Estimation of Daily Minimum Energy Expenditure in Free-moving Rats and Mice

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Summary In this paper, we presented a new procedure to determine daily minimum energy expenditure (MEE) in free-moving rats and mice kept in a chamber. Energy expenditure was measured for 23 h period and averaged every 10 min. Data were sorted in ascending order. MEE was estimated from the regression line with the smallest slope and the biggest intercept among the regression lines calculated between the sorted energy expenditure and the data ranking. Among three duplicate measurements in individual animals, MEE gave the smallest coefficient of variation (2.2%) as compared with actual measured-values: either the single lowest value (4.0%) or the average of the 6 lowest values (2.5%). Judging from diurnal patterns of energy expenditure and locomotor activity and from video tape observation of the rat’s performance, it was confirmed that MEE represented an energy expenditure at rest. MEE decreased with fasting from days 1 to 5. MEE per body weight also declined with age, but stayed around 71–72 kcal/day/kg^{3/4} at 18 and 34 weeks of age in male Wistar rats. MEE in mice increased at lower ambient temperatures between 16 and 32°C, but stayed fairly constant at the same temperature in repeated experiments. Thus, MEE estimated by the present regression procedure was highly reproducible and valid to determine the fundamental value of daily energy expenditure under free-moving conditions.

Key Words daily minimum energy expenditure, locomotor activity, fasting, age, temperature, rats, mice

Recently techniques and apparatuses for measuring continuous 24-h energy expenditure in small animals have been developed by using an indirect calorimeter with an open-circuit respirometer and computer (1–6). We have attempted to characterize daily activity from 24-h energy expenditure patterns (7). In studies of 24-h energy expenditure, it is important to estimate a baseline value of energy expenditure patterns such as the basal metabolic rate (BMR). BMR is defined as

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the energy expenditure at a neutral temperature in post-absorptive animals at rest (8). Thus, BMR has been generally measured under a fasting condition to exclude the thermic effect of diet (DIT) and energy expenditures due to physical activity are eliminated from the BMR calculation using equipment to measure locomotor activity such as ANIMEX (1, 9). The metabolic rate, however, falls under fasting conditions or undernourished conditions depending upon how long the animals are kept in such conditions (8). Thus, BMR itself is not always useful as an absolute index for describing the energy expenditure of animals, and small animals such as nibblers in particular (8).

The ultimate goal of our study was to describe daily energetic behavior of free-living rats and mice kept in a chamber. Therefore, we think that it is desirable not to set a fasting condition and the lowest metabolic rate observed under free-living conditions, not BMR, is the best parameter to represent a baseline value in daily energy expenditure. Thus, in this experiment we attempted to estimate daily minimum energy expenditure (MEE) by a regression procedure and examined its accuracy and validity as a baseline value in daily energy expenditure.

**METHODS**

*Animals.* Male Wistar rats and male ddy mice were individually housed in plastic cages (27.6×44.5×20.4 cm) in a room maintained at 24.0±0.5°C with a 12-h light-dark cycle. Each animal was allowed free access to water and food (Chow diet: MF type, Oriental Bioservice Co.).

*Measurement of energy expenditure.* Energy expenditure was measured continuously for 23 h by an open-circuit respirometer (Fig. 1) connected to four respiration chambers (24×46×18 cm) (10). Respiration gas from the four chambers and fresh air were vacuumed by pumps into a mass spectrometer (WSMR-1400 model, Westron Co.) through a sample controller. The flow rate of each line was controlled and monitored by a mass flowmeter. The flow rate was set to 4 liter/min for male rats and 2 liter/min for male mice to keep the CO2 concentration in each chamber at around 0.15%. The temperature in the chamber was not different from the room temperature: 24.0±0.5°C when a mouse was kept there, but it was 24.5±0.5°C in the case of a rat. The gas of the 4 chamber lines and the air line was transferred into the mass spectrometer in turn and analyzed for O2 and CO2 by the mass spectrometer. The data regarding flow rate and the gas concentration in each line were stored in a computer (PC-9801, NEC). Oxygen consumption (VO2) and CO2 production (VCO2) were summarized every 2 min by the computer on the basis of the difference in the concentration of O2 and CO2 between the air line and each chamber line. Energy expenditures were calculated according to the following equation deduced from Lusk's table (10, 11) and averaged every 10 min.

