±0.02^{c}
Upper hindleg	Gluteus medius	542±96^{c}	21.4±4.7^{b}	10.2±1.2^{a}	1690±111^{a}	849±33^{b}	25.5±1.4^{a}	0.27±0.03^{b,c}
Upper hindleg	Semitendinosus	319±44^{e}	12.5±1.9^{d}	7.8±1.2^{b}	1021±124^{b,c}	302±7^{d,e}	15.8±1.7^{b,c}	0.21±0.02^{c}
Lower hindleg	Gastrocnemius	680±79^{a,b}	26.7±3.0^{a}	6.4±0.5^{b}	755±105^{c}	498± 27^{c,e}	11.2±1.8^{c}	0.18±0.02^{c}
Tail	Coccygeus	119±13^{f}	4.6±0.2^{e}	5.7±1.2^{b}	1192±101^{b}	134±13^{d}	18.8±1.4^{b}	0.34±0.04^{b}

M_m is muscle mass, M_b is body mass, V_V(mt,f) is mitochondrial volume density, V(mt,m) is mitochondrial volume, J_V(c,f) is capillary length density, J(c) is total capillary length in a muscle, V(c)/gM_m is capillary volume per gram of muscle and V(c)/V(mt,m) is capillary volume per unit of mitochondrial volume.

Values are means ± S.E.M., N=4.

In columns, values that are significantly different have different superscript letters (P<0.05).

Body mass was 25.63±1.56 kg. Note: values for muscle masses are the total for the body, i.e. both sides where applicable. Capillary diameter was not significantly different between muscles; mean of mean muscle values was 4.51 μm, range 4.43–4.57 μm.

S_V(im,m), was measured for the m. multifidi lumborum and was 35.8±0.7 m^2 cm^3 (N=4).

Capillary characteristics also differ between muscles from different regions (Table 2). The capillary length density, J_V(c,f), is considered a good estimate of the capillary supply to muscles (Conley et al., 1987); it incorporates the tortuosity factor, c(K,0), which in this case was determined for five muscles from each animal. However, no significant difference was found between these muscles and the overall mean tortuosity (1.37±0.07, N=5) was used generally. Muscles that have significantly higher J_V(c,f) tended to have a high V_V(mt,f). Large muscles of the upper hindleg, m. gluteus medius, and the trunk, the m. erector spinae, have J_V(c,f) that are significantly higher than in other muscles. However, the pattern is not clearcut. While the J_V(c,f) of the m. triceps of the foreleg is significantly lower than in most other muscles, this is also the case for the m. gastrocnenius and the m. vastus lateralis. The pattern in J_V(c,f) was also reflected in the capillary volume per g of muscle, V(c)/gM_m, because no significant differences in capillary diameter were seen between muscles. The mean capillary diameter was 4.51 μm, with the range of muscle means being 4.43–4.57 μm. Consequently, the ratio V(c)/V(mt,m) also indicates the capillary blood supply to mitochondria in various muscles. The muscle with highest capillary volume per unit of mitochondria was the m. trapezius of the neck. This was followed by the m. triceps, m. coccygeus and the m. erector spinae. Overall there was a significant negative correlation (r=0.65, P<0.05) between V(c)/V(mt,m) and the V_V(mt,f) of various muscles (Fig. 3A).

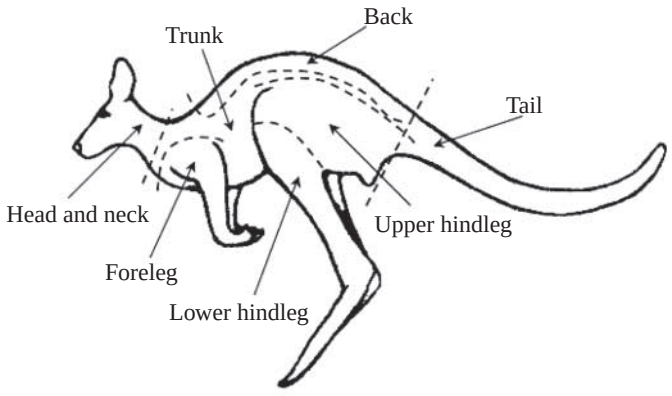

Fig. 1. The outline of a red kangaroo showing the delineation of the body regions from which muscle samples were taken.

The distribution of muscle in the regions of the body is shown in Table 3. The upper hindleg had significantly more muscle than other regions, containing 44.3% of the total skeletal muscle mass. This is followed by the trunk (25.6%), back (10.1%) and the lower leg (9.1%). The fore part of the body was lightly muscled. Notably, the foreleg carried only 3.9% of skeletal muscle. These patterns were also largely followed in the distribution of mitochondria and capillaries throughout the skeletal muscles, although the low V_V(mt,f) of the foreleg and the head and neck resulted in these regions containing only 1–2% of the total volume of skeletal muscle mitochondria. With respect to capillary volumes, the trunk appears to have a higher V(c) relative to its muscle mass than other sections.

