Energy budgets of lactating and non-reproductive Brown Long-Eared Bats (*Plecotus auritus*) suggest females use compensation in lactation

J. A. MCLEAN* and J. R. SPEAKMAN†

*Department of Biochemistry and Nutrition, Scottish Agricultural College, Auchincruive, Ayr KA6 5HW, UK and †Aberdeen Centre for Energy Regulation and Obesity, Department of Zoology, University of Aberdeen, Aberdeen AB24 2TZ, UK

Summary

1. The energy budgets of lactating and non-reproductive female Brown Long-Eared Bats fed primarily on noctuid moths (= 27·2 kJ g⁻¹) were constructed and compared in flight enclosures in captivity.
2. The average dry food consumption of non-reproductive individuals was 1·8 g bat⁻¹ day⁻¹ (gross energy intake = 48 kJ day⁻¹). The average food consumption throughout days 10–35 of lactation was 2·0 g bat⁻¹ day⁻¹ (gross energy intake = 53 kJ day⁻¹). Lactating females obtained six times more energy from increased food consumption than from mobilization of fat stores, compared with non-reproductive bats.
3. Milk export, calculated using the difference in water turnover between lactating and non-reproductive bats (measured using ²H turnover) averaged 2·6 ml bat⁻¹ day⁻¹ (22·9 kJ day⁻¹). This was similar to the average milk intake of sucklings estimated from ³H turnover (22·9 kJ day⁻¹).
4. Energy available for respiration from food and mobilization of fat stores was 18·2 kJ day⁻¹ for lactating females compared with 36·8 kJ day⁻¹ for non-reproductive females. In comparison, respiratory daily energy expenditure (DEE) of lactating and non-reproductive bats, measured by doubly labelled water (DLW), was 21·3 kJ day⁻¹ and 23·6 kJ day⁻¹, respectively. Hence, there was a discrepancy between respiratory DEE (measured by DLW) and net available energy estimates for non-reproductive bats but not for lactating bats.
5. Respiratory DEE for lactating bats was equal to or less than that of non-reproductive females, suggesting they used compensatory mechanisms in their energy budgets in lactation.

**Key-words:** Doubly labelled water, food intake, milk, reproduction, tritium


Introduction

For most small terrestrial mammals, lactation is the most energetically costly period of reproduction (Thompson 1993). For small mammals in captivity, energy demands in late lactation are between 2·5 and 5 times the level of non-reproductive energy expenditure (Clutton-Brock 1991; Thompson 1993; Poppitt, Speakman & Racey 1993). Most small terrestrial animals accommodate this increased requirement mainly by increasing food consumption (e.g. Hanwell & Peaker 1977; Randolph *et al*. 1977; Perrigo 1987; Konig, Reister & Markyl 1988; Hammond & Diamond 1992; Speakman & McQueenie 1996).

There have been relatively few studies of the energetics of reproduction in bats, and these have focused on animals in the wild, rather than in captivity. Some insectivorous bats in the wild also increase food consumption during lactation but not to the same extent as terrestrial mammals. For example, food consumption increased by about 45% from pregnancy to lactation in both *Myotis lucifugus* (Little Brown Bats) (Anthony & Kunz 1977) and *Myotis velifer* (Cave Bats) (Kunz 1974), and between early and mid lactation *Tadarida brasiliensis* (Mexican Free-Tailed Bats) increased energy intake by 82% (Kunz, Whitaker & Wadanoli 1995b). To support these increases in food consumption, flight durations of some bats increase between parturition and weaning (e.g. *Lasiurus cinereus* (Hoary Bats) Barclay (1989); *Plecotus townsendii* (Big-Eared Bats) Clark, Leslie & Carter (1993); *Eptesicus nilssonii* (Northern Bats) Rydell (1993); and *Eptesicus fuscus* (Big Brown Bats) Grinevitch, Holroyd & Barclay...
Energy budgets of Brown Long-Eared Bats

1995). Studies that have used the doubly labelled water (DLW) technique (Lifson & McClintock 1966) to investigate reproductive energetics in bats have found increased daily energy expenditure (DEE) in late lactation, a result consistent with increased food intake and foraging times (Kurta et al. 1989a; Kurta, Kunz & Nagy 1990).

Increased food consumption is not the only mechanism that might be employed to cope with the energy demands of reproduction (Racey & Speakman 1987). Costs could be met by reducing energy expenditure on some other component of the energy budget. Activity of small terrestrial mammals, for example, is known to decrease in pregnancy (Slonaker 1924; Randolph 1977) fully compensating the costs of the gestation. Some insectivorous bats may also compensate energy costs of reproduction. For example, activity of Rhinolophus ferrumequinum (Greater Horseshoe Bats) decreased in late gestation (Ransome 1973). Daily energy demands of three species of small insectivorous bats in NE Scotland measured by DLW did not increase between pregnancy and lactation (Speakman & Racey 1987a; Racey & Speakman 1987). This might reflect the use of torpor as a compensatory mechanism. In support of this hypothesis, Swift (1980) and Entwistle, Speakman & Racey (1996) found no increases in flight times of the same species between pregnancy and lactation. By contrast, Audet & Fenton (1988), Grinevitch et al. (1995) and Hamilton & Barclay (1994) all showed decreased utilization of torpor by lactating female Big Brown Bats compared with pregnant females or males. Variability in the results of studies using DLW may be due to differences in the way different species respond to lactation, or may reflect violation of different simplifying assumptions on which the DLW technique is based (Nagy 1980; Speakman 1997), in different species.

In Sorex araneus (the Common Shrew), estimates of DEE derived from DLW were 45% greater in mid lactation than DEE measured by indirect calorimetry (Poppitt et al. 1993). This discrepancy was attributed to an error in the DLW estimate caused by high ambient unlabelled CO₂ levels from the litter (Poppitt et al. 1993). During reproduction, some bats gather in maternity roosts and remain in prolonged close proximity with each other and with their young in small confined spaces. This may lead to elevated CO₂ levels which could compromise measures of energy demands made by DLW. Yet field estimates of energy expenditure using DLW can seldom be verified by comparison with indirect calorimetry and food intake as was performed in Poppitt et al.’s (1993) study of shrews.

