PHYSIOLOGY

# Skeletal muscle thermogenesis enables aquatic life in the smallest marine mammal

Traver Wright[1,2]*, Randall W. Davis[3], Heidi C. Pearson[4,5], Michael Murray[6], Melinda Sheffield-Moore[1,2]

Basal metabolic rate generally scales with body mass in mammals, and variation from predicted levels indicates adaptive metabolic remodeling. As a thermogenic adaptation for living in cool water, sea otters have a basal metabolic rate approximately three times that of the predicted rate; however, the tissue-level source of this hypermetabolism is unknown. Because skeletal muscle is a major determinant of whole-body metabolism, we characterized respiratory capacity and thermogenic leak in sea otter muscle. Compared with that of previously sampled mammals, thermogenic muscle leak capacity was elevated and could account for sea otter hypermetabolism. Muscle respiratory capacity was modestly elevated and reached adult levels in neonates. Premature metabolic development and high leak rate indicate that sea otter muscle metabolism is regulated by thermogenic demand and is the source of basal hypermetabolism.

Sea otters (*Enhydra lutris*) are the smallest of all marine mammals. Their small size, combined with the high thermal conductivity of water (23 times that of air) and the cold water temperatures of their North Pacific habitat (0° to 15°C), imposes a thermoregulatory challenge to maintain a core body temperature of 37°C. Unlike other marine mammals that have subcutaneous blubber for thermal insulation, sea otters rely on air trapped in their dense fur (the highest hair density of any mammal) for insulation (*1*). However, this is not adequate to offset heat loss, so an increase in metabolic heat production is required to maintain a stable core body temperature. As a result, sea otters have a basal metabolic rate (BMR) approximately three times that predicted for a eutherian mammal of similar size (*1*, *2*).

In endothermic mammals, BMR includes both the metabolism needed to produce energy for basic physiological functions and that necessary to maintain a constant core body temperature. Although BMR generally scales with body mass (i.e., smaller mammals have a greater mass-specific metabolic rate), deviation from the mass-predicted rate can indicate ecologically-driven adaptive metabolic remodeling (e.g., increased heat production in response to low ambient temperature) (*3*). Polar and small-bodied marine mammals are particularly vulnerable to heat loss and require increased heat production to maintain body temperature (*2*, *4*, *5*). This thermogenic hypermetabolism in thermally challenged polar mammals appears to be a result of reduced tissue-level mitochondrial efficiency, which may explain the deviation from predicted metabolic scaling (*6*).

Whole-body BMR emerges as an aggregate of individual tissue metabolism (*7*). Because of its large relative mass and high metabolic capacity, skeletal muscle is an important determinant of BMR and endothermy in mammals (*8*). As a result, skeletal muscle is the primary target of adaptive metabolic remodeling in animals, and deviation from the predicted BMR in endotherms primarily reflects changes to muscle mass and activity (*9*). Within muscle, heat production occurs either as a by-product of contractile work [adenosine triphosphate (ATP)–driven functional contraction or shivering] or directly via nonshivering thermogenesis from mitochondrial proton leak and other "futile" ion cycling (*8*). To better understand thermogenic hypermetabolism in cold-adapted mammals, we characterized sea otter muscle metabolism. Given the elevated thermogenic BMR of sea otters and the adaptive metabolic capacity of muscle, we hypothesized that sea otter skeletal muscle would have a high capacity for thermogenic mitochondrial proton leak, which may explain their elevated BMR. Using sea otters as an extreme example of thermogenic hypermetabolism, we explored how tissue-level metabolic plasticity enables homeotherms to inhabit thermally challenging cold environments.

At the cellular level, aerobic metabolism occurs within the mitochondria where oxygen is consumed in the final step of the electron-transfer system. Energy derived primarily from the breakdown of lipids and carbohydrates is used to pump protons across the inner mitochondrial membrane and establish a proton gradient. The energy released by allowing this proton gradient to dissipate back across the membrane can be either coupled to ATP generation to store energy within the cell, or uncoupled via proton leak, which produces metabolic heat without producing ATP to power functional work (*10*).

