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Review

Exercise-induced maximal metabolic rate scales with muscle aerobic capacity

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Summary

The logarithmic nature of the allometric equation suggests that metabolic rate scaling is related to some fractal properties of the organism. Two universal models have been proposed, based on (1) the fractal design of the vasculature and (2) the fractal nature of the 'total effective surface' of mitochondria and capillaries. According to these models, basal and maximal metabolic rates must scale as $M^{3/4}$. This is not what we find. In 34 eutherian mammalian species (body mass M_b ranging from 7 g to 500 kg) we found $\dot{V}_{O2max}$ to scale with the 0.872 (±0.029) power of body mass, which is significantly different from 3/4 power scaling. Integrated structure–function studies on a subset of eleven species (M_b 20 g to 450 kg) show that the variation of $\dot{V}_{O2max}$ with body size is tightly associated with the total volume of mitochondria and of the locomotor musculature capillaries. In athletic species the higher $\dot{V}_{O2max}$ is linked to proportionally larger mitochondrial and capillary volumes. As a result, $\dot{V}_{O2max}$ is linearly related to both total mitochondrial and capillary erythrocyte volumes, as well as to their surface areas. Consequently, the allometric variation of maximal metabolic rate is directly related to the scaling of the total effective surfaces of mitochondria and capillaries, thus confirming the basic conjecture of the second fractal models but refuting the arguments for 3/4 power scaling. We conclude that the scaling of maximal metabolic rate is determined by the energy needs of the cells active during maximal work. The vascular supply network is adapted to the needs of the cells at their working limit. We conjecture that the optimization of the arterial tree by fractal design is the result rather than the cause of the evolution of metabolic rate scaling. The remaining question is why the energy needs of locomotion scale with the 0.872 or 7/8 power of body mass.

Key words: metabolic rate, scaling, locomotor muscle, aerobic capacity, mitochondria, capillary, fractal design, vascular supply network, energy demand.

Maximal metabolic rate

Maximal metabolic rate (MMR) is elicited by a well-defined dominant process: the energy needs of locomotor activity at its sustainable maximum during a run. In contrast, other forms of MR have a more complex origin. Basal metabolic rate (BMR) reflects the lowest need of energy at rest in a postprandial state under thermoneutral conditions. It is the energy used to run basic cell functions in a large variety of organs, such as maintaining cell potentials, driving the heart and circulation, synthetic and housekeeping functions of all sorts, and maintenance of body temperature. BMR and MMR thus define the span over which the metabolic rate of the organism can vary. Their definitions are unambiguous and, as such, they are reproducible. They reflect the performance of the organism under these specific test conditions, but are not conditions that occur usually in life. Natural forms of MR are intermediate. Field metabolic rate (FMR) reflects the time-averaged MR elicited by all possible organismic functions at variable levels of activity, such as foraging or hunting for food, or digestion, and is commonly found to be about 4 times BMR. What is termed sustained MR refers to the metabolic rate that allows for maintaining body mass over longer time; it is a form of FMR mostly related to nutrient supply.

Whereas BMR appears to depend essentially on body mass, MMR shows large inter-individual and inter-species variability, related to the degree of work or exercise capacity. It is typically about tenfold higher than BMR. Well-trained human athletes can achieve MMR up to 20 times higher than their BMR. Even greater variation is found in animals. Athletic species such as dogs or horses increase their MR by 30-fold from rest to maximal exercise, and in race horses this factor can rise to 50, as in the pronghorn antelope, the world's top athletic mammal.

MMR reflects the limitation of oxidative metabolism of muscle cells. It is not sustainable for more than a few minutes because, under these limiting conditions, the incipient aerobic energy deficit must be covered by anaerobic glycolysis, which leads to lactate accumulation in the blood and thus to cessation of exercise.

All forms of MR in mammals and other taxa show some scaling with body mass M_b according to the allometric

equation $MR \propto aM_b^b$. One of the tasks of experimental biology is to sort out the effects of body size and of other determinants, such as athletic prowess, on this relationship, separating variations in the exponent b and in the coefficient a. This will be considered in setting up models for this analysis.

Models and hypotheses of MR scaling

When Max Kleiber stated that the standard metabolic rate of mammals scaled '*close to the three-fourth power of body mass*' (Kleiber, 1947), he argued on the basis of empirical studies in which he had first found a scaling exponent of 0.739±0.03 (Kleiber, 1932), and later one of 0.756±0.004. He suggested that 3/4 would be easier to remember. This was not meant to be 'Kleiber's law', as it is now often called, but rather an empirical observation that was confirmed repeatedly and eventually broadly accepted as a fact (Schmidt-Nielsen, 1984). Kleiber, however, attempted no mechanistic explanation of the 3/4 power exponent.

The logarithmic nature of the allometric equation $\log B=(3/4)\log M_b+\log a$, where B=metabolic rate, suggests that it may be based on some scale-invariant or self-similar features of the organism, and therefore it appeared attractive to search for the possibility that some fractal properties of the organism were related to metabolic rate scaling. This possibility was first suggested and explored by West et al. (1997). In an attempt to explain the '1/4 power law' from first principles they proposed two different models.

In their first model (West et al., 1997) they considered the fractal design of the network for nutrient and O_2 supply, the vascular system, and introduced two premises: (1) that blood vessels form a self-similar fractal network with the volume of blood V_{bl} proportional to body mass: $V_{bl} \propto M_b$; and (2) the capillaries as the terminal units of this network are invariant of fixed size whereas their number ($N_{(cap)}$) is proportional to the metabolic rate B of the cells with which they interact: $N_{(cap)} \propto B \propto M_b^b$. Applying the principles that (a) the network is volume-filling and (b) the energy needed to transport nutrients and O_2 is minimized, they derived that the conditions were fulfilled by b=3/4. This model has recently been criticized by Kozlowski and Konarzewski (2004), primarily on the basis of the internal inconsistency of the premises.

The second model of West et al. (1999) considers the fractal nature of the structural surfaces across which metabolic activity takes place: the capillaries and the mitochondria. The authors conjecture that metabolic rate B is limited by the geometry and scaling behaviour of what they call the total effective surface of the organism, a, which they take to be either the total capillary surface or the total mitochondrial surface (probably the area of their inner membranes where oxidative phosphorylation takes place). Their prediction that $B \propto a$ thus assumes that metabolic rate scales like the capillary and the inner mitochondrial surface areas. This tacitly implies that the rate of 'specific activity' of these membranes is size-independent.

