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Size effects on metabolic rate in cell, tissue, and body calorimetry ☆

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Abstract

Size effects on metabolic rate include the effects of body size (biological) and sample size (methodological) and are to be found in cell, tissue, and body calorimetry. The biological size effect, known as Kleiber's rule and consisting of a metabolic reduction with increasing body mass, is demonstrated by the size relationship of blood heat output in several mammalian species (cellular level) and by the differing metabolic behaviour of cardioplegic rat and dog heart samples (tissue level). The methodological size effect, known as the crowding effect and consisting of a metabolic decrease with increasing sample size, is demonstrated in human renal carcinoma cells (cellular level) and ischemic rat liver samples (tissue level) and is explained by a simple mathematical simulation. With respect to body calorimetry, it is stressed that metabolic size allometry may be temporarily inactivated in special biological adaptations (neonatal period, mammalian hibernation), and weight correction of metabolic rates may produce methodological problems when mass differences are mainly due to varying body fat content (circannual rhythms). In conclusion, careful size standardization is a prerequisite of comparability in biomedical calorimetry.

Keywords: Allometry; Calorimetry; Indirect calorimetry; Metabolic rate; Size effect

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1. Introduction

Size effects on metabolic rate are of interest in calorimetry both from a biological and from a methodological point of view. From a biological point of view, they reflect the relationship between body size and metabolic rate; from a methodological point of view, they demonstrate the role of sample size in heat output determination. Size effects are found in cell and tissue as well as in body calorimetry and may, if not properly considered, lead to severe misinterpretations of calorimetric results. In this paper, a short review of the occurrence of size effects in biomedical calorimetry is given, summarizing some recent observations in the field.

In Fig. 1, the influence of body size on metabolic rate, known as Kleiber's rule, is illustrated by the famous mouse-to-elephant curve, showing that the specific basal metabolic rate of mammals decreases with increasing body mass [1].

This is often explained by the decreasing surface-to-volume ratio and the lower heat loss in larger species (hence the term "surface rule"). However, for a surface dependence, a mass exponent of −0.33 would be expected, provided Heusner's

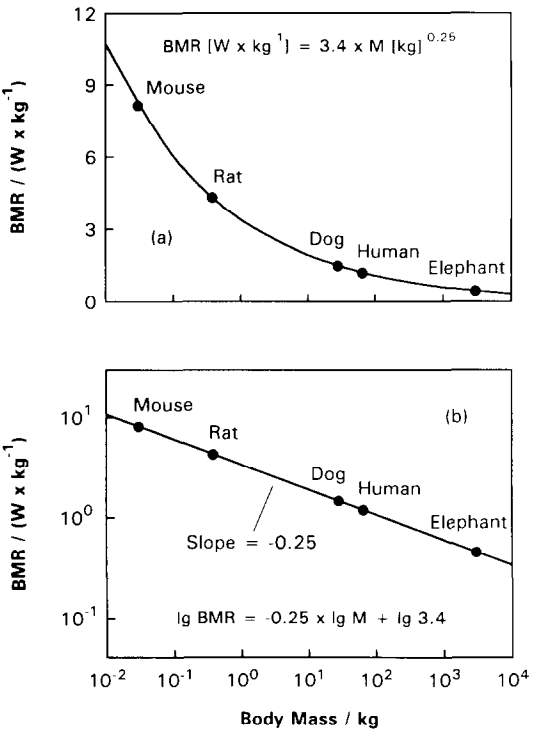


Fig. 1. Influence of body size on metabolic rate (Kleiber's rule). (a) As demonstrated by the "mouse-to-elephant" curve, the specific basal metabolic rate of mammals decreases with increasing body mass. (b) When plotted on log scales, a linear regression results with the exponent of the allometric power function (−0.25) being transformed into the slope of the line.