Discussion

Kangaroos are marsupials that belong to a large group of cursorial herbivores (Macropodoidea) that fill the ecological niche in Australia occupied by bovid antelopes in Africa and cervid deer in Laurasia. However, the locomotion and associated body shape of kangaroos and their relatives are unique for large mammals. How do the kangaroo's unique

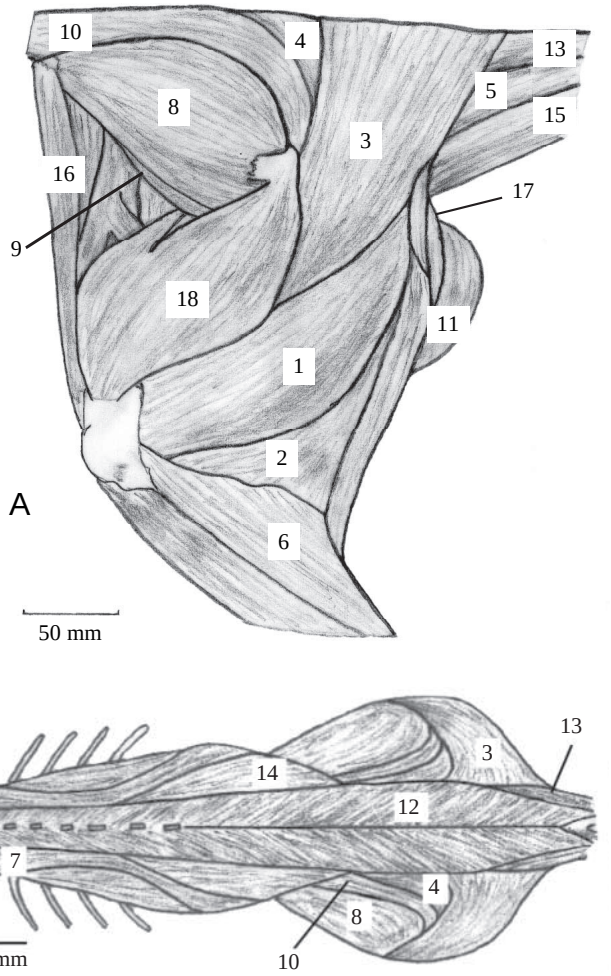

Fig. 2. The principal muscles of (A) the hindleg (superficial) and (B) the lumbar region of the red kangaroo. Numbers indicate muscles: 1, biceps femoris (cranial); 2, biceps femoris (caudal); 3, caudofemoralis (caudal); 4, caudofemoralis (cranial); 5, coccygeus; 6, crural muscles including gastrocnemius; 7, erector spinae; 8, gluteus medius; 9, gluteus profundus; 10, gluteus superficialis; 11, gracilis; 12, multifdi lumborum; 13, sacrocaudalis dorsalis; 14, sacrocaudalis dorsalis lateralis; 15, sacrocaudalis ventralis; 16, sartorius; 17, semitendinosus; 18, vastus lateralis.

locomotory characteristics relate to muscle distribution and aerobic potential? Are the kangaroos essentially different from placentals, or do their muscle characteristics simply reflect an extreme variation on a basic mammalian pattern? Studies on marsupial hearts indicate that the latter may be the case (Dawson et al., 2003), and this is supported by the results of the present study.

The core unit of metabolic capacity is the area of the inner mitochondrial surface, S_V(im,m); it has been consistently correlated with the activity of the terminal respiratory chain enzyme in several vertebrate groups (Else and Hulbert, 1981). In placental mammals the S_V(im,m) of muscle mitochondria appears relatively constant between muscles and across species, with a value of ~ 35 m^2 cm^{-3} (Else and Hulbert, 1985; Hoppeler et al., 1981a; Schwerzmann et al., 1989). Consequently, mitochondrial volume has been used to assess the aerobic potential of species (Hoppeler, 1990; Hoppeler and Weibel, 1998, 2000; Kayar et al., 1989). The S_V(im,m) of a red kangaroo skeletal muscle was the same as in placentals, 35.8±0.7 m^2 cm^{-3}. Essentially similar values (35–38 m^2 cm^{-3}) occur in the skeletal muscles of other marsupials (Webster, 2003) and in the heart muscles of the red kangaroos and other marsupials (Dawson et al., 2003). Assuming the aerobic capacity per unit of membrane surface area is relatively constant, the consistent S_V(im,m) between the two therian groups implies that we can use mitochondrial volume to assess the aerobic potential of kangaroos.

The volume densities of mitochondria V_V(mt,f) in the muscles of red kangaroos are variable, 3.7–10.6%, with the V_V(mt,f) for the diaphragm (10.8%) generally being significantly higher (Table 2). Generally, the V_V(mt,f) of red kangaroo muscles are higher than in comparable muscles of placental mammals (Else and Hulbert, 1985b; Hoppeler et al., 1981b; Mathieu et al., 1981) when body size is accounted for because V_V(mt,f) generally increases with decreasing mass. There are placental mammals that do have muscle V_V(mt,f) comparable to kangaroos; these are athletic species with a high

