The energetics of lactation in cooperatively breeding meerkats *Suricata suricatta*

M. Scantlebury^{1*†}, A. F. Russell², G. M. McIlrath³, J. R. Speakman^{4,5}
and T. H. Clutton-Brock²

¹Mammal Research Unit, School of Biological Sciences, University of Bristol, Bristol BS8 1UG, UK

²Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, UK

³Mammal Research Institute, University of Pretoria, 0002 Pretoria, Republic of South Africa

⁴Aberdeen Centre for Energy Regulation and Obesity (ACERO), Department of Zoology, University of Aberdeen, Aberdeen AB24 2TZ, UK

⁵Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, UK

Species may become obligate cooperative breeders when parents are unable to raise their offspring unassisted. We measured the daily energy expenditure of mothers, helpers and offspring during peak lactation in cooperatively breeding meerkats *Suricata suricatta* using the doubly labelled water technique. Lactating mothers expended more energy per day than allo-lactating subordinate females, non-lactating females or suckling offspring. Metabolizable energy intakes of lactating mothers were calculated from isotope-based estimates of offspring milk energy intake, and were not significantly different from the previously suggested maximal limit for mammals. Allo-lactating females were the only category of animals that lost weight during the period of study, probably because they spent more time babysitting than non-lactating females. Daily energy expenditure (DEE) of lactating mothers increased with litter size but decreased with the number of helpers. Calculations show that for every 10 helpers, even in the absence of allo-lactators, mothers are able to reduce their DEE during peak lactation by an amount equivalent to the energy cost of one pup. These results indicate that helpers have beneficial energetic consequences for lactating mothers in an obligate cooperatively breeding mammal.

Keywords: doubly labelled water; carnivore; maternal investment; kin selection; helpers; ecological physiology

1. INTRODUCTION

In cooperatively breeding species, young are raised by their parent(s) and assisted by a variable number of helpers (Stacey & Koenig 1990; Solomon & French 1997). In many of these species, parents are capable of breeding without helpers, but in a few, breeding is typically unsuccessful in their absence. One hypothesis for the occurrence of obligate cooperative breeding is that, following the evolution of cooperation, selection for female fecundity (and hence the energy required for reproduction) has increased such that breeders are unable to raise their offspring without help (Creel & Creel 1991). This hypothesis predicts that: (i) the daily energy intake of breeding females is high; (ii) helpers invest substantial amounts of energy in raising young; and (iii) the presence of multiple helpers reduces the energy costs to breeding females.

In this paper, we attempt to test whether or not these predictions are corroborated in the obligate cooperatively breeding meerkat *Suricata suricatta*. Meerkats are small (less than 1 kg) diurnal social carnivores that live in groups of 2–30 in arid regions of Southern Africa. Most reproduction is secured by a dominant female who produces up to four litters per year of one to eight pups per litter (Russell *et al.* 2002a). Our analysis focuses on the period

of peak lactation when pups are 21–25 days old, still at their natal burrow and whilst they are still dependent on milk for all of their nutritional requirements. During this time, helpers do not bring food to the pups or to the breeding female, however, helpers do guard pups at the natal burrow (Clutton-Brock *et al.* 1998). In addition, female helpers may also lactate to the dominant female's pups, most commonly when they have lost their own litter, although they also appear to lactate spontaneously (T. H. Clutton-Brock, A. F. Russell and L. L. Sharpe, unpublished data). Allo-lactators are defined here as females that had not given birth, but that showed enlarged nipples, from which milk was able to be expressed.

2. METHODS

Fieldwork took place during the breeding season (September 1999 to February 2000 and September to December 2000) on ranch land near Van Zyl's Rus in the South African Kalahari (25°58' S, 21°49' E). The area consists of sparsely vegetated sand dunes and river terraces, on either side of the dry Kuruman riverbed. Data were collected from 11 habituated groups ranging in size from 5–30 individuals, which occupied home ranges of 3–8 km². Animals were habituated to the researchers, visited daily, individually marked and at least one member of each group had a radio collar. Consequently, it was possible to locate a target animal at a desired time. Measurements of body mass were possible as the animals were trained to stand on electronic balances (Clutton-Brock *et al.* 1998). Target animals were

* Author for correspondence (m.scantlebury@bristol.ac.uk).

† Present address: Department of Biology, University of Haifa-Oranim, Tivon 36006, Israel.

captured in canvas bags and anaesthetized (intramuscularly with 0.08 ml kg⁻¹ ketamine hydrochloride and 0.06 ml kg⁻¹ dormitol). An antidote anaesthetic (antipamisol hydrochloride 0.06 ml kg⁻¹) was administered before they were released. Offspring were not anaesthetized. Statistical analyses were performed using MINITAB 12.1 software (McKenzie & Goldman 1998) unless otherwise indicated. All means are presented $\pm$ s.d.