hypothesis that the −0.25 in the interspecific comparison is only a statistical artifact of a "true" surface proportionality within each single species, is ignored [2,3]. Moreover, a similar allometric relationship also holds true for poikilothermic organisms, such as reptiles, indicating that it is due to some intrinsic metabolic needs rather than to thermoregulatory reasons [4–6]. Perhaps the exponent of −0.25 represents a fractal dimension of metabolism, as suggested by Sernetz and co-workers [7,8], looking at metabolism in terms of a bioreactor. However, even this theory leaves unexplained the physiological mechanisms that "tell" the cells and tissues whether they are in a small or in a large organism. Whatever may be its cause, metabolic size allometry is characterized by a linear fit with a negative slope of around −0.25, when plotted on log scales (for reviews see Refs. [9]–[12]).

In Fig. 2, the decrease of specific heat output with increasing sample size, often observed in biological calorimetry and known as the "crowding effect"

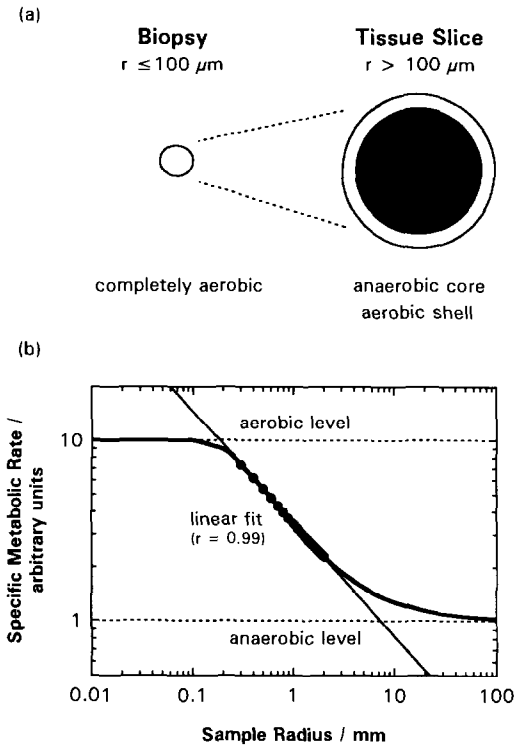


Fig. 2. Influence of sample size on metabolic rate (crowding effect). (a) Whereas a small biopsy is completely aerobic, a large tissue slice consists of an aerobic shell and an anaerobic core. (b) Thus, with increasing sample radius, the heat output per unit of volume shows a sigmoidal decrease from the higher aerobic to a lower anaerobic level. When plotted on log scales, the steep portion of this transition fits a linear regression. Both the critical depth of tissue aerobiosis (100 μm) and the relationship of anaerobic to aerobic metabolism (1:10) are rough assumptions.

[13–16], is simulated by a simple mathematical model, going back to the classical considerations of Krogh, Warburg, and others on oxygen supply to tissues [17–20].

Two tissue samples are given, a small biopsy and a large slice. It is assumed that these are spheres with an aerobic shell and an anaerobic core. Following the above-mentioned considerations of Warburg, the critical depth of tissue aerobiosis is tentatively set at 100 μm and considered to be constant. A transitional zone with a partially anaerobic metabolism is neglected. Thus, with decreasing sample size, the aerobic shell occupies an increasing part of the total volume, until the whole sample has become completely aerobic.

For simulation purposes, the sum of the metabolic rates of the anaerobic core and the aerobic shell is calculated and divided by the total volume in order to obtain the "specific" metabolic rate which is plotted against sample radius. If the aerobic metabolic rate is assumed to be ten times as high as the anaerobic one, the heat output per unit of volume will be "ten" in the purely aerobic biopsies and asymptotically approach "one" in large, mainly anaerobic tissue slices. Between these extremes, there is a reverse s-shaped decrease in the specific metabolic rate with increasing sample radius which, in its steep portion, can be approximated by a power function. This explains the intriguing observation that the crowding effect, if plotted on log scales, fits a linear regression similar to the allometric relationship between body size and specific basal metabolic rate (see below).