The scaling argument for B against body size runs as follows. If the effective surfaces were built on Euclidean geometry their surface area would scale with body volume V or body mass M_b as:

$$a \propto V^{2/3} \propto M_b^{2/3} . \quad (1)$$

Assuming that these surfaces show fractal design, however, the scaling exponents must be modified to include a factor related to the fractal dimension of the structure so that:

$$a \propto V^{(2+\varepsilon_a)/(3+\varepsilon_a+\varepsilon_l)} \propto M_b^{(2+\varepsilon_a)/(3+\varepsilon_a+\varepsilon_l)} , \quad (2)$$

where $2+\varepsilon_a$ is the fractal dimension of the surface whereby $0<\varepsilon_a<1$; $\varepsilon_a=0$ means Euclidean surface, whereas $\varepsilon_a=1$ is a space-filling fractal surface. West et al. (1999) also considered the fractal nature of the containing volume and of body mass with the result that the exponent of the volume takes the form $3+\varepsilon_a+\varepsilon_l$, where ε_l is a fractal dimension factor for the linear vascular tree. This new model is linked to the first one by two assumptions: that the terminal capillary units are invariant of fixed size across the size range of species, and that the vascular tree is fractal in nature. The authors then conjecture that organisms have evolved to maximize the scaling of the total effective surface a, which is achieved when $\varepsilon_a=1$ and $\varepsilon_l=0$. On these premises one finds:

$$a \propto M_b^{3/4} \propto B , \quad (3)$$

'*regardless of the details of branching architecture and dynamics governing the metabolic process and distribution of resources*' (West et al., 1999). This then is the basis of the 'universal scaling law' that holds for mammalian organisms, cells, mitochondria, and molecules (West et al., 2002). This is because '*although living things occupy three-dimensional space, their internal physiology and anatomy operate as if they were four-dimensional*' (West et al., 1999).

It appears to us that this model development is mistaken on two counts. (1) The volume containing the effective surface a is a real world volume of dimension 3, and therefore the denominator in Equation 2 should be 3 without fractal complement; and (2) in the real world, 'fractal surfaces' are geometric properties of membranes, physical structures of finite thickness that function in conjunction with other structures (in this case the mitochondrial matrix), and it is therefore questionable whether they can be totally space-filling, i.e. of dimension 3. If it is justified to consider inner mitochondrial membranes as fractals then their fractal dimension should be $2<D_f<3$. Considering this, the area-to-volume relationship of mitochondrial membrane surfaces becomes $a \propto V^{Df/3}$. If the exponent 3/4 should be explained by fractal properties of the effective surface its fractal dimension could be $D_f=2+\varepsilon_a=2.25$. This discussion will be resumed below.

These attractive models have been criticized on several grounds, but largely with the argument that fractal geometry is not necessary to explain the 3/4 power scaling of metabolic rate. Alternative explanations are found in network structures (Banavar et al., 1999), quantum mechanics (Demetrius, 2000) and topology (Bejan, 2000). It has also been pointed out that

the metabolic rate of an organism is not a simple coherent process, but rather appears as the sum of many partial contributions of various compartments and steps that may show very different scaling behaviour under different functional conditions (Darveau et al., 2002; Hochachka et al., 2003).

One important critique is that 3/4 power scaling is not a general or universal law (Darveau et al., 2002; Weibel, 2002). It is rather a frequently occurring empirical result pertaining to measurements of 'standard' or perhaps 'basal' metabolic rates in mammals of varying body size. But different scaling may be obtained if the conditions of basal metabolic rate are tightly applied (White and Seymour, 2003), or when the effects of intra-specific size variations are considered (Heusner, 1984). Even though approximate 3/4 power scaling may be 'preponderant' when considering the metabolic scaling under resting or field conditions (Nagy et al., 1999; Savage et al., 2004) there are consistent deviations from 3/4 power scaling. One case of special interest is the maximal metabolic rate, MMR, or $\dot{V}_{O2max}$, which was repeatedly found to scale with a power larger than 3/4 (Koteja, 1987; Taylor et al., 1988; Bishop, 1999).

There has been less effort towards deriving a theory for the scaling of MMR. Some early studies had suggested that MMR achieved in exercise was about 10 times BMR and would therefore scale also with $M_b^{0.75}$ (Hemmingsen, 1960; Pasquis et al., 1970; Lechner, 1978), so that no special consideration of this condition seemed warranted. There were, however, some observations that suggested the possibility of a different scaling of MMR induced by locomotion. Taylor et al. (1980) estimated the energetic cost of generating muscular force by running animals on a treadmill with or without a load of up to 27% of body mass and found that O_2 consumption increased in direct proportion to the mass supported by the muscles (Fig. 1). This observation suggested that $\dot{V}_{O2max}$ should scale with M_b^1. One of the main differences between BMR and MMR is that the latter is a limiting condition and may depend on O_2 supply from the lung and circulation of blood (Fig. 2). Studies on the design of the pathway for oxygen have shown that the pulmonary diffusing capacity, the potential limit for O_2 uptake, scales in fact with M_b^1, thus differently from BMR (Weibel, 1972, 1973). With these two observations in mind Taylor and Weibel (1981) undertook a study on the allometry of the respiratory system with the hypothesis that $\dot{V}_{O2max} \propto M_b^1$, on the grounds that the cost of running was proportional to M_b and that the design of the O_2 cascade is matched to the maximal energy demands of the organism, thus introducing the hypothesis of symmorphosis.

This latter argument preceded one of the central statements of the universal model, namely that '*the economy of design …[is such that] … structures and functions tend to just meet maximal demands*' (Brown et al., 2000), the essential postulate of symmorphosis (Taylor and Weibel, 1981; Weibel et al., 1991, 1992). This means that maximal metabolic rate, i.e. the condition under which the resource distribution network as well as the total effective surfaces must meet maximal demands, must abide to the universal scaling law and scale as $M_b^{3/4}$.

