Oxidative metabolism and body weight: inactive, active, and mitochondrial volumes

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In homeotherms, the standardized (basal) metabolic rate should not be expressed per kilogram of body weight (specific metabolic rate), nor per unit of body surface (square meters of body-ambient interface), since both mitochondrial thermogenesis and heat-loss mechanisms (radiation, conduction, convection, evaporation) are not uniform processes. On the contrary, each organism is an heterogeneous bioreactor, which is composed at least of two compartments: 1) a metabolically ***active*** *volume (aV), where oxidative phosphorylation takes place; and 2) a metabolically* ***inactive*** *volume (iV), where oxygen consumption is negligible. The ratio (aV/iV) is not invariant, since iV increases disproportionately with the scaling up of body size, and as shown by us, when the three main components of iV,* ***i.e.,*** *skeleton, fat deposits, and blood volume, are added together, a similar disproportionality is found.*

The aV was determined by substracting the iV from the total volume (V) of an organism, or by estimating the volume occupied by all mitochondria, or ***mitochondrial volume*** *(mtV). For this purpose two procedures are discussed: 1) the stereological or morphometric method; and 2) the oxygen consumption per unit time or physiometric method. The latter procedure is based on the equivalence between an* VO_2 *= 3 ml* $O_2 \cdot min^{-1}$ *and a mtV of 1 ml, whose oxidative phosphorylation yields an approximate power output of 1 watt.*

The correspondence between oxygen consumption, heat production, and electron flux at the respiratory chain of the mitochondrial cristae, is discussed.

From a physical point of view, the metabolic rate is a "power" function ($P = M L^2T^{-3}$*), where M = mass, L = length, and T = time. The dimensional analysis and the statistical treatment of the corresponding numerical values of more than 200 allometric equations yields the 3/4 power, law established by Kleiber (1961), for the relationship between basal metabolism and body weight.*

Instead of expressing the metabolic rate per unit body weight (kg^{-1}*) or per unit body surface (*m^{-2}*) structural and functional criteria should be taken into account as, for instance, the distinction between iV and aV, and particularly by emphasizing the paramount importance of the mtV where oxidative phosphorylation takes place.*

An allometric equation relating mtV and body weight (W) could be tentatively established for interspecies comparisons.

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Biol Res 26: 341-355 (1993)

GLOSSARY

ADP = Adenosine diphosphate.

ATP = Adenosine triphosphate.

CoQ = Coenzyme Q (ubiquinone).

Cyt = Cytochromes.

$FADH_2$ = Flavin adenine dinucleotide (reduced form).

M_b = Body mass (kg).

M_m = Muscle mass (kg).

ml mt = One milliliter of mitochondria.

mt = Mitochondria.

NADH = Nicotinamide adenine dinucleotide (reduced form).

P = Power (metabolic rate).

R_b = Basal metabolic rate, not measured as oxygen consumption.

R_{max} = Maximal metabolic rate.

V = Volume (milliliters).

aV = Active volume of the oxidative metabolism (oxygen consumption; heat production; power output).

iV = Inactive volume; oxygen consumption is zero or negligible.

iV_{st} = Subtotal inactive volume (adipose tissue, bones, and blood volume).

VO_2 = Oxygen consumption (ml O_2 per minute).

$VO_{2\,rest}$ = Oxygen consumption under resting conditions.

$VO_{2\,max}$ = Maximal oxygen consumption per unit time.

W = Body weight (kg).

INTRODUCTION

The quantitative relationship between body size and the metabolic rate of living beings can be established by utilizing size as the independent variable as, for instance, the measurements of a characteristic length (L), a given area ($A = L^2$), a volume ($V = L^3$), and also body mass (M) or body weight ($W = M \cdot g$), where g is the acceleration of gravity on earth. On the other hand, the *metabolic rate* can be measured as oxygen consumption (ml O_2 min^{-1}), as heat production (kcal min^{-1}), or as a power (watts), since these three energy fluxes per unit time are equivalent, thus:

$$1 \text{ watt} = 1 J s^{-1} = 0.24 \text{ cal } s^{-1} = 0.05 \text{ ml } O_2 \, s^{-1},$$

where: *J* = joules; *cal* = calories; *s* = seconds.

The phenomenon of scaling, or the structural and functional consequences of changes in size, is conveniently described by means of the allometric equation:

$$Y = a X^b \quad (1)$$

where Y is any physiological, morphological or ecological variable that appears to correlate with size (X), and in most cases with body weight (W, in kg); while a and b are two parameters. The statistical analysis of the empirical data is facilitated by the log-log plot of the data, which yields a straight line:

$$\log Y = \log a + b \log X \quad (2)$$

where the intercept (a), and the slope (b) can be obtained directly.

A STRUCTURAL THEORY OF METABOLISM

As already mentioned, the relationship between basal metabolic rate (P) and body weight (W) is usually defined by means of a power function:

$$P = a W^b \quad (3)$$

in which a and b represent the two parameters of the allometric equation (eqn 1).

Instead of the power function described above (eqn. 3), Spaargaren (1992) has

recently established an improved correlation between body weight (W) and basal metabolic rate (P), namely

$$W = k_1 P + k_2 P^{1.5} \quad (4)$$

where k_1 and k_2 are two constants. The first addend of eqn 4 represents the metabolically "active" volume (aV) of the organism, and corresponds to the aerobic metabolism of all mitochondria within the cells; while the second addend deals with the metabolically inactive volume (iV) whose oxygen consumption is almost negligible. This inactive volume (iV) serves three functions: 1) structural support (skeleton); 2) storage of nutrients (fat deposits); and 3) fluid transport and distribution system (blood volume).

Due to the fact that there is no fixed ratio between the metabolically active (aV) and the inactive (iV) compartments, the canonical relationship between standard metabolic rate (P) and body weight (W) should not be expressed simply as the basal metabolism (P) per kilogram body weight (P/W), since body mass is not an homogeneous entity. Moreover, with increasing size, the metabolically inactive volume (iV) increases disproportionately (Spaargaren, 1992).