In the following, some examples for both types of size effects, observed in a variety of experimental settings, will be given.

2. Methods and materials

Microcalorimetric measurements were performed on a 4-channel 2277 Thermal Activity Monitor (assembled by ThermoMetric AB, Järfälla, Sweden, and distributed in Germany by C3-Analysentechnik, Baldham) in the static ampoule mode under varying incubation conditions [21].

2.1. Cell microcalorimetry

Mammalian blood samples were obtained from veterinary routine sampling or from experiments of related working groups and were collected in heparinized cups or syringes. The sample volume was 0.5–2.0 ml, depending on the species investigated. Heat output was measured at an incubation temperature of 37°C and continuously recorded over six or ten hours. Details of the study have been described elsewhere [22].

Human renal carcinoma cells were harvested from the culture flasks and adjusted to cell numbers between 10^5 and 10^6 in a sample volume of 2 ml (corresponding to cell concentrations of between 5×10^4 and 5×10^5 per ml of sample volume). They were incubated in RPMI 1640 Medium at 37°C. Basal heat production was read 30 min after starting the experiment [23].

2.2. Tissue microcalorimetry

Rat and dog heart samples were taken from the left ventricular myocardium after perfusion with Bretschneider's cardioplegic solution (Custodiol) under appropriate experimental conditions. They were stored in the preservative solution at 25°C to measure the ischemic decline of heat output over six hours [24–26].

Rat liver samples came from Sprague Dawley rats and were dissected into small "biopsies" and large slices. They were incubated in balanced salt solution (Tutofusin) at 25°C and simultaneously measured in the microcalorimeter to compare the initial peak and the further decrease in heat output. Dry weights ranged from 0.3 to 7.0 mg in "biopsies" and from 118 to 323 mg in slices [27].

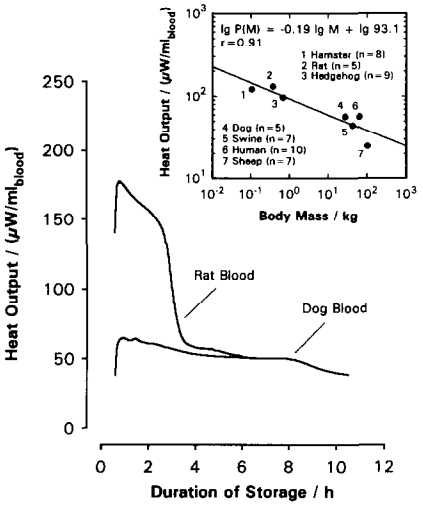
2.3. Body calorimetry

Metabolic rates of summer and winter hedgehogs (*Erinaceus europaeus*) were measured by indirect calorimetry. The animals were kept in the Institute garden and fed commercial cat food and fresh water with occasional additional vitamins. On the evening before a calorimetric experiment, they were transferred into a metabolic cage for collection of urine samples, so that at the time of measurement the summer animals (May–July) had fasted for one night and the winter animals (November/December) were fully aroused from hibernation. Indirect calorimetry was performed in a measuring box (Hugo Sachs Elektronik, March, Germany) perfused with humidified room air by a membrane pump (KNF Neuberger, Freiburg-Munzingen, Germany) at a constant flow rate of 350 ml min^{-1} (needle valve flowmeter by Fischer & Porter, Göttingen, Germany). The O_2 and CO_2 content in the outgoing air was measured by paramagnetic oxygen and infrared carbon dioxide analysers (Servomex Analyser Series 1400, Bühler, Ratingen, Germany). The O_2 consumption and CO_2 production rates were calculated from the differences in gas concentrations between outgoing and room air, multiplied by the flow rate (open-circuit system). Results were corrected to STPD conditions. Because the measuring box was installed in a climate testing cabinet (Tritec, Hannover, Germany), it was possible to observe the metabolic reactions of the animals to systematic variations in ambient temperature, and to determine the zone of thermal neutrality which was found to lie at ambient temperatures between 25 and 30°C independent of season. The oxygen consumption rates reported in this paper are the lowest ones measured in the thermoneutral range and, thus, correspond to "basal" conditions [28,29].