(a) *Doubly labelled water protocol*

We measured the daily energy expenditure (DEE) of six lactating dominant females (710–901 g, 2–7 years old), six subordinate female helpers (632–799 g, 1–2 years old) that were not lactating, three allo-lactating subordinate females (675–805 g, 1–2 years old) and six pups (87–124 g, 21–25 days old) during peak lactation using the doubly labelled water (DLW) technique (Lifson & McClintock 1966; Speakman 1997). Dominant females, allo-lactators and pups were measured at separate times, providing 15 instances in which breeding was observed. Three of the non-lactating subordinate females were measured concurrently with dominant lactating females, and three were measured during separate breeding events. Hence, the total number of times that breeding groups were observed was 18. We measured DEEs during peak lactation because it is generally the most demanding period of mammalian reproduction (Gittleman & Thompson 1988).

On day one, the animals were weighed (± 1.0 g Sartorius balance), and a 0.5 ml blood sample (0.2 ml in offspring) was taken from the jugular vein (cephalic vein in pups) to estimate the background isotope enrichments of ²H and ¹⁸O. Blood samples were collected in 2.5 ml heparinized vacutainers from which 3 $\times$ 100 μ l glass capillaries were immediately filled and heat-sealed. Afterwards, a known mass of DLW (2.3 parts 90% enriched ¹⁸O water (Enrichment Ltd., Rehovot, Israel) and one part 99.9% enriched ²H water (MSD Isotopes Inc., Pointe-Claire, Quebec, Canada)) was administered (intraperitoneally, 0.3 g kg⁻¹ body weight). Syringes were weighed before and after administration (± 0.001 g, Sartorius balance) to calculate the mass of DLW injected. Blood samples were taken after 1 h to estimate initial isotope enrichments. Adults remained anaesthetized throughout this duration. Following release, final blood samples were taken after 24 h in offspring and after 4–7 days in adults to estimate the isotope elimination rates (Speakman & Racey 1988).

(b) *Determination of isotope elimination curves in adults*

There were no previous data on isotope elimination rates in free-living meerkats, therefore we determined adult elimination curves of ¹⁸O in three subordinate males of similar age and weight to the measured subordinate females (600–763 g, 1–2 years old). A blood sample was taken to calculate background isotopic enrichments. The animals were then injected with DLW and an initial blood sample was taken before release. Animals were recaptured and blood sampled after 3 and 6 days. Elimination curves of ¹⁸O were estimated from the decrease in isotope enrichment measured from the blood samples. These results indicated a half-life of elimination for ¹⁸O of less than 4 days. Hence, we concluded that an optimum time to sample final isotope enrichment was greater than 4 days post-dose.

(c) *Isotope analyses*

Capillaries that contained blood samples were vacuum distilled into Pasteur pipettes (Nagy 1983). CO₂ and H₂ containing

the labelling isotopes from the blood were produced from the resulting distillate by small sample equilibration with CO₂ of known isotopic composition and by reduction with LiAlH₄, respectively (Speakman 1997; Ward *et al.* 2000). The isotope ratios ¹⁸O : ¹⁶O and ²H : ¹H were analysed using gas source isotope ratio mass spectrometers (Optima, Micromass IRMS and Isochrom μ G, Manchester, UK).

(d) *Calculation of daily energy expenditure*

Injectate enrichments of ¹⁸O and ²H (ppm), elimination rates (k_o and k_d , d⁻¹), dilution spaces (N_o and N_d , ml), and initial and final pool sizes (ml) were calculated following Speakman (1997) (table 1). The 'plateau' method was used to determine initial isotope enrichments with the 'percentage mass' method of calculating final pool sizes. Background levels of isotope enrichment across all individuals averaged 2043 $\pm$ 24 ppm for ¹⁸O and 185 $\pm$ 25 ppm for ²H. Mean ¹⁸O and ²H dilution spaces and elimination rates of adults and offspring are shown in table 1. The 'one-pool' calculation method was applied to the dataset to calculate CO₂ production (Speakman 1997, eqn 7.17). DEE was calculated using a computer program that took into account the effects of small deviations from 24 h for the sampling intervals and the effects of mass changes on body water pool size over the experimental period (Lemen & Speakman 1997).