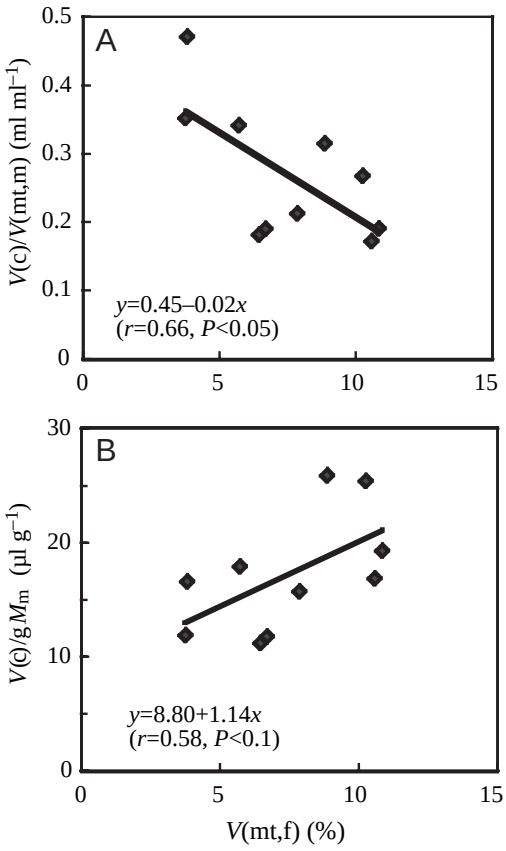

Fig. 3. Relationship in red kangaroo muscles between (A) mitochondrial volume density V(mt,f) and the capillary blood supply per ml of mitochondria V(c)/V(mt,m), and (B) mitochondrial volume density V(mt,f) and the capillary blood supply per g of muscle V(c)/gM_m. Data for individual muscles are derived from Table 2.

Table 3. *Distribution of muscle and muscle mitochondria and capillaries in the body of red kangaroos*

Body section	Mass (g)	% Total muscle mass	V(mt,m) (ml)	% Total V(mt,m)	V(c) (ml)	% Total V(c)
Head and neck	320±10^{d}	2.5±0.1^{d}	12±1^{e}	1.1±0.1^{e}	5.3±0.2^{d}	2.1±0.1^{d}
Foreleg	508±10^{d}	3.9±0.1^{d}	19±1^{e}	1.7±0.1^{e}	6.1±0.1^{d}	2.4±0.1^{d}
Trunk	3323±336^{b}	25.6±2.5^{b}	293±29^{b}	26.9±2.6^{b}	86.2±8.7^{b}	34.2±2.9^{b}
Back	1318±90^{c}	10.1±0.7^{c}	140±9^{c}	12.9±0.8^{c}	22.3±1.5^{c}	8.9±0.7^{c}
Upper hindleg	5772±260^{a}	44.3±1.3^{a}	496±22^{a}	45.7±1.4^{a}	102.0±4.6^{a}	40.6±1.7^{a}
Lower hindleg	1183±88^{c}	9.1±0.6^{c}	76±6^{d}	7.0±0.5^{d}	13.3±1.0^{c,d}	5.3±0.4^{c,d}
Tail	904±41^{c,d}	7.0±0.4^{c,d}	52±2^{d,e}	4.8±0.3^{d,e}	16.2±0.7^{c,d}	6.5±0.5^{c,d}
Total skeletal muscle	13327±270		1087±24		251.4±5.9	

Values are means ± S.E.M., N=4. In columns, significantly different values have different superscript letters (P<0.05).
V(mt,m) and V(c) values for the upper hindleg were derived from the mean densities of mitochondria and capillaries in the three muscles sampled from this region (Table 2).

aerobic capacity, i.e. a high $\dot{V}_{O_2max}$. This is reflected in the total body mitochondrial patterns (see Table 4), but also in V_V(mt,f) of comparable muscles such as the diaphragm (Hoppeler et al., 1987). This pattern of difference between athletic and sedentary placentals has been clearly demonstrated in several paired comparisons (Hoppeler and Weibel, 1998; Weibel et al., 1991; Weibel, 2000).

Across different regions of the body of red kangaroos variation in the V_V(mt,f) of muscles is obvious (Table 2). The muscles from the proximal parts of the body, the neck and foreleg had a significantly lower V_V(mt,f); those with significantly higher V_V(mt,f) were the large muscles of the back and trunk, the m. multifidi lumborum and m. erector spinae, together with the m. gluteus medius of the upper hindleg. However, other large muscles of the hindleg, such as the m. vastus lateralis, m. semitendinosus and m. gastrocnemius, had intermediate values. Such a variable pattern was also found by Hoppeler et al. (1981a) in two wild African bovids, wildebeest and dik-dik. The amount of mitochondria in a muscle presumably determines its longer term aerobic capacity rather than its total capacity to do work over short periods. Consequently, variation in V_V(mt,f) between muscles may reflect the mix of fibre types in muscles. Hoppeler et al. (1981a) found that fast-twitch-glycolytic fibres (type IIb) typically had low V_V(mt,f), about 1%, whereas in oxidative fibres, both slow-twitch-oxidative (type I) and fast-twitch-oxidative-glycolytic (type IIa) had V_V(mt,f) in the range 5–15%. Marsupial limb muscles also can express slow and fast myosin proteins, resulting in fibres of types I, IIa, IIx and IIb (Zhong et al., 2001). Dennington and Baldwin (1988) found that in the m. gastrocnemius of the western grey kangaroo (*Macropus fuliginosus*) a large majority of the muscle fibres were type IIa. The muscles of the trunk, back and hindleg of the red kangaroo which have higher V_V(mt,f) are similarly deep red in colour and presumably have large proportions of type IIa fibres. Data for the muscles of the foreleg and head and

Table 4. *Morphometry of total skeletal muscle of red kangaroo, goat, dog and pronghorn*

