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LIMITS TO SUSTAINED ENERGY INTAKE

II. INTER-RELATIONSHIPS BETWEEN RESTING METABOLIC RATE, LIFE-HISTORY TRAITS AND MORPHOLOGY IN *MUS MUSCULUS*

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Summary

Links between resting metabolic rate (RMR) and reproductive output have been previously sought at both inter- and intraspecific levels, but have only been found in some interspecific studies. We aimed to examine correlations between RMR measured both prior to breeding and at peak lactation with litter size and litter mass in *Mus musculus*. By manipulating the litter size of some females at birth, we aimed to establish the direction of causality in any correlation between litter size and RMR. Correlations between maternal morphology and RMR, litter size and litter mass were also examined. Neither pre-breeding RMR nor mass-independent pre-breeding RMR was correlated with litter size or litter mass. RMR at peak lactation, however, was positively correlated with litter size and negatively correlated with mean pup mass. After correcting for the effects of body mass, residual peak lactation RMR was not correlated with litter size or litter mass. Body size was the major morphological variable influencing litter mass, offspring mass and asymptotic food intake. Mammary tissue mass was correlated with litter size when only the data for mice raising unmanipulated litters were used. RMR at peak lactation was significantly related to the principal component of morphology dominated by carcass mass. This study confirms the findings of previous intraspecific and some interspecific studies that found no correlation between RMR and reproductive output after the effects of body mass had been removed.

Key words: energetics, maximal metabolic rate, sustained metabolic rate, pregnancy, lactation, reproduction, mouse.

Introduction

Variations in resting metabolic rate (RMR) have frequently been advocated as a potentially important factor influencing the reproductive output of female mammals (McNab, 1980; Henneman, 1983; Thompson and Nicoll, 1987; Thompson, 1992). This is because animals have a finite amount of available energy and, hence, those animals with a high RMR may have less energy remaining for allocation to reproduction (Gadgil and Bossert, 1970). Alternatively, animals with a high RMR may have a greater capacity for absorbing energy and, therefore, be able to devote more energy to reproduction (Thompson, 1992). This greater capacity to absorb energy in animals with a high RMR may be a consequence of the disproportionate effect that the masses of the intestines and associated organs (heart, liver and kidneys) have both on uptake capacity and on RMR (Daan et al., 1990; Konarzewski and Diamond, 1995; Speakman and McQueenie, 1996; but see Burness et al., 1998).

There is conflicting evidence, however, from interspecific studies over the correlation between RMR and reproductive output. McNab (McNab, 1980) found that basal metabolic rate (BMR) was positively correlated with the intrinsic rate of population increase across different species of mammals. However, other interspecific studies (Read and Harvey, 1989; Trevelyan et al., 1990; Harvey et al., 1991) failed to find significant effects of RMR on life-history traits when the lack of phylogenetic independence in the data was removed. Within closely related species, the evidence is also unclear. In *Peromyscus* spp. (Glazier, 1985a) and the Sorcidae (Genoud, 1988; Stephenson and Racey, 1995), RMR was highly correlated with the rate of energy use during lactation and with litter size. However, none of eight reproductive variables (including litter size and mass) was correlated with RMR in the Tenrecidae (Stephenson and Racey, 1995).

Although interspecific studies have the benefit of large ranges of RMR, body mass and life-history variables such as litter size and mass, results from these studies can be complicated by the lack of independence from phylogeny and ecology of the different species (Stearns, 1983; Felsenstein, 1985). Although this is largely reduced in studies of closely related species (Glazier, 1985b; Genoud, 1988; Stephenson

and Racey, 1995), it can be completely avoided only at the intraspecific level. Contrary to the conflicting results from interspecific studies, intraspecific studies have consistently failed to show any significant correlation between RMR and life-history traits in small mammals: laboratory mice *Mus musculus* (Hayes et al., 1992a), deer mice *Peromyscus maniculatus* (Earle and Lavigne, 1990), cotton rats *Sigmodon hispidus* (Derting and McClure, 1989), large-eared tenrecs *Geogale aurita* (Stephenson and Racey, 1993a) and shrew tenrecs *Microgale dobsoni* (Stephenson and Racey, 1993b).

The majority of both inter- and intraspecific studies have measured the RMR of pre-breeding females (Glazier, 1985a; Genoud, 1988; Derting and McClure, 1989; Earle and Lavigne, 1990; Hayes et al., 1992a; Stephenson and Racey, 1993a; Stephenson and Racey, 1993b) and examined correlations with subsequent reproductive output. However, RMR generally increases during pregnancy (Thompson and Nicoll, 1986; Speakman and Racey, 1987; Garton et al., 1994; Piers et al., 1995; Speakman and McQueenie, 1996) and increases even further during late lactation (Pennycuick, 1967; Thompson and Nicoll, 1986; Kenagy, 1987; Speakman and McQueenie, 1996; Kunkele and Kenagy, 1997). Speakman and McQueenie (Speakman and McQueenie, 1996) suggested that, although no relationships had been found between life-history traits and pre-breeding RMR (Derting and McClure, 1989; Hayes et al., 1992a), this might have been because of the flexibility in RMR, and relationships between RMR at peak lactation and life-history traits might be more likely (but see Stephenson and Racey, 1993a; Stephenson and Racey, 1993b).