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\text{Energy expenditure (kcal)} = 3.816 \times \text{VO}_2 \text{ (liter)} + 1.231 \times \text{VCO}_2 \text{ (liter)}
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Estimation of minimum energy expenditure by regression procedure. MEE was calculated as the theoretical minimal energy expenditure during a 23-h period by the following regression procedure. The 138 data of energy expenditure per 10 min were sorted in ascending order (Fig. 2A). Various regression equations \( y=ax+b \) between the sorted energy expenditures and their data ranking were calculated by increasing the data number one by one from the lower side of data. The slopes \( a \) and \( y \) intercepts \( b \) of the regression lines varied according to the data number as shown in Fig. 2B and 2C. The regression line with the smallest slope and the largest intercept was chosen to estimate MEE. Thus, MEE per 10 min was obtained as a \( y \) value, when 1 was substituted into the \( x \) of the regression equation. MEE (kcal/day) was simply calculated by multiplying the MEE per 10 min by 144. MEE
Fig. 2. Regression method for estimation of MEE using sorted data of 10-min energy expenditure. Regression lines were calculated between the sorted data and their data ranking. A) Sorted data of 10-min energy expenditure in ascending order. B) Changes in the slope of regression lines calculated by increasing the number of data one by one starting with the smallest. The smallest slope gave an $a$ value of 0.93 when the 56 lowest data were used. C) Changes in the intercept of regression lines. The largest intercept gave a $b$ value of 253 when the 56 lowest data were used.

was also expressed as kcal/day/kg$^{2/3}$ or kcal/day/kg$^{3/4}$ (12, 13).

*Description of performance.* The behavior of a rat was observed in the respiration chamber between 11:00 and 17:00 during the light period with a video camera. This daytime was chosen because MEE is generally found during this
period. Performance of the rat was classified into moving, eating, grooming and resting. Energy expenditure and locomotor activity (ANIMEX, Shimadzu Co.) were also measured simultaneously. MEE, the single observed minimum energy expenditure (MIN) and the average of the 6 lowest energy expenditures (AVG) were shown on the graph of the diurnal energy expenditure pattern and evaluated in reference to locomotor activity and visual behavior.

Effects of fasting, age and temperature on MEE. Seven 25-week-old rats weighing around 400 g were used to measure the 23-h energy expenditure on the day of feeding and on the first, third and fifth days of continuous fasting. During this fasting experiment, water was freely provided.

Six rats in each group of 5, 6, 7, 18 and 34 weeks of age were used to measure the 23-h energy expenditure and to examine changes in MEE with age.

Seven adult mice, 24 weeks of age and weighing around 45 g, were used to measure the 23-h energy expenditure to examine MEE under various temperature conditions where the temperature was altered daily in the following order: 24, 28, 32, 28, 24, 20 and 16°C.

Statistical analysis. Significant differences between treatments were examined by one-way ANOVA with Scheffe's F test.

RESULTS

The reliability of MEE estimated by the present regression procedure was assessed by the measurement of energy expenditure of 6 rats during 3 consecutive days. The mean MEE, MIN and AVG for 3 consecutive days were 39.18±0.48, 36.82±0.94 and 38.78±0.55 kcal/day, respectively (Fig. 3). The values of MEE and AVG were significantly larger than MIN (p<0.05). When expressed as per body weight, mean MEE, MIN and AVG were 66.22±0.56, 62.29±1.46 and 65.55±0.69 kcal/day/kg$^{2/3}$, respectively. They corresponded to 70.72±0.57, 66.53±1.54 and 70.01±0.71 kcal/day/kg$^{3/4}$, respectively. The MEE and AVG per metabolic mass were significantly different from the MIN (p<0.05).

The coefficients of variation (CV) among the three measurements were 2.4% for MEE, 4.0% for MIN and 2.6% for AVG. When the three values were expressed per kcal/day/kg$^{2/3}$ or kcal/day/kg$^{3/4}$, the CVs were 2.2% for MEE, 4.0% for MIN and 2.5% for AVG. These findings indicated that MEE was similar to AVG and that it remained fairly constant for the 3 consecutive days when measurement was repeated in the same animals.