Parameter	Unit	Red kangaroo	Goat	Dog	Pronghorn
M_b	kg	28.5	27.7	28.2	28.4
M_m/M_b	%	46.8	26.0	37.0	43.8
$\dot{V}_{O_2max}$	ml O_2 min^{-1} kg^{-1}	178	57	137	272
Mitochondria					
V_V(mt,f)	%	8.2	4.1	8.6	10.6
V(mt,m)/M_b	ml kg^{-1}	38.2	10.0	29.7	46.2
$\dot{V}_{O_2max}/V_V$(mt,m)	ml O_2 min^{-1} ml^{-1}	4.7	5.7	4.6	5.9
Capillaries					
J(c)/M_b	km kg^{-1}	546	244	453	653
V(c)/M_b	ml kg^{-1}	8.9	3.9	7.2	10.4
Hct	%	47.5	29.9	50.3	45.6
V(ec)/M_b	ml kg^{-1}	4.20	1.16	3.63	4.74
$\dot{V}_{O_2max}/V$(ec)	ml O_2 min^{-1} ml^{-1}	42.4	49.1	37.7	57.4

V(mt,m)/M_b is the mass-specific mitochondrial volume, Hct is haematocrit and V(ec) is the capillary erythrocyte volume. Values are means.
For the red kangaroo, the $\dot{V}_{O_2max}$ value is from Kram and Dawson (1998) and Hct value is from Buffenstein et al. (2001); all other data are from the present study.
All values for placentals are from Weibel (2000).

neck are lacking, apart from the jaw-closing muscles, which express a relatively slow cardiac myosin (Hoh, 2002).

Because of their fundamental role in aerobic metabolism it was anticipated that mitochondria in muscles would have a matching supply of oxygen *via* a proportional volume of capillaries and that the capillary network of the body would match aerobic capacity, i.e. $\dot{V}_{O_2max}$. However, while previous studies with quadrupedal placental mammals have shown this to be broadly the case, there is much variability (Conley et al., 1987; Hoppeler et al., 1981b). A similar pattern also emerges in the red kangaroo (Table 2). The m. erector spinae and m. gluteus medius have a significantly higher volume of capillaries per g of muscle (V(c)/gM_m) but the large aerobic m. multifidi lumborum of the back has a V(c)/gM_m similar to the m. trapezius. There was only a trend toward a positive correlation between V(c)/gM_m and V_V(mt,f) for red kangaroo muscles (r=0.58, P=0.1) (Fig. 3B), though when expressed as capillary density the values overlapped closely the data of Hoppler et al. (1981b), who report a statistically significant correlation from a larger data set.

Per unit volume of muscle mitochondria, the capillary supply was on average 16 km of capillaries, or 0.27 ml of capillary blood; this was similar to that in muscles of several placental quadrupeds, the values being 14 km and 0.22 ml, respectively (Conley et al., 1987). However, in both kangaroo muscles and the muscles of the placentals the capillary supply is variable. For kangaroos the range was 11–29 km of capillaries and 0.17–0.47 ml of capillary blood per ml of mitochondria; the equivalent values for placentals being 17–25 km and 0.18–0.45 ml. In fact, for the kangaroo muscles there was a significant negative correlation between V(c)/V_V(mt,m) and V_V(mt,f) (Fig. 3A). The mitochondria in m. trapezius had the highest blood supply while that to the mitochondria in the large muscles of back and hindleg was significantly lower (Table 2). Conley et al. (1987) found a tendency for the muscles of aerobic species to have a lower capillary supply than those of less aerobic species. They explain this apparent paradox by the muscles of more aerobic (athletic) species being supplied with blood with a higher hematocrit, i.e. a higher hemoglobin concentration. This explanation would not seem to apply for the muscles in a single species. Does the answer lie in a modulation of the hematocrit as an animal increases its aerobic metabolism? As in placentals, the spleen in marsupials contains a large reserve of erythrocytes (Dawson and Denny, 1968). Could these be metered into the circulation as a mammal increases energetic output, such that when the large mitochondria-dense locomotor muscles are fully active they are supplied with hemoglobin-rich blood? An explanation based on differential fibre types (Hoppeler et al., 1981a) would not seem to be appropriate if the kangaroo locomotor muscles have few type IIb (fast-glycolytic) fibres.

How do these data on the mitochondrial and capillary characteristics of red kangaroo muscles translate into a picture of the aerobic capacity of the whole animal? Red kangaroo females shot in the field comprised 46.8±1.7% skeletal muscle. Values of 47–52% have been reported previously for red kangaroos; variability can occur due to variable fill of the large foregut (Table 1) and sexual dimorphism (Grand, 1990; Hopwood, 1981; Hopwood and Griffiths, 1984; Tribe and Peel, 1963). A value near 50% places kangaroos among the most muscular of mammals (Table 4) (Grand, 1990). The muscle mass of the red kangaroo is concentrated around the pelvis, particularly in the upper hindleg (Fig. 1; Table 3). Not only does the upper hindleg make up 44.3% of all skeletal muscle, when the trunk and back, which have their bulk posteriorly located (Fig. 1), are included some 80% of skeletal muscle is so positioned. This pattern does not just reflect the energetic needs of a kangaroo's hopping. During slow-speed locomotion, often called pentapedal locomotion because the tail is used as a 'fifth leg', the hindlegs are moved forward in unison when the body is supported by the tail and the forelegs. J. M. Donelan, S. Rodoreda, A. Grabowski, R. Kram and T. J. Dawson (unpublished observations) examined pentapedal locomotion and showed that the small forelegs act only as a brake, while the hindlegs and the tail successively provide the propulsive forces. Apparently the muscles of the tail and back provide the propulsive forces during both forms of kangaroo locomotion.