The aims of the present study were to determine whether pre-breeding RMR (RMR_{PB}) and RMR during late lactation (RMR_L) were correlated with litter size or litter mass in laboratory mice *Mus musculus*. As both RMR (Brody, 1945; Kleiber, 1961; Haysson and Lacy, 1985; Daan et al., 1990; Weiner, 1989) and reproductive output (Blueweiss et al., 1978; Western, 1979; Western and Ssemakula, 1982; Clutton-Brock and Harvey, 1983; Harvey et al., 1989; Harvey, 1990) are correlated with body mass, we calculated residuals to fitted regression equations to obtain mass-independent data. This enabled correlations between mass-independent pre-breeding and peak lactation RMR and the parameters of reproductive output to be examined. If RMR_L is correlated with reproductive output, it is important to establish the direction of causality in the relationship. By manipulating the litter size at birth, the potential effects of experimental variation in litter size on RMR were also determined.

Materials and methods

Animals and housing

Virgin female laboratory mice *Mus musculus* L. (outbred MF1), 8–9 weeks old, were housed individually with sawdust and paper bedding for nest-building. Food [CRM(P), Special Diet Services, BP Nutrition, UK] and water were available *ad libitum*. The environment was regulated at 21±1 °C on a 12 h:12 h L:D photoperiod. Females were paired with males for 6 days. Pregnancy was detected by an increase in body mass over the following 7 days. After parturition (day 0 of lactation), a group of 71 mice was allowed to raise natural litters to peak lactation (day 18). The litters of a further 37 experimental mice were manipulated on day 0, by cross-fostering, so that they raised more or fewer pups than they gave birth to. Litter size manipulations ranged from −7 to +5 offspring of the birth litters. Data from these two groups of mice are presented in a companion paper (Johnson et al., 2001).

Body mass and food intake

Maternal body mass was measured (Sartorius top-pan balance 0.01 g) prior to breeding and daily throughout lactation (between 09:00 h and 11:00 h). Following parturition (=day 0), the number of pups and the total mass of the litter were also recorded. Food intake was measured daily throughout lactation and was calculated as the weight of food missing from the hopper each day. Sorting through the bedding revealed that spillage of food was negligible (less than 2 %; Johnson et al., 2001). The asymptotic food intake was calculated as the mean daily food intake between days 13 and 16 of lactation (Johnson et al., 2001).

Resting metabolic rate (RMR)

Resting metabolic rate (RMR) was quantified as the rate of oxygen consumption, using an open-flow respirometry system (as described previously; Hayes et al., 1992b; Speakman and McQueenie, 1996). Air was pumped (Charles Austin Pumps Ltd) through a sealed Perspex chamber within a constant-temperature incubator (INL-401N-010, Gallenkamp) set at 30 °C (within the thermoneutral zone; Speakman and Rossi, 1999). A flow rate of 500–700 ml min^{-1} was metered using an Alexander Wright flowmeter (DM3A) upstream of the chamber. A sample of air (approximately 150 ml) in the excurrent stream was dried (silica gel) and directed through a paramagnetic oxygen analyser (Servomex 1100A) (as described previously; Johnson et al., 2001). Previous measurements of repeatability of oxygen consumption in MF1 mice using this system indicate that the measurement-to-measurement repeatability (coefficient of variation) was 8 % for mice measured on consecutive days (E. Krol and J. R. Speakman, unpublished data). We did not absorb CO_2 in the outflow stream prior to gas analysis as this minimised error in the conversion of oxygen consumption to energy expenditure when respiratory quotient is unknown (Koteja, 1996; Speakman, 2000).

The conditions under which these measurements were made were the same as for measuring basal metabolic rate (Kleiber, 1961), with the exception that the mice were not necessarily post-absorptive because they were not deprived of food prior to the measurements. All the female mice were measured prior to breeding (RMR_{PB}) and at peak lactation (RMR_L) (day 18).

Morphology

Thirty-five of the control mice and 10 of the experimental mice were killed at peak lactation (day 18) and dissected; all

their organs were dried (Gallenkamp oven at 60 °C) for 14 days before being weighed. The dry masses of tissues were accurate to 0.0001 g (Ohaus Analytical plus balance) except for the carcass, which was accurate to 0.01 g (Sartorius top-pan balance). The stomach and intestines were rinsed with Ringer's solution to eliminate all the gut contents, before being dried and weighed. The small and large intestines were also straightened, but not stretched, and measured to the nearest 5 mm (method after Koteja, 1996).

Statistical analyses

Least-squares regression analysis was used to determine relationships between RMR and female body mass, litter size and litter mass. Reduced-major-axis regression analysis was used to determine the relationship between RMR pre-breeding and at peak lactation. Mass-independent data were calculated as the residual values from least-squares regression analysis of each trait on body mass. Variables that were not normally distributed were log-transformed to normalise them. Morphological data from both groups of mice were combined and, because of the inter-correlations of organ masses, a principal components analysis (PCA) was performed to redefine the morphological variability as a series of orthogonal axes. The scores along each principal component (PC) were used as independent predictors in multiple regression analyses (Joliffe, 1986) with stepwise backward deletion, using litter size, litter mass, mean pup mass, food intake and maternal mass as dependent variables. All statistical analyses were performed using commercially available software (Minitab; Ryan et al., 1985).

