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Review

Allometry of mammalian cellular oxygen consumption

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Abstract. In the 1930s, Max Kleiber and Samuel Brody established that the interspecies correlation between mammalian body mass and metabolic rate ($\alpha M^{0.75}$) cannot be explained (solely) by whole body surface area ($\alpha M^{0.66}$) to volume ratios. Metabolic considerations must also be taken into account. Decreases in the proportion of visceral organ mass to whole body mass can account for some of the whole body metabolic differences. However, superimposed upon these anatomical differences, the metabolism of tissues and cells has been demonstrated to decrease with increasing body mass. These decreases in oxygen consumption rates (with increasing body mass) in cells and tissues can be explained by a decrease in ATP turnover and mitochondrial density and an increase in mitochondrial functional efficiency (decrease in proton leak). The majority of the proton leak differences reflect differences in mitochondrial inner membrane surface area. Indeed, liver metabolism correlates directly with liver mitochondrial inner membrane surface area. Apart from being a significant contributor (~25%) to basal metabolism, mitochondrial proton leak is a major factor determining the differences in basal metabolism between mammals of different body mass.

Key words. Standard metabolic rate; mitochondria; proton leak; oxidative phosphorylation; allometry; body mass.

Introduction

The first whole animal calorimetry experiments were conducted by Antoine Lavoisier and his collaborator Pierre Laplace in 1783 [reviewed in refs 1, 2]. A guinea pig (named Gina) was placed in an ice machine (designed by Laplace and built by Lavoisier) and for various period over 24 h, the heat produced was determined by measuring the amount of ice that had melted. During the latter part of the 18th century, Lavoisier and Laplace were able to combine direct (heat loss) and indirect (gas exchange) calorimetry to demonstrate that the quantity of heat produced was directly proportional to the amount of oxygen consumed. Lavoisier also looked at allometric relationships. He was able to demonstrate that the heat produced by large animals was greater than that produced by smaller animals and, within a given species, oxygen consumption was proportional to body size. Since then, scientists have been endeavouring to account for the unique relationship that exists between body mass and metabolic rate. The literature in this area is fascinating and includes some excellent books [1, 3–6] and monographs [7–9]. In this review, the focus is on tissue, cellular and subcellular allometry and the fundamental role played by mitochondria and, in particular, a phenomenon known as mitochondrial proton leak in deterrnining the differences in the standard metabolic rate of mammals of different body mass. Aspects of the topic have been reviewed elsewhere [1, 4–6, 10, 11]

Allometry of the whole animal

Basal metabolism can be measured in mammals using calorimetry (heat production), protein turnover rate and, quite accurately, using oxygen consumption rate [1, 6,

12]. Basal metabolism is usually defined as the steady-state rate of heat production by a whole organism under a set of standard conditions (standard metabolic rate). In mammals, these conditions are that the individual is an adult, resting but not asleep, stress free, postabsorptive and maintained at a temperature that elicits no thermoregulatory effects on heat production [1]. The resulting basal energy expenditure can be divided into two main catagories: (i) heat produced from 'service functions', that is to say, energy expenditure required for the organism as a whole, as exemplified by the operating heart and lungs in bringing oxygen and carbon dioxide to and from the body and (ii) heat produced from 'cellular maintenance functions', an umbrella term, which includes maintenance of ion gradients and turnover of protein, lipids and other cellular components [13].

As mentioned in the introduction, since the late 18th century larger mammals have been known to produce more heat and consume more oxygen than smaller mammals and that per unit body mass, small mammals consume more oxygen (and produce more heat) than larger mammals. To explain these differences, early researchers focused their experimental approaches on animal surface area (to volume ratio). The first systematic experimental approach of this type is accredited to Rubner [14]. He was able to demonstrate that the fasting metabolic rate, per unit body surface area, of seven dogs, ranging in body mass from 3.2 to 31.2 kg, was approximately constant. The logic of a decreased whole body surface area to volume ratio to explain differences in metabolic rate between mammals of different body mass seems perfectly plausible even today. However, as techniques for measuring metabolic rate and animal surface area improved, it became clear to Brody and Kleiber, in 1932 [reviewed in refs. 3, 4], that whole body surface area alone could not explain the differences in basal metabolism. Cumulative evidence has shown that the relationship between basal (standard) metabolic rate (E/t, heat production per unit time) and body mass (M) in an interspecies comparison of eutherian mammals is given by the following equation: $E/t = aM^{0.75}$, where a is the elevation constant and has a value of 293 when units of kilojoules per day are used. Whole body surface area is proportional to $\alpha M^{0.67}$. Thus a 200-kg horse has a metabolic rate ~1000 times that of a 20-g mouse. When metabolic rate is expressed per unit body mass, resting oxygen consumption is $\alpha M^{-0.25}$ and therefore 20-g mice have ~10 times the mass-specific metabolic rate of the 200-kg horses (fig. 1).

The basis of the 0.75 relationship (exponent 0.75) between body mass and metabolic rate is still a matter of investigation. However, allometric differences in internal organ size can account for some of the differences.