Using high-resolution respirometry, we measured respiratory capacity in cranial tibial skeletal muscle samples taken from both northern and southern sea otters encompassing a broad range of body mass (1.4 to 44.5 kg) and representing age classes from neonate to adult (table S1). Steady-state oxygen flux was measured at five induced respiratory states (detailed in table S2), including the three primary states of LEAK (native mitochondrial proton leak), OXPHOS (native proton leak in addition to oxidative phosphorylation), and ETS (maximal flux through the electron-transport system), which reflects mitochondrial density (*11*) [see supplementary materials for detailed methods and Texas Data Repository for DatLab respirometry data files (*12*)].

We found that OXPHOS and ETS respiratory capacities in sea otter skeletal muscle are modestly elevated compared with values measured in the muscle of other active mammals but do not reach the extreme levels observed in some elite performance animals such as Alaskan husky Iditarod dogs (Table 1 and table S1). The 15.7% additional ETS capacity beyond OXPHOS results in a flux control

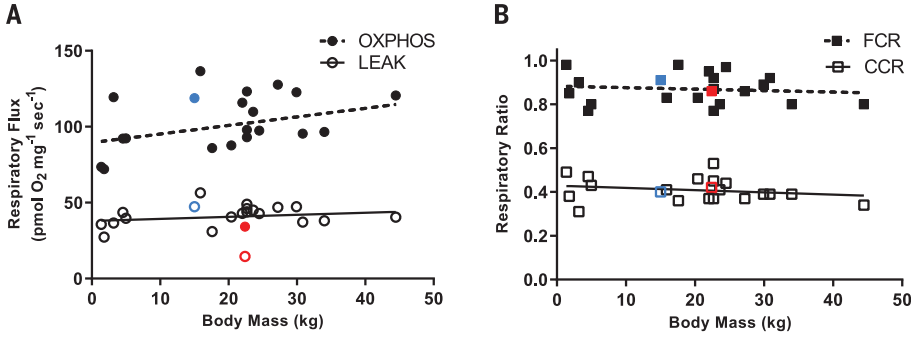


**Fig. 1. Sea otter skeletal muscle respiratory flux.** (**A**) In individuals ranging from 1.4 to 44.5 kg, body mass was not predictive of OXPHOS or LEAK respiratory capacity. (**B**) Body mass was not predictive of CCR (LEAK/OXPHOS) or FCR (OXPHOS/ETS). Respiratory capacity in a rehabilitated, captive-raised individual (infancy to adulthood) (blue) was indistinguishable from that of wild conspecifics but was drastically reduced in one emaciated, stranded adult (red).

[1]Department of Health and Kinesiology, Texas A&M University, College Station, TX, USA. [2]Department of Internal Medicine, University of Texas Medical Branch, Galveston, TX, USA. [3]Department of Marine Biology, Texas A&M University at Galveston, Galveston, TX, USA. [4]Department of Natural Sciences, University of Alaska Southeast, Juneau, AK, USA. [5]College of Fisheries and Ocean Sciences, University of Alaska Fairbanks, Juneau, AK, USA. [6]Monterey Bay Aquarium, Monterey, CA, USA.
*Corresponding author. Email: traywright@gmail.com