Results

Females with unmanipulated litters

Prior to breeding, the RMR of the females averaged 21.51±0.72 kJ day^{-1} (mean ± S.E.M., N=71). This increased significantly (paired t=16.43, P<0.0001) by, on average, 2.2±0.11 kJ day^{-1} (N=71) to a mean of 47.05±1.63 kJ day^{-1} (N=71) at peak lactation (Fig. 1). Pre-breeding RMR (RMR_{PB}) was significantly positively related to pre-breeding body mass, but only weakly (r^2=0.073, $F_{1,69}$=5.4, P=0.023) (Table 1; Fig. 2A). Females with the highest RMR_{PB} also had the highest RMR_L: there was a positive relationship between RMR_{PB} and the RMR at peak lactation (RMR_L) (r^2=0.107, $F_{1,69}$=8.29, P=0.005) (Fig. 3). RMR_L was also significantly related to body mass (r^2=0.206, $F_{1,69}$=17.5, P<0.001) (Fig. 2A). We combined the pre-breeding and lactation data, avoiding pseudoreplication by randomly assigning the mice to one or other of the groups. In this pooled data set, RMR was positively related to body mass (r^2=0.682, $F_{1,69}$=148.09, P<0.001). The difference in RMR between the pre-breeding females and the lactating females was only a function of the difference in body mass between these two groups (analysis of covariance, ANCOVA; mass effect, $F_{1,68}$=10.2, P=0.002; reproductive-state effect, P=0.845).

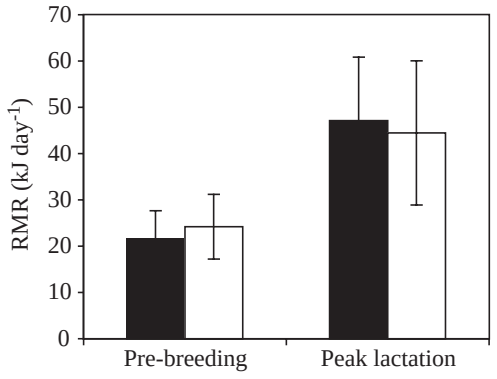

Fig. 1. Resting metabolic rate (RMR) prior to breeding and at peak lactation for females raising natural (filled columns, N=71) and manipulated (open columns, N=37) litters. Values are means ±1 S.E.M.

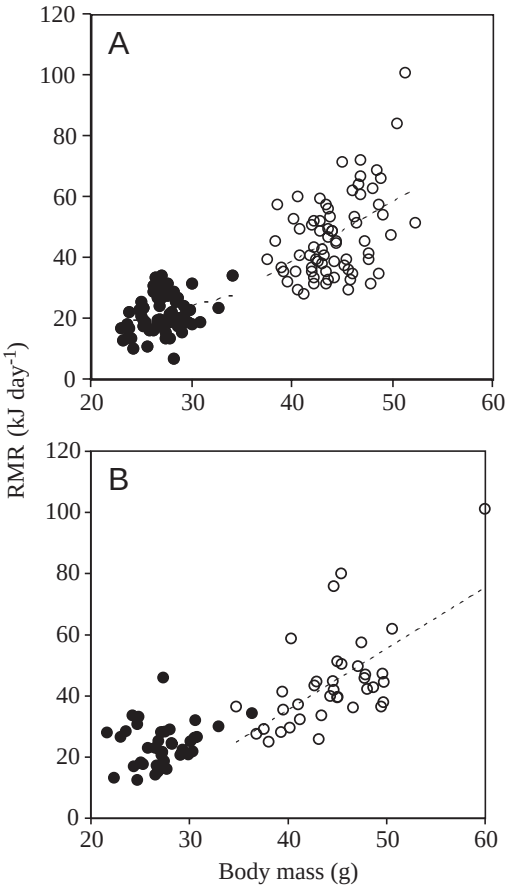

Fig. 2. Relationship between resting metabolic rate (RMR) and body mass prior to breeding (filled circles) and at peak lactation (open circles) of females with unmanipulated (A) and manipulated (B) litters. Least-squares regression lines fitted to the two sets of data are shown and are described by (A) y=0.79x+0.1 ($F_{1,69}$=5.4, P=0.023) prior to breeding and y=1.92x–38.0 ($F_{1,69}$=17.5, P=0.001) at peak lactation, and (B) relationship prior to breeding not significant (P=0.156) and y=2.00 x–44.7 ($F_{1,35}$=22.16, P=0.001) at peak lactation.

Table 1. *Results of regression analyses on the relationships between resting metabolic rate (RMR) or residual RMR and body mass, litter size, litter mass, mean pup mass and maternal food intake of the females with unmanipulated litters (*N*=71)*

	RMR		Residual RMR	
	Pre-breeding	Peak lactation	Pre-breeding	Peak lactation
Pre-breeding RMR	–	P=0.005	–	–
Pre-breeding residual RMR	–	–	–	P=0.005
Body mass	P=0.023	P<0.001	–	–
Litter size at birth	NS	P=0.019	NS	NS
Residual litter size at birth	NS	NS	NS	NS
Litter size at peak lactation	NS	P=0.004	NS	NS
Residual litter size at peak lactation	NS	NS	NS	NS
Litter mass at birth	NS	P=0.012	NS	NS
Residual litter mass at birth	NS	NS	NS	NS
Litter mass at peak lactation	NS	NS	NS	NS
Residual litter mass at peak lactation	NS	NS	NS	NS
Mean pup mass at peak lactation	NS	P=0.041	NS	NS
Residual mean pup mass at peak lactation	NS	NS	NS	NS
Asymptotic food intake	NS	P=0.021	NS	NS
Residual asymptotic food intake	NS	NS	NS	NS

NS, P>0.05.