I. *The metabolically active part of the organism*

The common final pathway of aerobic metabolism is located inside mitochondria. Atmospheric oxygen is transported to the tissue level by means of two-carrier systems, namely, the respiratory and the circulatory systems, which have a serial arrangement. Furthermore, both carrier systems are arranged as dichotomic branching systems: the first originates from the trachea and ends at the alveolar sacs, while the second is initiated at the aorta or at the main pulmonary artery and terminates at the corresponding capillary networks, where an enormous surface is available for the exchange of O_2, CO_2, heat, and water. It is worth mentioning, that these dichotomic branching systems can be analyzed from a "geometric" point of view (Murray, 1926; Spaargaren, 1992; Weibel, 1963), or a "fractal" (self-similar) design can be applied (Sernetz *et al.*, 1985).

The metabolically active part (aV) of the intracellular respiratory chain is located at the cristae of the internal mitochondrial membrane, which operates as a bioreactor with immobilized enzymes, and which is supplied –at a constant rate– with the corresponding substrates by means of the above mentioned two mass-transport systems (Sernetz *et al.*, 1985). In consequence, the whole organism can be considered as a "fractal area-volume hybrid", whose fractal dimension (D_F) is $2 < D_F < 3$. The same authors obtained a fractal dimension of $D_F = 2.25$ for organisms, a value which is significantly greater than the topological dimension (D_T) for any area ($D_T = 2.0$.) Inasmuch as the area-volume relationship (A/V) is 2/3 for all geometric bodies (b= 0.66), for fractal structures the A/V ratio will be 2.25/3 = 0.75, *i.e.*, equal to Kleiber's 3/4 power law (Sernetz *et al.*, 1985).

It is worth mentioning that the theory of elastic similarity (McMahon and Bonner, 1983) predicts that the cross-sectional area of any anatomical feature is proportional to $W^{3/4}$.

II. *The metabolically inactive part of the organism*

As already mentioned, Spaargaren (1992) has emphasized that the metabolically inactive infrastructure (iV) increases disproportionately with increasing body size. This is evident when the inactive volumes (iV) are expressed as percentages of the corresponding body weights, as shown in his Fig. 5 on p. 503.

From Spaargaren's data on homeothermic animals we obtained a linear equation for the volume (%) of the metabolically inactive component (iV) as a function of the logarithm of body weight (*W*) given in kg,

$$iV\ (\%) = 18 \log W + 68 \quad (5)$$

This equation is valid for a range which comprises a minimal body weight of 10g (mouse) to a maximal and constant value

(95%) for body weights beyond 100 kg. It should be emphasized that the metabolically inactive infrastructure (iV) does not consume significant amounts of oxygen, and that (iV) is formed by body water, dissolved substances, as well as by minerals and organic deposits (Spaargaren, 1992).

Instead of the "total" inactive volume (iV) already mentioned in this study, we will now analyze three of the main components of the metabolically inactive volume (iV), and whose allometric equations are specified in Table I. From the numerical data given in Table I it is possible to calculate the percentage of each component as a function of body weight, and when these percentages are added, an exponential increase of the metabolically inactive volume (iV) is obtained (Fig. 1).

It is noteworthy, that the final percentages are almost identical with Spaargaren's data, particularly in animals whose weights exceeded 100 kg. It is also worth mentioning, that the sum of the three inactive volumes (subtotal, *st*) is again an allometric relationship:

$$(iV)_{st} = 0.205\ W^{1.1} \quad (6)$$

where: iV is expressed in kg, or in liters; W = body weight, in kg.

A comparison between the inactive volumes (iV) and the metabolically active volumes (aV)

From Spaargaren's numerical data it was possible to calculate the metabolically inactive volumes (iV). The differences correspond to the metabolically active volume or mass (Table II).

On the other hand, the standard metabolic rate expressed as a function of body mass was obtained from Benedict's (1938) allometric equation, which comprises measurements in homeotherms from 0.01 to 3000 kg; the corresponding equation reads as follows:

$$\text{Heat production (watts)} = 4.22\ W^{0.65} \quad (7)$$

where W is the body weight in kg.

TABLE I

Allometric equations for three representative components of metabolically inactive volumes (iV), in accordance with Calder (1984)

Items	Parameter (a). Intercept at 1 kg bw	Exponent (b). Slopes
Blood volume	0.069	1.02
Skeleton	0.061	1.09
Fat deposits	0.075	1.19
TOTAL	0.205	1.10 (mean)

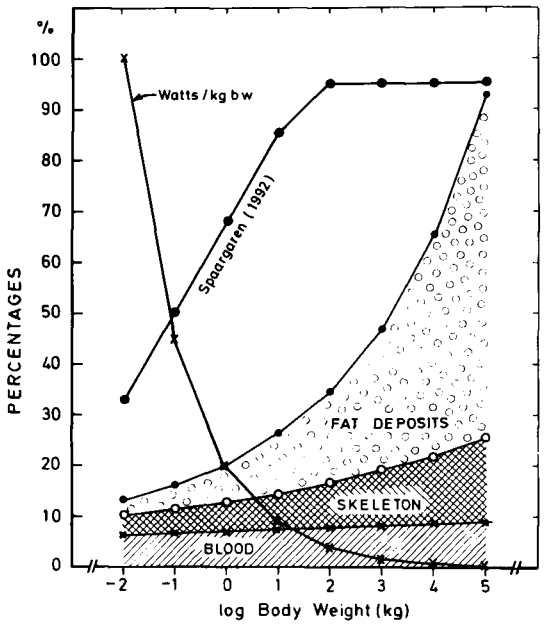

Fig. 1: Volume of the inactive infrastructure (iV) as a percentage of total body weight (in semilogarithmic plot). Spaargaren's data (1992) are compared with the subtotal inactive volumes (iV_{st}), which are represented by the volumes of blood , skeleton, and fat deposits. The «specific metabolic rate» (watts/kg bw) is shown on the left hand curve.