Allometry of metabolic organs

Many excellent studies have focused on the anatomy and physiology of oxygen delivery to tissues, cells and mitochondria at an appropriate rate to fuel basal and maximal metabolic rate [1, 6]. Animals with higher mass-specific rates have faster oxygen delivery systems [15, 16]. In addition, differences in the proportion of highly metabolic visceral organs to whole body mass can account for some of the differences in whole animal metabolic rate allometry [17, 18]. The liver, for example, accounts for ap-

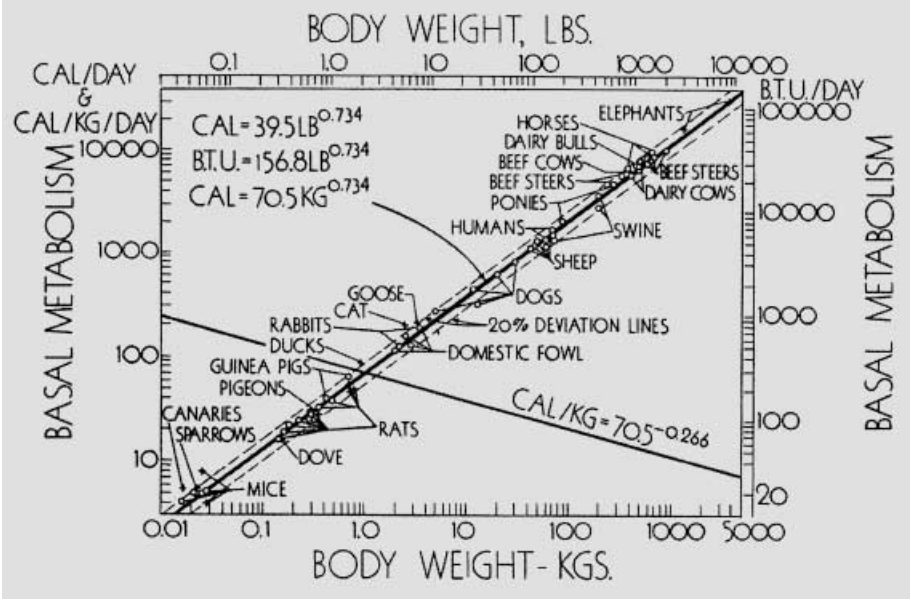

Figure 1. Log-log plot of basal metabolic rate and mass-specific metabolic rate as a function of body weight, for animals and birds. Cumulative evidence has resulted in the modification of the exponent from 0.734 (shown here) to 0.75. Reproduced from Brody [3].

proximately 4% of the body mass of a rat but 20% of its metabolic rate [19]. However, one can calculate that the liver comprises 5.5% of the body mass of the 20-g mouse, whereas it comprises only 0.5% of the body mass of a 200-kg pony [17, 18]. Therefore, assuming (incorrectly) that the metabolic activity of a given mass of liver is the same for mammals of different body mass, the proportion of the whole body mass that is liver (or other organs) can explain some of the difference in whole body metabolic rate as a function of body mass.

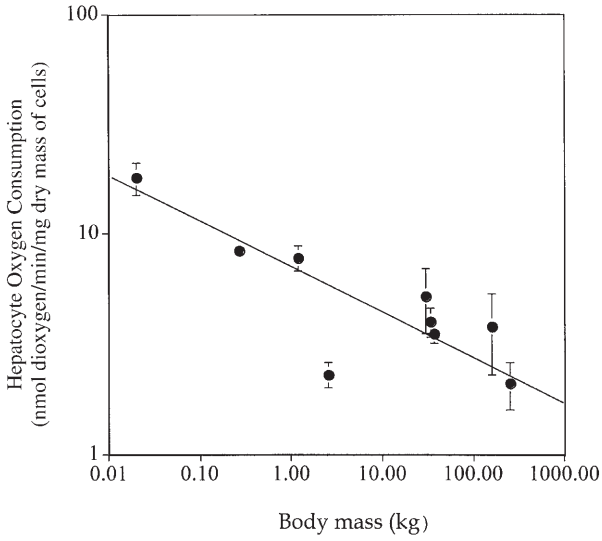

Figure 2. Log-log plot of hepatocyte oxygen consumption rates as a function of body mass. Data are taken from table 1 [21]. The line through the points was fitted by linear regression. The slope of the line is −0.18 ($p < 0.01$). Reproduced with permission from the American Physiology Society.

Allometry of tissue and cell oxygen consumption

Superimposed upon the decrease in the proportion of highly metabolic organs (with increasing body mass) are differences in the metabolic activity of the tissues. Krebs [20] was able to demonstrate that the oxygen consumption rate of liver slices from mice was ~ 7.4 times greater than that of liver slices from horses. The increase in oxygen consumption rate in tissues slices from smaller animals is also reflected at the cell level. Total oxygen consumption was shown to be greater in hepatocytes from small mammals over a range of body masses from 20 g mice to 200 kg horses [21] (table 1, fig. 2). To quote the extremes, hepatocytes from mice consume oxygen at ~ 5.5 times the rate of hepatocytes from horses. The allometric relationship for hepatocyte oxygen consumption rate was calculated as $\alpha M^{-0.18}$ (per unit mass or number of cells). This exponent is similar to the value for liver slices obtained by Couture and Hulbert [22] of −0.21 and practically identical to the exponent of −0.17 obtained when plotting log of oxygen consumption rate for liver slices against log of body mass from the data in table 7 of Krebs [20]. Therefore, most if not all the decrease in oxygen consumption rate in the liver is due to the decrease in cellular activity with increasing body mass. A similar relationship between tissue slice oxygen consumption and body mass has been demonstrated by Krebs [20] and Couture and Hulbert [22] for kidney cortex (−0.11 to −0.07), brain (−0.07), spleen −0.14) and lung (−0.10).