There were no significant relationships between RMR_{PB} and any of the life-history traits measured (Table 1). When the effect of body mass on RMR_{PB} was removed, residual RMR_{PB} was still not significantly related to any of the life-history traits or to the mass-independent life-history traits (Table 1). Five life-history traits were significantly related to RMR_L: litter size at birth (r^2=0.078, $F_{1,69}$=5.80, P=0.019) (Fig. 4A), litter size at peak lactation (r^2=0.112, $F_{1,69}$=8.72, P=0.004) (Fig. 4B), the mass of the litter at birth (r^2=0.087, $F_{1,69}$=6.61, P=0.012) (Fig. 5A), the asymptotic daily food intake at peak lactation (r^2=0.075, $F_{1,69}$=5.60, P=0.021) (Fig. 5B) and the mean mass of the pups at peak lactation (r^2=0.059, $F_{1,69}$=4.33, P=0.041) (Fig. 5C). Even after the data for low litter sizes of five and six pups, which may have had undue leverage on the regressions, had been removed, all the above relationships remained significant. High RMR_L was therefore associated with more, lighter pups and with greater maternal food intake. Litter size, offspring mass and maternal food intake are all inter-correlated. When all the above variables were included in a multiple regression, RMR_L was significantly related only to litter size at peak lactation.

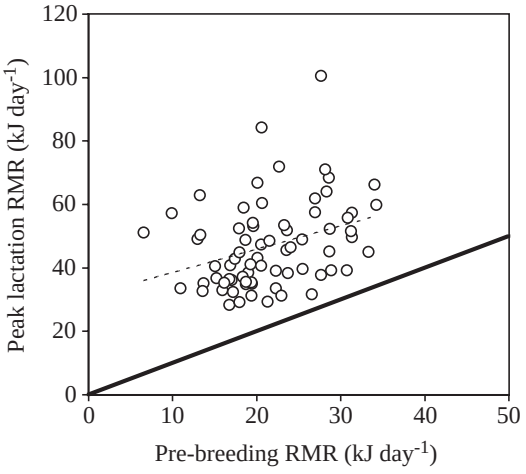

Fig. 3. Relationship between pre-breeding resting metabolic rate (RMR) and RMR at peak lactation in females with unmanipulated litters. The dashed line shows the fitted regression, described by the equation y=0.74x+31.2, and the solid line is the line of equality.

Body mass at peak lactation was significantly related to four life-history traits: litter size at birth (r^2=0.087, $F_{1,69}$=6.54, P=0.013), litter mass at birth (r^2=0.135, $F_{1,69}$=10.74, P=0.002), litter size at peak lactation (r^2=0.111, $F_{1,69}$=8.57, P=0.005) and the asymptotic daily food intake (r^2=0.097, $F_{1,69}$=7.44, P=0.008). Consequently, the positive relationships between RMR_L and the same life-history traits might reflect covariation of the traits and RMR_L with body mass. Once the effect of mass on RMR_L had been removed, residual RMR_L was not related to any of the life-history traits measured or to mass-independent life-history traits (Table 1).

Females with manipulated litters

Females with manipulated litters significantly increased their mean RMR (paired t=8.43, P<0.0001) by 1.8±0.12-fold (N=37) from 24.19±1.15 kJ day^{-1} (N=37) prior to breeding to 44.4±2.56 kJ day^{-1} (N=37; means ± S.E.M.) at peak lactation (Fig. 1). In this manipulated group, there was no significant

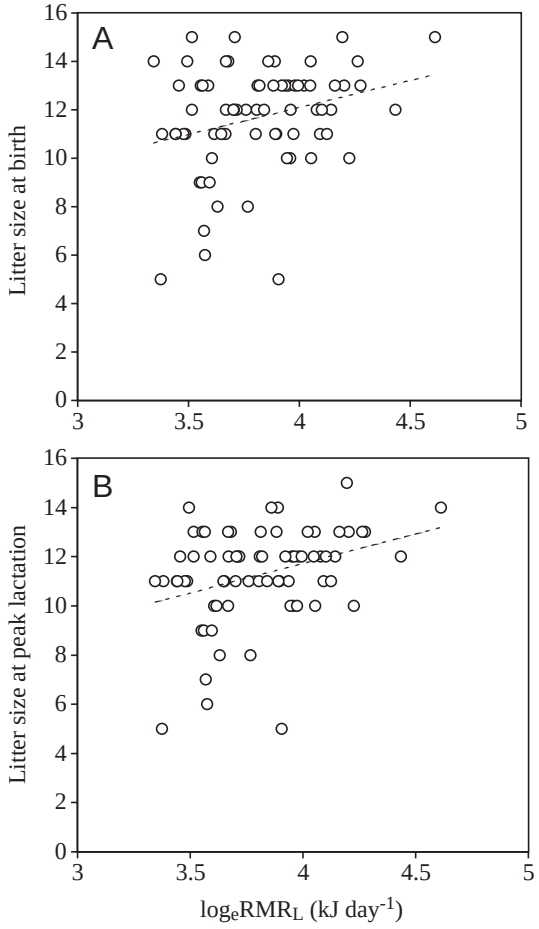

Fig. 4. Relationship between resting metabolic rate at peak lactation (RMR_L) and (A) litter size at birth (y=2.23x+3.2) and (B) litter size at peak lactation in females with unmanipulated litters (y=2.41x+2.1).