Most studies of reproductive energetics in bats have concentrated on wild animals because of questions concerning the validity of energy cost measurements of captive bats which are often deprived of flight and fed on artificial prey. Yet there are considerable advantages connected with studying the reproductive energetics of animals in captivity (as is routinely done in small terrestrial mammals) because this allows simultaneous quantification of components of the energy budget which would be logistically difficult in the field. However, the cost of locomotion in free-ranging bats may exceed those recorded in captivity.

The aim of the present study was to construct and compare the energy budgets of lactating and non-reproductive Plecotus auritus L. (Brown Long-Eared Bats) kept in captivity in flight enclosures and fed their natural prey. We tested the hypothesis that if females used compensation during lactation, their daily respiratory energy expenditure would be equal to or less than that of non-reproductive females. Conversely, if females did not use compensation during lactation, we expected a large increase in food consumption and respiratory energy expenditure compared to non-reproductive females.

Materials and methods

Palpably pregnant (Racey 1988) P. auritus were obtained from nursery roosts in the Grampian and Highland regions of Scotland (57°N) and housed in outdoor flight enclosures (12 m² and 36 m³) subjected to a natural photoperiod. Bats roosted in a single, heated roost box (0·4 m × 0·4 m × 0·3 m) provided in each enclosure. In the wild, P. auritus live in small groups (10–30 individuals), which include reproductive and non-reproductive females (Swift 1991; Entwistle et al. 1996). The role of non-reproductive females in these groups is not fully understood, but they may contribute to group thermoregulation (Perez 1995). Ideally, to measure food consumption of lactating females, groups comprising only lactating bats would be housed separately and their food intake compared with groups comprising only non-reproductive females. However, such groups are not representative of conditions in the wild. We aimed to set up two groups containing a mix of reproductive and non-reproductive bats, and to compare the food intake of these bats to a group containing only non-reproductive females. Unfortunately, difficulties determining reproductive status, and the possibility that some females may have reabsorbed their foetuses after capture, led to: group 1 containing four mother–young pairs and five non-reproductive females, group 2 containing nine non-reproductive females and one mother–young pair (juvenile died of unknown causes aged 20 days), and group 3 containing eight non-reproductive females and one mother–young pair. Bats were checked twice daily for the presence of newly born young. Hence the birth time of all young was known (± 12 h).

To construct energy budgets measures of food consumption and changes in body mass were used in combination with measurements of DEE and milk production. Food consumption was measured by sub-
tracting dry masses of uneaten food from the dry masses of food supplied. DLW was used to measure DEE. Milk production of females was calculated from the difference in $^2$H turnover between non-reproductive and lactating females. The $^3$H estimates of milk production were cross-checked using $^3$H turnover in offspring.

FOOD CONSUMPTION

Bats were fed on free-flying noctuid moths caught locally. When moth-trap catches yielded less than 20 moths per bat, the diet was supplemented with 10–50 g of live mealworms (Tenebrio molitor) on which the bats were trained to feed on arrival in captivity (method after Racey 1970).

Measurements of food consumption were made from June to September. Daily food intake was measured, on 63 out of 73 days (group 1), 64 out of 73 days (group 2), and 57 out of 64 days (group 3). Daily dry food consumption was calculated 'per bat' for groups 1–3 over time. It is probable that the juvenile contribution to the group food consumption was negligible. Juvenile P. auritus in the wild do not begin to fly until 4–5 weeks of age (Swift 1981; De Fanis & Jones 1995) and it may take 2–4 weeks after the onset of flight before food consumption matches that of adults (e.g. M. velifer, Kunz 1974). Juveniles were therefore excluded in the calculation of food consumption per bat.

Intake of moths

Live moths (30–400) caught the previous night were counted and weighed (± 0·001 g) and a random subsample of 10 moths was reweighed and dried to constant mass (5–7 days at 60 °C). The remaining moths were released into the flight enclosure. The dry:wet mass ratio for the subsample was used to calculate the dry mass of moths supplied to the bats. Flight enclosures were completely lined with 3-mm mesh net to contain the moths. In addition, the floors were covered with heavy-duty polythene sheeting. Each morning, any uneaten whole moths and remains were collected, weighed and dried to constant mass. The dry mass of the moths consumed was calculated from the difference between dry masses supplied and remaining.

Since the number of moths released into the flight enclosures each night was known and the culled remains, along with any uneaten moths, were collected the next day, it was possible to estimate the maximum proportion of moths which may have escaped from the flight enclosures uneaten. This estimate is a maximum because it assumes that no wings were eaten. On average 88·3% (SE = 2·02, n = 15 nights) of those released were recovered. This suggested a maximum of 12% of the moths escaped. We therefore subtracted 12% from the estimated mass intake of moths.

Intake of mealworms

On days that mealworms were supplied, a sample (10–50 g) was weighed (± 0·001 g) and a subsample of 15 mealworms was reweighed and dried to constant mass. The dry mass of mealworms supplied to the bats was calculated in the same way as for the moths. The following morning, any uneaten mealworms were removed and a subsample of 15 mealworms was weighed and dried to calculate the dry mass of uneaten mealworms. The dry mass consumed was calculated by difference.

Food consumption of lactating bats

Group 1 contained five non-reproductive females and four lactating females. The daily food consumption of the five non-reproductive females in group 1 was predicted by multiplying the mean daily food consumption 'per bat' in groups 2 and 3 by a factor of 5. The predicted values of food ingested by non-reproductive bats was subtracted from the total food consumption by group 1, and divided by 4 to give the predicted food consumption 'per bat' of each lactating bat.