There is, however, no *a priori* reason why MMR should scale the same way as BMR with body size. There are distinct differences in the performance of the system under the two conditions. (1) At BMR, O_2 is consumed in all cells of the body mainly for maintenance of cell polarity, protein synthesis etc.; at MMR, over 90% of O_2 is consumed in a single organ system, the locomotor muscles, for ATP resynthesis related to work output (Fig. 2). (2) At BMR, blood flow is distributed equitably through the (fractal) vascular tree to all organs of the body; at MMR, over 90% of blood flow is directed to contracting

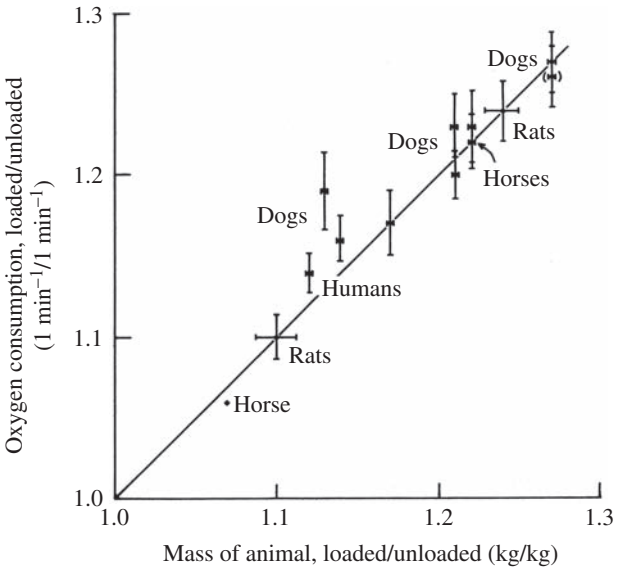

Fig. 1. Oxygen consumption in mammals carrying different loads. From Taylor et al. (1980).

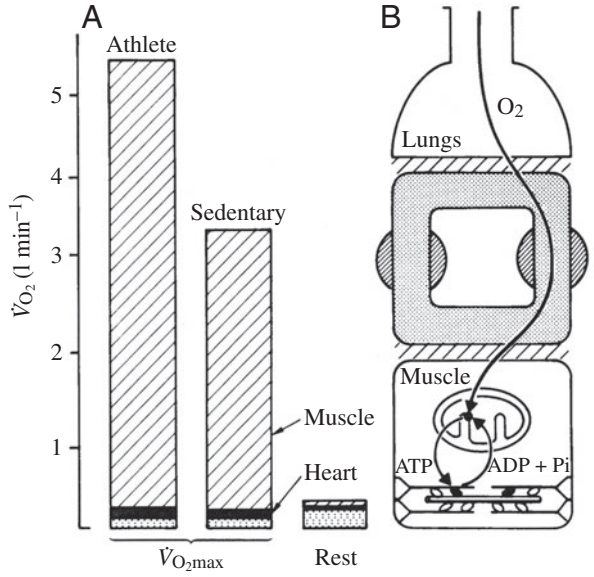

Fig. 2. (A) The distribution of oxygen consumption at rest and at $\dot{V}_{O2max}$. (B) Model of pathway for oxygen from the lung to muscle.

muscle tissue in response to increased energy demand. (3) Among species with similar BMR, there are great differences in the capacity to increase MR in support of exercise, e.g. in athletic *vs* sedentary species (Taylor et al., 1987; Jones et al., 1989).

In this paper we shall first critically review the existing data base for MMR scaling in mammals. From the systems physiology point of view, we then ask what factors may be determining MMR and how this will cause the scaling of MMR to be different from that of BMR. An important characteristic of this analysis is that body size is considered as only one of several factors determining MR.

We defined MMR as $\dot{V}_{O_2max}$ estimated by the accepted standardized method, which is to run animals on a treadmill at varying work intensity (speed and incline) measuring $\dot{V}_{O_2}$ and plasma lactate concentration when steady state is achieved (Seeherman et al., 1981); for this, speed is kept constant during each run but varied between runs. Under such experimental conditions $\dot{V}_{O_2max}$ is achieved when a further increase in work output does not cause $\dot{V}_{O_2}$ to rise further but the additional energy is provided by anaerobic metabolism, as reflected by an increase in plasma lactate concentration.

The scaling of MMR

In an extensive review of the literature, Weibel et al. (2004) found 57 estimates of $\dot{V}_{O_2max}$ conforming to the above-stated conditions, representing 34 mammalian species ranging in body mass from the pigmy mouse at 7 g to the horse at over 500 kg. It includes a representative range of mammalian species, wild and domesticated. The data set covers five orders of magnitude and therefore encompasses the vast majority of terrestrial mammalian species.

In Fig. 3, $\dot{V}_{O_2max}$ is plotted against body mass on a double-logarithmic scale. The power law regression calculated for the entire data set is:

$$\dot{V}_{O_2max} = 118.2 M_b^{0.872\pm0.03} \quad (4)$$

This slope is significantly different (F=17.472, d.f.=1, 64, P<0.01) from 3/4 that characterizes BMR and it is also different from 1.

Fig. 3 also reveals the great variability of $\dot{V}_{O_2max}$ in relation to body mass. For example, in the size class of 25 kg the range of $\dot{V}_{O_2max}$ is almost one order of magnitude between the goat on one hand and the pronghorn antelope on the other. This is related to differences in the aerobic exercise capacity of different species, which is higher in athletic mammals, such as horse, dog and pronghorn, compared to the majority of 'normal' or more sedentary species (Taylor et al., 1987; Jones et al., 1989; Lindstedt et al., 1991). These athletic species are marked by large, open triangles whereas small, filled triangles mark the 'normal' species; the separation is made on the grounds of a high mass-specific $\dot{V}_{O_2max}$ of athletic species.