Table 1. Mammalian hepatocyte oxygen consumption rates and volumes.

	Mouse	Rat	Ferret	Rabbit	Dog	Sheep	Pig	Pig	Horse
Gender	females	mixture	mixture	males	males	females	females	females	females
Body mass (kg)*	0.02 ± 0.00 (11)	0.27 ± 0.02 (4)	1.2 ± 0.1 (10)	2.5 ± 0.1 (7)	30 ± 4 (2)	33.8 ± 0.4 (5)	37 ± 3 (7)	160+ (3)	250+ (3)
Calculate standard metabolic rate [293.$M^{0.75}$] (kJ day^{-1})	16	110	336	583	3756	4107	4397	13,181	18,421
Calculated mass-specific metabolic rate 293.$M^{-0.25}$] (kJ day^{-1} kg^{-1})	779	406	280	233	125	122	118	82	74
Hepatocyte oxygen consumption rates (nmol O_2 min^{-1} mg^{-1}) dry mass of cells)*	18 ± 3 (4)	8.4 ± 0.6 (4)	7.8 ± 1.0 (10)	2.3 ± 0.3 (7)	5.2 ± 0.3 (2)	4.0 ± 0.6 (5)	3.5 ± 0.3 (7)	3.8 ± 1.5 (3)	2.1 ± 0.5 (3)
Hepatocyte volume (µl mg^{-1} dry mass of cells)*	1.9 ± 0.5 (4)	1.2 ± 0.2 (4)	1.5 ± 0.2 (10)	1.3 ± 0.2 (7)	2.2 ± 0.1 (2)	1.4 ± 0.3 (5)	1.9 ± 0.3 (7)	1.0 ± 0.4 (3)	1.5 ± 0.4 (3)

* Values are expressed as mean ± SE (numer of mammals). Hepatocyte oxygen consumption rates and volumes were measured at least in duplicate for each animal.

+ Approximate values.

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Table 2. Allometric exponents for mammalian liver

Liver mass [17] $\alpha M^{0.82}$	Liver mass [17] $\alpha M^{0.82}$	Liver mass [17] $\alpha M^{0.82}$
Oxygen consumption per unit mass of liver cells [21] $\alpha M^{0.82}$	Mitochondrial density per unit mass of liver cells [40] $\alpha M^{-0.09}$	Mitochondrial density per unit mass of liver cells [40] $\alpha M^{-0.09}$
	Inner membrane surface area per unit volume of mitochondria [40] $\alpha M^{-0.10}$	Mitochondrial proton leak rate per unit volume of mitochondria [49] $\alpha M^{-0.13}$
Derived liver oxygen consumption $\alpha M^{0.64}$	Derived liver mitochondrial inner membrane surface area $\alpha M^{0.63}$	Derived liver mitochondrial proton leak rate $\alpha M^{0.60}$

Since relative mass of the liver scales $\alpha M^{0.82}$ [17], then, based on the above hepatocyte data, the metabolic activity of the liver can be derived as $\alpha M^{0.64}$ (table 2). This value is clearly dissimilar to the whole body metabolism ($\alpha M^{0.75}$). Presumably, variations in the allometric relationships of metabolism in the other organs counterbalance the equation. Interestingly, cells from a given tissue from mammals of different body mass would appear to be of similar size [5, 6], with liver cells being no exception (table 1) [21]. Clearly, an explanation for differences in tissue and cellular oxygen consumption rates must centre on the quantity of mitochondria in those cells, the efficiency of the oxidative and phosphorylating functions of mitochondria in those cells, and ATP demand of those cells.

Oxidative phosphorylation

The consensus of opinion in the literature is that oxidative phosphorylation is correctly described by the chemiosmotic theory of Mitchell [23; reviewed in ref. 24] Reducing equivalents such as $NADH_2$ and $FADH_2$, derived from the oxidation of reduced carbon compounds (the carbohydrate or fatty acid molecules derived directly from the diet or released from the fuel stores of the body), feed electrons into the electron transport chain. This chain is situated in the mitochondrial inner membrane. As electrons pass down the chain, protons are translocated across the mitochondrial inner membrane from the matrix space to the intermembrane space thus setting up a delocalised transmembrane proton electrochemical gradient (Δp). Δp can drive useful work such as (i) metabolite transport across the inner membrane (e. g. ADP/ATP exchange and phosphate/proton symport) and (ii) ATP synthesis from matrix ADP and phosphate by driving protons through the ATP synthase which is also located in the inner membrane and extends into the matrix. Hence, ATP synthesis is coupled to oxygen consumption via Δp. This is the principle source of ATP at rest. However, it has long been known from H^+/O stoichiometric studies of the electron transport chain and H^+/ATP stoichiometric studies of the ATP synthase that ATP synthesis is not perfectly coupled to oxygen consumption in intact mitochondria [25]. The reason for this discrepancy is that the mitochondrial inner membrane is not absolutely impermeable to protons, i. e. there is an inefficiency in the system, namely a leakage of protons across the mitochondrial inner membrane.