The diurnal patterns of energy expenditure (cal/10 min) and locomotor activity (count/10 min) in a rat are shown in Fig. 4. In addition, the levels of MEE, MIN and AVG (cal/10 min) are shown and the rat's visual performance is described on the same graph. A significant correlation was observed between energy expenditure (cal/10 min) and locomotor activity (cal/10 min) during the 23-h period in the rat (n=138, r=0.775, p<0.001). MEE clearly represented the lowest side of energy expenditure at rest.
Fig. 3. Day-to-day variability of MEE, MIN and AVG in rats expressed as kcal/day, kcal/day/kg^{2/3} and kcal/day/kg^{3/4}. MEE, minimum energy expenditure estimated by our regression procedure; MIN, single observed minimum energy expenditure; AVG, average of the 6 lowest energy expenditures. Values are M±SD for 3 days (n=6). Means with the same superscript letter were not significantly different at p<0.05.

MEEs estimated under feeding and fasting conditions are shown in Fig. 5. MEE decreased gradually with fasting of up to 5 days. There were significant differences among the MEEs during this experiment (F[3, 24]=31.978, p<0.0001). MEE on the first fasting day was 10% lower than that on the previous feeding day. When MEE was expressed per body weight (BW)^{2/3} or ^{3/4}, the fall of MEE was 8% on the first fasting day.

Changes in MEE with age in rats are shown in Fig. 6. MEEs at 5, 6, 7, 18 and 18 and
Fig. 4. Diurnal variations of energy expenditure (cal/10 min) and locomotor activity (count/10 min) with a description of rat performance. MEE, MIN and AVG were calculated from 23-h energy expenditure as described in the text.

34 weeks of age were 26.2±3.9, 27.2±2.8, 33.6±2.5, 35.0±2.8, and 40.0±2.9 kcal/day, respectively. The MEEs (kcal/day) were significantly different among the different age groups ($F[4,25]=21.6, p<0.001$). MEEs (kcal/day) were not significantly different between rats aged 5 and 6 weeks, between rats aged 7 and 18 weeks or between rats aged 18 and 34 weeks. MEE (kcal/day) clearly increased with age because of the increase in body mass. MEE per metabolic body weight, however, decreased with age, with the MEEs in rats aged 5, 6, 7, 18 and 34 weeks being 103.2±11.0, 93.7±6.2, 93.1±5.2, 66.5±3.0 and 66.9±2.6 kcal/day/kg^{2/3}, respectively. They corresponded to 122.5±12.6, 109.4±6.8, 105.7±5.8, 72.0±3.0 and 71.4±2.6 kcal/day/kg^{3/4}, respectively. The MEEs (kcal/day/kg^{2/3}) were significantly different among the groups of different ages ($F[4,25]=42.2, p<0.001$). The MEEs (kcal/day/kg^{3/4}) were also significantly different among the groups of different ages ($F[4,25]=63.8, p<0.001$). The MEEs expressed as kcal/day/kg^{2/3} or kcal/day/kg^{3/4} in the rats at 18 and 34 weeks of age were significantly smaller than
Fig. 5. Effect of fasting condition on MEE. MEE is expressed as kcal/day, kcal/day/kg^{2.3} and kcal/day/kg^{3.4}. Values are M±SD for 7 rats. Means with the same superscript letter were not significantly different at p<0.05.

those in the younger rats at 5, 6 and 7 weeks of age.

The effects of room temperature on MEE in mice are seen in Fig. 7. The MEEs were significantly different among the different temperatures (F[4, 30] = 189.8, p<0.0001 for MEE kcal/day, F[4, 30] = 213.1, p<0.0001 for MEE kcal/day/kg^{2.3}, F[4, 30] = 209.2, p<0.0001 for MEE kcal/day/kg^{3.4}). Exposure to 16 and 20ºC significantly increased MEE. There was a clear relationship between MEE and temperature (r=0.96, n=35). In repeated measurements at the same temperature, 24 or 28ºC, MEE gave similar values at the same temperature. In the first measurement at 24ºC, MEE was 8.94±1.27 kcal/day; in the second measure-
Fig. 6. Changes in MEE with age in rats. Values are $M \pm SD$ for 6 rats. Means with the same superscript letter were not significantly different at $p < 0.05$.

ment at $24^\circ C$, it was $8.69 \pm 0.65$ kcal/day. At $28^\circ C$, MEE in the first measurement was $5.99 \pm 0.66$ kcal/day and that in the second measurement was $6.03 \pm 0.76$ kcal/day. MEE (kcal/day) at $32^\circ C$ was not significantly different from that at $28^\circ C$.