The volume of mitochondria in the body regions is primarily determined by regional muscle masses (Table 3). However, the upper hindleg, trunk and back regions also generally have high V_V(mt,f) and contain 86% of all skeletal muscle mitochondrial volume. Thus, the majority of the aerobic power output associated with locomotion will be generated in this region. By contrast, the foreleg has only 2% of the total muscle mitochondrial volume. The lower hindleg, with its large Achilles tendon, has been the focus of interest in relation to the elastic conservation of energy during hopping, but it contains only 7% of muscle mitochondria. The distribution of capillary blood broadly follows the distribution of muscle mass, with the trunk being more endowed with capillaries (Table 3). The V(c) of the lower hindleg and the foreleg are relatively low. Combining these regional data establishes the overall aerobic characteristics of the red kangaroo (Tables 3 and 4).

To examine the possible phylogenetic and adaptive influences on muscle morphometry in the red kangaroo, we compared our data and the $\dot{V}_{O_2max}$ data of Kram and Dawson (1998) with data from placental species of similar size (Weibel, 2000), both sedentary (goat) and athletic (dog and pronghorn) (Table 4). The use of animals of similar size removes effects due to allometry (Weibel et al., 1987). It is obvious that the marsupial red kangaroo closely resembles the dog and pronghorn in its $\dot{V}_{O_2max}$, muscle mass and the morphometry of its muscle mitochondria and capillaries (Table 4). However, the ratio of $\dot{V}_{O_2max}$ to V_V(mt,m) is similar for the red kangaroo and all the placental species, whether athletic or sedentary (Table 4). The close relationship between $\dot{V}_{O_2max}$ and total volume of skeletal muscle mitochondria holds for a broader range of placental mammals, including those of different sizes (Hoppeler et al., 1990), as well as the kangaroo (Fig. 4). The mitochondrial oxygen consumption at $\dot{V}_{O_2max}$ calculated for

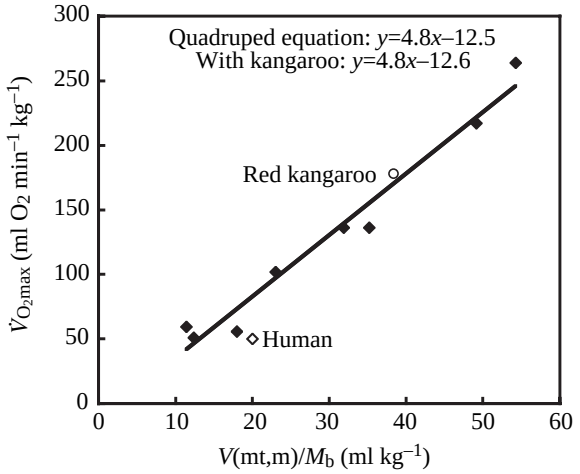

Fig. 4. The relationship between the total mitochondrial volume of skeletal muscle [V(mt,m)/M_b] and maximum aerobic capacity $\dot{V}_{O_2max}$ in a series of mammalian species. Values for red kangaroo and human are indicated. The values for human and other placental species are from Hoppeler (1990); value for red kangaroo is from Table 4. The addition of the red kangaroo data to the quadruped data does not significantly change the relationship between $\dot{V}_{O_2max}$ and V(mt,m)/M_b.

the red kangaroo was 4.7 ml O_2 min^{-1} ml^{-1} of mitochondria, which is the same as the average for placentals in general (Hoppeler, 1990; Hoppeler and Weibel, 1998). This feature is apparently conserved among therian mammals. Because S_V(im,mt) is the same in both therian groups (~35 m^2 ml^{-1} mitochondria), the maximum oxygen consumption per unit area of inner mitochondrial membrane appears also invariant at ~0.13 ml O_2 min^{-1} m^{-2}.

The whole body capillary volume of the red kangaroo is also related to $\dot{V}_{O_2max}$ in the manner seen in placental quadrupeds (Table 4). As indicated by Conley et al. (1987), to appreciate the full picture of the oxygen supply to muscle mitochondria the concentration of haemoglobin in the blood has to be considered. The difference in V(c)/M_b between sedentary species and athletic species (including the kangaroo) is not equivalent to the difference in $\dot{V}_{O_2max}$. However, when hematocrit during exercise is taken into account (Table 4) the capillary erythrocyte volume V(ec)/M_b matches the various levels of $\dot{V}_{O_2max}$. This is a pattern seen generally in placental mammals (Hoppeler and Weibel, 1998; Jones et al., 1989; Weibel et al., 1991). The heart is intimately involved with the flux of oxygen through the body and the red kangaroo's heart is also matched to a high $\dot{V}_{O_2max}$ in a manner comparable to that seen in placental mammals (Dawson et al., 2003).