Previous research has shown that isotope recycling between the lactating female and dependent offspring is unlikely to introduce errors into the estimate of maternal metabolic rate, but may have large effects on estimates of water turnover and energy expenditure estimates in suckling offspring (Scantlebury *et al.* 2000a). Therefore, adults and offspring were labelled at separate times and only one pup per litter was labelled, with another sampled as an unlabelled control to correct for any effects of isotope recycling. There was no significant increase in enrichment of either isotope in the six unlabelled pups of six litters (mean change = -2.4 ± 7.67 ppm, Wilcoxon matched-pairs signed-ranks test, Wilcoxon statistic = 5, $p > 0.05$ for ¹⁸O; and mean change = -8.8 ± 33.6 ppm, Wilcoxon statistic = 6, $p > 0.05$ for ²H). This compared with average decreases in enrichment of 192 ± 46 ppm ($T = 0$, $p < 0.05$) for ¹⁸O and 85 ± 28 ppm ($T = 0$, $p < 0.05$) for ²H in the six labelled pups. Hence, we concluded that isotope recycling between pups was negligible and that no corrections to estimates of DEE were required.

(e) *Milk production and milk energy output*

Milk samples were obtained from anaesthetized dominant lactating females (which had not fed their pups for at least 8 h) and facilitated by an injection of oxytocin (0.3 IU kg⁻¹ (intramuscularly)). Milk was expressed from all nipples, and all glands were evacuated as fully as possible (Ofstedal 1984). This produced 1–2 ml of milk per female. Milk water content was determined by drying samples to constant weight (at 70 °C), and gross energy content was determined by subsequent adiabatic bomb calorimetry (semi-micro calorimeter: 1425 Parr, USA). All females were at the same stage of lactation (21–25 days post partum). Offspring had no access to free water and only consumed milk. Mean offspring daily water efflux (rH₂O, g d⁻¹) was calculated as

$$rH_2O \times F = k_d N_d \quad (2.1)$$

(Lifson & McClintock 1966; Nagy & Costa 1980), where F is the fractionation factor of the isotope (0.941; Speakman 1997). Offspring milk intake (g d⁻¹) was calculated as

Table 1. Isotope dilution spaces and elimination rates.

(Mean and s.d. of ^{18}O dilution space (N_o , ml), ^2H dilution space (N_d , ml), dilution space ratio (N_d/N_o), ^{18}O elimination rate (k_o , h^{-1}), ^2H elimination rate (k_d , h^{-1}) and isotope elimination ratio (k_o/k_d) for lactating dominant mothers (dom-fem), subordinate non-lactating helper female (sub-fem), subordinate allo-lactating females (allo-fem) and offspring. n denotes sample sizes).

	n	N_o	s.d.	N_d	s.d.	N_d/N_o	K_o	s.d.	K_d	s.d.	K_o/K_d
dom-fem	6	587	73	591	64.1	1.010	0.015	0.002	0.012	0.002	1.320
sub-fem	6	497	34.4	509	49.5	1.024	0.011	0.001	0.008	0.002	1.390
allo-fem	3	484	56.9	530	41.9	1.040	0.009	0.002	0.007	0.002	1.314
offspring	6	77	10.3	75	9.9	1.061	0.012	0.004	0.010	0.005	1.290

$$\text{MI} = 100 \times (\text{TWI} + 1.07F_D + 0.42\text{Pr}_D) / (\%W_M + 1.07\%F_M + 0.42\%\text{Pr}_M + 0.58\%S_M) \quad (2.2)$$

(Ofstedal & Iverson 1987), in which total water intake ($\text{TWI} = (\text{rH}_2\text{O} + G)$; where G is the daily increase in body water as a result of offspring growth. F_D and Pr_D are fat and protein deposition (g d^{-1}) and $\%W_M$, $\%F_M$, $\%\text{Pr}_M$ and $\%S_M$ are percentages of water, fat, protein and total sugar, respectively, in the ingested milk. We used the measured values of N_d to calculate increases in body water space of offspring during the experimental period. We made the assumption that the composition of meerkat milk was similar to that of other terrestrial carnivores (for example arctic fox, striped skunk, domestic cat) with a similar dry matter and gross energy content, collected at peak lactation (we used the mean values $\pm$ s.d. for these three species of per cent fat, per cent protein and per cent sugar of 12.7 ± 1.7 , 10.5 ± 0.6 and 3.2 ± 0.4 , respectively (Gittleman & Ofstedal 1987)). This approximation is justified because the values from the three species above varied very little despite their taxonomic differences, and because the final calculation of pup energy intake does not depend critically on the value of milk composition that we assume. For example, a 10% increase or decrease in fat or protein content of the milk only have effects of 1.5% and 0.5%, respectively, on the calculated milk energy intake of pups. We did not measure deposition rates of fat and protein in offspring. However, we assumed that the change in offspring mass was due to an increase in lean tissue, based on previous measurements of offspring growth and body composition change in domestic dogs at the same stage of lactation (Scantlebury *et al.* 2000b). Protein deposition was calculated as the increase in $N_d/0.8$ (Sawicka-Kapusta 1974).