TABLE II

Comparison between the metabolically inactive (iV) and active (aV) volumes as functions of body weight (W)

W (kg)	log W	iV*		aV (kg)	Metabolic rate (watts)**		
		%	kg		Watts	Watts per unit W (kg)	Watts per unit aV (kg)
0.01	-2	33	0.0033	0.0067	0.21	21.2	31.34
0.10	-1	50	0.050	0.050	0.95	9.45	19.0
1.0	0	68	0.680	0.320	4.22	4.22	13.18
10	1	85	8.50	1.5	18.85	1.88	12.56
100	2	95	95.0	5.0	84.20	0.84	16.84
1 000	3	95	950	50	376.00	0.38	7.52
100 000	5	95	95 000	5 000	7 510.00	0.075	1.50

* Spaargaren, 1992
** After eqn 7.

An alternative reference system for the standardized (basal) metabolic rate is Kleiber's (1961) allometric equation :

$$P = 73.3\ W^{0.75} \quad (8)$$

where P = power in kcal/day; and W = body weight in kg (see Appendix B & C). When both parameters are rounded off (Schmidt-Nielsen, 1984), the following relationship is obtained:

$$P = 70\ W^{0.75} \quad (9)$$

The only difference between eqns 7 and 9 is the slope (b) of the "specific metabolic rate" (kcal/kg); in the first case the slope is $b = 0.65 - 1 = -0.35$, and in the second $b = 0.75 - 1 = -0.25$.

The "specific metabolic rate" (Table II) can now be expressed either as a function of body weight (kg^{-1}), or as a function of the active volume (aV), also expressed in kg. In both cases, the specific metabolic rates decrease exponentially (see Fig. 1), albeit less markedly in the second case, when the aV is used as the corresponding reference system (Table II). Furthermore, in the latter case, the exponential decrease of the specific metabolic rate is not uniform (see last column of Table II).

III. *The mitochondrial volume (mtV) of an organism*

Instead of starting out by establishing the numerical values for the inactive volume (iV) in order to calculate then the remainder, *i.e.*, the active volume (aV), we have intended to directly define the active volume (aV) as represented by the volume of all mitochondria (mtV) within the organism, irrespective of their heterogeneous distribution in different organs.

The *mitochondrial volume* (mtV) can be established quantitatively by means of two procedures, namely:

i) the stereological determination (morphometry); or ,
ii) through the measurement of oxygen consumption per unit of time (ml $O_2 \cdot s^{-1}$), and the subsequent calculation of the volume of mitochondria (ml mt) involved.

A. *The morphometric estimation of the mitochondrial volume (mtV)*

The allometric parameters of liver, skeletal muscle, and heart muscle mitochondria are known.

In the case of the liver mitochondria of different species, Smith (1956) has established the following allometric relationships:

1) the *number of mitochondria per gram* liver = $W^{-0.1}$
2) the *liver mass* is proportional to $W^{0.82}$
3) the total number of *mitochondria in the liver* is therefore $W^{0.72}$, and
4) the number of *liver mitochondria per gram* of body mass is $W^{-0.28}$.

In conclusion, from Item 1) it can be deduced that the number of mitochondria decreases progressively with increasing size (slope = –0.1), and from Item 2) it follows that the liver mass, as that of other viscera, has an exponent (b) less than unity; nevertheless, the product of items 1) and 2) yields an exponent *b* of 0.72, which is similar to Kleiber's (1961) value (b = 0.75) for the relationship of the basal metabolic rate with body weight. The same is valid for the correlation between "specific metabolic rate" of homeotherms (b = 0.75^{-1} = –0.25) and for the number of liver mitochondria per unit of body mass (b = –0.28).

With regards to muscle mitochondria, Mathieu *et al.* (1981) have found that the parameter *a* for the total volume of mitochondria (mtV) in different muscles is similar, with the exception of the diaphragm, but that the allometric exponent *b* decreases consistently with increasing body size (Table III).

Finally, the volume density of *heart* mitochondria of 11 mammalian species, ranging from the shrew (3g) to cattle (929 kg) was determined stereologically by Hoppeler *at al.*, 1984. The relationship between heart mass and body mass scaled with an allometric exponent *b* of 0.97, while that of the volume density of mitochondria was found to scale with a *b* of –0.044. In consequence, the total volume of heart mitochondria scales with b = 0.927. The slope of this allometric regression is significantly greater than the regression for VO_{2max}, whereas a similar exponent (b = 0.90) was obtained for the work rate (power) of the heart under resting conditions. Thus, the mitochondrial volume of the heart could be defined morphometrically in accordance to the following allometric equation:

$$mtV = 1.85\ W_b^{0.927} \qquad (10)$$

where: mtV is mitochondrial volume (ml); W_b is body weight (kg).

TABLE III

Allometric parameters of the mitochondrial volumes (ml) in different muscles of 13 species of mammals. (Mathieu *et al.*, 1981; Peters, 1983).

Muscle	Parameter a or intercept (Vol. %) at 1 kg W	Slope b ($b \pm S_b$)
Semitendinosus	0.065	–0.231 ± 0.025
Longissimus dorsi	0.066	–0.163 ± 0.024
Vastus medialis	0.071	–0.139 ± 0.070
Diaphragm	0.131	–0.055 ± 0.028

B. *The physiometric estimation of the mitochondrial volume (mtV)*

Under VO_{2max} conditions, skeletal and heart muscle consume 3.5 ml $O_2 \cdot min^{-1} \cdot ml^{-1}$ of mitochondria (mt); the corresponding value per second is 0.058 ml $O_2 \cdot s^{-1} \cdot (ml\ mt)^{-1}$, roughly 0.06 ml $O_2 \cdot s^{-1} \cdot (ml\ mt)^{-1}$.

The mitochondrial volume (mtV) can also be correlated with the oxygen consumed per

second due to the fact that an oxygen consumption of 0.05 ml $O_2 \cdot s^{-1}$ is equivalent to 1 watt.

As summarized in Table IV and in Appendix A, the quantitative analysis of this process yielded a power function equivalent to about 80 watts for an oxygen consumption of 250 ml $O_2 \cdot min^{-1}$ of a human being under resting condition. On the other hand, when the equivalent power (watts) is based on the oxygen consumption, where 0.05 ml $O_2 \cdot s^{-1}$ is equivalent to 1 watt, we obtained for a value of 4.17 ml $O_2 \cdot s^{-1}$ a total power of 82.0 watts (see Appendix A).