Bipedal humans diverge most from the relationship between V_V(mt,m) and $\dot{V}_{O_2max}$ (Fig. 4). Humans are capable of reaching $\dot{V}_{O_2max}$ with a subset of their body musculature. Bergh et al. (1976) found that $\dot{V}_{O_2max}$ obtained by concurrent arm and leg work did not result in a $\dot{V}_{O_2max}$ higher than during running, and the $\dot{V}_{O_2max}$ was lower than would be predicted by adding separately measured arm and leg $\dot{V}_{O_2max}$. Unlike humans, the red kangaroo involves almost its whole musculature to achieve $\dot{V}_{O_2max}$ during exercise, despite its bipedal posture (Fig. 4).

In summary, the red kangaroo has characteristics fundamental to highly aerobic mammals. The heart is large and levels of muscle mitochondria, capillaries and hematocrit are essentially the same as those of athletic placental mammals like dogs, horses and pronghorn antelope (Weibel, 2000). These features support the symmorphosis model of Taylor and Weibel (1981), Weibel et al. (1991) and Weibel (2000). The red kangaroo has considerable muscle mass, about 50% of total body mass M_b, which is largely situated around the pelvis and the upper hindlegs. The locomotor force for both forms of locomotion, 'walking' and hopping, is provided by this region. Although its predominate gait is bipedal, and its locomotor characteristics are unusual (Dawson and Taylor, 1973), this marsupial seems to be just an extension of a basic mammalian skeletal/muscular energetic pattern. The evolution of such a pattern obviously predates the divergence of the marsupials and placentals before 125 million years ago. Of note, it has been suggested that the basic mammalian aerobic pattern was set with the mammal-like reptiles in the Triassic (220 million years ago), before the actual evolution of the mammals (Ruben, 1995). Recent findings regarding monotreme evolution (Musser, 2003) would support this contention, monotremes having heart and muscle mitochondrial characteristics which are essentially mammalian (Else and Hulbert, 1985b).

List of symbols and abbreviations

A(c)	capillary cross-sectional area
BMR	basal metabolic rate
c(K,0)	tortuosity of the capillary bed
d	density of muscle
Hct	haematocrit
J_V(c,f)	length density of capillaries
J(c)	capillary length
M_b	body mass
M_m	muscle mass
N_A(c,f)	numerical density of capillaries
r_c	capillary radius
S(im,m)	surface area of inner mitochondrial membranes
S_V(im,mt)	surface density of inner mitochondrial membranes per volume of mitochondria
V(c)	capillary volume
V(c)/gM_m	capillary volume per unit of muscle mass
V(ec)	capillary erythrocyte volume
V(mt,f)	mitochondrial volume density
V(mt,m)	mitochondrial volume
$\dot{V}_{O_2max}$	maximal rate of oxygen consumption
V_V(f,m)	proportion of muscle volume taken up by muscle fibres
V_V(mt,f)	mitochondrial volume fraction

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References

Baudinette, R. V., Snyder, G. K. and Frappell, P. B. (1992). Energetic cost of locomotion in the tammar wallaby. *Am. J. Physiol.* **262**, R771-R778.

Bennett, M. B. and Taylor, G. C. (1995). Scaling of elastic strain energy and the benefits of being big. *Nature* **378**, 56-59.

Bergh, U., Kanstrup, I. and Ekblom, B. (1976). Maximal oxygen uptake with various combinations of arm and leg work. *J. Appl. Physiol.* **41**, 191-196.

Buffenstein, R., McCarron, H. C. K. and Dawson T. J. (2001). Erythrocyte osmotic fragility of red (*Macropus rufus*) and grey (*Macropus fuliginosus* and *Macropus giganteus*) kangaroos and free-ranging sheep of the arid regions of Australia. *J. Comp. Physiol. B* **171**, 47-47.

Conley, K. E., Kayar, S. R., Roesler, K., Hoppeler, H., Weibel, E. R. and Taylor, C. R. (1987). Adaptive variation in the mammalian respiratory system in relation to energetic demand: IV. Capillaries and their relationship to oxidative capacity. *Respir. Physiol.* **69**, 47-64.

Cooper, C. E. and Withers, P. C. (2003). Ventilatory physiology of the numbat (*Myrmecobius fasciatus*). *J. Comp. Physiol. B* **174**, 107-111.

Dawson, T. J. (1989). Responses to cold of monotremes and marsupials. In *Adaptation of Vertebrates to Cold. Advances in Comparative and Environmental Physiology* (ed. L. C. Wang), pp. 255-288. Berlin: Springer-Verlag.

Dawson, T. J. (1995). *Kangaroos: The Biology of the Largest Marsupial.* Sydney: University of New South Wales.

Dawson, T. J. and Dawson, W. R. (1982). Metabolic scope and conductance in response to cold of some dasyurid marsupials and Australian rodents. *Comp. Biochem. Physiol.* **71A**, 59-64.

Dawson, T. J. and Denny, M. J. S. (1968). Influence of the spleen on blood volume and haematocrit in the brush-tailed possum (*Trichosurus vulpecula*). *Aust. J. Zool.* **16**, 603-608.

Dawson, T. J. and Hulbert, A. J. (1970). Standard metabolism, body temperature, and surface areas of Australian marsupials. *Am. J Physiol.* **218**, 1233-1238.

Dawson, T. J. and Needham, A. D. (1981). Cardiovascular characteristics of two resting marsupials: an insight into the cardio-respiratory allometry of marsupials. *J. Comp. Physiol.* **145**, 95-100.