We calculated gross energy intake per pup as milk intake multiplied by milk energy density, and we calculated maternal milk energy output (MEO) as pup energy intake multiplied by litter size. Because mothers and pups had to be labelled at separate times, we could not measure maternal and offspring energy expenditures simultaneously. We used the relationship between the estimated energy intake of the litter and litter size, measured in the offspring energy expenditure experiments to determine MEO for each lactating mother. Hence, the metabolizable energy intake (MEI) was approximated as the DLW measurement of DEE plus the calculated MEO. For lactating mothers, we made the assumption that, except for lactation, they were in energy balance (i.e. no growth, fat storage or use of stored energy). We also assumed that the DEE estimate was robust to recycling (Scantlebury *et al.* 2000a) and there was no significant loss of energy in any other manner (e.g. non-milk secretions). For suckling offspring, we assumed that errors involved in milk energy transfer calculations were minimal, because there was no significant respiratory exchange of isotopes between pups (which would cause errors in calculations of water turnover (Nagy

1980)) and that the measured pup from each litter was representative of the rest of the pups from that litter.

3. RESULTS

(a) Energy expenditure and energy intake of pups

Across all 18 monitored litters, litter size ranged from one to six pups (mean = 4.4 ± 1.3). Milk energy averaged $7.43 \pm 0.84 \text{ kJ gross g}^{-1}$ ($n = 8$ samples). For the six mothers whose DEE was measured, mean maternal body mass, litter size and parity were $828 \pm 78 \text{ g}$, 4.8 ± 0.98 pups and 4.2 ± 3.2 previous litters, respectively. Similarly, for the six mothers whose offspring's DEE was measured, mean maternal body mass, litter size and parity were $790 \pm 98 \text{ g}$, 4.2 ± 0.18 pups and 6.8 ± 4.5 previous litters, respectively. These pups initially weighed $108 \pm 21 \text{ g}$ and increased in weight by $6 \pm 4 \text{ g d}^{-1}$ (Wilcoxon statistic = 0, $p < 0.05$) and expended $69 \pm 21 \text{ kJ d}^{-1}$. Using measures of pup milk intake, the estimated gross energy intake per pup and per litter was $184 \pm 48 \text{ kJ d}^{-1}$ and $837 \pm 620 \text{ kJ d}^{-1}$, respectively. There was no relationship between individual pup gross energy intake and litter size (Spearman's rank correlation $r_s = 0.21$, $n = 6$, $p > 0.05$). However, the estimated gross energy intake per litter increased with litter size ($r_s = 0.95$, $n = 6$, $p < 0.05$) and was described by the equation:

$$\text{energy intake of litter (kJ d}^{-1}\text{)} = 202 \times \text{litter size.} \quad (3.1)$$

(b) Energy expenditure of breeding females

Dominant lactating females were heavier than non-breeding subordinates (Mann-Whitney U -test, $W = 3$, $n = 6, 6$, $p < 0.05$; table 2) and did not change in body mass over the study period (Wilcoxon statistic = 6, $p > 0.05$). Residual DEE (from the least-squares regression on mass) was positively correlated with litter size, and negatively correlated with the number of helpers, when these variables were entered as covariates (ANCOVA, $F_{1,3} = 18.21$, $p < 0.05$ for litter size and $F_{1,3} = 22.63$, $p < 0.05$ for number of helpers). MEO and MEI values are shown in table 2. MEI was not significantly different from the maximal prediction for lactating mammals (Wilcoxon statistic = 4, $p > 0.05$) (Weiner 1992).

(c) Energy expenditure of helpers

The number of helpers over six months varied from 3 to 18 (mean = 10.2 ± 5.1). Although helpers were lighter than dominant females (table 2), there was no difference

Table 2. Mass, energy expenditure and energy intake.