The 'proton leak rate' can be measured indirectly by measuring the rate of oxygen consumption by mitochondria in the absence of any ATP. A non-ohmic relationship between 'proton leak rate' and its driving force, Δp, is observed in isolated mitochondria from all tissues studied so far: skeletal muscle, heart, liver, kidney and brain mitochondria [10, 26]. Furthermore, proton leak is not an artefact of isolated mitochondria. A similar non-ohmic dependence of oxygen consumption rate on Δp is observed for non-phosphorylating mitochondria in situ in a variety of cells (hepatocytes, thymocytes and lymphocytes) and tissue (skeletal muscle) [10, 27–29]. In fact, proton leak accounts for ~25% of the resting oxygen consumption rate of cells, up to ~30% of the resting oxygen consumption rate of rat hind-quarter skeletal muscle and ~20% of the whole body resting oxygen consumption rate of a mammal (rat) [30, 31]. The majority of the remaining ~70–80% reflects the oxygen consumption rate due to ATP turnover.

Apart from being quantitatively important, proton leak seems to vary in accordance with factors that determine standard metabolic rate, such as thyroid status, phylogeny and body mass [10, 11]. Hyperthyroid rats have higher liver mitochondrial proton leak rates than their euthyroid controls and, vice versa, hypothyroid rats have lower protein leak rates [32–35]. Lizards of equivalent mass to rats have lower whole body resting metabolic rates and lower proton leak rates [36]. This review will focus on the proton leak changes as a result of body mass differences and discuss their implications to whole energy metabolism.

It should be noted that an alternate view in the literature argues that the non-ohmic relationship between mitochondrial oxygen consumption rate and mitochondrial membrane potential is not due to proton leak but that slippage of the proton pumps (reduced H^+/O stoichiometry) of the inner membrane is occurring at the high membrane potential [for a review see ref 37]. On both sides, there is no contesting the argument that there is an inefficiency in the oxidative phosphorylation system, only the mechanism (proton leak/pump slippage) is the contentious issue. I will return to this issue later.

Mitochondria and body mass

The main site of oxygen consumption in mammalian cells is the mitochondria. The changes in the amount of metabolically active tissue and in the oxygen consumption per unit mass of tissue are paralleled by changes in the content of mitochondria and mitochondrial components. For example, the cytochrome c content has been shown to be $\alpha M^{0.70}$ [38] and cytochrome oxidase activity is $\alpha M^{0.76}$ [39]. In addition, mitochondrial number, per gram of liver, decreases with increasing body mass with exponent −0.1 [17]. A similar relationship (exponent −0.09) was found for the mitochondrial density of hepatocytes from mammals ranging in body mass from 20 g to 250 kg (table 2) [40]. Maximal metabolic rate studies have also shown striking correlations with mitochondrial properties. Maximal metabolic rate is approximately ten times standard metabolic rate and thus scales with similar exponent to body mass [41, 42]. During exercise, most oxygen consumption occurs in the skeletal muscle. Mathieu et al. [43] were able to show that the mitochondrial density of skeletal muscle was proportional to maximal aerobic rate and hence standard metabolic rate. Jansky [44, 45] found a similar relationship for cytochrome oxidase activity in a number of tissues and the whole body. Interestingly, scaling persists down to the molecular level. Estimates of power generated by mammalian cells, mitochondria and cytochrome oxidase when compared with cell mass, mitochondrial mass and cytochrome oxidase molecular mass show that these entities fall on the body mass/metabolic rate curve quite well [46]. Differences in metabolic rate between mammals of different body mass may well be a function of the number of fundamentally sized oxygen consuming 'units' within it and this in turn is argued to be limited by the rate at which essential resources that sustain them can be supplied [46].

A fundamental oxygen-consuming entity inside a cell is the mitochondrion and, intuitively, the metabolic activity of a given tissue or cell is related to the proportion of that tissue or cell that is mitochondrial. However, as can been seen from the information presented, decreases in hepatocyte oxygen consumption rate with increasing body mass (exponent −0.18) are not directly proportional to decreases in hepatocyte mitochondrial density with increasing body mass (exponent −0.09) (table 2) [40]. It is as if hepatocyes, from larger mammals, contain more mitochondria than they need for the oxygen consumption that is occurring. The only explanation that makes sense is that superimposed upon the decrease in the physical presence of mitochondria per gram of tissue or cell (with increasing body mass), there would appear to be a decrease in the activity of mitochondria for a given tissue (in this case liver) with increasing body mass.