DOUBLY LABELLED WATER MEASUREMENTS

Estimates of energy expenditure using DLW were made on three occasions for the bats in group 1. Females were injected twice during lactation (days 10–35 postpartum, giving a total of eight measurements on four individuals). Simultaneous injections were given to non-reproductive adult females in the same group, and in addition, these bats were injected on a third occasion (equivalent to days 55–63 postpartum in reproductive females) (n = 15 estimates on five individuals). Measures of six non-reproductive individuals housed indoors were also made. However, the interval between initial and final blood samples was 144 h in these bats, by which time the $^{18}$O isotope had declined to background levels. It was still possible, however, to estimate water turnover for this group.

Bats were weighed (± 0·01 g) and labelled with a single, subcutaneous, dorsal injection of = 0·15 ml water, containing enriched $^2$H (9 atom percentage excess, APE) and $^{18}$O (18 APE). Syringes were weighed (± 0·0001 g) before and after injection. Ninety minutes were allowed for equilibration (Speakman & Racey 1987a). After equilibration 35–75 μl of blood was collected by puncturing the interfemoral vein (Kunz & Nagy 1988). Samples were immediately flame sealed into calibrated pipettes (Vitrex, Camlab Ltd, Cambridge, UK). The bats were then returned to the roost box. Approximately 72 h later, the bats were reweighed and a second series of blood samples were collected. Background isotope enrichments were estimated from the drinking water (Speakman & Racey 1987b).
Water from the blood samples was vacuum distilled and reacted with zinc to provide hydrogen (Wong & Klein 1986) or with guanidine hydrochloride to produce CO₂ (Wong, Lee & Klein 1987). Stable isotope analyses were performed using a dual inlet gas-source isotope-ratio mass spectrometer (Micromass Optima, Manchester, UK). The 2H:1H and 18O:16O ratios were measured by the mass spectrometer and expressed in relation to known working standards. At least two subsamples of blood were independently analysed to produce each measure of isootope enrichment. DEE was calculated from CO₂ production assuming a respiratory quotient (RQ) of 0·85 (Speakman & Racey 1987a).

Lean body mass and fat mass

Total body water (TBW, ml) estimated by oxygen iso- tope dilution was used to calculate lean (wet) body mass (LBM) (Mathews & Gilker 1995). Fat mass (FM) was calculated by subtracting LBM from total body mass.

DEUTERIUM TURNOVER

Water flux was determined from 2H turnover (Nagy & Costa 1980) using blood samples obtained during the DLW technique (described above). The main components of water influx in lactating and non-reproductive females were identified by combining 2H turnover measurements with estimates of food consumption. Preformed water intake was estimated by assuming dietary water content of 62% (mid way between moths and mealworms: present study). Metabolic water production was calculated from our DLW estimates of CO₂ production using 0·583 ml of metabolic water produced for each litre of CO₂ produced (see Kurta et al. 1989b). Preformed and metabolic water production was subtracted from the total water flux to estimate the amount of water intake through drinking.

TRITIATED WATER METHOD

Measurements of water flux of sucklings in group 1 were obtained on a single occasion using 3H turnover to cross-check the milk production estimates based on 2H turnover of the mothers. Tritiated water (HTO, Radiochemical Centre, Amersham, UK) was diluted with distilled water to obtain an activity of 250 microcuries (= 9·25 MBq) per ml. Each juvenile bat (aged 27–35 days at the time of injection) was weighed and then injected with a weighed quantity of solution (± 0·0001 g) containing = 12·5 microcuries (i.e. 0·05 ml). Equilibration took 90 min during which the juveniles were reunited with their mothers, and housed in their normal roosting box. The interval between initial and final blood samples was 72 h. Simultaneous blood samples were collected from the mothers, to establish if there was any transfer of 3H from the juveniles back to the lactating females (Baverstock & Green 1975).

The specific activity of 3H was measured for duplicate 5-μl blood samples using a liquid scintillation counter (Packard, model 1600TR; Packard Instrument Company, CT, USA). Each blood sample was transferred from the pipette into a standard liquid scintillation vial containing 3 ml of scintillation fluid (hionic fluor, ICN Ltd, Hampshire, UK) and one drop of solvent. Samples were decolorized by adding one drop of hydrogen peroxide. Five duplicate standards were prepared from the injection solution as described for the blood samples, except that blood was replaced with = 0·05 ml (syringes weighed with a precision of 0·0001 g) of the diluted injection solution. Background levels of radioactivity were quantified using a set of five duplicate vials containing 3 ml scintillation fluid, one drop solvent and one drop hydrogen peroxide. Specific activity of 3H was expressed as DPM (disintegrations per minute) per μl of water corrected for background counts. Since whole blood was analysed, the volume of blood was corrected assuming it consisted of 90% water (Kimball 1983; Campbell 1990).

Conversion of total daily water intake of sucklings to milk intake required knowledge of metabolic rate, water content of milk consumed, and metabolic water production from catabolism of milk fat and carbohydrate. Metabolic rate (litres O₂ consumed per day) was predicted for each juvenile (Table 2) using a regression equation describing the curvilinear relationship between resting metabolic rate (RMR) and body mass of juveniles (log₁₀ RMR = – 3·20 + 0·0809 × body mass, r² = 0·13, F = 7·73, df = 1,52, P = 0·008; unpublished data). The milk of vespertilionid bats contains around 68–75% water, 6–20% fat, 3–11% carbohydrate and 4–9% protein (Jenness 1974; Jenness & Studier 1976; Kunz, Stack & Jenness 1983; Kunz et al. 1995a). Milk protein is probably mostly incorporated in tissue growth. In the absence of specific data on milk composition for P. auritus it was assumed that bats utilized fat and carbohydrate in the proportions present in bat milk (3 : 1 average from published data for vespertilionids) which gave a metabolic water production of 0·633 g l⁻¹ oxygen consumed (Bassett & Studier 1988). Maximum and minimum limits for metabolic water production were also calculated assuming 100% fat utilization or 100% carbohydrate utilization, and incorporating 95% confidence intervals for the estimates of RMR in the calculations. Milk intake (ml day⁻¹) was calculated by subtracting metabolic water production from total water intake (ml day⁻¹) using 72% water content for milk (average for vespertilionids, Kunz et al. 1995a).