(Mean $\pm$ 1 s.d. of body mass (g) and DEE (kJ d^{-1}) for dominant lactating females, subordinate non-breeding female helpers, allo-lactating subordinate females and offspring. Predicted DEE (kJ d^{-1} (Nagy *et al.* 1999)) is shown for dominant lactating females, subordinate non-breeding female helpers and allo-lactating subordinate females. MEO (kJ d^{-1}) and MEI, (kJ d^{-1}) are shown for dominant lactating females. Predicted MEI (kJ d^{-1} (Weiner 1992)) is shown for dominant lactating females and subordinate non-breeding female helpers. *n* denotes sample sizes.)

	dominant lactating females	subordinate female helpers	allo-lactating subordinate females	offspring
<i>n</i>	6	6	3	6
mass (g)	830 $\pm$ 75	697 $\pm$ 68	755 $\pm$ 70	108 $\pm$ 21
DEE	709 $\pm$ 301	468 $\pm$ 89	391 $\pm$ 93	69 $\pm$ 21
DEE (predicted)	543	485	510	—
MEO	976 $\pm$ 198	—	—	—
MEI	1685 $\pm$ 493	—	—	—
MEI (predicted)	1604	1320 ^a	—	—

^a Denotes a significant difference between the measured value and the predicted value.

in body mass between subordinate non-breeding females and allo-lactating subordinate females (Mann–Whitney, $W = 14$, $n = 6, 3$, $p > 0.05$). During the study period, lactating mothers and non-breeding female helpers did not change significantly in body mass. However, allo-lactating females decreased in mass by $8 \pm 2 \text{ g d}^{-1}$ (paired *t*-test, $t = 8.69$, $p < 0.05$). Mean DEEs of subordinate non-lactating and allo-lactating females were significantly lower than dominant females (table 2) (Mann–Whitney, $W = 3$, $n = 6, 4$, $p < 0.05$ and $W = 0$, $n = 4, 3$, $p < 0.05$ for non-lactating and allo-lactating subordinate females, respectively). However, these values were not significantly different from the allometric prediction for same-sized free-ranging eutherians (Speakman 2000, eqn 4; table 2), and differences were not significant when body mass was included as a covariate (ANCOVA $F_{1,9} = 0.08$, $p > 0.05$). In contrast to dominant lactating females, DEEs of subordinate females were significantly less than the prediction for maximal energy budgets for same-sized mammals (Wilcoxon statistic = 0.0, $p < 0.05$) (Weiner 1992).

(d) Effect of helpers on energy expenditure of dominant females

There was a negative relationship between the residual DEE of dominant lactating females (from the least-squares regressions on mass and litter size) and the number of helpers. The least-squares fitted regression

$$\text{residual DEE} = 235 - 26.6 \times \text{number of helpers}, \quad (3.2)$$

explained 90.3% of the residual variation ($F_{1,4} = 37.1$, $p < 0.01$; figure 1). By contrast, there was no relationship between the residual DEE of subordinate helpers (from the least-squares regression on mass) with number of helpers or litter size (ANCOVA, $F_{1,4} = 0.15$, $p > 0.05$ for number of helpers, $F_{1,4} = 0.06$, $p > 0.05$ for litter size).

4. DISCUSSION

Although DEEs of dominant lactating females were close to allometric predictions for same-sized free-ranging eutherians (Speakman 2000, eqn 4), calculated values of MEI were not significantly different from maximal suggested limits (Weiner 1992). Our findings thus support Creel & Creel's (1991) contention that obligate cooperat-

Figure 1. Mean plus residual DEE (from the least-squares regressions on mass and litter size, mean + residual DEE, kJ d^{-1}) of dominant lactating females against number of helpers.

ive breeding may be associated with females investing close to maximal amounts of energy in their offspring. Limitations are unlikely to act on the energy expenditure of lactating females, because the observed values of DEE were not high compared with other measurements of field metabolic rates of free-ranging mammals (Nagy *et al.* 1999; Speakman 2000). However, limits may act on the mothers' ability to assimilate sufficient energy, or to produce enough milk, as has been observed in laboratory mice *Mus musculus* (the 'central' and 'peripheral' limitations hypotheses (Weiner 1987, 1992; Hammond *et al.* 1996)).

Contrary to our expectations, DEEs of allo-lactating and non-lactating subordinate females were not significantly different, possibly because of the low sample sizes. As a result, we were unable to assess the effects of allo-lactation on DEE. However, during the study period, allo-lactating females decreased in mass, while the other categories of animals (dominant lactating females and non-lactating subordinate females) did not. This mass loss was equivalent to an energy deficit of 264 kJ d^{-1} , or 35 g milk d^{-1} (assuming an efficiency of milk production from body reserves of 0.85 (Commonwealth Agricultural Council 1980; English 1985)), which is potentially enough resources to support at least one extra pup per allo-lactator. Bearing in mind that babysitting and personal foraging are mutually exclusive activities (Clutton-Brock