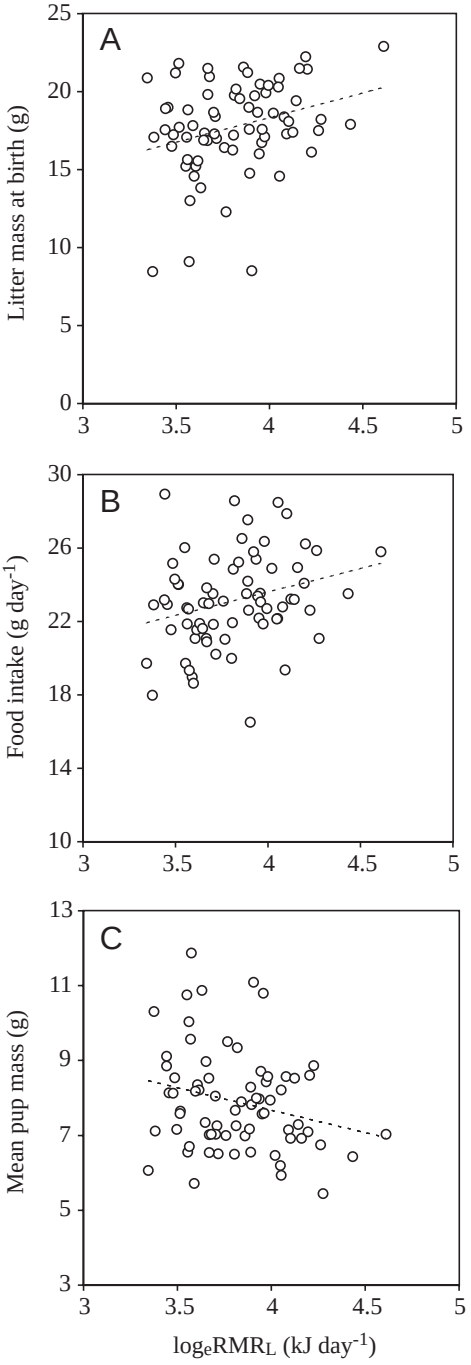

Fig. 5. Relationship between (A) the mass of the litter at birth and the maternal resting metabolic rate at peak lactation (RMR_L) (y=3.16x+5.7), (B) the asymptotic food intake and maternal RMR_L (y=2.55x+13.4) and (C) the mean pup mass and maternal RMR_L (y=1.2x+12.5) for females raising unmanipulated litters.

relationship between RMR_{PB} and body mass, but there was a significant positive relationship between RMR_L and body mass (r^2=0.388, $F_{1,35}$=22.16, P<0.001) (Fig. 2B). One mouse with a high RMR and body mass (Fig. 2B) had a strong influence on this relationship. Excluding this datum, the relationship was weakened but remained significant (r^2=0.192, $F_{1,34}$=8.06, P=0.008). We combined the data for pre-breeding and lactating mice avoiding pseudoreplication again by randomly assigning each mouse either to the pre-breeding or lactating groups. In this pooled data set, the relationship to body mass was strengthened (r^2=0.644, $F_{1,35}$=63.4, P<0.001), mirroring the effect in the unmanipulated group.

RMR_L was not related to any of the life-history traits measured in the manipulated group (Table 2). Body mass at peak lactation in mice with manipulated litters was significantly related to six life-history traits: litter size at birth (r^2=0.118, $F_{1,35}$=4.70, P=0.037), manipulated litter size at birth (r^2=0.371, $F_{1,35}$=20.63, P<0.001), litter size at peak lactation (r^2=0.310, $F_{1,35}$=15.74, P<0.001), the mean mass of pups at peak lactation (r^2=0.319, $F_{1,35}$=16.42, P<0.001), litter mass at peak lactation (r^2=0.154, $F_{1,35}$=6.38, P=0.016) and the asymptotic daily food intake (r^2=0.205, $F_{1,35}$=9.0, P=0.005). As with the females raising unmanipulated litters, mass-independent RMR_L was also not significantly related to any of the life-history traits or to the same traits with the effects of body mass removed (Table 2).

There was a positive relationship between the extent of

Table 2. *Results of regression analyses of resting metabolic rate at peak lactation (RMR_L) or residual RMR_L and body mass, maternal food intake, litter size, litter mass and mean pup mass of the manipulated females (*N=*37)*

	RMR_L	Residual RMR_L
Body mass	$P<0.001$	–
Litter size at birth	NS	NS
Residual litter size at birth	NS	NS
Premanipulated litter size	NS	NS
Litter size at peak lactation	NS	NS
Residual litter size at peak lactation	NS	NS
Litter mass at birth	NS	NS
Residual litter mass at birth	NS	NS
Litter mass at peak lactation	NS	NS
Residual litter mass at peak lactation	NS	NS
Mean pup mass at peak lactation	NS	NS
Residual mean pup mass at peak lactation	NS	NS
Asymptotic food intake	NS	NS
Residual asymptotic food intake	NS	NS

NS, $P>0.05$.

manipulation of litter size and the increase in body mass between pre-breeding and peak lactation (r^2=0.217, $F_{1,35}$=9.69, P=0.004) (Fig. 6). Adding or removing a pup changed maternal mass by 0.5 g on average. There was, however, no significant relationship between the increase in RMR and the extent of manipulation (P=0.147). When the analysis was repeated using only the litters in which there was no pup mortality, there was still a significant effect of the extent of manipulation on the increase in maternal body mass

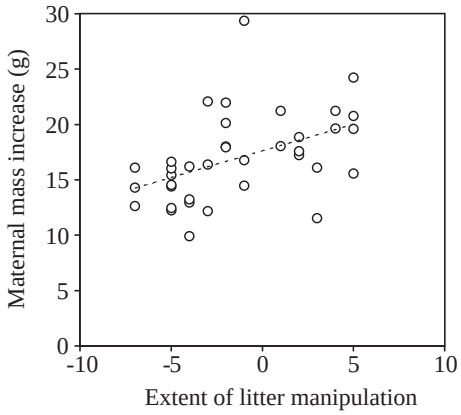

Fig. 6. Relationship between the extent of litter manipulation (number of pups added or removed) and the increase in maternal mass from pre-breeding to peak lactation. The relationship is described by y=0.48x+17.6.

(r^2=0.262, $F_{1,25}$=8.88, P=0.006) but not on the increase in RMR (P=0.217). Females given more pups to raise gained more mass than females that had pups removed.