Could the slope be steeper than that of BMR because of the presence of athletic species, which are prevalent in the larger size classes? In Fig. 3 the scaling of athletic and 'normal' species is also plotted. We find the regression for the non-athletic species alone to be:

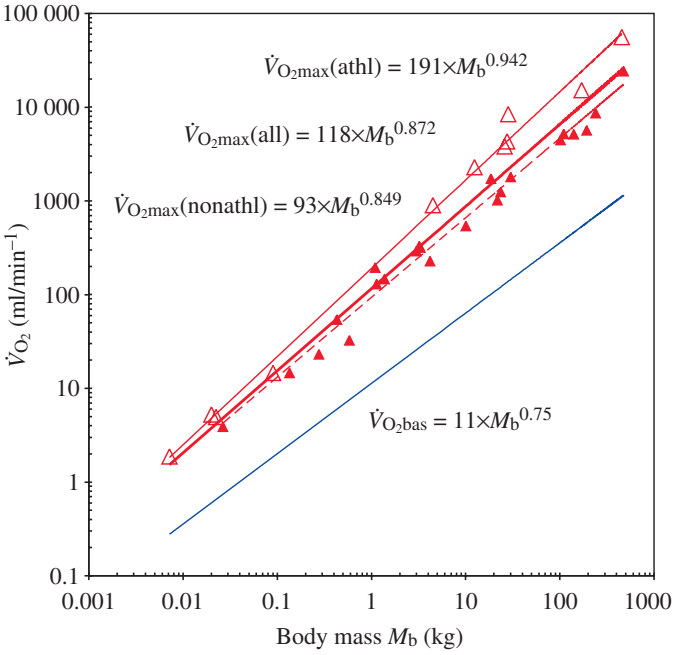

Fig. 3. $\dot{V}_{O_2max}$ plotted against body mass M_b for the 34 mammalian species separated into athletic (open triangles) and non-athletic (filled triangles) species. The heavy line represents the allometric regression for all animals with a slope of 0.872±0.029 (95% confidence limits 0.813, 0.932. F=890, d.f.=1,32, P<0.00001). The mass exponent of the allometric regression of athletic species (thin solid line) is 0.942 (95% confidence limits 0.889, 0.995, F=1609, d.f.=1,?; that of non-athletic species (broken line) is 0.849 (95% CL 0.799, 0.900; F=1231, d.f.=1,21), P<0.00001 for both. The slope of athletic species is significantly larger than that for the non-athletic species (F=38.3, P<0.00001). Data from Weibel et al. (2004). For reference, the curve for BMR or $\dot{V}_{O_2bas}$ (blue line) is plotted after Kleiber (1947).

$$\dot{V}_{O_2max} = 93.4 M_b^{0.849\pm0.024}, \quad (5)$$

with 95% confidence limits CL=0.799–0.899. The coefficient a is lower by 20% but the slope b is not different from that of the overall population (F=0.265, d.f.= 24, P=0.609). From this we conclude that the difference between the power law slopes of BMR and $\dot{V}_{O_2max}$ is not due to the inclusion of athletic species.

The MMR of athletic species scales according to:

$$\dot{V}_{O_2max} = 191 M_b^{0.942\pm0.024}, \quad (6)$$

which is significantly different from the non-athletic regression both with respect to the coefficient a and the exponent b (95% CL for b=0.889–0.995).

A steeper allometric slope of $\dot{V}_{O_2max}$ compared to BMR suggests that larger mammals have a greater relative capacity to increase metabolic rate above the resting state than small mammals. Weibel et al. (2004) estimated the so-called factorial aerobic scope (fAS), the ratio of $\dot{V}_{O_2max}$ to BMR, to be on the order of 6–10 for normal species and 10–60 for

athletic species; it also increases with body mass, particularly in athletic species where it scales in proportion to $M_b^{0.18}$. The fAS is therefore related both to body mass and to aerobic capacity, and the range is greater in large than small mammals, potentially giving large animals an evolutionary advantage.

$\dot{V}_{O_2max}$ and muscle mitochondria

One of the reasons for the conjecture that MMR may scale differently from BMR was the fact that the energetic cost of muscle activity increased linearly with load (Fig. 1). Since MMR as here defined reflects $\dot{V}_{O_2max}$ as elicited by muscle work, we must first look for characteristics of the locomotor muscle system that could cause the variation of $\dot{V}_{O_2max}$ to be partly independent of BMR. Essential factors to consider are muscle mass and aerobic capacity of muscle fibres.

Weibel et al. (2004) report the results of a series of in-depth studies where correlated data were obtained on $\dot{V}_{O_2max}$ and muscle structure in a set of mammals ranging from the woodmouse of 20 g to the horse and steer of 500 kg. The characteristic of these studies was that $\dot{V}_{O_2max}$, whole body muscle mass and the complete morphometry of locomotor muscle, as well as lung morphometry, were estimated on the same animals (Hoppeler et al., 1987). It was first found that muscle mass, M_m, constituted 36% of body mass M_b on average, ranging from 25% in the goat to 45% in the pronghorn, with athletic animals having a larger muscle mass, but without any dependence on body size as M_m scales with $M_b^{1.01}$ (r^2=0.997). Interestingly, the smallest animal, the (athletic) woodmouse, had one of the highest relative muscle masses, 42%.

It has been noted for a long time that the higher aerobic capacity of athletes is related to a higher mitochondrial content of the locomotor muscles, both in humans (Hoppeler et al., 1973) and in athletic mammals (Hoppeler et al., 1987). This suggests the hypothesis that the mitochondria of muscle could also determine $\dot{V}_{O_2max}$ with respect to allometric variation. To test this hypothesis requires a study design where the physiological and morphometric measurements are congruent, i.e. relate to the same compartment and are obtained on the same animals. In quadrupedal mammals running at $\dot{V}_{O_2max}$, almost the entire muscle mass is engaged and oxidative phosphorylation accordingly occurs in all muscles, which may be different in humans using bipedal locomotion (Hoppeler, 1990). The estimate of muscle mitochondrial content must therefore reflect the musculature of the whole body.

Estimates of whole body muscle mitochondrial volume $V_{(mt)}$ have been obtained in 11 mammalian species, for which $\dot{V}_{O_2max}$ was also estimated on the same animals (Weibel et al., 2004). Fig. 4 shows these data in a log-log plot against body mass. We note that $\dot{V}_{O_2max}$ and $V_{(mt)}$ are proportional in all instances, i.e. the overall regression lines have identical slope of 0.96 (F=0.168, d.f.=1, P=0.683), and the data points for athletic species are consistently higher in both data sets.