Figure 2. Contribution to babysitting by subordinate allo-lactators and non-lactators (Wald statistic, $\chi^2 = 4.81$, d.f. = 1, $p < 0.05$). Contributions were calculated as the proportion of days in which individuals were observed to babysit pups aged between 20 and 25 days old out of the total number of potential days. Data were collected between 1997 and 2001 and included 496 contributions to babysitting, by 156 individuals across 88 litters and 10 groups. Data were analysed in GENSTAT (GENSTAT 1993) using a generalized linear mixed model, with binomial error structure and logit link function, controlling for individual age, group size and repeated measures within individuals, litters and groups.

et al. 1998), one possible explanation as to why allo-lactators lost weight during the week of peak lactation is that they may have babysat more than other categories of females did. Using a larger dataset collected from the same site and on the same groups but over a greater time period (1997–2001 inclusive), we found that allo-lactators did indeed babysit more frequently than non-lactators of a similar age during the period of peak lactation (figure 2). Allo-lactators had not given birth, but were more likely to have aborted litters, be in good condition and be closely related to the dominant pair (T. H. Clutton-Brock, A. F. Russell, L. L. Sharpe, unpublished data). If an allo-lactator can provide enough milk to feed an extra pup, allo-lactation may be one of the most significant ways in which helpers could assist dominants in reproduction. However, why allo-lactators should not only provide milk to offspring of the dominant female, but also guard their pups frequently, may be viewed as surprising. There are several potential explanations. For example, if helper contributions are dependent on their body condition, allo-lactators may babysit more because they are in good condition or in better condition than other group members. In addition, the most effective time for allo-lactators to provide milk to offspring may be during the day, as dominant females will provide milk during the night. Alternatively, it may be possible that intensive babysitters are simply suckled more frequently, and that the action of suckling itself stimulates lactation (Wilkinson 1992).

Moehlman & Hofer (1997) describe two extremes of helper effects in cooperative breeding systems. Given assistance from helpers: (i) the breeder's input remains unchanged and allo-parental care leads to an increased amount of food being received by offspring; and (ii) the breeder's input decreases and the total amount of food received by offspring remains largely unchanged. Hatchwell (1999) suggested that helpers should cause reductions in parental investment rather than increases in offspring food acquisition when fitness benefits from this strategy are greater. Comparative evidence from birds

Figure 3. Correlation between the number of females over one year old and the number of allo-lactators per litter (Wald statistic, $\chi^2 = 11.23$, d.f. = 1, $p = 0.001$). Analysis was conducted using a residual maximum-likelihood model on numbers collected across 88 litters in 10 groups between 1997 and 2001. Analysis controls for repeated measures within the 10 groups (GENSTAT 1993).

showed that helpers were associated with reductions in parental investment when offspring mortality was low and presumably little benefit would be derived by the provisioning of extra food to offspring (Hatchwell 1999). In our observations, although the DEE of helpers was unaffected by the number of helpers, the DEE of lactating mothers was lower in the presence of many helpers. Our results thus indicate, for an obligate cooperative breeder during lactation, that non-lactating helpers primarily serve to reduce the DEE of lactating mothers (see Creel & Creel 1991). This suggestion is supported by the fact that increased numbers of helpers do not significantly affect pup mass around the time of weaning, but do influence maternal mass around the time of the mothers' subsequent conception (Russell *et al.* 2002a).

In meerkats, helpers may provide energetic contributions to reproduction in several ways. For example, many helpers may simply reduce the thermoregulatory costs of the group overnight (Russell *et al.* 2002b), or they may increase the feeding rate of the dominant female by their contributions to sentinel duty whilst she is foraging (Clutton-Brock *et al.* 1999). In larger groups, there are also a greater number of older, more experienced babysitters that may allow dominant females to become emancipated from babysitting duties (Clutton-Brock *et al.* 1998). However, perhaps the most dramatic way in which helpers assist dominant females is allo-lactation. Using the larger dataset outlined above (see also figure 2), we found that the number of subordinate lactators that provide milk to the pups of the dominant female increased with the number of females in the group over the age of one year (figure 3). Future studies should investigate the relative importance of these alternatives for subordinates' contributions to breeding.

The predicted DEE of lactating dominant females without helpers was 235 kJ d^{-1} above the measured average (equation (3.2)). This compares with a cost per individual pup of 202 kJ d^{-1} calculated for a lactating mother. Therefore, an average of 10 helpers would potentially enable a typical breeding female to reduce her own DEE, whilst maintaining maximal MEI, and thereby support one extra pup (assuming a milk production efficiency of 85% (Commonwealth Agricultural Council 1980; English 1985)), resulting in a litter size of four; a 25% fitness bene-

fit to the breeding female. It is important to bear in mind that these values were recorded for groups in the absence of allo-lactators. If the effects of allo-lactators are included, the energetic importance of helpers to dominants is likely to be even more significant. These calculations assumed that mothers were in energy balance, and that the water turnover and hence milk consumption estimates in offspring were accurate. We are confident that these conditions applied, as we observed no significant change in maternal body mass over the study period, and measured no isotope recycling in offspring.