Dawson, T. J. and Taylor, C. R. (1973). Energetic cost of locomotion in kangaroos. *Nature* **246**, 313-314.

Dawson, T. J., Webster, K. N., Mifsud, B., Raad, E., Lee, E. and Needham, A. D. (2003). Functional capacities of marsupial hearts: size and mitochondrial parameters indicate higher aerobic capabilities than generally seen in placental mammals. *J. Comp. Physiol. B* **173**, 583-590.

Dennington, S. and Baldwin, J. (1988). Biochemical correlates of energy metabolism in muscles used to power hopping by kangaroos. *Aust. J. Zool.* **36**, 229-240.

Else, P. L. and Hulbert, A. J. (1981). Comparison of the 'mammal machine' and the 'reptile machine': energy production. *Am. J. Physiol.* **240**, R3-R9.

Else, P. L. and Hulbert, A. J. (1983). A comparative study of the metabolic capacity of hearts from reptiles and mammals. *Comp. Biochem. Physiol.* **76A**, 553-557.

Else, P. L. and Hulbert, A. J. (1985a). An allometric study of the mitochondria of mammalian and reptilian tissues: The implications for the evolution of endothermy. *J. Comp. Physiol. B* **156,** 3-11.

Else, P. L. and Hulbert, A. J. (1985b). Mammals: an allometric study of metabolism at tissue and mitochondrial level. *Am. J. Physiol.* **248**, R415-R421.

Gehr, P., Mwangi, D. K., Ammann, A., Maloiy, G. M. O., Taylor, C. R. and Weibel, E. R. (1981). Design of the mammalian respiratory system. V. Scaling morphometric pulmonary diffusing capacity to body mass: wild and domestic animals. *Respir. Physiol.* **44**, 61-86.

Grand, T. I. (1990). Body composition and the evolution of the Macropodidae (*Potorous*, *Dendrolagus*, and *Macropus*). *Anat. Embryol.* **182**, 85-92.

Hinds, D. S., Baudinette, R. V., MacMillen, R. E. and Halpern, E. A. (1993). Maximal metabolism and the aerobic factorial scope of endotherms. *J. Exp. Biol.* **182**, 41-56.

Hoh, J. Y. (2002). 'Superfast' or masticatory myosin and the evolution of jaw-closing muscles of vertebrates. *J. Exp. Biol.* **205**, 2203-2210.

Hoppeler, H. (1990). The different relationship of $\dot{V}_{O_2max}$ to muscle mitochondria in humans and quadrupedal animals. *Respir. Physiol.* **80**, 137-146.

Hoppeler, H., Kayar, S. R., Classen, H., Uhlmann, E. and Karas, R. H. (1987). Adaptive variation in the mammalian respiratory system in relation to energetic demand: III. Skeletal muscles: setting the demand for oxygen. *Respir. Physiol.* **69**, 27-46.

Hoppeler, H. and Lindstedt, S. L. (1985). Malleability of skeletal muscle in overcoming limitations: structural elements. *J. Exp. Biol.* **115**, 355-364.

Hoppeler, H., Lindstedt, S. L., Uhlmann, E., Niesel, A., Cruz-Orive, L. M. and Weibel, E. R. (1984). Oxygen consumption and the composition of skeletal muscle tissue after training and inactivation in the European woodmouse (*Apodemus sylvaticus*). *J. Comp. Physiol. B* **155**, 51-61.

Hoppeler, H., Mathieu, O., Krauer, R., Classen, H., Armstrong, R. B. and Weibel, E. R. (1981a). Design of the mammalian respiratory system. VI. Distribution of mitochondria and capillaries in various muscles. *Respir. Physiol.* **44**, 87-111.

Hoppeler, H., Mathieu, O., Weibel, E. R., Krauer, R., Lindstedt, S. L. and Taylor, C. R. (1981b). Design of the mammalian respiratory system. VIII. Capillaries in skeletal muscles. *Respir. Physiol.* **44**, 129-150.

Hoppeler, H. and Weibel, E. R. (1998). Limits for oxygen and substrate transport in mammals. *J. Exp. Biol.* **201**, 1051-1064.

Hoppeler, H. and Weibel, E. R. (2000). Structural and functional limits for oxygen supply to muscle. *Acta Physiol. Scand.* **168**, 445-456.

Hopwood, P. R. (1974). The intrinsic musculature of the pectoral limb of the eastern grey kangaroo (*Macropus major* (Shaw) *Macropus giganteus* (Zimm). *J. Anat.* **118**, 445-468.

Hopwood, P. R. (1976). The quantitative anatomy of the kangaroo. PhD thesis, University of Sydney, Australia.

Hopwood, P. R. (1981). Carcass muscle weight distribution and yield: a comparison between the grey kangaroo *Macropus giganteus*, and red kangaroos, *M. rufus. Aust. Wildl. Res.* **8**, 263-268.

Hopwood, P. R. and Butterfield, R. M. (1976). The musculature of the proximal pelvic limb of the eastern grey kangaroo *Macropus major* (Shaw) *Macropus giganteus* (Zimm). *J. Anat.* **121**, 259-277.

Hopwood, P. R. and Butterfield, R. M. (1990). The locomotor apparatus of the crus and pes of the Eastern Grey Kangaroo, *Macropus giganteus*. *Aust. J. Zool.* **38**, 397-413.