3. Results and discussion

3.1. Influence of body size on cell and tissue metabolic rate (biological size effect)

To test the influence of body size on cellular metabolism, whole blood samples from different mammalian species were studied by microcalorimetry (Fig. 3).



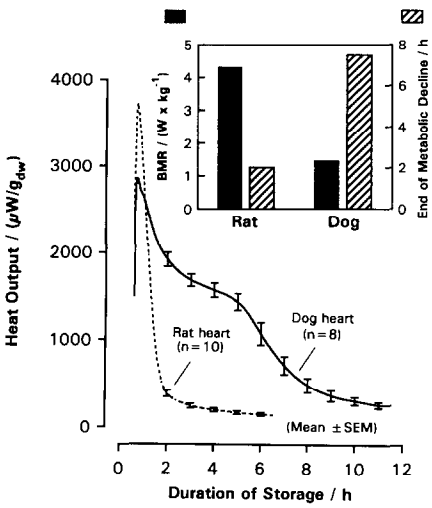


Fig. 3. (left) Influence of body size on heat output in cell microcalorimetry. Whole blood heat output is lower in the larger (dog) than in the smaller species (rat). The thermal power values of blood samples from several mammalian species fit a regression line similar to Kleiber's rule (inset).

Fig. 4 (right). Influence of body size on heat output in tissue microcalorimetry. Cardioplegic myocardium exhibits a much better preservation of heat output per unit of dry weight (dw) in the larger (dog) than in the smaller species (rat). When compared to the specific basal metabolic rates of the species, a reciprocal relationship is evident (inset).

A typical microcalorimetric record of a blood sample consists of a more or less steady "plateau" and a final "breakdown" of metabolism, the latter being correlated to the exhaustion of glucose reserves in the ampoule. Mean heat output can be calculated from the total heat liberated during the plateau period (determined by integration of the thermal power curve), divided by the duration of the plateau.

As can be seen in the comparison between typical rat and dog records, the blood of the smaller species has a much higher heat production, and, thus, a shorter plateau duration than the blood of the larger species. This effect could be verified in a number of different species, leading to the surprising result that mammalian blood heat output bears an allometric body size relationship similar to Kleiber's rule. The same relationship also holds true (with an even better correlation coefficient) when the heat output rates are corrected for the slightly differing packed cell volumes (hematocrit values) in different species [22]. Moreover, in a recent study, a similar body size relationship was also found in the blood heat output from human neonates as compared with adults [30].

The unexpected occurrence of a metabolic size relationship in blood samples may offer a novel approach to the underlying physiological mechanisms of Kleiber's rule. At any rate, it is another convincing argument against a thermoregulatory

cause of metabolic size allometry and invalidates earlier assumptions that this is limited to whole organisms or intact tissues, with single cell metabolism being unaffected by body size [7,8].

The influence of body size on tissue metabolism is demonstrated in Fig. 4, showing mean microcalorimetric records of rat and dog myocardial samples which were pretreated by a preservative solution to slow the anaerobic tissue damage.

It is obvious that in the larger species, the heat output is better preserved, and the final breakdown of anaerobic metabolism occurs later than in the smaller species. If the time interval from the induction of artificial cardiac arrest (called cardioplegia) until the end of metabolic decline is correlated to the specific metabolic rate of the corresponding species, a reciprocal relationship is seen, indicating the influence of the allometric size relationship on tissue metabolic rate. This is in accordance with earlier results from Krebs [31] on the occurrence of size allometry in tissue metabolism. Of course, the different protectibility of tissues from different species is a rather indirect sign of size influence on tissue metabolic rate which, perhaps, cannot be reproduced in all tissues to the same degree. However, even if more data are needed to establish a regularity, this example proves that calorimetric results obtained with small animal tissues must not unreservedly be taken as representative of organs from larger species, such as for human transplants [32].