Morphology

There were many intercorrelations between the dry organ masses across individuals (Table 3). These intercorrelations compromised any attempt to relate RMR directly to morphological variation at the level of each organ. We therefore refined the morphology using a principal components analysis (see also Speakman and McQueenie, 1996). The results of the principal components analysis are shown in Table 4. The first principal component (PC1) explained 54 % of the variation in the morphological data and appeared to be a general size component. Both PC2 and PC3 were dominated by the lungs and organs of the alimentary tract, PC2 being

Table 3. *Pearson product-moment correlation coefficients relating the dry mass of different organs*

	Carcass	Skin	Heart	Lungs	Liver	Spleen	Uterus	Pancreas	Tail	Stomach	SI	LI	Ms.fat	Abd.fat	Mammary
Skin	0.841**														
Heart	0.617**	0.519**													
Lungs	0.106	0.315*													
Liver	0.777**	0.637**	0.526**												
Spleen	0.665**	0.585**	0.454**	0.349*	0.507**										
Uterus	0.669**	0.668**		0.349*	0.556**	0.487**									
Pancreas	0.588**	0.493**			0.665**	0.339*	0.415**								
Tail	0.852**	0.664**	0.552**		0.725**	0.672**	0.473**	0.541**							
Stomach	0.445**	0.376**	0.332*		0.477**	0.462**	0.425**	0.393**	0.441**						
SI															
LI	0.460**	0.533**	0.439**	0.413**	0.505**	0.499**	0.468**	0.346*	0.462**	0.483**	0.342*				
Ms.fat	0.791**	0.694**	0.515**		0.620**	0.697**	0.499**	0.307*	0.747**	0.331*		0.573**			
Abd.fat	0.847**	0.834**	0.501**		0.665**	0.594**	0.695**	0.513**	0.709**	0.361*		0.507**	0.805**		
Mammary	0.671**	0.475**	0.353*		0.727**	0.543**	0.358*	0.614**	0.681**	0.472**		0.445**	0.650**	0.592**	
BAT	0.817**	0.769**	0.429**	0.322*	0.628**	0.620**	0.653**	0.422**	0.717**	0.517**		0.503**	0.788**	0.839**	0.679**

SI, small intestine; LI, large intestine; Ms.fat, mesenteric fat; Abd.fat, abdominal fat; BAT, brown adipose tissue.
*$P<0.05$ ($r>0.288$); **$P<0.01$ ($r>0.372$).

Table 4. *Principal components analysis of morphological variation in dry masses of 16 organs measured across 45 mice (both unmanipulated and manipulated)*

	PC1	PC2	PC3	PC4	PC5
Eigenvalue	8.571	1.660	1.303	0.938	0.802
Proportion	0.536	0.104	0.081	0.059	0.050
Eigenvectors					
Carcass	**–0.319**	0.137	–0.057	0.101	0.160
Pelage	0.291	–0.069	–0.139	0.020	**0.349**
Heart	–0.206	0.007	0.176	**0.694**	0.073
Lungs	–0.123	**–0.502**	**–0.436**	–0.251	–0.181
Liver	–0.278	0.202	0.259	–0.020	0.077
Spleen	–0.258	–0.138	–0.084	0.097	**–0.337**
Uterus	–0.245	–0.178	–0.049	–0.281	**0.484**
Pancreas	–0.206	0.292	**0.327**	**–0.425**	0.153
Tail	–0.291	0.137	0.035	0.156	–0.130
Stomach	–0.199	–0.135	**0.403**	–0.223	–0.294
SI	0.029	**–0.564**	**0.488**	0.071	0.151
LI	–0.225	**–0.361**	0.235	0.008	–0.115
Ms.fat	–0.289	–0.067	–0.195	0.218	–0.163
Abd.fat	**–0.305**	0.041	–0.190	0.007	0.241
Mammary	–0.258	0.236	0.054	–0.198	**–0.460**
BAT	**–0.301**	–0.195	–0.195	–0.093	–0.036

SI, small intestine; LI, large intestine; Ms.fat, mesenteric fat; Abd.fat, abdominal fat; BAT, brown adipose tissue; PC, principal component.

Dominant variables influencing the principal components as revealed by eigenvectors (>0.3 or <–0.3) are in bold type.

influenced by the small and large intestines, while PC3 was dominated by the small intestine and stomach. The masses of the heart and pancreas dominated PC4, whereas PC5 was influenced mostly by the mammary tissue and uterus masses. Therefore, in the first five principal components, which together explained 83 % of the morphological variation, there was a general 'body size' component (PC1), two alimentary components (PC2 and PC3) and a mammary tissue component (PC5). We used scores on these five components as independent predictors in regression analyses.

RMR_L was significantly related to PC1, the general 'body size' component, which was dominated by carcass and fat mass (r^2=0.372, $F_{2,41}$=12.94, P<0.001). As expected from PC1 being a general size component, this relationship disappeared when the effect of mass on RMR_L was removed by using residual RMR_L as the dependent variable.

Litter size at peak lactation was significantly related to PC3 (r^2=0.278, $F_{2,42}$=8.08, P=0.001), which was one of the alimentary components. The general size component (PC1) was significantly related to the mean mass of pups at peak lactation (r^2=0.301, $F_{1,43}$=18.48, P<0.001). Litter mass at peak lactation was significantly related to PC1 and PC3 (r^2=0.531, $F_{4,40}$=11.32, P<0.001). The asymptotic daily food intake was significantly (but weakly) related to the general 'body size' component (PC1) (r^2=0.097, $F_{1,42}$=4.53, P=0.039), but not to the alimentary components PC2 and PC3. None of the life history traits was correlated with PC5, the mammary tissue component.