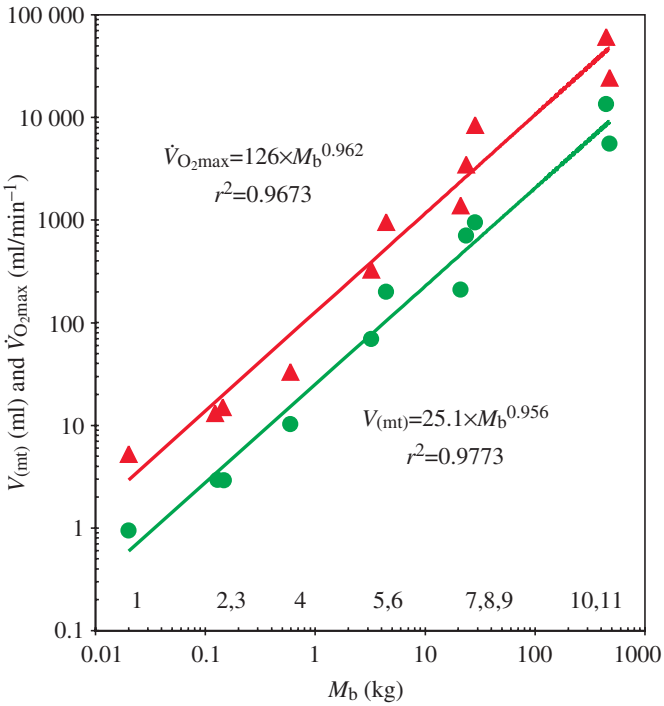

Fig. 4. $\dot{V}_{O_2max}$ (triangles) and morphometric estimate of total volume of muscle mitochondria $V_{(mt)}$ (circles) in 11 species based on whole body sampling. The slope is 0.962 for $\dot{V}_{O_2max}$ (95% CL=0.829–1.096; F=265, d.f.=1,9, P<0.00001), and 0.956 for $V_{(mt)}$ (95% CL=0.846–1.066; F=388, d.f.=1,9, P<0.00001); the two regressions are identical (r^2=0.168, d.f.=1, P=0.683). Numbers at the bottom identify species: 1, woodmouse; 2, mole rat; 3, white rat; 4, guinea pig; 5, agouti; 6, fox; 7, goat; 8, dog; 9, pronghorn; 10, horse; 11, steer. From Weibel et al. (2004).

$\dot{V}_{O_2max}$ and capillary blood supply

Mitochondria can only perform their high rate of oxidative phosphorylation if they receive an adequate supply of O_2 from capillary blood (Fig. 5). This can be a limiting factor for aerobic metabolism. We must therefore ask whether the design of muscle microvasculature is matched to the varying demands observed in large and small mammals. In previous studies comparing athletic with sedentary mammals whose $\dot{V}_{O_2max}$ differs by a factor of 2.5, it was found that the volume of the capillary network was higher in the athletic species, but only by a factor of 1.7. However, athletic species have a 1.8 times greater hematocrit (Conley et al., 1987; Kayar et al., 1994) so that, as a consequence, the volume of capillary erythrocytes in the musculature is 2.5 times larger in athletic species, and hence matched to their higher $\dot{V}_{O_2max}$ (Weibel et al., 1991). This is also seen in Fig. 6, based on the same animals as for the study of mitochondria (Fig. 4): the data points for capillary volume show the same distribution with respect to the regression line as those of $\dot{V}_{O_2max}$. We note that the scaling exponent of capillary volume of 0.98 does not differ from that of $\dot{V}_{O_2max}$, nor from that of the volume of muscle mitochondria.

Structures of aerobic capacity and $\dot{V}_{O_2max}$

The large scatter of data points observed in Figs 4 and 6 results from the fact that aerobic capacity is determined by two properties, body size and athletic prowess. These affect $\dot{V}_{O_2max}$ and the morphometric characteristics of muscle, in parallel. If $\dot{V}_{O_2max}$ is plotted against $V_{(mt)}$ it becomes evident that the two variables are tightly associated (Fig. 7). The 'noise' in the allometric relations due to the presence of athletic and sedentary species in all size classes (Fig. 4) disappears and all data points lie tightly around the linear regression

$$\dot{V}_{O_2max} = 4.876 V_{(mt)}^{1.01} . \quad (7)$$

$\dot{V}_{O_2max}$ and $V_{(mt)}$ show a high positive association (Spearman R=0.909, t=6.547, d.f.=9, P<0.001), independent of their relationship to M_b. This result also means that, in all mammalian species considered, whether athletic or sedentary, whether small or large, 1 ml mitochondria consumes 4.9±0.43 ml O_2 min^{-1} at $\dot{V}_{O_2max}$, confirming the observation of Hoppeler and Lindstedt (1985).

A similar association of functional and structural variables is found for capillaries. What counts here, however, is the volume of capillary erythrocytes that deliver O_2 to the muscle cells, which is the volume of capillaries times the hematocrit or the cells' volume fraction of blood. It is found that the hematocrit is invariant with body mass, averaging 0.42, but it is higher in the athletic species. We then find that the volume of capillary erythrocytes and $\dot{V}_{O_2max}$ are linearly related across the entire size range as shown in Fig. 7.

We thus conclude that, in all mammalian species whether small or large, athletic or sedentary, 1 ml of capillary erythrocytes delivers 45 ml O_2 min^{-1} at $\dot{V}_{O_2max}$. We also note that muscle tissue contains about 1 ml of erythrocytes in capillaries for every 10 ml of mitochondria in the muscle fibres. From this regression analysis we cannot decide whether it is capillaries or mitochondria that set the limit for O_2 flow through the respiratory system.