3.2. Influence of sample size on cell and tissue metabolic rate (methodological size effect)

The crowding effect, originally described for lymphocytes [13–15], has been reproduced by many authors in various types of cell suspensions including cultured tissue cells [33,34]. To add another example, in an investigation on human renal carcinoma cells, the basal heat output per cell was shown to decrease with increasing cell number in the ampoule (Fig. 5). This is probably due to the fact that, starting from purely aerobic metabolism in "monolayer-like" conditions, "crowding" the ampoule leads to an increasing degree of anaerobiosis in cellular metabolism. Thus, by recalculating metabolic data reported by Nässberger et al. [35] for hybridoma cells, Kemp and Gnaiger [36] were able to demonstrate alterations in the calorimetric-respirometric ratio, indicating impaired oxygen supply with increasing cell density. This means that the above mathematical simulation, although developed for tissue samples, apparently holds true for cell suspensions.

An analogous phenomenon has been observed in tissue samples from ischemic rat livers (Fig. 6), in accordance with the results of other authors [16]. In this case, the heat output per gram dry weight at the onset of measurement was shown to increase with decreasing sample size. This can be explained by an increasing relative contribution of the aerobic shell to the total metabolic rate in "biopsy-like" samples, in contrast to the mainly anaerobic conditions in large tissue slices. From this figure, it becomes clear that in working with biopsies, absence of standardization can lead to considerable errors in the determination of tissue heat output although such effects may be diminished when oxygen supply to the sample is

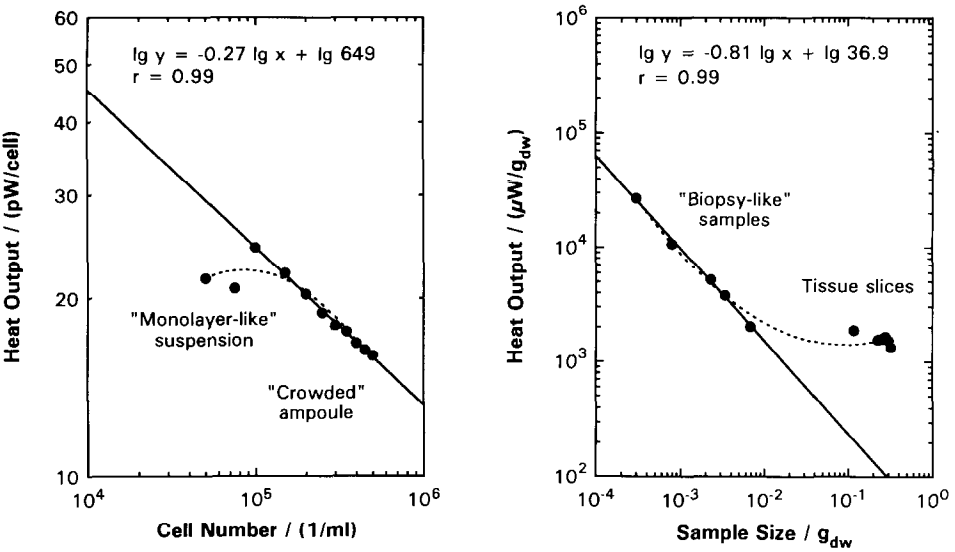


Fig. 5 (left). Influence of sample size on heat output in cell microcalorimetry. In a suspension of human renal carcinoma cells, the heat output per cell decreases with increasing cell number in the ampoule. This may be explained by a continuous transition from aerobiosis in a "monolayer-like" suspension to increasingly anaerobic conditions in a "crowded" ampoule.