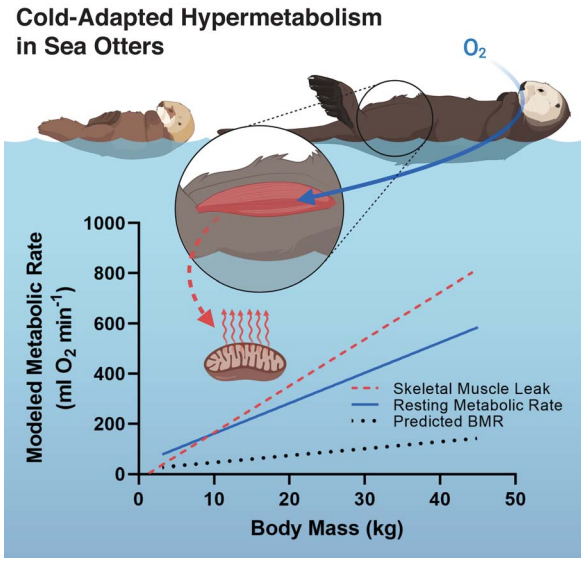


**Fig. 2. Whole-body estimates of sea otter metabolism scaled to body mass.** Estimated scaled whole-body metabolic rates include predicted [BMR; predicted on the basis of scaled rate for eutherian mammals (*3*)], sea otter resting metabolic rate, and whole-body skeletal muscle leak capacity.

ratio (FCR; OXPHOS/ETS) of 0.87 (Table 1 and table S1), indicating that phosphorylation capacity limits maximum OXPHOS respiration (*13*). The LEAK respiratory capacity in sea otter skeletal muscle resulted in a coupling control ratio (CCR; LEAK/OXPHOS) of 0.41 (Table 1 and table S1), indicating that leak respiratory capacity accounts for up to 41% of OXPHOS capacity. This mitochondrial LEAK capacity is elevated compared with that of other mammals (Table 1); absolute LEAK capacity and CCR are, respectively, two and four times that of comparably sized Alaskan husky Iditarod dogs. These values are also higher than comparable measures reported for any known mammal, excluding extremely small mice species with body mass ranging from 5 to 22 g (*14*). Increased thermogenic mitochondrial leak is predicted for polar animals relying on metabolic heat production to maintain body temperature (*6*) and is described here in sea otter skeletal muscle.

Because of the scaling of surface area to body mass, smaller neonatal mammals are particularly susceptible to heat loss (*4*, *15*). In addition, metabolically active skeletal muscle constitutes a smaller relative portion of total body mass in younger, less physically developed individuals (*16*), and their immature skeletal muscle tends to be less thermogenic (*17*). Sea otters are altricial; born relatively inactive, they depend on their mothers for feeding and grooming and become progressively more active as they mature during the first 3 months of life (*18*, *19*). Given the broad ranges of body mass and age classes included in our data, we determined how sea otter skeletal muscle respiratory capacity changed throughout ontogenetic development.

Respiratory capacity was surprisingly consistent across all age classes, and Pearson correlation indicated that body mass was not correlated with respiratory measures of LEAK ($r$ = 0.172; $P$ = 0.457; Fig. 1A), OXPHOS ($r$ = 0.274; $P$ = 0.229; Fig. 1A), or ETS ($r$ = 0.294; $P$ = 0.197). Body mass was likewise not predictive of FCR ($r$ = −0.112; $P$ = 0.628) or CCR ($r$ = −0.220; $P$ = 0.339) (Fig. 1B). In altricial mammals, skeletal muscle is underdeveloped at birth and is delayed in developing adult measures of mechanical function (*20*) as well as metabolic and thermogenic capacity (*21*). However, sea otter muscle metabolic capacity appears fully developed in even the smallest neonates, although other measures of muscle maturity, including muscle mass and myoglobin concentration, do not reach adult levels until animals are ~2 years of age (*16*). The consistent skeletal muscle mitochondrial leak capacity and CCR across a broad range of body mass and age classes indicate that mitochondrial leak does not constitute a greater portion of total OXPHOS capacity in smaller, less mature individuals as might be predicted.

Samples collected from two animals in unusual circumstances provided additional insights. Included in our study was one captive-raised, rehabilitated juvenile. This individual was housed using a flow-through system of water from its natural habitat, ensuring an endemic temperature range. Despite reduced functional demand on skeletal muscle for foraging, diving, and swimming, this sea otter was metabolically indistinguishable from its wild counterparts (Fig. 1, A and B; blue). This is consistent with a skeletal muscle metabolic capacity that is modulated by thermogenic demand and not by contractile muscle load. Other thermally challenged marine mammals, including Weddell seals (*Leptonychotes weddellii*) (*22*) and northern elephant seals (*Mirounga angustirostris*) (*23*), also demonstrate similarly high neonatal muscle metabolic capacity (*5*).