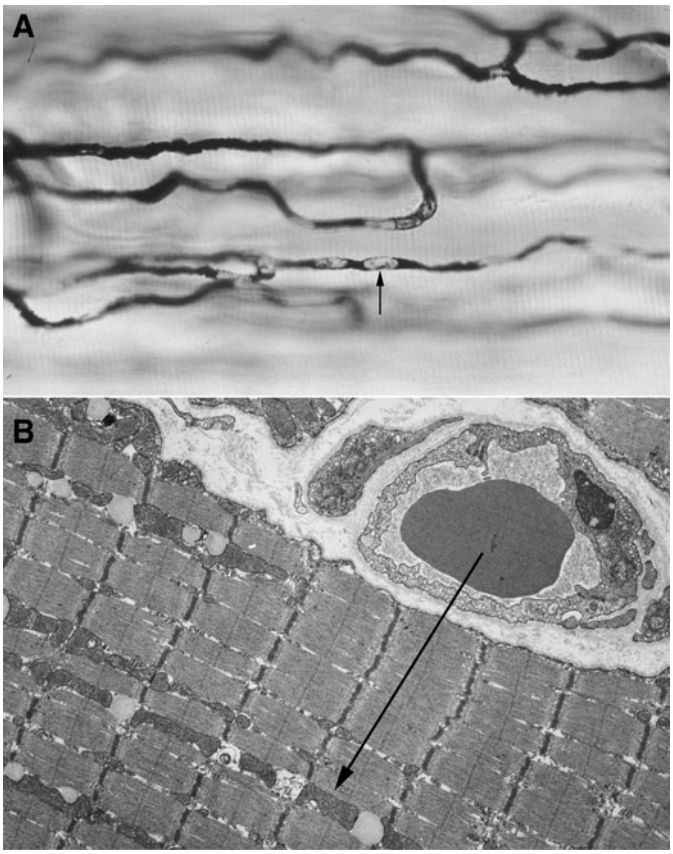

Fig. 5. Light micrograph of capillary network in muscle (A) with arrow pointing to an erythrocyte in stained plasma. Electron micrograph (B) shows path for oxygen from capillary erythrocyte to mitochondria in muscle cell. From Weibel and Hoppeler (2004).

MMR and the scaling of active surfaces

In the subset of correlated species we have shown that MMR scales with the same exponent, 0.96, for both the mitochondrial and capillary (or capillary erythrocyte) volumes that represent the active surfaces of musculature. Is it justified to generalize these findings obtained on a subset of species to the entire range of mammals for which MMR was found to scale with the 0.87 power of body mass? We believe so, for several reasons. First, the scaling exponent of the subset is not statistically different from that of the overall population. Second, the subset is evenly distributed over nearly the entire

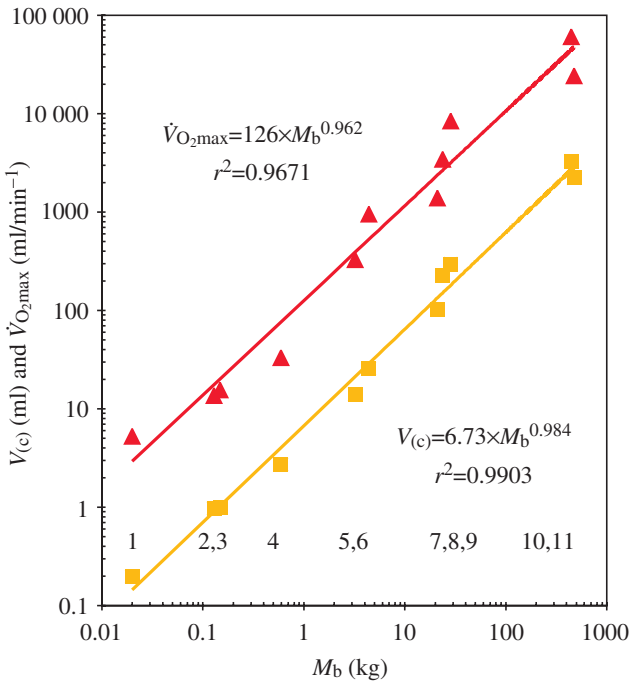

Fig. 6. $\dot{V}_{O_2max}$ (triangles) and morphometric estimate of total volume of muscle capillaries $V_{(c)}$ (squares) in 11 species based on whole body sampling. The slope is 0.962 for $\dot{V}_{O_2max}$ (95% CL=0.829–1.096; F=265, d.f.=1,9, P<0.00001), and 0.984 for $V_{(c)}$ (95% CL= 0.909–1.056; F=916, d.f.=1,9, P<0.00001); the two regressions are identical. Numbers at the bottom identify species, as in Fig. 4. From Weibel et al. (2004).

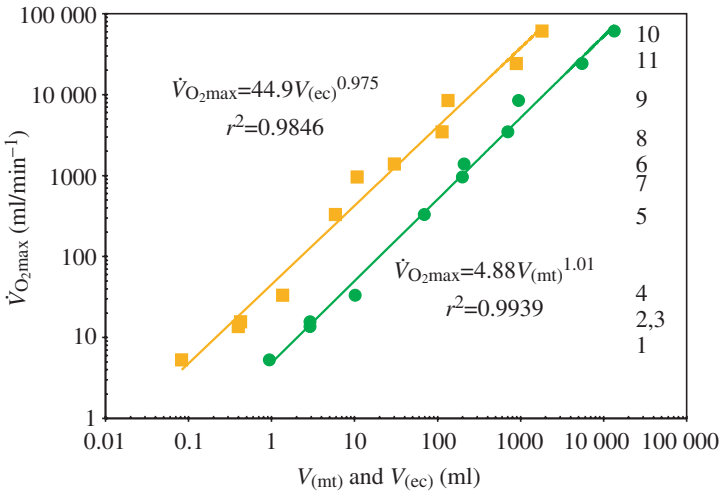

Fig. 7. $\dot{V}_{O_2max}$ plotted as function of total muscle mitochondrial volume $V_{(mt)}$ (squares) and capillary erythrocyte volume ($V_{(ec)}$ (squares) in 11 species. The exponent for the regression to $V_{(mt)}$ is 1.009; 95% CL=0.949,1.068, F=1463, d.f.=1,9, P<0.00001; that for $V_{(ec)}$ is 0.975; 95% CL=0.893,1.074, F=604, d.f.=1,9, P<0.00001. Numbers at right identify species, as in Fig. 4.

size range, but it excludes the smallest species of the overall set. Furthermore the subset includes both athletic and non-athletic species, but with an overweight of athletic species in the larger size classes. In addition, we have obtained approximate estimates of muscle mitochondrial and capillary volume in 10 additional mammalian species with a similar result. We therefore conclude that the scaling of the aerobic capacity of locomotor muscle is strictly proportional to the scaling of $\dot{V}_{O_2max}$ with a scaling exponent close to 0.87 for the entire population.

This agrees in principle with the conjectures of the second model of West et al. (1999) that the scaling of metabolic rate is determined by the scaling of what they called the 'active surfaces', specifically mentioning mitochondria and capillaries. We found that MMR scales in parallel with the mitochondrial and capillary (or capillary erythrocyte) volumes.