ENERGY BUDGETS

The energy absorption efficiency of several species of insectivorous bats fed on mealworms ranges from 88
to 91% and energy contents of mealworms vary from 23.3 to 28.6 kJ g\(^{-1}\) dry mass (Brisbin 1966; Neuhauser & Brisbin 1969; O’Farrell, Studier & Ewing 1971; Kunz 1988; Barclay, Dolan & Dyck 1991; Webb 1992). In comparison, mean energy absorption efficiencies for three species of insectivorous bats fed exclusively on moths were 75–78% and the energy content of moths was 23.2 kJ g\(^{-1}\) dry mass (Barclay et al. 1991). However, the energy content of moths may be higher than this. Kunz et al. (1995b) reported an average energy density for stomach contents of *T. brasiliensis* of 31.2 kJ g\(^{-1}\) dry mass. This was attributed to the consumption of insects high in fat (including the abdomens of culled moths). Thus, we used a mean energy absorption efficiency of 89.8% (average of all studies) and a mean energy content of 26.2 kJ g\(^{-1}\) dry mass for mealworms, with a mean energy absorption efficiency of 76.5% and a mean energy content of 27.2 kJ g\(^{-1}\) dry mass for moths. The total daily energy intake was calculated by applying these assumed energy contents and absorption efficiencies to the amounts of moths and mealworms eaten. Limits were defined for our estimates of energy intake using the minimum and maximum published values of energy contents and absorption efficiencies for each prey item. It was assumed that 10% of assimilated energy was lost as urea (Kurta et al. 1989a: calculation based on a typical insect diet for *M. lucifugus*).

**STATISTICS**

Two-way analysis of variance (ANOVA) was used to test for differences in patterns of food consumption between groups. Directionality in the patterns of food consumption in relation to time was examined using least squares linear regression. Regression was also used to describe the relationship between DEE and body mass, and water flux and body mass in non-reproductive animals. The DLW data contained repeated measures on four lactating females on two occasions and five non-reproductive females on three occasions. This was necessary because we could not bring more breeding bats into captivity for logistical and licensing reasons. In all cases individual values were plotted for comparative purposes but all statistical tests were performed on mean values for each individual. *T*-tests were used to compare body mass (including lean and fat mass), water flux and DEE between non-reproductive and lactating bats.

**Results**

**FOOD CONSUMPTION METHOD**

Moths consisted on average of 64.2% (± SE = 0.13) water (n = 127). Mealworms put into the enclosures consisted on average of 60.2% (± SE = 0.14) water (n = 185 samples). In comparison, the mean percent-age of water contained in subsamples of uneaten mealworms was slightly, but significantly, lower at 59.6% (± SE = 0.22) (paired sample *t*-test, *n* = 185, mean difference = 0.78% ± SE 0.22, *t* = 3.51, *P* < 0.001). This suggested a slight decrease in the water content of the mealworms in the feeding pots overnight, due to dehydration.

**Food consumption**

There was no significant effect of time (days) on the pattern of dry food consumption per bat for groups 2 and 3 which each contained eight to nine non-reproductive individuals with only one lactating female (two-way ANOVA, *P* > 0.05). There was also no significant group effect on food consumption (two-way ANOVA, *P* > 0.05). The mean daily dry food consumption per bat for both groups 2 and 3 over the entire experimental period was 1.8 g (± SE = 0.06, group 2; ± SE = 0.08, group 3). When the dry food consumption per bat for group 1 (which contained four lactating and five non-reproductive females) was included in the analysis of variance, there was no significant time effect but there was a significant group effect (two-way ANOVA (time), *P* > 0.05; (group), *F* = 5.52, df = 2.32, *P* < 0.01). There was a significant increase in the level of daily dry food consumption per bat in relation to time for groups 1 and 3 (least squares regression analysis: *P* < 0.001, *P* < 0.01, respectively: Fig. 1). There was no significant relationship between food consumed per bat and time for group 2 (least squares regression analysis: *P* > 0.05).

**Factors affecting food consumption**

Multiple regression analysis was performed on dry food consumption (g bat\(^{-1}\) day\(^{-1}\)) for groups 1, 2 and 3 using the following factors as predictors: (1) daily maximum temperature in the flight enclosure; (2) daily minimum temperature in the flight enclosure; (3) number of moths released into the flight enclosure each day; (4) length of night (sunset to sunrise); (5) the mean of individual daily mass changes for the group; and (6) the mean daily mass of bats on the morning before feeding. Factors significantly related to food consumption are documented in Table 1. Length of night was significantly correlated with food consumption in all three groups. In addition mean daily mass change and mean mass of bats were significantly related to food consumption in group 3.

**Food consumption in lactating bats (group 1)**

There was a significant increase in predicted dry food consumption per lactating bat over time (least squares regression analysis: food intake = 1.49 + 0.0237 day of measurement, *r*\(^2\) = 0.18, *F* = 4.06, df = 1.47, *P* = 0.05) from 1.7 g bat\(^{-1}\) day\(^{-1}\) on day 10 of the measurement period (about day 1 of lactation) to 3.0 g
Energy budgets of Brown Long-Eared Bats

The average food consumption of lactating females over the period of DLW measurements was 2.0 g bat\(^{-1}\) day\(^{-1}\) (SE = 0.1, n = 17). This represented an increase of 11% compared with non-reproductive bats (2.0–1.8/1.8 \times 100).