Whereas the mass-specific muscle mass in homeotherms is almost invariant ($M_m/M_b = 0.385\ M_b^{-0.0082}$), and equivalent to 38.5% of body weight, irrespective of body size, the total mitochondrial volume in muscles ($mtV/M_b = 0.0238\ M_b^{-0.175}$) decreases with a slope $b = -0.175$ when sedentary species are considered (Weibel *et al.*, 1992). The same authors came to the conclusion that mitochondria operate at the same rate at VO_{2max} in all mammals, *i.e.*, the overall capacity for maximal oxygen consumption is related to the total quantity of mitochondria (mtV). The ratio (mtV/VO_{2max}) is invariant.

It should be emphasized that mtV is always a maximal when the stereological methods are employed (Mathieu *et al.*, 1981; Weibel, 1987). On the contrary, the physiometric determination yields variable mtV values, due to the fact that oxygen consumption (VO_2) determines mtV. Thus, during exercise there is an increase in pulmonary ventilation, in cardiac output, in

TABLE IV

Morphometry and physiometry of mammalian mitochondria.

A	MORPHOMETRY (Stryer, 1975; Taylor, 1987)
	The *mitochondrion* is an ellipsoid of revolution, whose length is 1.5 μm, whose
A_1	width is 0.5 μm, and whose volume is 0.8μm^3.
A_2	A volume of *1 ml* contains 1.25×10^{12} mitochondria (mt).
A_3	The *surface area of the inner membrane* per volume of mitochondria is 20-40 m^2 ml (mt); with an average value of 30 m^2 / ml mt.
A_4	The *respiratory chain enzymes* occupy 40% of the surface of the inner membrane,
A	or $30 \times 0.4 = 12\ m^2/ml\ mt^{-1}$.
B	PHYSIOMETRY (Taylor, 1987)
B_1	At maximal oxygen consumption (VO_{2max}), *oxygen uptake* per unit of inner membrane surface is: 0.156 ml $O_2/min^{-1} \cdot m^{-2}$.
B_2	In *skeletal muscle*, and under VO_{2max} conditions, 1 ml of mitochondria consumes between 3 and 6 ml $O_2 \cdot min^{-1} \cdot ml^{-1}$.
B_3	In *the heart muscle* O_2 consumption is 3.5 ml $O_2\ min^{-1} \cdot$ ml mt^{-1}
C	MICROCIRCULATION AND MITOCHONDRIA (Weibel, 1987)
C_1	*Capillary density* is directly proportional to mitochondrial volume density. Amount of O_2 supplied by capillaries closely matches that required by mitochondria.
C_2	*1 ml of capillary blood* supplies O_2 to 3 ml of mitochondria.
C_3	*Mean transit time* in a capillary is 0.5 s.
C_4	*Total capillary length* is directly proportional to total mitochondrial volume: 13 km of capillaries per 1 ml of mitochondria.

capillarization of the muscular tissue, and also in the total volume of the mitochondria (mtV) involved.

Mitochondrial volume density in different organs: a comparison of mitochondrial thermogenesis at rest and during heavy work

Cellular thermogenesis is located primarily inside the mitochondria (Himms-Hagen, 1976). Only 25% of the free energy released from the substrates is conserved as ATP; the remaining 75% appears as heat. The regulation of oxidation phosphorylation is determined by the levels of ADP (respiratory control); when the ADP level increases (due to ATP consumption), oxygen uptake increases. Electrons do not flow from fuel to oxygen unless ATP needs to be synthesized.

Table V specifies the intensity of heat production (mitochondrial thermogenesis) for different organs (%), at rest and during heavy work. These values are compared with the relative weight (%) of the organs involved. It is interesting to note that under resting conditions (see Fig. 2) visceral thermogenesis, including the brain, accounts for 72% of all heat production, despite the fact that thoracic and abdominal viscera and the brain represent only 8% of body weight, and that the heat production of the mitochondria in skin and striated musculature is only 18% of the total. These relationships are completely reversed during heavy work, where the main heat production is produced in the musculature and the skin (73%), while visceral and cerebral thermogenesis is reduced to 25%. In consequence, during rest mitochondrial oxidative metabolism is concentrated in the visceral organs, whilst during heavy exercise over 90% of the oxygen consumed occurs in the muscle cells (Weibel *et al.*, 1992).

The allometric relationships between mitochondrial and capillary densities

The interspecies variation in the density of mitochondrial, as well as the capillary density in different muscles, were studied by

TABLE V

Relative intensity of the thermogenesis in human is given for different organs, both at rest and during heavy work. (Adapted from Aschoff, 1971)

Organs	Organ weight (% W)	Thermogenesis (%) At rest	Thermogenesis (%) Heavy work
Brain	**2**	**16**	3
Thoracic and abdominal viscera	**6**	**56**	22
Skin and musculature	52	18	**73**
Other tissues	40	10	2
TOTAL	100	100	100

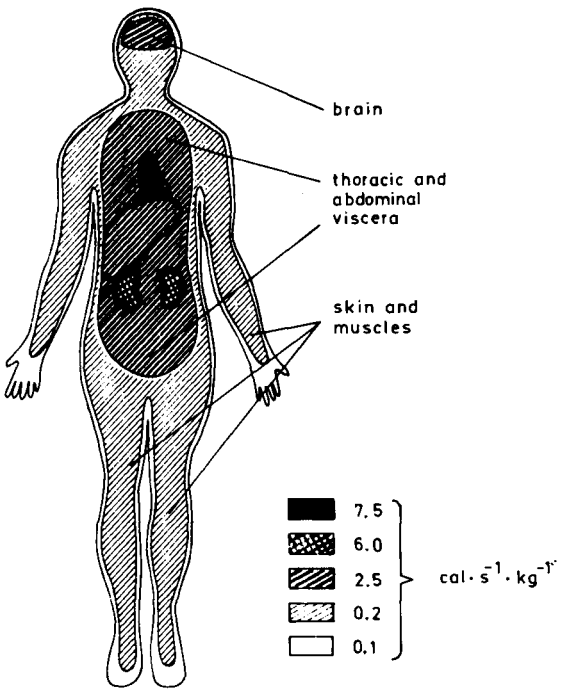

Fig. 2: Topography of the different intensities of the specific thermogenesis (cal $s^{-1}kg^{-1}$) in different organs of a human being under resting conditions. (Modified after Aschoff, 1971).