Allometry of mitochondrial proton leak

The first of two reasons for the decrease in mitochondrial activity of cells and tissues (with increasing body mass) could be a decrease in ATP demand by cells and tissues from larger mammals and the evidence is there in the literature. Cell total oxygen consumption studies, have shown that the ATP turnover of hepatocytes decreases with increasing body mass [40]. A specific example of such an ATP consumer is the plasma membrane Na^+/K^+-ATPase. The Na^+/K^+-ATPase is one the major ATP consumers in resting cells, accounting for ~10% of the resting oxygen consumption rate of thymus cells (in addition to ~20% due to proton leak) [29]. The reason for an oxygen consumption rate of such proportion can be assigned to a sodium leak across the plasma membrane [47] (a process which parallels the leak of protons across the mitochondrial inner membrane). Couture and Hulbert [22] have shown that Na^+/K^+-ATPase activity decreases with increasing body mass in liver ($\alpha M^{-0.14}$) and kidney cortex slices ($\alpha M^{-0.13}$).

However, the decrease in ATP turnover is matched by a decrease in the other major oxygen consuming reaction of mitochondria, namely the proton leak (fig. 3) [48, 49]. Mitochondrial proton leak in liver per mitochondrial volume, measured at a given membrane potential, decreases with increasing body mass $\alpha M^{-0.13}$ [49]. One has to assume that the resulting proportionate decrease in the ATP turnover and mitochondrial proton leak rate in hepatocytes ensures that the effective P/O remains relatively constant for hepatocytes over a range of body mass differences [40]. One obvious result of this scenario is that the in situ mitochondria (in resting hepatocytes) are poised closer to state 4 than state 3 as body mass increases. This is reflected in the increase in resting in situ mitochondrial membrane potential in hepatocytes from larger mammals [40].

Allometric basis of mitochondrial proton leak

The mechanism behind the 'proton leak' is not that clear. Differences in proton leak rate (at any given membrane potential) correlate with differences in mitochondrial inner membrane surface area as shown from thyroid status,

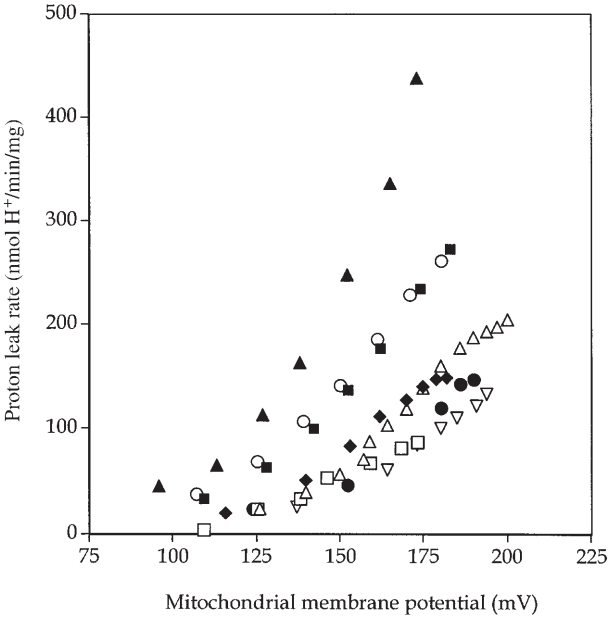

Figure 3. Proton leak kinetics for isolated liver mitochondria from mammals of different body mass. Proton leak kinetics were measured as described in Porter and Brand [48]. Proton leak rates at any given membrane potential (e. g. 150 mV) are significantly greater in mitochondria from small mammals compared to larger mammals ($\alpha M^{-0.14}$, r = –0.77). Symbols: mouse (▲) rat (■), hamster (◆), ferret (○), rabbit (▽), pig (△), sheep (●) and horse (□). Reproduced with permission of Macmillam Magazines Ltd.

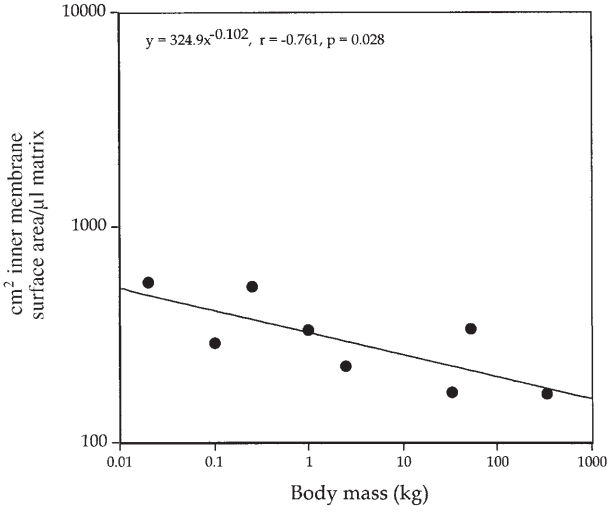

Figure 4. Measures of mitochondrial surface area as a function of body mass. Log-log plot of mitochondrial inner membrane surface area per microlitre matrix volume. The line through the points was fitted by linear regression. Its slope is –0.129 (p = 0.034). Reproduced with permission from the American Physiology Society.

mammal/reptile comparisons and body mass [10, 36, 49]. The mitochondrial inner membrane surface area decreases with increasing body mass, per unit volume of mitochondria $\alpha M^{-0.10}$ (table 2, fig. 4). The non-ohmic nature of the proton leak kinetics is characteristic of diffusion processes [50, 51] and certainly if proton leak occurs by delocalised diffusion, then inner membrane surface area differences can account for the majority of differences in proton leak rate [10, 36, 49].