DOUBLY LABELLED WATER MEASUREMENTS

**Body mass and composition**

The mean initial body masses (g) of lactating and non-reproductive bats involved in DLW measurements were 9.72 g (± SE = 0.09, n = 4 individuals, 8 measurements) and 8.66 g (± SE = 0.16, n = 5 individuals, 15 measurements), respectively. Initial body mass was significantly greater in lactating than in non-reproductive females (t-test, t = 4.15, df = 5, P < 0.01). There was no significant difference between mean initial body mass (9.03 g ± SE 0.15) and mean final body mass (8.95 g ± SE 0.14) across all individuals involved in DLW measurements (paired t-test, mean difference = 0.11 g, SE = 0.07, n = 9, t = -1.54, P = 0.16).

Mean total body water estimated from the \(^{18}O\) dilution space (N\(_O\)) was 5.67 ml (range 4.82–6.71, SE = 0.11, n = 23 measurements). On average this represented 63% of initial body mass (range 51–68%, SE = 0.9, n = 23).

Lean body masses of lactating and non-reproductive bats were on average 8.53 g (± SE = 0.12, n = 4 individuals, 8 measurements) and 7.35 g (± SE = 0.14, n = 5 individuals, 15 measurements), respectively. Lactating females had a significantly greater lean body mass than non-reproductive females (t-test, t = 7.44, df = 4, P < 0.001). The inferred mean fat masses of lactating and non-reproductive bats were 1.19 g (± SE = 0.12) and 1.31 g (± SE = 0.17), respec-

### Table 1. Multiple regression analysis for factors related to daily dry food consumption (g bat\(^{-1}\) day\(^{-1}\)) for groups 1, 2 and 3. Variables include length of night (sunset to sunrise), the mean of individual daily mass changes for the group, and mean daily mass of bats on the morning before feeding

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-2.95</td>
<td>0.582</td>
<td>-0.27</td>
</tr>
<tr>
<td>Night length</td>
<td>0.012</td>
<td>0.00294</td>
<td>0.00534</td>
</tr>
<tr>
<td>Mass change</td>
<td>NS</td>
<td>NS</td>
<td>1.02</td>
</tr>
<tr>
<td>Mean mass</td>
<td>NS</td>
<td>NS</td>
<td>0.042</td>
</tr>
<tr>
<td>(\hat{r}^2)</td>
<td>0.68</td>
<td>0.18</td>
<td>0.45</td>
</tr>
<tr>
<td>(F)</td>
<td>82.59</td>
<td>11.04</td>
<td>7.64</td>
</tr>
<tr>
<td>(df)</td>
<td>1.39</td>
<td>1.52</td>
<td>3.28</td>
</tr>
<tr>
<td>(P)</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01</td>
<td>0.001</td>
</tr>
</tbody>
</table>

NS = \(P > 0.05\)
tively. There was no significant difference in fat mass between these groups of bats (t-test, \( t = -0.39, \) df = 5, \( P = 0.72 \): estimated mean fat mass for each individual was used in the analysis).

**Daily energy expenditure**

Four different equations were used for calculating DEE (a single pool model, Lifson & McClintock 1966; and variations of the two-pool model, Coward et al. 1985; Speakman 1993; Speakman 1997). The effect of equation on estimates of DEE was small (average absolute deviation of DEE estimates calculated by a particular equation from the mean across all four equations ranged from 0.3 to 1.5 kJ). Therefore the most recent version of the two-pool model was used (Speakman 1997) for all further analyses of DEE.

A scatterplot of DEE (kJ bat\(^{-1}\) day\(^{-1}\)) in relation to mean body mass (initial + final body mass/2) for all measurements (\( n = 23 \)) on all individuals (\( n = 9 \)) in group 1 is given in Fig. 3. Mean DEE (kJ bat\(^{-1}\) day\(^{-1}\)) of non-reproductive females was 23.6 (± SE = 1.5) (\( n = 15 \) measurements on 5 individuals). There was a significant increase in DEE with increasing mean body mass for non-reproductive females (least squares regression analysis using the mean of three measurements for DEE and mean body mass for each individual: \( DEE = (5.56 \times \text{mean mass}) – 24.2, r^2 = 0.80, F = 11.60, \) df = 1,3, \( P = 0.042 \)).

The regression equation obtained for DEE and mean body mass of non-reproductive bats were used to predict DEE of lactating individuals from their mean body masses. Mean DEE predicted from body mass was 29.8 kJ bat\(^{-1}\) day\(^{-1}\) (SE = 0.76, \( n = 4 \)). In comparison, mean observed DEE of lactating females was significantly lower at 21.3 kJ bat\(^{-1}\) day\(^{-1}\) (SE = 1.91, \( n = 4 \)) (t-test, \( t = -3.58, \) SE = 2.36, \( n = 4, P = 0.037 \)).

**\(^2\)H turnover technique**

A scatterplot of water flux (ml bat\(^{-1}\) day\(^{-1}\)) measured by \(^2\)H turnover in relation to mass for all individuals and measurements is given in Fig. 4. The mean water flux for non-reproductive adult females (21 measurements on 11 individuals, including 7 measures on 6 non-reproductive females housed in an indoor flight enclosure) was 3.2 ml bat\(^{-1}\) day\(^{-1}\) (SE = 0.1, range = 2.2–4.3). Analysis of covariance on water flux data for non-reproductive bats (grouped according to individual and with mass as a covariate) revealed no significant difference in the level of water flux between individuals (individual effect: \( F = 1.02, \) df = 4,5, \( P = 0.478 \)). There was also no significant difference in the slope of the relationship between water flux and body mass between non-reproductive individuals (individual × mass effect: \( F = 1.10, \) df = 4,5, \( P = 0.449 \)). There was a significant decline in water flux with increasing body mass (least squares regression analysis using mean values for water flux and body mass for each individual (\( n = 4 \)) and six independent measures of flux and mass from bats which were sampled only once: water flux (ml bat\(^{-1}\) day\(^{-1}\)) = 7.87–0.513 mean body mass (SE = 0.1, range = 5.2–1.1).

**Fig. 2.** Dry food consumption (g bat\(^{-1}\) day\(^{-1}\)) for lactating bats in group 1. Values represent the difference between predicted levels of food intake for the five non-reproductive individuals and whole group food consumption levels for group 1, divided by a factor of 4 to give an estimate of food consumption per lactating female. The regression equation is provided in the text.