Mathieu *et al.* (1981) and Hoppeler *et al.* (1984); the corresponding allometric exponents (b) are summarized in Table VI. The exponents for mitochondrial densities vary between b = –0.055 and b = –0.231. On the other hand, the slopes of the capillary densities (b) vary from b = –0.045 to b = –0.138.

In consequence, the decline of the specific metabolic rate (b = –0.25) is in part due to the progressive reduction of the mitochondrial density in different muscles, and which is parallel by decreasing capillarization.

Hoppeler and Kayar (1988) established a linear correlation between oxidative capacity (mitochondrial volume density) and capillary length density for the heart, diaphragm, and locomotor muscles.

TABLE VI

Allometric exponents (b) of mitochondrial and capillary densities in different muscles of mammals. (After Mathieu *et al.*, 1981, and Hoppeler *et al.*, 1984)

Muscle	Mitochondrial density (Mathieu *et al.*, 1981)	Capillary density (Hoppeler *et al.*, 1981)
Semitendinosus	–0.231	–0.138
Longissimus dorsi	–0.163	–0.100
Vastus medialis	–0.139	–0.097
Diaphragm	–0.055	–0.045

A comparison between the "mammalian" and "reptile" machines

Extensive interspecific measurements of standardized metabolic rates of homeotherms and their comparisons with poikilotherms can be found in the literature (Hemmingsen, 1960). The slopes (b) of the corresponding allometric exponents are almost identical ($b \approx 0.75$), while parameter **a** is four to five times greater in homeotherms than in poikilotherms.

In order to investigate the possible causes for these differences, Else and Hulbert (1981) compared animals (*Mus musculus* and *Amphibolurus nuchalis*) of the same body size (≈ 30g) and at the same body temperature (37°C). They found that the difference between mammalian and reptile models was due to three factors: 1) mammals have relatively larger internal organs; 2) the same organs have a greater proportion of mitochondria; and 3) the corresponding mitochondria have a greater relative membrane surface area. These differences are attributed to a greater thyroid gland activity in mammals than in reptiles.

ATP production per day in different organs of an adult human being under resting condition

In order to visualize the real magnitude of ATP production in man, we have calculated the approximate ATP production in different organs (Table VII), based on empirical data which are commonly expressed in micromoles of ATP per gram tissue per minute. This information was utilized to calculate ATP production in moles per day, then in kg ATP per day, and finally in kg of ATP per organ weight.

For the human brain, whose average weight is 1.4 kg, the daily output of ATP is 10 kg. Nevertheless, the highest ATP producing organ is the heart, since it produces 22 times its own weight in ATP per day; whereas the striated musculature –under resting condition– has an ATP output equivalent to 50% of its own weight.

It is noteworthy that this daily ATP production does not affect the weights of the corresponding organs, the reason being that, as soon as ATP is produced it is utilized instantaneously; thus, a steady–state in organ masses is achieved.

Total body ATP production is approximately 200 moles per day (Aschoff, 1971), which is equivalent to 100 kg ATP per day. This amount is produced by a mitochondrial volume (mtV) of around 100 ml, as the resultant of the oxidative phosphorylation of an equivalent of about 500 g of glucose per day, which yields 25% as ATP, and 75% as heat (see Appendix A).

TABLE VII

Quantitative appraisal of ATP production (kg/day) in an adult human being under resting condition. A comparison between different organs. MW of ATP = 507.2

Organ	Organ weight g	ATP-production (approximate values)			
		$\mu mol \cdot g^{-1} \cdot min^{-1}$	$mol \cdot d^{-1}$	$kg \cdot d^{-1}$	kg ATP $\cdot d^{-1}$ (kg organ weight)$^{-1}$
Heart	300	30	13	6.5	22
Kidneys	300	25	11	5.5	18
Brain	1,400	10	20	10.0	7
Musculature (striated)	27,000	0.7	27	13.5	0.5

DISCUSSION

The relationship between metabolic rate and body size has been discussed for more than a century in the quest of obtaining some uniforming principle capable of quantitatively describing the scaling of metabolism (power) and body size (mass).

When oxidative metabolism is expressed per unit body mass (kcal/kg) this "specific metabolic rate" decreases exponentially with increasing body mass (Fig. 1). On the other hand, when the metabolic rate is expressed as a function of body surface ($kcal/m^2$) in accordance with Rubner's (1883) law, an almost constant value is obtained, both for intra- or interspecific comparisons, despite the fact that from a physical point of view, the four main heat-loss mechanisms (radiation, evaporation, conduction, and convection) vary from point to point, since each square centimeter of the skin (interface between the organism and the surrounding medium) is different from a thermodynamic point of view.

Moreover, organisms are not homogeneous bioreactors (Semetz *et al.*, 1985), since each living being is an open, dissipative system, at steady-state, in which the heterogeneous catalysis takes place on immobilized enzymes inside mitochondria, specifically at the respiratory chain located at the cristae, while the substrate solution is supplied at a constant rate. The products and the unconsumed substrates are released at the outlet. The distribution volume (V) of this enzyme reactor model, is equivalent to the body mass (M) of each living being.

On the contrary, Spaargaren's (1992) has emphasized the necessity to distinguish between a metabolically "active" volume (aV) and an "inactive" volume (iV) in which oxygen consumption is negligible ($VO_2 \approx 0$). Due to the fact that the inactive volume (iV) increases disproportionally with increasing body mass, the corresponding active volume (aV) must diminish in a similar manner.

A second interspecies estimation of the inactive volume (iV) of organisms was based on the allometric equations of the three inactive tissues (skeleton, fat deposits, blood), from which we could directly obtain the scaling of the inactive volume (iV) in animals of different sizes. The notorious differences between Spaargaren's data (1992) and our estimations are due to the fact that the former author probably considered the total water content –about 70% of body weight in an adult man– whereas in our estimations we only considered the water

content of the "blood volume". With regards to the skeleton, as part of iV, we have included the hydroxyapatite crystals and the organic matrix (collagen fibrils) of the bone, without considering the cells (osteoblasts and osteoclasts) and blood vessels responsible for active bone turnover.