Thus, liver mass ($\alpha M^{0.82}$) [17] combined with hepatocyte oxygen consumption ($\alpha M^{-0.18}$) [21] gives a derived liver metabolism $\alpha M^{0.64}$ (table 2). Liver mass ($\alpha M^{0.82}$) [17] combined with mitochondrial density per unit mass hepatocytes ($\alpha M^{-0.09}$) [40] and inner membrane surface area per unit volume of mitochondria ($\alpha M^{-0.10}$) [40] gives a derived liver mitochondrial inner membrane surface area $\alpha M^{0.63}$, a value similar to that calculated independently by Else and Hulbert [52] and remarkably similar to the allometric relationship derived for liver metabolism ($\alpha M^{0.64}$) (table 2). Alternatively, one could substitute inner membrane surface area with the proton leak rate (albeit calculated at a single membrane potential) ($\alpha M^{-0.13}$) (Table 2) [49] and get a correlation between derived liver metabolism ($\alpha M^{0.64}$) and derived liver mitochondrial proton leak rate ($\alpha M^{0.60}$). Clearly, from the information presented, the amount of mitochondrial inner membrane surface area for liver parallels liver metabolism. Interestingly, Else and Hulbert [53] independently showed that mitochondrial inner membrane surface area for the whole body correlated with whole body metabolism. As the surface area of the mitochondrial inner membrane correlates with proton leak rate, the differences in inner membrane surface area with body mass suggest that proton leak rate is an important determinant of the differences in metabolic rate observed between large and small mammals.

Differences in proton leak rate have also been correlated with differences in mitochondrial inner membrane phospholipid fatty acid composition in thyroid status, mammal/reptile comparisons and body mass [36, 49, 54]. In general, phospholipids from leakier membranes have more polyunsaturated fatty acids. However, there is no significant difference in permeability of deproteinised phosphoplipid unilamellar vesicles made from mitochondria in which proton leak differences do occur [55], suggesting a role for proteins in proton leak. Recent research has shown that there is a family of mitochondrial inner membrane proteins with homology to the uncoupling protein occurring in mitochondria of brown adipose tissue [56]. These novel putative uncoupling proteins may play a role in determining differences in mitochondrial proton leak.

As mentioned earlier, there is an argument that potential-dependent slippage of the proton pumps and not proton leak accounts for the non-ohmic relationship between mitochondrial oxygen consumption and mitochondrial membrane potential [37]. Methodology has been devised to distinguish between potential dependent slippage of the proton pumps and proton leak in isolated mitochondria. The application of this technique suggests that pro-

ton leak (and not potential-dependent slippage of the pumps) is indeed occurring in isolated mitochondria incubated at 37 °C, the temperature used in all the allometric studies quoted in this review [57, 58]. The means to discriminate between pump slippage and proton leak for mitochondria in situ in cells are not as well defined [59]. In that context, it may be that slippage and not proton leak is the 'inefficiency' of interest, thus the aforementioned differences in inner membrane surface area would probably not play a part in accounting for differences in slippage between mitochondria from large and small mammals. A plausible argument could be made to suggest that the observed changes in inner membrane phospholipid fatty acid composition affect pump activity. Either way, the proton leak/pump slippage inefficiency is real and varies with body mass and other factors that affect basal metabolic rate.

Summary

In the 1930s, Max Kleiber and Samuel Brody established that the interspecies correlation between mammalian body mass and metabolic rate ($\alpha M^{0.75}$) cannot be explained (solely) by whole body surface area ($\alpha M^{0.66}$) to volume ratios. Metabolic considerations must also be taken into account. Decreases in the mass of highly metabolic internal organs, relative to whole body mass, can account for some of the whole body metabolic differences, for example liver mass $\alpha M^{0.82}$ [17]. However, superimposed upon these anatomical differences are metabolic differences. Tissue slice and isolated cell studies have shown that the oxygen consumption rate decreases with increasing body mass. These decreases can be explained by a decrease in ATP turnover, a decrease in cellular mitochondrial density and an increase in the efficiency with which mitochondria make ATP (i.e. a decrease in particular proton leak) in these cells and tissues. The majority of the proton leak differences can be accounted for by differences in mitochondrial inner membrane surface area which itself correlates with liver metabolism. Mitochondrial proton leak is a significant contributor (~25%) to basal metabolism and is a major factor determining the differences in basal metabolism between mammals of different body mass, as was predicted by Brand [60].