Hopwood, P. R. and Griffiths, D. A. (1984). Carcass muscle weight distribution and yield: a comparison between male and female grey kangaroos, *Macropus giganteus*. *Aust. Wildl. Res.* **11**, 299-302

Howard, C. V. and Reed, M. G. (1998). *Unbiased Stereology: Three-Dimensional Measurement in Microscopy*. Oxford: BIOS Scientific Publishers Ltd.

Ji, Q., Luo, X., Yuan, C.-X., Wible, J. R., Zhang, J.-P. and Georgi, J. A. (2002). The earliest known placental mammal. *Nature* **398**, 326-330.

Jones, J. H., Longworth, K. E., Lindholm, A., Conley, K. E., Karas, R. H., Kayar, S. R. and Taylor, C. R. (1989). Oxygen transport during exercise in large mammals. I. Adaptive variation in oxygen demand. *J. Appl. Physiol.* **67**, 862-870.

Kayar, S. R., Hoppeler, H., Lindstedt, S. L., Claassen, H., Jones, J. H., Essen-Gustavsson, B. and Taylor, C. R. (1989). Total muscle mitochondrial volume in relation to aerobic capacity of horses and steers. *Pflüg. Arch.* **413**, 343-347.

Kinnear, J. E. and Brown, G. D. (1967). Minimum heart rates of marsupials. *Nature* **215**, 1501.

Kram, R. and Dawson, T. J. (1998). Energetics and biomechanics of locomotion by red kangaroos (*Macropus rufus*). *Comp. Biochem. Physiol.* **120B**, 41-49.

Mathieu, O., Cruz-Orize, L.-M., Hoppeler, H. and Weibel, E. R. (1983). Estimating length density and quantifying anisotropy in skeletal muscle capillaries. *J. Microsc.* **131**, 131-146.

Mathieu, O., Krauer, R., Hoppeler, H., Gehr, P., Lindstedt, S. L., Alexander, R. M., Taylor, C. R. and Weibel, E. R. (1981). Design of the mammalian respiratory system. VII. Scaling mitochondrial volume in skeletal muscle to body mass. *Respir. Physiol.* **44**, 113-128.

Mendez, J. and Keys, A. (1960). Density and composition of mammalian muscle. *Metabolism* **9**, 184-188.

Musser, A. M. (2003). Review of the monotreme fossil record and a comparison of palaeontological and molecular data. *Comp. Biochem. Physiol.* **136A**, 927-942.

Ruben, J. (1995). The evolution of endothermy in mammals and birds: from physiology to fossils. *Annu. Rev. Physiol.* **57**, 69-95.

Schwerzmann, K., Hoppeler, H., Kayar, S. R. and Weibel, E. R. (1989). Oxidative capacity of muscle and mitochondria: Correlation of physiological, biochemical, and morphometric characteristics. *Proc. Natl. Acad. Sci. USA* **86**, 1583-1587.

Suarez, R. K. (1996). Upper limits to mass-specific metabolic rates. *Annu. Rev. Physiol.* **58**, 583-605.

Taylor, C. R., Maloiy, G. M. O., Weibel, E. R., Langman, V. A., Kamau, J. M. Z., Seeherman, H. J. and Heglund, N. C. (1981). Design of the mammalian respiratory system. III. Scaling maximum aerobic capacity to body mass: wild and domestic mammals. *Respir. Physiol.* **44**, 25-37.

Taylor, C. R. and Weibel, E. R. (1981). Design of the mammalian respiratory system. I. Problem and strategy. *Respir. Physiol.* **44**, 1-10.

Tribe, D. E. and Peel, L. (1963). Body composition of the kangaroo (*Macropus* sp.) *Aust. J. Zool.* **11**, 273-289.

Webster, K. N. (2003). Locomotory performance and aerobic capacity of the small hopping marsupial, *Bettongia penicillata*. PhD thesis, University of New South Wales (Sydney), Australia.

Webster, K. N. and Dawson, T. J. (2003). Locomotion energetics and gait characterisitics of a rat-kangaroo, *Bettongia penicillata*, have some kangaroo-like features. *J. Comp. Physiol.* **173**, 549-557.

Weibel, E. R. (2000). *Symmorphosis: On Form and Function in Shaping Life.* Cambridge, Massachusetts: Harvard University Press.

Weibel, E. R., Taylor, C. R. and Hoppeler, H. (1991). The concept of symmorphosis: a testable hypothesis of structure-function relationship. *Proc. Natl. Acad. Sci. USA* **88**, 10357-10361.

Weibel, E. R., Taylor, C. R., Hoppeler, H. and Karas, R. H. (1987). Adaptive variation in the mammalian respiratory system in relation to energetic demand: I Introduction to problem and strategy. *Respir. Physiol.* **69**, 1-6.

Withers, P. C., Thompson, G. G. and Seymour, R. S. (2000). Metabolic physiology of the northeastern marsupial mole, *Notoryctes caurinus* (Marsupialia: Notoryctidae). *Aust. J. Zool.* **48**, 241-258.

Zhong, W. W. H., Lucas, C. A., Kang, L. H. D. and Hoh, J. F. Y. (2001). Electrophoretic and immunochemical evidence showing that marsupial limb muscles express the same fast and slow myosin heavy chains as eutherians. *Electrophoresis* **22**, 1016-1020.