Nevertheless, the two procedures mentioned above are of an indirect nature, since they are exclusively based on structural criteria, but neglect the main point, namely, the metabolic rate, which is determined either as oxygen consumption or as heat production per unit time.

The metabolic range

It should be emphasized that the "standardization" of metabolic rate measurements (ambient temperature at the thermal neutral zone; fasting or postabsorptive condition; awake; inactive; unexcited; healthy; and at a nonreproductive period) means that, in order to attain reproducible values for basal metabolism, the organisms must be submitted to a set of very unusual conditions. Furthermore, many basal metabolism measurements contain a systematic bias since circadian rhythms are neglected (Prothero, 1984).

During normal life the metabolic rate varies constantly, depending –among other circumstances– upon muscular activity, which may attain values approximately ten times higher than the basal metabolic rate when VO_{2max} is reached. In mammals, under *basal* conditions, the metabolic rate is $R_b = 3.28\ W^{0.756}$, where R_b is given in watts and W in kg; while the *maximal* metabolic rate (R_{max}) is $23.4\ W^{0.73}$ in ordinary mammals, and in wild mammals Taylor (1987) found $R_{max} = 39.0\ W^{0.79}$ (see Peters, 1983), which is more than 10 times the basal values. Recently, Weibel *et al.* (1992) have described that "athletic" species (racehorses and pronghorn antelopes) are able to increase their metabolism by 30 to 60 times above resting rates.

Thus, *the basal metabolic rate* is a measure of oxygen consumption or heat production of organisms under highly defined conditions of measurement; in addition, these values are not minimal (Schmidt-Nielsen, 1984). However, it is important to consider that they are obtained under strictly reproducible and standardized conditions.

On the other hand, *maximal oxygen consumption* ($VO_{2\ max}$) represents either a metabolic bursts or a sustained activity, in which the organisms can attain more than a tenfold increase in oxygen consumption. The maximum metabolic rate has great biological significance, since during "fight or flight" the survival of an organism depends upon the capacity to markedly increase its muscular power, and simultaneously to augment the main transport functions (respiration, circulation, as well as oxygen delivery from the blood).

Symmorphosis

A comparison of a given function in animals of different sizes, for instance, oxygen transport at different levels of the "respiratory cascade", namely 1) external respiration; 2) diffusion at the alveoli; 3) cardiovascular transport of blood; 4) diffusion at the capillaries; and 5) oxidative metabolism inside the mitochondria, is also of interest.

In accordance with the hypothesis of *symmorphosis* (from the Greek *symmorphos*, which means "alike" or "of the same form"), postulated by Weibel and Taylor (1981), at all levels, "*structures supporting a function should be no larger than needed to satisfy the fundamental demands at the limit of performance.*".

With regards to mitochondria in muscle cells, it was found that the mitochondrial volume (mtV) is directly proportional to VO_{2max}.

The same consideration is valid for the capacity for O_2 delivery of the microvasculature, as well as for the oxygen transport capacity of the circulatory system. Nevertheless, the principle of symmorphosis is only partially fulfilled in the lungs, since the allometric variation of the "pulmonary diffusing capacity" varies directly with body mass (b = 1.0), and is thus not commensurate with VO_{2max} whose allometric exponent is close to b = 0.75. Furthermore, it is interesting to note that the internal steps in the transport of oxygen (mitochondria,

capillaries, blood, and heart) are matched to functional demands during VO_{2max} conditions, whereas the lung maintains a variable excess of morphometric diffusing capacity, which seems to indicate that the respiratory system of mammals was designed with an appreciable "safety margin", in order to cope with bursts of maximal demands of oxygen when life is in danger.

It is also worth mentioning that the allometric equation for the basal metabolic rate has the same exponent (b = 0.75) as the corresponding equation for VO_{2max}, in the latter case there is only a tenfold change of *parameter a.* The ratio between maximal oxygen consumption (VO_{2max}) and oxygen consumption under resting conditions (VO_{2rest}) has been studied in wild mammals (Stahl, 1967; Taylor, 1987; Calder, 1984), and the corresponding allometric equations have yielded the following quantitative information:

$$VO_{2max}/VO_{2rest} =$$

$$116.4W^{0.79}/11.6W^{0.76} = 10\ W^{0.03} \qquad (11)$$

In consequence, the ratio between VO_{2max} and VO_{2rest} is equal to ten, irrespective of the size of the animals considered.

FINAL COMMENTS

It is interesting to note that, five decades ago, Benedict (1938), in his classical monograph entitled "Vital Energetics", summarized the main problems in the following statement:

> "It is believed that greater progress will be made by discarding all thoughts of a uniformity in heat loss and emphasizing the non-uniformity in heat production, seeking to associate differences in configuration and body make-up, and above all differences in the morphology, chemistry, and quantity of blood, with greatest emphasis upon the differences in the minute-volume as being the great regulator of metabolic intensity".

In conclusion, it should be emphasized that oxidative metabolism takes place exclusively in the mitochondria of a given organism, and that oxygen consumption (ml O_2 • min) and heat production (kcal • min) are closely correlated with the volume of mitochondrial (mtV), which, in fact, represents the active volume (aV) of each organism.

The mtV can be determined *in vitro* by stereological methods or *in vivo* by measuring VO_{2max}. In an adult human being of 65 kg body weight VO_{2max} is ten times greater than VO_2 under standardized condition, and therefore $VO_{2max} \approx 2500$ ml $O_2 \cdot min^{-1}$, which is equivalent to about 800 watt and to a mtV of 800 ml. This value (0.8 kg) represents an aV equal to 1.2% of body weight and, in consequence, the inactive volume will be iV = 98.8%.

Finally, for interspecies comparisons we would suggest a tentative allometric equation for mitochondrial volume (mtV), namely:

$$mtV = 36.4W^{0.75} \qquad (12)$$

where: mtV is given in ml; W is body weight in kg.