**Fig. 3.** DEE (kJ bat\(^{-1}\) day\(^{-1}\)), estimated using DLW, in relation to mean body mass. Data represents 8 measurements on four reproductive bats and 15 measurements on five non-reproductive bats. ○; lactating, ●; non-reproductive females. The fitted line represents the least squares regression equation for non-reproductive data. Estimates do not include milk export.
Energy budgets of Brown Long-Eared Bats

The regression equation obtained for water flux and mean body mass of non-reproductive bats was used to predict water flux in lactating individuals from their mean body masses. Mean water flux predicted from body mass was 2.88 ml bat\(^{-1}\) day\(^{-1}\) (SE = 0.06, \(n = 4\)). In comparison, mean observed water flux in lactating females was 4.81 ml bat\(^{-1}\) day\(^{-1}\) (SE = 0.24, \(n = 4\)), yielding a difference of 1.93 ml which was significant (\(t\)-test, \(t = 7.80\), SE = 0.25, \(n = 4\), \(P < 0.01\)).

Assuming the increased water flux in reproductive females compared with non-reproductive females was entirely due to milk export, this represented a milk production of 2.6 ml bat\(^{-1}\) day\(^{-1}\) (assuming a 72% water content for bat milk, average for vespertilionid bats, Kunz et al. 1995a).

Non-reproductive bats had a preformed water intake of 2.94 and lactating bats 3.26 ml day\(^{-1}\). Metabolic water production was 0.58 and 0.53 ml bat\(^{-1}\) day\(^{-1}\) for non-reproductive and lactating bats, respectively. Preformed and metabolic water was subtracted from the total water flux to estimate the amount of water intake via drinking. This difference was negative for non-reproductive females, indicating they did not need to drink, and 1.02 ml day\(^{-1}\) for lactating females.

### \(^3\)H Turnover Technique

Mean water intake of sucklings was 2.1 ml bat\(^{-1}\) day\(^{-1}\) (SE = 0.4, range = 1.1–2.7, \(n = 4\)). Calculated metabolic water production was 0.19 ml day\(^{-1}\) (SE = 0.01, \(n = 4\); Table 2). Minimum and maximum limits for metabolic water production (assuming 100% fat utilization or 100% carbohydrate utilization, and incorporating 95% confidence intervals for individual RMR estimates in the calculation) were 0.15 ± SE 0.01 ml day\(^{-1}\) and 0.27 ± SE 0.01 ml day\(^{-1}\) (Table 2). Using the estimate of 0.19 ml day\(^{-1}\) for metabolic water production, mean milk intake was 2.63 ± SE 0.48 ml day\(^{-1}\). Limits calculated for milk intake were 2.52 ± SE 0.48 ml day\(^{-1}\) and 2.69 ± SE 0.48 ml day\(^{-1}\) (Table 2). Using an energy content of 8.8 kJ g\(^{-1}\) for bat milk (average for vespertilionid milk, Jenness 1974; Jenness & Studier 1976; Kunz et al. 1983; Kunz et al. 1995a), this represents 22.2–23.7 kJ day\(^{-1}\).

Mean DPM/\(\mu l\) averaged across four lactating females taken simultaneously with the final blood sampling in juveniles was 23.9 (SE = 2.9, \(n = 8\), range = 14–36). This represented 1% of the mean DPM/\(\mu l\) obtained for final blood samples of the juveniles.

### Table 2

<table>
<thead>
<tr>
<th>Bat no.</th>
<th>Water turnover (ml day(^{-1}))</th>
<th>RMR (l (O_2) day(^{-1}))</th>
<th>95% CIs for RMR</th>
<th>M water 1 (ml day(^{-1}))</th>
<th>M water 2 (ml day(^{-1}))</th>
<th>M water 3 (ml day(^{-1}))</th>
<th>Milk intake 1 (ml day(^{-1}))</th>
<th>Milk intake 2 (ml day(^{-1}))</th>
<th>Milk intake 3 (ml day(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>8766</td>
<td>2.183</td>
<td>0.292</td>
<td>(0.288–0.374)</td>
<td>0.208</td>
<td>0.161</td>
<td>0.301</td>
<td>2.743</td>
<td>2.808</td>
<td>2.614</td>
</tr>
<tr>
<td>8756</td>
<td>2.371</td>
<td>0.295</td>
<td>(0.262–0.323)</td>
<td>0.187</td>
<td>0.147</td>
<td>0.259</td>
<td>3.033</td>
<td>3.089</td>
<td>2.933</td>
</tr>
<tr>
<td>8751</td>
<td>2.700</td>
<td>0.299</td>
<td>(0.267–0.331)</td>
<td>0.189</td>
<td>0.149</td>
<td>0.266</td>
<td>3.487</td>
<td>3.543</td>
<td>3.381</td>
</tr>
<tr>
<td>8760</td>
<td>1.078</td>
<td>0.277</td>
<td>(0.245–0.310)</td>
<td>0.175</td>
<td>0.137</td>
<td>0.249</td>
<td>1.254</td>
<td>1.307</td>
<td>1.151</td>
</tr>
</tbody>
</table>

Table 3. Energy budgets of non-reproductive and lactating bats. Values represent energy (kJ) available for respiration (DEE predicted) calculated from measurements of food consumption (N = 4 lactating females, 17 non-reproductive females) and milk export (from tritium turnover in juveniles N = 4, and deuterium turnover in lactating females N = 8 measures on 4 females), compared with estimates from doubly labelled water (DLW) (N = 8 measures on 4 lactating females, 15 measures on 5 non-reproductive females). Values in parentheses are limits for energy intakes calculated by applying the minimum and maximum published values of energy contents and absorption efficiencies for moths and mealworms to the amounts eaten.