In conclusion, Creel & Creel (1991) suggested that some mammals might be obligate cooperative breeders because of energetic constraints. This hypothesis predicts that: the daily energy intake of breeding females is high; helpers invest substantial amounts of energy in raising young; and the presence of helpers reduces the energy costs to breeding females. Although it is difficult to test these predictions definitively without experimental manipulation, our results are consistent with these predictions. First, we found that MEI values of dominant mothers during peak lactation were close to allometric predictions for maximal energy intake (Weiner 1992). This indicates that mothers were energetically stressed in an attempt to produce enough milk for their growing offspring. Second, although the energy throughput of helpers was significantly less than that of dominants, allo-lactating helpers lost weight and were likely to have provided significant quantities of milk. Third, non-lactating helpers allowed dominant females to reduce their DEE during peak lactation, and hence channel more of their resources into milk. Helpers may have achieved this by relieving dominant lactators from babysitting (Clutton-Brock *et al.* 1998), by improving their foraging efficiency (Clutton-Brock *et al.* 1999) and by reducing their (or their pups') thermoregulatory costs at night (Russell *et al.* 2002b). Hence, the contributions of helpers are significant as they may not only allow mothers to breed more frequently (Russell *et al.* 2002a), but may also allow them to raise more offspring (this study). The challenge for the future will be to determine the effects of helpers on the nature of the fitness functions of breeding females as well as for their offspring.

We are grateful for the kind help and support of all those who worked on this project. In particular, we thank Mr and Mrs H. Kotze and the Northern Cape Conservation Authority for permission to work on their land at Van Zyl's Rus. The following people provided valuable assistance in the field: D. Allsop, L. Cattini, G. Telford, F. Dalerum, D. Wright, Z. Hodgeson, J. White, W. Linklater, E. Cameron and L. Sharpe. We thank P. Thomson for the mass spectrometry, J. McLaren for isotope analysis and U. Shanas for the bomb calorimetry. I. Cuthill and S. Ward made valuable contributions to earlier versions of the manuscript. We also thank three anonymous referees for their valuable and constructive comments. This research was funded by a British Ecological Society Small Ecological Project research grant (no. 1643) and a Carnegie Trust grant to M.S. and by grants from Natural Environment Research Council and Biotechnology and Biological Sciences Research Council to T.H.C.-B. and J.R.S.