From eqn 12 it is possible to calculate the mitochondrial volume (mtV) of the smallest (3 g shrew) to the largest living mammal (100 tons blue whale). The mtV of the shrew is equal to 15% of its body weight, while that of the whale is only 0.2% of its body weight. The mass-specific metabolic rate of the shrew is extremely high (15% W), and in this regard it is worth mentioning the recent work of Suarez (1992), who stereologically determined the volume density (%) of the mitochondria in the flight muscles of 2 g hummingbirds to be 35%. This latter result is in agreement with the corresponding oxygen consumption, which is 2-fold higher than that estimated in the skeletal muscles of mammals running at VO_{2max}.

Summarizing, per day *one milliliter of mitochondria,* whose power output is 1 watt:

1) consumes 4.32 liters of oxygen;

2) produces around 20 kcal of heat;

3) synthesizes about 1.25 kg of ATP; and

4) metabolizes the equivalent of 5 g of glucose.

ACKNOWLEDGMENTS

The authors are grateful to Prof Dr Peter H. Ward, Department of Physiology, University of Concepción, for helpful discussions and comments, and to Prof Jorge Alé, Department of Physics, University of Concepción, for valuable suggestions concerning the dimensional analysis discussed in Appendix C.

APPENDIX A

The transport of oxygen (respiration and circulation) leads to the "oxygen sink", which is represented by the enzyme chains located at the mitochondrial cristae, where the oxidation phosphorylation of the substrate takes place (Stryer, 1975). The sequence of the electron carriers in the respiratory assembly is:

$NADH$ or $FADH_2$ —> Co Q —> Cyt b —> Cyt c_1 —> Cyt c —> Cyt $(a+a_3)$ —> O_2

1) An oxygen consumption of a human being, under basal conditions (about 250 ml O_2 min^{-1}), is equivalent to an uptake of 4.17 ml O_2 s^{-1}.
2) Each molecule of O_2 accepts 4 electrons, in accordance with the following process:

$$O_2 + 4H^+ + 4e^- \longrightarrow 2H_2O$$

3) 1 mol of O_2 under STPD conditions occupies a volume of 22.4 liters;
4) 1 mol O_2 contains 6.02×10^{23} molecules (Avogadro's number).
5) An oxygen consumption of about 4.2 ml $O_2 \cdot s^{-1}$ is equivalent to 11.2×10^{19} molecules of O_2 per second;
6) Since each atom of O_2 accepts $4e^-$, about 45×10^{19} electrons are mobilized per second.
7) One Ampere is equivalent to an electron flux of 6.24×10^{18} electrons per second, therefore, the total electron flux in the human organism is equal to 72 Amperes.
8) The redox potential between the substrate NADH or $FADH_2$ and molecular oxygen is 1.14 Volts ($E'_o = -0.32$ Volts for NADH, and $E'_o = +0.82$ Volts for oxygen).
9) The equivalent electric power of the whole organism is 72A x 1.14 V = 82.0 Watts.
10) Essentially the same result can be obtained from the oxygen consumption per unit of time (ml $O_2 \cdot s^{-1}$) and the equivalence of 1 watt, which is (4.167 ml $O_2 \cdot s^{-1}$) / (0.05 ml $O_2 \cdot s^{-1}$) = 83.3 watts. On the other hand, since 1 watt is equivalent to 0.24 cal s^{-1}, 83.3 watts yield around 20 cal s^{-1}; therefore, the total heat production will be 20 cal s^{-1} x 86400 s = 1728 kcal per day.

APPENDIX B

From a statistical point of view, and based on the theory of biological similarity and the dimensional analysis (Günther *et al.*, 1992), we have examined the correlation between 203 empirical allometric exponents (b) and the theoretically calculated reduced exponent (b). For twelve different functions of biological interest the general equation is:

$$b_R = 0.958\alpha + 0.346\beta + 0.296\gamma \qquad (B_1)$$

where:

b_R = the estimated allometric exponent, which were obtained from the regression analysis;
α = mass exponent (M = mass);
β = length exponent (L = length);
γ = time exponent (T = time).

Since the metabolic rate is a "power function (P), whose physical dimensions are:

$$[P] = ML^2 T^{-3} \qquad (B_2)$$

It is possible to estimate the most probable exponent (b) for a power (P) as a function of body weight (W), namely:

$$b_R = 0.958\,(1) + 0.346\,(2) + 0.296\,(-3) \qquad (B_3)$$

which yields:

$$P = a \cdot W^{0.762} \qquad (B_4)$$

Thus, the theoretically expected allometric exponent is very close to the empirical 3/4 exponent established by Kleiber (1961).

APPENDIX C

The statistical analysis of Kleiber's 3/4 interspecific mass exponent for basal metabolism in mammals has led to a lively controversy (Heusner, 1982; Feldman & McMahon, 1983), as has the dimensional analysis for obtaining the mass exponent for metabolic scaling (see Butler *et al.*, 1987).

With regards to the problem of dimensional analysis and its application to biology, it is necessary to emphasize that Newton's reduction coefficient (χ_q) should always be based on a physical law of the form:

$$q = k \cdot m^{\alpha} \cdot l^{\beta} \cdot t^{\gamma} \qquad (C_1)$$

where:

q is a physical function;
k is a numerical constant;
m is a mass;
l is length; and
t is time.