One stranded, emaciated adult was an exception to the consistent high metabolic capacity seen in this study. Owing to its extensive dental wear, this individual was estimated to be >12 years of age; it was dehydrated and emaciated at the time of stranding, which suggests that it may have had difficulty feeding. This geriatric sea otter demonstrated a universally profound reduction in skeletal muscle metabolic capacity, with OXPHOS and LEAK respiratory capacity only 34% and 36% of the average value, respectively (Fig. 1A, red). Despite the metabolic disturbance, LEAK, OXPHOS, and ETS respiratory capacities were uniformly reduced and respiratory ratios of FCR and CCR remained constant (Fig. 1B, red). Although the cause of the emaciation and stranding cannot be deduced, metabolic factors may have affected thermogenic capacity and contributed to stranding.

To estimate the potential metabolic contribution of skeletal muscle to sea otter resting metabolic rate and thermogenesis throughout life, we combined our measures of skeletal muscle leak capacity with published measures of scaled sea otter muscle mass to determine whole-body skeletal muscle leak capacity (Fig. 2; see supplementary methods for calculations). In sea otters with body mass greater than ~9 kg, skeletal muscle leak capacity exceeds whole-body resting oxygen consumption. However, at less than ~9 kg, skeletal muscle leak is not independently adequate to fully account for the resting metabolic rate owing to the lower relative muscle mass. For smaller animals to maintain body temperature, maximal use of thermogenic leak from skeletal muscle and other tissues may be critical. Smaller animals may also require additional muscle thermogenesis via either shivering or sarcoplasmic reticulum calcium leak to use a greater portion of skeletal muscle respiratory capacity (fig. S1). Notably, neonatal pups have dense natal fur and spend most of their time resting on the mother's abdomen, so there is little heat loss to water until the age of 1 month (*24*).

Thermogenic leak metabolic capacity in sea otter muscle is elevated across all age and size classes and, if extrapolated to whole-body muscle mass, is adequate to explain the elevated resting metabolic rate of adult sea otters. However, smaller, less mature individuals with lower relative muscle mass require either non-leak muscle thermogenesis (e.g., shivering, active grooming) or other metabolically active tissues to account for their elevated resting metabolism. Although mass-specific LEAK respiratory capacity does not change throughout ontogenetic development in sea otter skeletal muscle (Fig. 1A), skeletal muscle leak capacity constitutes a greater portion of resting metabolic rate in larger animals owing to larger relative muscle mass (Fig. 2).

**Table 1. Skeletal muscle respiratory capacity in various mammals.** Respiratory flux (picomoles $O_2$ per second per milligram) includes similarly derived measures of LEAK, OXPHOS, and ETS. Leak respiration was determined using substrates without adenylates. In the current study, LEAK was induced with both mitochondrial complex I and complex II substrates, whereas others included only complex I substrate. Respiratory ratios of CCR (LEAK/OXPHOS) and FCR (OXPHOS/ETS) were reported directly in the cited study or calculated on the basis of reported respiratory measures. Respiratory measures from the current study represent the combined average of all age classes from both northern and southern subspecies. Measures for American quarter horses were estimated from published graphs. For Alaskan huskies, humans, mice, and rats, CCR and FCR were calculated from published respiratory measures.