<table>
<thead>
<tr>
<th></th>
<th>Non-reproductive</th>
<th>Lactating</th>
<th>Difference (lact. – non-rep.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingested</td>
<td>47·7 (41·9–53·1)</td>
<td>53·1 (46·6–59·0)</td>
<td>+5·4</td>
</tr>
<tr>
<td>Assimilated</td>
<td>40·6 (35·0–45·8)</td>
<td>45·3 (38·9–51·0)</td>
<td>+4·7</td>
</tr>
<tr>
<td>Mobilization of fat stores</td>
<td>0·2</td>
<td>0·9</td>
<td>+0·7</td>
</tr>
<tr>
<td>Urea</td>
<td>4·0 (3·5–4·6)</td>
<td>4·5 (3·9–5·1)</td>
<td>+0·5</td>
</tr>
<tr>
<td>Milk export</td>
<td>0</td>
<td>23·0</td>
<td>+23·0</td>
</tr>
<tr>
<td>Respiration (DEE pred.)</td>
<td>36·8 (31·7–41·4)</td>
<td>18·7 (12·9–23·8)</td>
<td>–18·1</td>
</tr>
<tr>
<td>Respiration (DLW)</td>
<td>23·6</td>
<td>21·3</td>
<td>–2·3</td>
</tr>
</tbody>
</table>

ENERGY BUDGETS OF NON-REPRODUCTIVE AND LACTATING BATS

The average food intakes of non-reproductive and lactating bats involved in DLW measurements were converted to ingested energy and corrected for losses due to assimilation and urea production (Table 3). Lactating females ingested 5·4 kJ more than non-reproductive bats. The body mass of non-reproductive bats decreased by 0·3 g over this period. If this mass loss represented the mobilization of fat, and using an energy content for bat fat of 39·4 kJ g⁻¹ (Ewing, Studier & O’Farrell 1970), a total of 10·6 kJ or 0·2 kJ day⁻¹ was obtained from fat stores. Lactating females lost 0·6 g over the measurement period, representing a total of 23·6 kJ or 0·9 kJ day⁻¹ obtained from fat stores. Energy obtained from fat stores represented 0·5% and 2% of daily assimilated energy for non-reproductive and lactating females, respectively. The energy exported in milk (23·0 kJ, midway between ⁴H and ³H turnover estimates) was subtracted from the energy available from assimilated food and fat utilization, leaving between 12·9 and 23·8 kJ available for respiration in lactating bats. The average respiratory DEE of lactating females measured using DLW (21·3 kJ) fell within this range. In contrast, the energy available for respiration in non-reproductive bats estimated from food intake and fat stores (between 31·7 and 41·4 kJ) was significantly greater than the DLW measure (23·6 kJ) and was = 18·6 kJ greater than that available to lactating females.

Discussion

FOOD CONSUMPTION

The average food intake of lactating P. auritus (mean mass 9·72 g) was equivalent to assimilation of 45 kJ day⁻¹ (limits: 39–51 kJ day⁻¹). In comparison, the average assimilated energy for free-ranging M. lucifugus (mean mass = 7·9 g) was similar at 41·3 kJ day⁻¹ (Kurta et al. 1989a). These data indicate our observations in captivity may be broadly representative of demands placed on wild female bats. Using the observed mean wet mass of 0·25 g for a typical noctuid moth and a mean water content of 64%, the dry food consumption of non-reproductive bats represented consumption of 20 whole moths per night. The average level of food consumption throughout days 10–35 of lactation (2·0 g bat⁻¹ day⁻¹) represented an intake of 22 whole moths per night. However, the wings of noctuid moths are culled by the bats before ingestion and so these values are underestimates of required capture rates. Keeler & Studier (1992) reported a reduction in wet mass of 5% due to culling. In comparison, Barclay et al. (1991) noted that M. lucifugus rejected on average 25% of the dry mass of moths offered to them. If 15% (mean of these two values) of the dry mass is rejected when culling, an individual P. auritus, based on our estimates of food intake, would have to ingest 23 culled moths per night when not reproducing and 26 culled moths during lactation. Entwistle et al. (1996) reported median flight times of 253 and 239 min for free-ranging non-reproductive and lactating P. auritus, respectively. To meet the predicted levels of consumption, a non-reproductive bat would have to capture one moth every 11 min (253/23) and a lactating bat would have to capture one moth every 9 min (239/26). Reported insect capture success for bats in the wild is variable. For example, Hickey & Fenton (1990) observed an average capture success of 40% for Lasiusus borealis (Red Bats). Hickey (1992) reported that 50–56% of attacks on insects by L. cinereus were successful. In comparison, Anderson & Racey (1991) observed capture success of 68–79% in free-flying, captive P. auritus. If capture success was only 40% (lowest of the above values), then to meet the predicted insect capture rate, a lactating female would have to attack one moth every 4 min when foraging.

DOUBLY LABELLED WATER MEASUREMENTS

Daily energy expenditure

DEEs, measured by DLW, for captive P. auritus were consistent with previous DLW measures in the field for this species, ranging from 16 to 30 kJ day⁻¹.
Energy expenditure during lactation (21 kJ day\(^{-1}\)) was significantly less than expected for non-reproductive females of similar body mass (29.8 kJ day\(^{-1}\)). A daily milk production equivalent to 23 kJ day\(^{-1}\) was estimated. Thus the total energy budget averaged 44 kJ day\(^{-1}\) for *P. auritus* during lactation. This was almost identical to the calculated energy assimilated from food (45 kJ day\(^{-1}\)). Based on measurements of oxygen consumption for day and night-roosting, along with allometric predictions for flight costs, Kurta, Johnson & Kunz (1987) estimated a similar total energy requirement (including milk export) of 49 kJ bat\(^{-1}\) day\(^{-1}\) during peak lactation for *M. lucifugus*. The correspondence between DEE measurements for reproductive and non-reproductive individuals in our study with previous studies in the wild suggests our captive housing conditions were broadly representative of the wild.