DISCUSSION

In this paper, we presented a new regression procedure to determine the theoretical MEE in free-moving rats and mice. When energy expenditure is measured during a 24-h period in free-living rats and mice, the lowest energy
Fig. 7. Changes in MEE in mice at different temperatures between 16 and 32°C. MEE is expressed as kcal/day, kcal/day/kg^{2/3} and kcal/day/kg^{3/4}. Values are M±SD for 7 mice. Means with the same superscript letter were not significantly different at p<0.05.

expenditure actually measured or an average of several lowest energy expenditures is widely taken as the minimum energy expenditure (2, 3, 5). This value is, in some cases, generally regarded as BMR under a fasting condition or the resting metabolic rate under a feeding condition. However, it depends on the accuracy of a calorimeter used, and the adoption of a small number of data may involve a risk of some random measurement error. Any animal experiment is subject to some degree of variability due to both individual differences and measurement errors. So, it is worth considering an appropriate way to minimize experimental variability, partic-
ularly, in the measurement of energy expenditure in animals.

To avoid adopting as MEE an unstable datum or data which may be due to some measurement error on the lowest side of daily energy expenditure, we estimated MEE by the regression procedure which employed as many stable data of energy expenditure at rest as possible. The stable group of resting data followed by a relatively sharp increase in energy expenditure can be easily recognized on a graph of sorted data (Fig. 2A). The highest point of datum in this group can be determined by calculating regression equations employing data from the lower rank up to a data rank where b value of a regression equation gives a peak (Fig. 2C). As data at ranks higher than this point are added in the calculation of regression equations, the b values begin to decrease. The change in energy expenditure around this point is so sharp that any data of higher level than this point are judged to belong to another group of elevated energy expenditure level due to physical activity and thereby they were excluded from the estimation of MEE. The MEE calculated by our procedure was found to be more reliable than the actual lowest measurement in terms of daily variability. The average of 6 lowest data was also stable in this experiment, but its variability was higher than that of MEE.

The rationale of our MEE was proved by comparing energy expenditure, locomotor activity and visual observation of the rat's performance on VTR. MEE represented an energy expenditure in a post-absorptive rat at rest and did not represent any energy expenditure elevated due to physical activity. In addition, the present regression method is, on a theoretical basis, valid for calculating MEE especially when data on the lowest side vary considerably, as is often observed. Moreover, in this regression procedure, it is not necessary either to measure locomotor activity or to remove meal during energy expenditure measurement. This advantage is important in our study which aims at analyzing daily behavior in free-living animals from the view point of energy metabolism.

We decided not to adopt BMR as the baseline value of daily energy metabolism because BMR determination requires measuring energy expenditure under such unusual conditions as fasting or energy restriction and relatively higher temperatures. Energy-restricted animals have lower energy metabolic rate than freely feeding animals (3), as indicated in the present study where MEE on the first day of fasting was 10% lower than that on a free feeding day. Rodents including rats and mice are usually 'nibblers' ingesting their food in many small meals. Therefore, it is assumed that the thermic effect of food on the MEE measured in the present experiment must be small in nibblers.

It is known that the metabolic rate varies with body surface area. Thus, MEE needs to be normalized according to body mass when compared, for instance, between different ages (12, 13). To normalize data, BW$^{3/4}$ is used rather than BW$^{2/3}$ in comparisons of BMR between animals (6, 13, 14). The equation, $\text{BMR} = 70 \times \text{BW kg}^{0.75}$, was suggested for interspecies comparison of BMR in animals (15). Previously, BMR was actually found to rise first and then fall with age (13). In our study, the MEE (kcal/day/kg$^{3/4}$) declined with age from 5 weeks to 18 weeks, but it...
stayed around 70 at 18 and 34 weeks in Wistar rats. The alteration pattern of the MEE with age was similar to that of BMR.

The relationship between energy expenditure and ambient temperature at 5°C to 30°C was linear in obese and non-obese rats (16). Our results suggested that exposure to cold temperature clearly stimulated the metabolic rate, giving higher MEEs in mice. This type of thermoregulation with altered ambient temperature can be explained by the contribution of non-shivering thermogenesis (17). Good reproducibility of MEE at the same temperature was clear in the present experiment.

In conclusion, the present regression procedure provides a reliable and reproducible daily MEE under free-living conditions. MEE estimated without any food restriction can be used as a baseline value for analyzing daily energy expenditure pattern of small animals under free-living conditions.

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REFERENCES


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