Fig. 6 (right). Influence of sample size on heat output in tissue microcalorimetry. In rat liver tissue samples, the heat output per unit of dry weight (dw) increases with decreasing sample size. This is apparently due to a continuous transition from mainly anaerobic conditions in large tissue slices to an increasing amount of aerobiosis in "biopsy-like" samples.

The two curves shown in Figs. 5 and 6 complement each other giving a sigmoidal relationship, whose steep portion fits a linear regression when plotted on log scales (see Fig. 2).

improved by stirring the medium [33]. However, biopsies, because of their diffusive gas and substrate exchange with the incubation medium, are definitely unsuitable for the examination of ischemic tissue metabolism [32].

The two plots (Figs. 5 and 6) complement each other as a reverse s-shaped curve, leading from purely aerobic conditions in cell suspensions to mainly anaerobic conditions in large tissue samples, as predicted by the above mathematical simulation of the crowding effect. In both cases, the relationship between specific metabolic rate and cell number or sample size fits a linear relationship, when plotted on log scales. Remarkably, in the cultured cells, the slope of the regression line is -0.25 and thus coincides with the one found in metabolic size allometry, reinforcing the analogy between the reduction law of metabolism and the conditions of a bioreactor, as put forward by Sernetz and co-workers [7,8].

3.3. True and apparent variations in specific metabolic rate

In view of the general importance of the metabolic size relationship, it is worth mentioning that this can be inactivated in special biological adaptations.

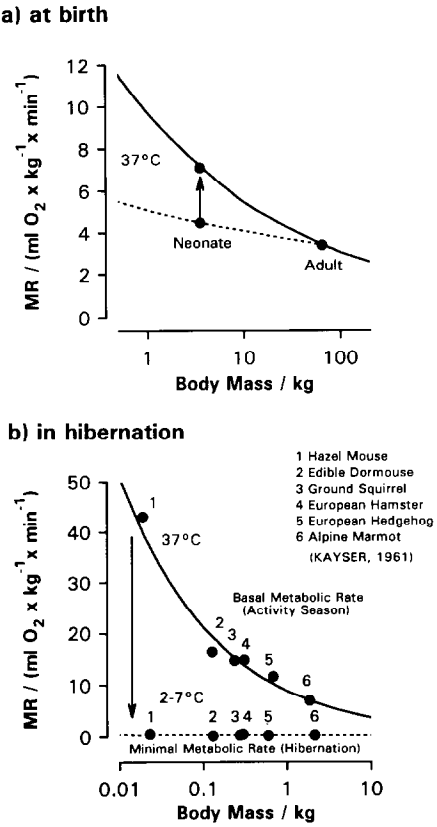


Fig. 7. Temporal inactivation of metabolic size allometry in special adaptation strategies. (a) At birth, the specific metabolic rate of the human neonate is closer to that of the mother than to the value which would be expected from its small body size. (b) In hibernation, the specific metabolic rate is reduced to a uniform lower level which, in the smallest species, cannot be explained by the pure temperature effect and, thus, suggests an additional endogenous metabolic reduction.

This is known from hibernating mammals which, following the results of Kayser [37], reduce their metabolism to a uniform minimal specific metabolic rate (Fig. 7(b)). Hence, smaller species have a greater metabolic advantage which, as recently confirmed by Geiser [38], may even exceed the pure temperature effect and, thus, requires some additional endogenous metabolic suppression [12,39–41].

Remarkably, there seems to be a similar adaptive mechanism immediately after birth in that the (human) neonate, before activating its metabolism, starts at a specific turnover rate which is much lower than would be expected from its small body size (Fig. 7(a)). The disproportionately low metabolic rate, although convincingly described in the sixties [42–44], has been considered only by a few authors [45–47]. Yet, it might be one of the reasons for the still unexplained hypoxia tolerance observed in newborn mammals including man [19,48].