The comparison of measurable physical quantities of a given phenomenon (q), both in prototype (p) and model (m), yields:

$$q_p = k \cdot m_p^{\alpha} \cdot l_p^{\beta} \cdot t_p^{\gamma} \qquad (C_2)$$

$$q_m = k \cdot m_m^{\alpha} \cdot l_m^{\beta} \cdot t_m^{\gamma} \qquad (C_3)$$

and the corresponding ratio ($\chi_q = q_p/q_m$) is:

$$\chi_q = \frac{q_p}{q_m} = \left(\frac{m_p}{m_m}\right)^{\alpha} \left(\frac{l_p}{l_m}\right)^{\beta} \left(\frac{t_p}{t_m}\right)^{\gamma} \qquad (C_4)$$

Instead of using the corresponding physical dimensions (m, l, t), it is more convenient to establish the following ratios:

$$m_p/m_m = \mu \qquad (C_5)$$

$$l_p/l_m = \lambda \qquad (C_6)$$

$$t_p/t_m = \tau \qquad (C_7)$$

When these three ratios are introduced into Newton's reduction coefficient (χ_q), we obtain:

$$\chi_q = \mu^{\alpha} \lambda^{\beta} \tau^{\gamma} \qquad (C_8)$$

The meaning of the proportionality constant (k), which appeared in eqn. C_1, is the following

$$k = \frac{q}{m^{\alpha} \cdot l^{\beta} \cdot t^{\gamma}} = \Pi_B \qquad (C9)$$

where: Π_B is a dimensionless number of Buckingham (see Günther, 1975) and prototype (p) and model (m) are similar when $\Pi_p = \Pi_m$, or else, when:

$$\frac{qp}{(m_p)^{\alpha} (l_p)^{\beta} (t_p)^{\gamma}} = \frac{qm}{(m_m)^{\alpha} (l_m)^{\beta} (t_m)^{\gamma}} \qquad (C10)$$

Since the dimensionless numbers are numerically constant, irrespective of body size, they are particularly valuable for interspecies comparisons.

REFERENCES

ASCHOFF J (1971) Temperaturregulation. In: Gauer O, Kramer K, Jung R, eds. Physiologie des Menschen. München: Urban & Schwarzenberg. Bd 2. pp 43-116.

BENEDICT FG (1938) Vital Energetics. A Study in Comparative Basal Metabolism. Washington DC: Carnegie Institute.

BUTLER JP, FELDMAN A, FREDBERG JY (1987) Dimensional analysis does not determine a mass exponent for metabolic scaling. Am J Physiol *253:* R195-R199.

CALDER WA III (1984) Size, Function, and Life History. Cambridge: Harvard University Press.

ELSE PL, HULBERT AG (1981) Comparison of the "mammal machine" and the "reptile machine": energy production. Am J Physiol *240:* R3-R9.

FELDMAN HA, MCMAHON TA (1983) The 3/4 mass exponent for energy metabolism is not a statistical artifact. Respir Physiol 52: 149-163.

GÜNTHER B (1975) Dimensional analysis and theory of biological similarity. Physiol Rev 55: 659-699.

GÜNTHER B, GONZALEZ U, MORGADO E (1992) Biological similarity theories: a comparison with the empirical allometric equations Biol Res 25:7-13.

HEMMINGSEN AM (1960) Energy metabolism as related to body size and respiratory surfaces, and its evolution. Reports of the Steno Memorial Hospital and Nordinsk Laboratorium 9: 6-110.

HEUSNER AA (1982) Energy metabolism and body size. II. Dimensional analysis and energetic non-similarity. Respir Physiol 48:13-25.

HIMMS-HAGEN J (1976) Cellular thermogenesis. Annu Rev Physiol 38:315-351.

HOPPELER H, MATHIEU O, WEIBEL ER, KRAUER R, LINDSTEDT S, TAYLOR CR (1981) Design of the mammalian respiratory system. VIII. Capillaries in skeletal muscles. Respir Physiol 44:129-150.

HOPPELER H, LINDSTEDT SL, CLAASSEN H, TAYLOR CR, MATHIEU O, WEIBEL ER (1984) Scaling of mitochondrial volume in heart to body mass. Respir Physiol 55:131-137.

HOPPELER H, KAYAR SR (1988) Capillarity and oxidative capacity of muscles. News Physiol Sci 3:113-116.

KLEIBER M (1961) The Fire of Life. New York: Wiley.

MATHIEU O, KRAUER R, HOPPELER H, GEHR P, LINDSTEDT SL, ALEXANDER RM, TAYLOR CR, WEIBEL ER (1981) Design of the mammalian respiratory system. VII. Scaling of mitochondrial volume in skeletal muscle to body mass. Respir Physiol 44:113-128.

MCMAHON TA, BONNER JT (1983) On Size and Life. New York: Scientific American Books.

MURRAY CD (1926) The physiological principle of minimum work. I. The vascular system and the cost of blood volume. Proc Nat Acad Sci USA 12:207-214.

PETERS RH (1983) The Ecological Implications of Body Size. Cambridge: Cambridge University Press.

PROTHERO J (1984) Scaling of standard energy metabolism in mammals: I. Neglect of circadian rhythms. J Theor Biol 106:1-8.

RUBNER M (1883) Ueber den Einfluss der Körpergrösse auf Stoff-und Kraftwechsel. Z Biol 19:535-562.

SERNETZ M, GELLERI B, HOFFMANN J (1985) The organism as bioreactor. Interpretation of the reduction law of metabolism in terms of heterogeneous catalysis and fractal structure. J Theor Biol 117:209-230.

SCHMIDT-NIELSEN K (1984) Scaling: Why is Animal Size so Important? Cambridge: Cambridge University Press.

SMITH RE (1956) Quantitative relations between liver mitochondria, metabolism and total body weight in mammals. Ann NY Acad Sci 62/17:403-422.

SPAARGAREN DH (1992) Transport function of branching structures and the "surface law" for basic metabolic rate. J Theor Biol 154: 495-504.

STAHL WR (1967) Scaling of respiratory variables in mammals. J Appl Physiol 22:453-460.

STRYER I (1975) Biochemistry. San Francisco: Freeman.

SUAREZ RK (1992) Hummingbird flight: sustaining the highest mass-specific metabolic rates among vertebrates. Experientia 48:565-570.

TAYLOR CR (1987) Structural and functional limits to oxidative metabolism: insights from scaling. Annu Rev Physiol 49:135-146.

WEIBEL ER (1963) Morphometry of the Human Lung. Berlin: Springer.

WEIBEL ER, TAYLOR CR (1981) Design of the mammalian respiratory system. Respir Physiol 44:1-164.

WEIBEL ER (1987) Scaling of structural and functional variables in the respiratory system. Annu Rev Physiol 49:147-159.

WEIBEL ER, TAYLOR CR, HOPPELER H (1992) Variations in function and design: Testing symmorphosis in the respiratory system. Respir Physiol 87:325-348.