1 Blaxter K. (1989) Energy Metabolism in animals and man. Cambridge University Press, Cambridge, UK
2 Prentice A. (1995) Le Symposium Lavoisier. Proc. Nutr. Soc. **54:** 1–8
3 Brody S. (1945) Bioenergetics and Growth, Reinhold, New York
4 Kleiber M. (1961) The Fire of Life, Wiley, New York
5 Calder W. A. (1984) Size function and life history. Harvard University Press, Cambridge, Mass.
6 Schmidt-Nielsen K. (1984) Scaling: Why Is Animal Size So Important? Cambridge University Press, Cambridge, UK
7 Benedict F. G. (1938) Vital Energetics: A Study in Comparative Basal Metabolism. Carnegie Institute, Washington DC
8 Zeuthen E. (1953) Oxygen uptake as related to body size in organisms. Q. Rev. Biol. **28:** 1–12
9 McNab B. K. (1988) Complications inherent in scaling the basal rate of metabolism in mammals. Q. Rev Biol. **63:** 25–54
10 Brand M. D., Chien L.-F., Ainscow E. K., Rolfe D. F. S. and Porter R. K. (1994) The causes and functions of mitochondrial proton leak. Biochim. Biophys. Acta **1187:** 132–139
11 Rolfe D. F. S. and Brown G. C. (1997) Cellular energy utilisation and molecular origin of standard metabolic rate in mammals. Physiol. Rev. **77:** 731–758
12 Waterlow J. C., Garlick P. J. and Millward D. J. (1978) Protein Turnover in Mammalian Tissues and the Whole Body. Elsevier, Amsterdam
13 Baldwin R. L., Smith N. E., Taylor J. and Sharp M. (1980) Manipulating metabolic parameters to improve growth rate and milk secretions. J. Anim. Sci. **51:** 379–387
14 Rubner M. (1883) Über die Einflus der Körpergrösse auf Stoff und Kraftwechsel. Z. Biol. **19:** 535–562
15 Weibel E. R., Taylor C. R,. Gehr P., Hoppeler H., Mathieu O. and Maloiy G. M. O. (1981) Design of the mammalian respiratory system IX. Functional and structural limits for oxygen flow. Resp. Physiol. **44:** 151–164
16 Weibel E. R. (1987) Scaling of the structural and functional variables in the respiratory system. Annu. Rev. Physiol. **49:** 147–159
17 Smith R. E. (1956) Quantitative relations between liver mitochondria metabolism and total weight in mammals. Ann. N.Y. Acad. Sci. **62:** 403–422
18 Stahl W. R. (1965) Organ weights in primates and other mammals. Science **150:** 1039–1042
19 Jansky L. (1965) Adaptability of heat production mechanisms in homeotherms. Acta Univ. Carol. Biol. **1:** 1–91
20 Krebs H. A. (1950) Body size and tissue respiration. Biochim. Biophys. Acta **4:** 249–269
21 Porter R. K. and Brand M. D. (1995) Cellular oxygen consumption depends on body mass. Am. J. Physiol. **269:** R226–R228
22 Couture P. and Hulbert A. J. (1995) On the relationship between body mass tissue metabolic rate and sodium pump activity in mammalian liver and kidney cortex. Am. J. Physiol. **268:** R641–R650
23 Mitchell P. (1961) Coupling of phosphorylation to electron and hydrogen transfer by a chemiosmotic type of mechanism. Nature **191:** 144–148
24 Nicholls D. G. and Ferguson S. J. (1992) Bioenergetics, 2. Acedemic Press, London
25 Brand M. D., Lehninger A. L. and Reynafarje B. (1977) The stoichiometric coupling of H^+ efflux during electron transport to H^+ influx during ATP synthesis by mitochondria. FEBS Symp. **42:** 520–534
26 Rolfe D. F. S., Hulbert A. J. and Brand M. D. (1994) Characteristics ot the mitochondrial proton leak and control of oxidative phosphorylation in the major oxygen consuming tissues in the rat. Biochim. Biophys. Acta **1188:** 405–416
27 Nobes C. D., Brown G. C. and Brand M. D. (1990) Non-ohmic conductance of the mitochondrial inner membrane of hepatocytes. J. Biol. Chem. **265:** 12903–12909
28 Rolfe D. F. S. and Brand M. D. (1996a) Proton leak and control of oxidative phosphorylation in perfused, resting rat muscle. Biochim. Biophys. Acta **1276:** 45–50
29 Buttgereit F. and Brand M. D. (1995) A hierarchy of ATP-consuming processes in mammalian cells. Biochem. J. **312:** 163–167
30 Rolfe D. F. and Brand M. D. (1996b) The contribution of mitochondrial proton leak to skeletal muscle and to standard metabolic rate. Am J. Physiol. **271:** C1380–C1389