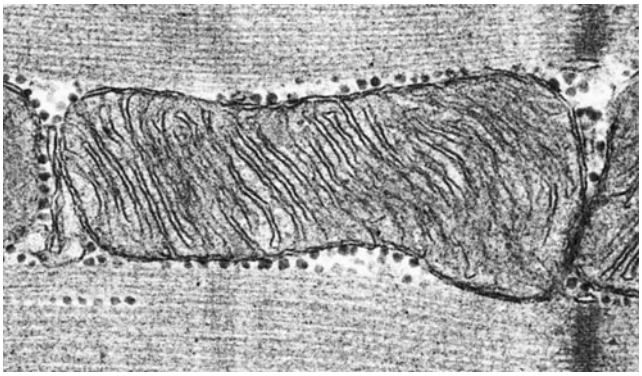

Fig. 8. Electron micrograph of muscle mitochondrion shows the packing of inner mitochondrial membranes where oxidative phosphorylation takes place.

It must be noted that the active mitochondrial surface, the inner mitochondrial membrane (Fig. 8), where oxidative phosphorylation takes place, shows invariant density in the mitochondria both with respect to body mass and aerobic capacity (Hoppeler and Lindstedt, 1985; Schwerzmann et al., 1989) so that the active surface is directly proportional to mitochondrial volume. We therefore conclude that MMR indeed scales with the surface area of inner mitochondrial membranes such that for each ml O_2 consumed per minute at $\dot{V}_{O_2max}$ the muscle contains 7 m^2 of active membrane. The capillary volume is essentially determined by the total length of the capillary network; since the capillary diameter varies weakly with body mass ($\propto M^{0.01}$) the capillary surface across which O_2 is delivered also scales about in proportion to $\dot{V}_{O_2max}$. This therefore suggests that the conjecture of West et al. (1999) of a direct proportionality between 'active surfaces' and MMR is correct, although the scaling exponent is not 0.75 but 0.87.

Could this be explained if the fractal model was corrected to account for the fact that the containing volume has dimension 3 and the fractal surface is not completely space-filling, so that D_f<3, as mentioned above? A scaling exponent b=0.87= D_f/3 is obtained if D_f =2.6. Even though it is uncertain whether this is a realistic value for the fractal dimension of the inner mitochondrial membrane surface it may be interesting that Paumgartner et al. (1981) estimated the fractal dimension of inner mitochondrial membranes of liver cells at 2.54. This was a microscopic study at the level of the liver cell and it is not really justified to extend this to the fractal properties of the entire membrane system of skeletal muscle.

However, it may be fundamentally problematic to invoke fractal properties of real physical membranes in finding an explanation for the scaling of metabolic rate. We have seen that the surface density of membranes within the mitochondria is invariant with body size, so the observed allometric variation of aerobic capacity with $\dot{V}_{O_2max}$ is fully explained by the proportional variation of the volume of structurally and functionally invariant mitochondria. It is thus not necessary to involve fractal properties in this analysis. Nevertheless an in depth study of the putative importance of fractal geometry in setting up efficient functional systems at the organismic level may be warranted.

The scaling of the O_2 supply cascade

In our concept of the pathway for oxygen (Taylor et al., 1981; Weibel et al., 1991, 1992) $\dot{V}_{O_2max}$ is the functional parameter that defines the limiting flow rate through all steps of the respiratory system, from the lung to the muscle mitochondrial respiratory chain (Fig. 2; Table 1). The flow rates are determined by the product of a stepwise driving force and a set of design parameters that essentially determine the conductance. We found that design parameters of the last two steps in this cascade, the mitochondrial and

Table 1. *Model for structure–function relation in the respiratory system*

		Function	•	Design
(1)	$\dot{V}_{O2max}$ =	$(P_{AO_2}-P_{bO_2})\{t_c,\theta_{O_2}\}$	•	$D_{LO_2}\{S_A,S_c,V_c,V_{V(ec)},\tau_{hb}\}$
(2)	$\dot{V}_{O2max}$ =	$(\sigma_a P_{aO_2}-\sigma_v P_{vO_2})f_H$	•	$V_S\{V(LV)\}V_{V(ec)}$
(3)	$\dot{V}_{O2max}$ =	$(P_{bO_2}-P_{cO_2})\{t_c,\theta_{O_2}\}$	•	$D_{TO_2}\{S_c,V_c,V_{V(ec)}\delta_{(c-mi)}\}$
(4)	$\dot{V}_{O2max}$ =	$\dot{v}_{O_2}\{\dot{m}_{ATP}\}$	•	$V(mt)\{S_{V(im,m)}\}$

The model relates $\dot{V}_{O2max}$ to functional and design variables for (1) the pulmonary gas exchanger, (2) the heart, (3) muscle capillaries and (4) muscle mitochondria.

The O_2 flow rate $\dot{V}_{O_2}$ is expressed as the product of functional and design parameters; parameters that affect these factors placed in brackets { }.

The functional parameters include: O_2 partial pressures P_{O_2}, coefficients of 'hematocrit-specific' O_2 capacitance σ, which depend on O_2-hemoglobin dissociation, O_2 binding rate θ, heart frequency f_H, capillary transit time t_c, and unit mitochondrial O_2 consumption rate as a function of ATP flux $\dot{v}_{O_2}\{\dot{m}_{ATP}\}$.

Design parameters include: diffusion conductances D of lung L and tissue T gas exchangers, which depend on alveolar and capillary exchange surface areas S_A and S_c, respectively, capillary volumes V_c, hematocrit $V_{V(ec)}$, harmonic mean barrier thickness τ_{hb}; capillary–mitochondrial diffusion distance $\delta_{(c-mi)}$ and mitochondrial volume V(mi) with inner membrane surface density $S_{V(im,m)}$.

Modified after Weibel et al. (1991).

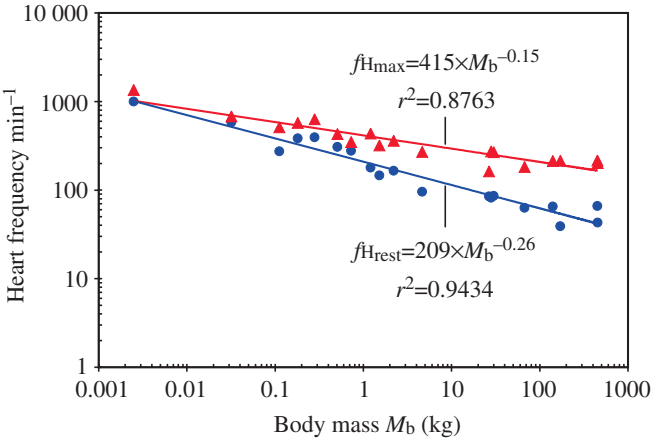

Fig. 9. Allometric plot of resting (circles) and maximal heart frequencies (triangles) in mammalian species. From Weibel and Hoppeler (2004).

capillary volumes and surfaces, scale strictly in proportion to MMR. What about the other two steps, the heart and the lung?