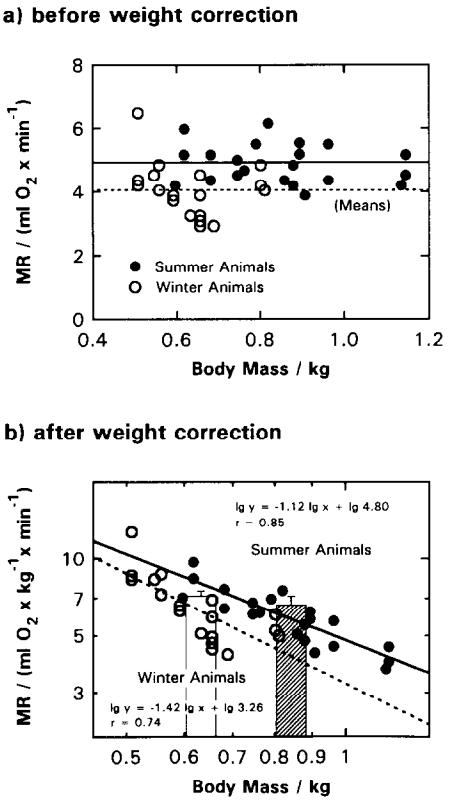


Fig. 8. Problems in body weight correction of metabolic rates, as demonstrated by indirect calorimetry in waking summer and winter hedgehogs (*Erinaceus europaeus*, $n = 5$). (a) The absolute oxygen consumption is somewhat higher in summer than in winter animals and hardly depends on body weight. (b) When corrected for individual body weights, an apparent size dependence results, proving that at a given body mass the specific metabolic rate is somewhat lower in winter than in summer animals. Because in this study the winter animals were smaller than the summer ones, a direct comparison of the mean values (bar chart) would have suggested a "paradoxical" increase of specific metabolic rate (MR).

In view of the metabolic reduction of the neonate, the additional metabolic reduction found in small hibernators might be a reactivation of a protective mechanism common to all mammals at birth.

Some years ago, we carried out an investigation on European hedgehogs addressing the question of whether the additional metabolic suppression in hibernation, at least partly, may be explained by a prehibernatory metabolic reduction in the autumn. This study is a good example of the methodological problems arising with the weight correction of metabolic rates in body calorimetry.

Contrary to earlier data of Hildwein and Malan [49], we found a small but significant decrease in absolute oxygen consumption between waking summer and winter animals (Fig. 8(a)), in good accordance with the results of Tähti [50]. Remarkably, however, there was little, if any, difference in the O_2-consumption

rates between small and large individuals. This means that the weight differences are mainly due to different amounts of metabolically inactive fat deposits.

Nevertheless, if the metabolic rates are divided by the body mass, a strong apparent dependence of specific metabolic rate on body mass results, with the mass exponent being lower than -1.0 (Fig. 8(b)). Hence, if the winter animals were larger, their specific metabolic rates would necessarily be lower than in the summer animals, suggesting an endogenous metabolic reduction although the winter animals might not be more economical, but simply more obese. In our study, however, the winter animals were smaller than the summer ones, leading, for the same mathematical reasons, to a "paradoxical" increase in specific metabolic rate. This could be refuted by a comparison of the apparent metabolic size relationships of summer and winter animals, proving that, even in the waking state and independent of size errors, the specific metabolic rate of the winter hedgehogs is lower than in the summer animals. In other words, winter hedgehogs, when compared with summer individuals, seem to have lowered their metabolic rate to a level from which the metabolic reduction in hibernation is explainable by the pure temperature effect.

4. Conclusions

In summary, size effects on metabolic rate should be considered in calorimetry for methodological and biological reasons:

Firstly, because sample size, or cell number, respectively, may affect the heat output in a non-proportional way, and because changes in body weight may influence the calculation of specific metabolic rates, care must be taken to standardize.

Secondly, because the metabolic size allometry (or Kleiber's rule) is present not only at the body but also at the tissue and cellular level, caution is necessary in generalizing calorimetric results of small animal experiments.

Acknowledgements

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