31 Rolfe D. F. S., Newman J. M., Buckingham J. A., Clark M. G. and Brand M. D. (1999) Contribution of mitochondrial proton leak to respiration rate in working skeletal muscle and liver and to standard metabolic rate. Am. J. Physiol. **276:** C692–C699
32 Hafner R. P., Nobes C. D., McGown A. D. and Brand M. D. (1988) Altered relationship between proton motive force and respiration rate in non-phosphorylating liver mitochondria isolated from rats of different thyroid status. Eur. J. Biochem. **178:** 511–518
33 Hafner R. P., Leake M. J. and Brand M. D. (1989) Hypothyroidism in rats decreases mitochondrial innermembrane cation permeability. FEBS Lett. **248:** 175–178
34 Harper M.-E. and Brand M. D. (1993) The quantitative contributions of mitochondrial proton leak and ATP turnover reactions to changed respiration rates of hepatocytes from rats of different thyroid status. J. Biol. Chem. **268:** 14850–14860
35 Harper M.-E. and Brand M. D. (1994) Hyperthyroidism stimulates mitochondrial proton leak and ATP turnover in rat hepatocytes but does not change the overall kinetics of substrate oxidation reactions. Can. J. Physiol. Pharmacol. **72:** 899–908
36 Brand M. D., Couture P., Else P. L., Withers K. W. and Hulbert A. J. (1991) Evolution of energy metabolism. Biochem. J. **275:** 81–86
37 Brown G. C. (1992) The leaks and slips of bioenergetic membranes. FASEB J. **6:** 2961–2965
38 Drabkin D. L. (1950) The distribution of the chromoproteins, hemoglobin, myoglobin, and cytochrome c, in the tissues of different species, and the relationship of the total content of each chromoprotein to body mass. J. Biol. Chem. **182:** 317–333
39 Kunkel H. O., Spalding J. F., Franciscis G. and Futrell M. F. (1956) Cytochrome oxidase activity and body weight in rats and in the species of large animals. Am. J. Physiol. **186:** 203–206
40 Porter R. K. and Brand M. D. (1995) Causes of the difference in respiration rates from mammals of different body mass. Am. J. Physiol. **269:** R1213–R1224
41 Taylor C. R., Maloiy G. M. O., Weibel E. R., Langman V. A., Kamau J. M. Z., Sheeherman H. J. et al. (1981) Design of the mammalian respiratory system. III. Scaling maximum aerobic capacity to body mass: wild and domestic mammals. Resp. Physiol. **44:** 25–37
42 Hoppeler H. P. and Turner D. L. (1989) Plasticity of aerobic scope: adaptation of the respiratory system in animals, organs and cells. In: Energy Transformation in Cells and Organisms: Proceedings of the 10th Conference of the European Society for Comparative Physiology and Biochemistry, pp 116–122, Weisser W. and Gnaiger E. (eds.), Thieme, Stuttgart
43 Mathieu O., Krauer R., Hoppeler H., Gehr P., Lindstedt S. L., Alexander R. M. et al. (1981) Design of the mammalian respiratory system. VII. Scaling mitochondrial volume in skeletal muscle to body mass. Resp. Physiol. **44:** 113–128
44 Jansky L. (1961) Total cytochrome oxidase activity and its relation to basal and maximal metabolism. Nature **189:** 92–922
45 Jansky L. (1963) Body organ cytochrome oxidase activity in cold and warm acclimated rats. Can. J. Biochem. Physiol. **41:** 1847–1854
46 West G. B., Brown J. H. and Enquist B. J. (2000) The origin of universal scaling laws in biology. In: Scaling in Biology, pp 87–112, Brown J. H. and West G. B. (eds.), Oxford University Press, Oxford
47 Else P. L. and Hulbert A. J. (1987) Evolution of mammalian endothermic metabolism: leaky membranes as a source of heat. Am. J. Physiol. **253:** R1–R7
48 Porter R. K. and Brand M. D. (1993) Body mass dependence of H^+ leak in mitochondria and its relevance to metabolic rate. Nature **362:** 628–630
49 Porter R. K., Hulbert A. J. and Brand M. D. (1996) Allometry of mitochondrial proton leak: influence of surface area and fatty acid composition. Am. J. Physiol. **271:** R1550–R1560
50 Garlid K. D., Beavis A. D. and Ratkje S. K. (1989) On the nature of ion leaks in energy-transducing membranes. Biochim. Biophys. Acta **976:** 109–120
51 Brown G. C. and Brand M. D. (1986) Changes in the permeability of protons and other cations at high proton motive force in rat liver mitochondria. Biochem. J. **234:** 75–81
52 Else P. L. and Hulbert A. J. (1985) An allometric comparison of the mitochondria of mammalian and reptilian tissues: the implications for the evolution of endothermy. J. Comp. Physiol. B **156:** 3–11
53 Else P. L. and Hulbert A. J. (1985) Mammals: an allometric study of metabolism at tissue and mitochondrial level. Am. J. Physiol. **248:** R415–R421
54 Hoch F. L. (1992) Cardilipins and biomembrane function. Biochim. Biophys. Acta **1113:** 71–133
55 Brookes P. S., Rolfe D. F. S. and Brand M. D. (1997) The proton permeability of liposomes made from mitochondrial inner membrane phospholipids: comparison with isolated mitochondria. J. Membr. Biol. **155:** 167–174
56 Ricquier D. and Bouillaud F. (2000) The uncoupling protein homologues: UCP1, UCP2, UCP3, StUCP and AtUCP. Biochem. J. **345:** 161–179
57 Brand M. D., Chien L.-F. and Diolez P. (1994) Experimental discrimination between proton leak and redox slip during mitochondrial electron transport. Biochem. J. **297:** 27–29
58 Canton M., Luvissetto S., Schmehl I. and Azzone, G. F. (1995) The nature of mitochondrial respiration and discrimination between membrane and pump properties. Biochem. J. **310:** 477–481
59 Porter R. K. and Brand M. D. (1995) Mitochondrial proton conductance and H^+/O ratio are independent of electron transport rate in isolated hepatocytes. Biochem. J. **310:** 379–382
60 Brand M. D. (1990) The contribution of the leak of protons across the mitochondrial inner membrane to standard metabolic rate. J. Theor. Biol. **145:** 267–286