We must first ask whether and how the functional and structural properties of the heart are matched to the variation in O_2 supply to muscle microcirculation with variations in body size. The two key parameters of blood flow, heart frequency f_H and stroke volume V_S (Table 1, line 2), must be considered. Stroke volume is proportional to heart size, which is known to be larger in athletes but invariant with body mass (Prothero, 1979). In contrast, heart frequency depends on body size as it is higher in small than in large species. At BMR, heart frequency is found to scale with body mass to the power –0.25 (Fig. 9). Resting heart frequency therefore scales in parallel with mass-specific basal metabolic rate. During exercise, heart frequency increases to reach a maximum at $\dot{V}_{O2max}$. We find that maximal heart frequency scales with an exponent –0.15 (Bishop, 1999; Weibel and Hoppeler, 2004), which agrees with the scaling of mass-specific MMR, for which we obtain an exponent –0.13 from Fig. 1. It is thus evident that cardiac output is adjusted to the needs of working muscle in animals of varying body mass.

Such a close match is not easily found in the lung. This organ at the interface to the environment maintains a variable excess capacity for O_2 uptake so that the scaling of its diffusing capacity does not follow the simple relationship observed in the internal parts of the respiratory pathway: the pulmonary diffusing capacity scales linearly with body mass (Weibel et al., 1991; Weibel, 2000). However, considering also the structure of the pulmonary acinar airways that distribute O_2 to the alveolar surface, one finds that the scaling of the membrane conductance is tightly associated with that of $\dot{V}_{O2max}$, both with exponents of 0.9. The effect of O_2 screening in the acinar airways reduces the effective conductance at rest to scale with 0.735, similar to the scaling of basal metabolic rate (Sapoval et al., 2002).

Conclusions

Can we now attempt some conclusions on the mechanistic explanation of the scaling exponents observed for MMR as elicited by exercise? Two observations stand out.

The first is that scaling of MMR and BMR differs in three respects: (1) the scaling exponent b for MMR of 0.87 differs significantly from that of 0.75 for BMR; (2) the coefficient a is larger in MMR than BMR by a factor of around 10 that characterises the aerobic scope of 'normal' mammals; and (3) this factor ranges from about 8 in small mammals to 50 in large athletic mammals and thus causes the range of MMR to be much larger than that for BMR. The factorial aerobic scope, fAS, is thus also a function of body mass, varying with $M_b^{0.1}$ in normal and $M_b^{0.18}$ in athletic species. This means that MMR can be expressed as a product of BMR and fAS such that for normal species $MMR \propto BMR \cdot fAS \propto 11.3 M_b^{0.75} * 8.3 M_b^{0.1} = 93 M_b^{0.85}$. For athletic species one will have to consider, in addition, an 'athleticity' factor that also depends partly on M_b, as larger species show a greater potential for athletic trait than small species.

Is there an explanation for the scaling exponent of 0.87 for MMR in contrast to 0.75 for BMR? The cascade model of Darveau et al. (2002) and Hochachka et al. (2003) conceives the scaling exponent of metabolic rate as a resultant of the weighted partial scaling of the sequential functions that determine metabolic rate and that this is different for BMR and MMR. The problem is that a sum of power functions cannot be converted into an overall power function. This is not so

important, however, as we have shown here that the relevant scaling exponents for all the steps involved (Table 1), except the lung, are identical and the same as the scaling exponent for $\dot{V}_{O2max}$. Most significantly we have found that the scaling of $\dot{V}_{O2max}$ is tightly correlated with the aerobic capacity of the locomotor muscles determined by the complex of mitochondria and capillary blood. These are stressed to the limit at $\dot{V}_{O2max}$. The heart also adjusts to the needs for oxygen supply determined by this aerobic capacity of muscle: it does so by increasing heart frequency in proportion to the factorial aerobic scope of the animal, which is greater in large than in small animals.

The mechanistic explanation of the scaling of MMR appears easy because it relates to conditions where metabolism occurs predominantly in one functionally homogenous compartment, the locomotor musculature, which is dominant in the sense that under these conditions this organ system receives 90% of the blood flow and consumes over 90% of the oxygen taken up in the lung. Further since the key elements of all the steps between the lung and the muscle cells show tightly related scaling relationship this may explain the observed scaling of the overall MMR.

Interestingly, we found that the scaling of MMR could be explained by the fractal nature of the effective active surface according to the notions proposed by West et al. (1999), but only after modifying their fractal model to account for realistic conditions. On the other hand, we pointed out that it is questionable to invoke fractal properties of real membranes, and that this is not necessary to explain the association of $\dot{V}_{O2max}$ and mitochondrial surface. We also found the capillaries in muscle to scale the same as MMR and mitochondria, but here it is particularly problematic to invoke a fractal design of the capillary network as this is incompatible with the structure of this network. Capillaries may be simply adjusted to the demand of the mitochondria for O_2 supply (Vock et al., 1996), just as the heart adjusts its frequency to meet the different demands in blood flow. By all that, however, the fact that MMR, or the muscles capacity for aerobic work, scale with 0.87 or 7/8 power of body mass rather than with M_b^1 is not explained.

We conclude that the scaling of maximal metabolic rate is determined by the energy needs of the cells that are active during maximal work, which determines the quantity of oxidative enzymes and mitochondria as well as the capillary volume and surface needed for energy supply. The terminal units of the vascular supply network, the capillaries, are thus not invariant structures but are rather quantitatively adapted to the needs of the muscle cells at their working limit. This leads us to suggest that the optimization of the supplying vascular network, judiciously designed as a fractal tree, occurs as an adaptation of the oxygen supply system to meet the demands of working cells. It would thus be the effect rather than the cause of the evolution of metabolic rate scaling. Nevertheless, the question remains as to why the energy needs of locomotion scale with the 0.872 or 7/8 power of body mass.

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