REFERENCES

- Clutton-Brock, T. H., Gaynor, D., Kansky, R., MacColl, A. D. C., McIlrath, G., Chadwick, P., Brotherton, P. N. M., O'Riain, J. M., Manser, M. & Skinner, J. D. 1998 Costs of cooperative breeding in suricates (*Suricata suricatta*). *Proc. R. Soc. Lond. B* **265**, 185–190. (DOI 10.1098/rspb.1998.0281.)
- Clutton-Brock, T. H., O'Riain, M. J., Brotherton, P. N. M., Gaynor, D., Kansky, R., Griffin, A. S. & Manser, M. 1999 Selfish sentinels in cooperative mammals. *Science* **284**, 1640–1644.
- Commonwealth Agricultural Council 1980 Requirements for energy. In *The nutrient requirements of ruminant livestock* pp. 73–119. Slough, UK: Commonwealth Agricultural Bureaux.
- Creel, S. R. & Creel, N. M. 1991 Energetics, reproductive suppression and obligate communal breeding in carnivores. *Behav. Ecol. Sociobiol.* **28**, 263–270.
- English, R. M. 1985 Breast milk production and energy exchange in human lactation. *Br. J. Nutr.* **53**, 459–466.
- GENSTAT 1993 *GENSTAT 5 release 3 reference manual*. Oxford, UK: Clarendon.
- Gittleman, J. L. & Oftedal, O. T. 1987 Comparative growth and lactation energetics in carnivores. *Symp. Zool. Soc. Lond.* **57**, 41–77.
- Gittleman, J. L. & Thompson, S. D. 1988 Energy allocation in mammalian reproduction. *Am. Zool.* **28**, 863–875.
- Hammond, K. A., Lloyd, K. C. & Diamond, J. 1996 Is mammary output capacity limiting to lactation performance in mice? *J. Exp. Biol.* **199**, 337–349.
- Hatchwell, B. J. 1999 Investment strategies of breeders in avian cooperative breeding systems. *Am. Nat.* **154**, 205–219.
- Lemen, C. & Speakman, J. R. 1997 DLW program and DLW userguide. See <http://www.abdn.ac.uk/zoology/speakman.html>.
- Lifson, N. & McClintock, R. 1966 Theory of the turnover rates of body water for measuring energy and material balance. *J. Theor. Biol.* **12**, 46–74.
- McKenzie, J. & Goldman, R. N. 1998 *MINITAB handbook for Windows 95 and Windows NT*. Boston, MA: Addison-Wesley.
- Moehlman, P. D. & Hofer, H. 1997 Cooperative breeding, reproductive suppression, and body mass in canids. In *Cooperative breeding in mammals* (ed. N. G. Solomon & J. A. French), pp. 91–95. New York: Cambridge University Press.
- Nagy, K. A. 1980 CO₂ production in animals: an analysis of potential errors in the doubly labelled water technique. *Am. J. Physiol.* **238**, R466–R473.
- Nagy, K. A. 1983 *The doubly labeled water (³HH¹⁸O) method: a guide to its use*. UCLA publication no. 12-1417. Los Angeles, CA: University of California.
- Nagy, K. A. & Costa, D. P. 1980 Water flux in animals: an analysis of potential errors in the tritiated water method. *Am. J. Physiol.* **238**, 454–465.
- Nagy, K. A., Girard, I. A. & Brown, T. K. 1999 Energetics of free-ranging mammals, reptiles and birds. *A. Rev. Nutr.* **19**, 247–277.
- Oftedal, O. T. 1984 Milk composition, milk yield and energy output at peak lactation: a comparative review. *Symp. Zool. Soc. Lond.* **51**, 33–85.
- Oftedal, O. T. & Iverson, S. J. 1987 Hydrogen isotope methodology for the measurement of milk intake and the energetics of growth in suckling young. In *Marine mammal energetics*, special publication 1 (ed. A. C. Huntly), pp. 67–96. Lawrence, KS: Society for Marine Mammalogy.
- Russell, A. F., Brotherton, P. N. M., McIlrath, G. M., Sharpe, L. M. & Clutton-Brock, T. H. 2002a Breeding success in cooperative meerkats: effects of helper number and maternal state. *Behav. Ecol.* (In the press.)
- Russell, A. F., Clutton-Brock, T. H., Brotherton, P. N. M., Sharpe, L. L., McIlrath, G. M., Dalerum, F. D., Cameron, E. Z. & Barnard, J. A. 2002b Factors affecting pup growth and survival in cooperatively breeding meerkats *Suricata suricatta*. *J. Anim. Ecol.* **71**, 700–709.

- Sawicka-Kapusta, K. 1974 Changes in the gross body composition and energy value of bank voles during their postnatal development. *Acta Theriol.* **19**, 27–54.
- Scantlebury, M., Hynds, W., Booles, D. & Speakman, J. R. 2000a Isotope recycling in lactating dogs (*Canis familiaris*). *Am. J. Physiol.* **278**, R669–R676.
- Scantlebury, M., Butterwick, R. & Speakman, J. R. 2000b Energetics of lactation in domestic dog (*Canis familiaris*) breeds of two sizes. *Comp. Biochem. Physiol. A* **125**, 197–210.
- Solomon, N. G. & French, J. F. 1997 *Cooperative breeding in mammals*. Cambridge University Press.
- Speakman, J. R. 1997 *Doubly labelled water, theory and practice*. London: Chapman & Hall.
- Speakman, J. R. 2000 The cost of living: field metabolic rates of small mammals. *Adv. Ecol. Res.* **30**, 177–297.
- Speakman, J. R. & Racey, P. A. 1988 Consequences of steady state for accuracy of the doubly labelled water technique: the importance of the recapture interval. *Comp. Biochem. Physiol. A* **90**, 337–340.
- Stacey, P. B. & Koenig, W. D. 1990 *Cooperative breeding in birds*. Cambridge University Press.
- Ward, S., Scantlebury, M., Krol, E., Thomson, P. J., Sparling, C. & Speakman, J. R. 2000 Preparation of hydrogen from water by reduction with lithium aluminium hydride for the analysis of delta H-2 by isotope ratio mass spectrometry. *Rapid Commun. Mass Spectrom.* **14**, 450–453.
- Weiner, J. 1987 Maximum energy assimilation rates in the Djungarian hamster (*Phodopus sungorus*). *Oecologia* **72**, 297–302.
- Weiner, J. 1992 Physiological limits to sustainable energy budgets in birds and mammals: ecological implications. *Trends Ecol. Evol.* **7**, 384–388.
- Wilkinson, G. S. 1992 Communal nursing in the evening bat *Nycticeius humeralis*. *Behav. Ecol. Sociobiol.* **31**, 225–235.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.