The mean length of the small intestine at peak lactation was 59.9±0.51 cm and that of the large intestine 13.9±0.21 cm (means ± S.E.M., N=45). There were no significant relationships between the length of the small intestine and maternal mass (P=0.206), asymptotic food intake (P=0.316), litter size at peak lactation (P=0.902), litter mass at peak lactation (P=0.374) or the mean mass of the pups (P=0.745). There was a significant relationship between the length of the large intestine and maternal mass (r^2=0.153, $F_{1,43}$=7.77, P=0.008), but not with asymptotic food intake (P=0.711), litter size (P=0.184), litter mass (P=0.719) or the mean mass of the pups (P=0.092).

Discussion

Relationship between metabolic rate and life-history traits

RMR has been shown previously to increase between pre-breeding and peak lactation (Pennycuick, 1967; Garton et al., 1994; Spaaij et al., 1994; Speakman and McQueenie, 1996). Speakman and McQueenie (Speakman and McQueenie, 1996) suggested that this flexibility in RMR might explain why several previous studies have failed to establish links between variation in RMR_{PB} and life-history traits at the intraspecific level (e.g. cotton rats *Sigmodon hispidus*, Derting and McClure, 1989; and a different strain of *Mus musculus*, HSD/ICR, Hayes et al., 1992a).

In the present study, we also found no relationship between RMR_{PB} and the life-history traits, either with or without removing the effects of body mass on both variables. We did find, however, that RMR_L was significantly correlated with litter size and pup mass, suggesting that RMR_{PB} may indeed be an inappropriate trait for seeking correlations between energetics and life history because of the temporal flexibility in RMR. The significant effects of RMR_L on life-history traits appear to support the suggestion that high RMR potentiates reproductive performance (Henneman, 1983; McNab, 1980; Glazier, 1985a; Genoud, 1988; Thompson, 1992; Stephenson and Racey, 1995). However, when the effect of body mass on RMR_L was removed, none of the relationships remained significant, in agreement with previous intraspecific studies (Derting and McClure, 1989; Earle and Lavigne, 1990; Hayes et al., 1992a; Stephenson and Racey, 1993a; Stephenson and Racey, 1993b). Correlations between life-history traits and RMR_L, but not mass-independent RMR_L, suggest that the effects of RMR_L were only a consequence of the shared variation of both life-history traits and RMR_L with body mass. Our data do not, therefore, support the suggestion that individual variation in RMR (either pre-breeding or during peak lactation) potentiates individual variation in reproductive performance.

Relationship between morphology and metabolic rate

Variations in body size (PC1) had the largest influence on variation in RMR_L, probably because the body size component included the skeletal muscle which, because of its large mass, has previously been found to contribute most, in terms of tissue metabolic rate, to RMR, despite its low mass-specific

metabolic rate (Field et al., 1939; Blaxter, 1989). Some previous studies have also established links between muscle mass and RMR; for example, McNab (McNab, 1994) found a significant positive association between basal metabolic rate (BMR) and pectoral muscle mass in flightless birds. These data were unexpected because previous studies have found that the increase in RMR from pre-breeding to lactation is strongly correlated with hypertrophy in the alimentary tract (Speakman and McQueenie, 1996), and strain differences in RMR of mice have also been attributed to variations in the size of the alimentary tract and associated organs (Konarzewski and Diamond, 1995) (for similar data across species of birds, see also Daan et al., 1990).

There are at least two potential explanations for why we did not detect an effect of the alimentary components in the present study. First, error in our determinations of oxygen consumption may have masked the links between metabolic rate and alimentary morphology. This explanation, however, seems unlikely because the reported individual variation in RMR_L was over 30 times greater than the day-to-day repeatability of measurements of given individuals in our system. A more likely explanation was that variations in the alimentary components between individuals during late lactation were relatively small compared with the differences between individuals, strains and species reported in previous studies. For example, between pre-breeding and late lactation, we previously found that liver mass increased by an average of 273% and gut mass by 189% (Speakman and McQueenie, 1996), but in the present study the range of values across individuals was much smaller (106% difference between the smallest and largest livers and 76% difference in total gut mass). Because variation in the alimentary components between individuals was much lower, it was less likely to emerge as a significant factor driving individual variation in RMR. Several other recent studies have also failed to find an effect of variation between individuals in mass of the alimentary tract on variation in RMR (e.g. Koteja, 1996; Burness et al., 1998; Corp et al., 1997), probably also because variation in the size of the gut, despite its high tissue-specific metabolic rate (Field et al., 1939; Krebs, 1950), was relatively small compared with variation in other body components. When individual variation in morphology is relatively small, variations in RMR may be related mostly to other factors. Recent genetic studies, for example, have indicated very strong links between RMR and a quantitative trait locus (QTL) including the UCP-2 and UCP-3 genes (Bouchard et al., 1997). Thus, some of the variation in RMR may be linked to individual polymorphisms at these loci, which would not necessarily bear any relationship to morphological differences (or incidentally provide any potentiating effects for reproductive performance). This would be consistent with the low percentage variation in RMR that is explained by variation in the morphological traits and the correlation between RMR_{PB} and RMR_L.