| Condition | LEAK | OXPHOS | ETS | CCR | FCR | Reference |
|---|---|---|---|---|---|---|
| *Sea otter (cranial tibial)* | | | | | | |
| Average | 40.6 | 100.6 | 116.4 | 0.408 | 0.87 | Current study |
| *Northern elephant seal (longissimus dorsi)* | | | | | | |
| Pre-molt females | 13.0 | 100.5 | 116.5 | 0.13 | 0.87 | (*28*) |
| *American quarter horse (triceps brachii)* | | | | | | |
| Young | 6 | 85 | 115 | 0.068 | 0.74 | (*29*) |
| Old | 6.5 | 80 | 116 | 0.075 | 0.68 | (*29*) |
| *Alaskan huskies (biceps femoris)* | | | | | | |
| Raced | 14 | 245 | 253 | 0.057 | 0.97 | (*30*) |
| Nonraced | 24 | 237 | 254 | 0.101 | 0.93 | (*30*) |
| *Human, young healthy male (vastus lateralis)* | | | | | | |
| Untrained | 10.7 | 54.5 | 64.0 | 0.196 | 0.85 | (*31*) |
| Trained | 21.8 | 75.6 | 104.4 | 0.288 | 0.72 | (*31*) |
| *Mouse, male BALB/c (spinotrapezius)* | | | | | | |
| Sham | 33.9 | 63.9 | 69.7 | 0.531 | 0.92 | (*32*) |
| *Rat - male Long-Evans (soleus)* | | | | | | |
| Control | 5.8 | 101.7 | 110.8 | 0.057 | 0.92 | (*33*) |

As a group, carnivorous marine mammals (e.g., whales, dolphins, and seals) have a 2.3-fold increase in BMR relative to the allometric prediction for eutherian mammals (*2*). This consistently elevated BMR is noteworthy given that this group evolved from different phylogenetic orders (Carnivora and Artiodactyla) to live in differing marine habitats. In comparison, the mean BMR for six other species of terrestrial and semiaquatic Mustelidae (mean body mass: 1.8 kg), including river otters, is 1.6 times greater than the allometric prediction (*3*), which is less than that of fully aquatic sea otters. Only shrews (*Sorex sp.*; mean body mass 5.6 g) have an elevated BMR (3.1 times higher than the allometric prediction) comparable to that of sea otters. Hence, the high BMR in sea otters is not exclusive, but it is the highest reported for any mammal with body mass >1 kg.

For homeothermic mammals, basal metabolism and tissue-level muscle metabolism are each shaped by both environmental acclimation and evolutionary adaptation (*25*–*27*). Although other tissues may also contribute to sea otter thermogenesis (including brown adipose tissue that is not well documented in sea otters), we show here that skeletal muscle leak capacity alone is adequate to account for adult sea otter hypermetabolism. Sea otter skeletal muscle metabolic capacity is indistinguishable between adults and neonates, but it is not clear if this development occurs in utero (precocial) or rapidly after birth (precocious). Premature development of skeletal muscle metabolic capacity combined with high thermogenic capacity for mitochondrial leak throughout life suggests that thermogenesis is a fundamental determinant in the development and lifelong regulation of sea otter muscle metabolism in response to the thermal demands of the marine environment. Some combination of adaptive and acclimative forces shapes sea otter skeletal muscle for thermogenic hypermetabolism.

The generally elevated BMR in most marine mammals indicates similar metabolic remodeling as an adaptation for aquatic life. Instead of the strict allometric relationship between BMR and body mass historically ascribed to mammals (as well as birds), our results indicate that metabolic plasticity may be fundamental for mammals to adapt to varied thermal environments for enhanced efficiency, performance, and fitness. Enhanced thermogenesis may have been a critical adaptation enabling the terrestrial ancestors of marine mammals to overcome the thermoregulatory challenges of aquatic life and inhabit the world's oceans.

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## SUPPLEMENTARY MATERIALS

science.sciencemag.org/content/373/6551/223/suppl/DC1
Materials and Methods
Fig. S1
Tables S1 and S2
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View/request a protocol for this paper from *Bio-protocol*.

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**Keeping warm when small**

Several mammal species live in cold-water environments, enabled by adaptations such as blubber and large size. A notable exception to this rule is the sea otter, a species that is orders of magnitude smaller and skinnier than the others. It is known that the sea otter's unusually thick fur helps, but Wright *et al.* show that they are also internally warmed by thermogenic leak from skeletal muscle, a process that elevates their metabolic rate three times above that expected for their size. This mechanism is present even in infants with immature muscles, providing these animals with internal warmth from birth.

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