### WATER FLUX MEASURED BY \(^{3}H\) TURNOVER

Daily water flux for captive non-reproductive *P. auritus* was 1.2–4 times greater than previously reported for captive non-reproductive bats (*M. lucifugus* O’Farrell et al. 1971; Coutts, Fenton & Glen 1973; *Myotis thysanodes* (Fringed Bat) O’Farrell et al. 1971; and *E. fuscus* Coutts et al. 1973). In these previous studies, bats were housed individually and denied flight activity. The greater level of water flux observed was probably because the bats were allowed free-flight and pursued flying insect prey. Our measurements for non-reproductive *P. auritus* (3.2 ml bat\(^{-1}\) day\(^{-1}\)) represented a turnover of 56% total body water and fell in the range of previous field measurements for non-reproductive individuals (1.8–5.4 ml bat\(^{-1}\) day\(^{-1}\), Bell et al. 1986; Ellis, Marples & Phillips 1991).

In the present study, lactating *P. auritus* had a mean water flux of 4.8 ml bat\(^{-1}\) day\(^{-1}\) which was 66% greater than expected for non-reproductive bats of similar mass. A greater water flux during lactation than in pregnant or non-reproductive bats was also measured in *E. fuscus* (Kurta et al. 1990) and *N. geoffroyi* (Ellis et al. 1991). The greater water flux during lactation in the present study was accounted for by the increased water efflux due to milk secretion as it was almost identical to the estimated milk intake of sucklings measured using \(^{3}H\) turnover. Of the total daily water flux for lactating bats, 68% represented preformed water in food, 11% represented metabolic water and 21% came from drinking water. Our estimates of drinking water requirements for lactating females are similar to those predicted for free-ranging pregnant and lactating *M. lucifugus* and *E. fuscus* (20–26%, Kurta et al. 1989b, 1990). These data suggest lactating bats must drink to stay in water balance and may explain why proximity to water appears to be an important factor in the selection of maternity roost sites (Speakman et al. 1991).

### ENERGY BUDGETS

Compared with non-reproductive females, the energy available to lactating females from increased food consumption was 6 (4.7/0.7, see Table 3) times greater than from the use of stored energy. This is consistent with previous studies on bats, which have suggested that maternal fat reserves are of limited importance in sustaining the costs of lactation, since they are not large enough to provide more than a few days total energy requirements (Kurta & Kunz 1987; Racey & Speakman 1987; Speakman & Racey 1987a; Kurta et al. 1989a). In general, this is also the case in most other small mammals (e.g. Mattingly & McClure 1982, 1985; McClure 1987; Millar 1987; Kenagy & Barnes 1988; but see Randolph et al. 1977). In contrast, energy stores may be very important in covering reproductive energy costs in larger species such as pinnipeds (Anderson & Fedak 1987; Oftedal, Boness & Tedman 1987).

After subtracting the energy exported in milk from the energy available from food and from fat stores, lactating bats had 13–24 kJ for respiration compared with 32–41 kJ for non-reproductive bats (Table 3). In comparison, respiratory DEE of non-reproductive and lactating bats measured by DLW was 21 and 24 kJ day\(^{-1}\), respectively. The estimate of DEE of non-reproductive bats derived from the DLW technique was lower than the estimate derived from food consumption. It is unclear why the two estimates differed. It is possible that the bats balanced their energy budgets over a greater period than was measured during DLW samples. If this occurred, the net available energy estimate would not necessarily be consistent with the DLW estimate since the former was measured continuously over the measurement period, but DLW samples were taken twice over a relatively short timescale within this measurement period. However, the food consumption of bats over the days when DLW was performed was not significantly different from that expected from the overall regression of food intake and time obtained for bats in group 1 (\(t\)-test, mean difference = 0.40 g (expected – observed), \(t = 1.47\), SE = 0.27, \(n = 8, P = 0.18\)). This suggested that food consumption was not significantly affected by the DLW protocol and that our DLW estimates of energy expenditure should be directly comparable to the net available energy estimates.
COMPENSATION

Both food intake and DLW methods suggested that lactating females were compensating energy costs because, independent of the method employed, respiratory DEE for lactating bats was equal to or less than that of non-reproductive females (Table 3). Since lactating females would have incurred costs of milk synthesis (Blaxter 1989) in addition to other respiratory energy demands connected to their greater size, it would be expected that their respiratory DEE would be greater than that of non-reproductive bats if they did not use compensation. The current evidence strongly indicates the lactating bats were using compensating mechanisms in their energy budgets. The mechanism for this compensation is unclear. Lactating *P. auritus* substantially reduce their grooming activity compared with non-reproductive bats (McLean & Speakman 1997), which may contribute to the energy saved. Many mammals, including bats, have the ability to enter torpor and this might represent a powerful mechanism for compensating energy costs during lactation. However, it is likely that a decrease in body temperature would reduce the rate of milk secretion (Wilde et al. 1995). Thus, frequent use of torpor during lactation could compromise growth rates of the offspring. In other mammals, torpor appears not to be a major feature during lactation (Geiser & Baudinette 1987; Darrow et al. 1989).

In conclusion, lactating *P. auritus* increased food consumption above non-reproductive levels but to a smaller degree than expected from studies of small terrestrial mammals. The bats did withdraw some energy from body stores but this represented only one-sixth of the energy they obtained from increased food consumption. There was an unexplained discrepancy between respiratory DEE measured by DLW and net available energy estimated from food intake and milk production. Both food consumption and DLW measurements suggested the use of compensation by lactating females.

Acknowledgements

Our bats were taken into captivity under licence from Scottish Natural Heritage (SNH) during the summers of 1991 and 1992. We are grateful to all the householders who gave access to their homes to catch bats. We would also like to thank Ian Mitchell, Paul Thompson and Gareth Jones for providing useful comments on earlier drafts of the manuscript.

References


CIP-gegevens Koninklijke Bibliotheek, den Haag.


Received 25 August 1997; revised 2 October 1998; accepted 15 October 1998