Relationship between morphology and life-history traits

The dominant morphological factor that correlated with variation in the life-history traits was overall body size (PC1), which was significantly correlated with mean pup mass, total litter mass and asymptotic food intake. The alimentary component linked to variation in the stomach and small intestine (PC2) was not correlated with any of the life-history traits, but variation in the component influenced by the small and large intestines (PC3) was correlated with litter size and, hence, litter mass. The nature of this link, however, was unclear because there was no significant relationship between variation in this component and variations in asymptotic daily food intake. Moreover, the lengths of the small and large intestines were unrelated to any of the life-history variables. The overall impression from these data was, therefore, that individual variation in the alimentary tract among late-lactating mice did not influence their asymptotic daily food intake or have any major effects on their life histories. This suggests that hypertrophy of the tract and associated organs between pre-breeding and late lactation (Speakman and McQueenie, 1996) is sufficient to accommodate the large increase in food intake between these two phases, but that the ultimate level of the asymptotic daily food intake (Johnson et al., 2001) and associated life-history traits are regulated by other factors. Why maternal body mass rather than the mass of the alimentary tract or mammary tissue appears to play a pivotal role in these relationships is currently unclear.

By manipulating litter sizes (and hence also total litter mass and mean offspring mass), we were able to show that at least some of the variation in maternal body size during late lactation is driven by variation in the life-history traits (and not the reverse). When mothers were given larger litters to raise than they were anticipating, they increased their mass more between pre-breeding and late lactation; when they were given fewer offspring, they increased their mass less. This effect is consistent with the correlations between body mass and life-history traits of unmanipulated litters. Why such changes and correlations occur, however, has not been clarified by our analyses.

Although the mass of the mammary tissue was a dominant factor influencing PC5, variation in this component was not correlated with RMR_L or any of the litter variables. It was, however, significantly correlated with litter size when the data from the manipulated females were removed. Previous studies have suggested that the mass of the mammary tissue is determined by the litter size at birth (Bateman, 1957). If this is the case, then the development of the mammary tissue would be driven by the number of developing foetuses; hence, when litter sizes were increased, the females were unable to increase the size of the mammary tissue to match this increased demand. The presence of an effect in the unmanipulated litters but not the manipulated litters is consistent with this suggestion.

Limits on sustained energy intake

There has been considerable debate in the literature over where the limit in sustained energy intake (SusEI) during lactation (presumed in some studies to equal sustained metabolic rate) is acting (Peterson et al., 1990; Hammond and Diamond, 1992; Hammond and Diamond, 1994; Hammond

and Diamond, 1997; Weiner, 1992; Koteja et al., 1994; Hammond et al., 1994; Hammond et al., 1996; Koteja, 1996; Speakman and McQueenie, 1996). Is this limit set centrally by aspects of the morphology of the gut and the associated energy-processing 'machinery' or peripherally at sites where the energy is ultimately utilised, e.g. by the mammary tissue? Since variations in the masses or lengths of the alimentary tract were not reflected in variations in the asymptotic daily food intake of the mice in the present study, it seems unlikely that the females were limited centrally during lactation. However, the masses of the mammary glands were also not correlated with variations in asymptotic daily food intake, suggesting that the level of intake was unlikely to be regulated to match a peripheral limit set by the mammary glands. In this study, we only measured the mass of the mammary glands, and no measures of actual milk production or activity within the mammary glands were made. It is possible that, within our sample, milk production might have been related to asymptotic food intake; however, this was unfortunately not measured.

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References

Bateman, N. (1957). Some physiological aspects of lactation in mice. *J. Agri. Sci.* **49**, 60–77.

Blaxter, K. (1989). *Energy Metabolism in Animals and Man*. Cambridge: Cambridge University Press.

Blueweiss, L., Fox, H., Kudzma, V., Nakashima, D., Peters, R. and Sams, S. (1978). Relationships between body size and some life history parameters. *Oecologia* **37**, 257–272.

Bouchard, C., Perusse, L., Chagnon, Y. C., Warden, C. and Ricquier, D. (1997). Linkage between markers in the vicinity of the uncoupling protein 2 gene and resting metabolic rate in humans. *Human Mol. Gen.* **6**, 1887–1889.

Brody, S. (1945). *Bioenergetics and Growth*. New York: Reinhold.

Burness, G. P., Ydenberg, R. C. and Hochachka, P. W. (1998). Interindividual variability in body composition and resting oxygen consumption rate in breeding tree swallows, *Tachycineta bicolor*. *Physiol. Zool.* **71**, 247–256.

Clutton-Brock, T. H. and Harvey, P. H. (1983). The functional significance of variation in body size among mammals. In *Advances in the Study of Animal Behavior* (ed. J. F. Eisenberg and D. G. Kleiman), pp. 632–663. New York: American Society of Mammalogists.

Corp, N., Gorman, M. L. and Speakman, J. R. (1997). Seasonal variation in the resting metabolic rate of male wood mice *Apodemus sylvaticus* from two contrasting habitats 15 km apart. *J. Comp. Physiol.* B **167**, 229–239.

Daan, S., Masman, D., Strijkstra, A. and Verhulst, S. (1990). Intraspecific allometry of basal metabolic rate: relations with body size, temperature, composition and circadian phase in the kestrel (*Falco tinnunculus*). *J. Biol. Rhythms* **4**, 11–23.

Derting, T. L. and McClure, P. A. (1989). Intraspecific variation in metabolic rate and its relationship with productivity in the cotton rat, *Sigmodon hispidus*. *J. Mammal*. **70**, 520–531.

Earle, M. and Lavigne, D. M. (1990). Intraspecific variation in body size, metabolic rate and reproduction of deer mice (*Peromyscus maniculatus*). *Can. J. Zool.* **68**, 381–388.

Felsenstein, J. (1985). Phylogenies and the comparative method. *Am. Nat.* **125**, 1–15.

Field, J., Belding, H. S. and Martin, A. W. (1939). An analysis of the relation between basal metabolism and summated tissue respiration in the rat. I. The post-pubertal albino rat. *J. Cell. Comp. Physiol*. **14**, 143–157.

Gadgil, M. and Bossert, W. H. (1970). Life historical consequences of natural selection. *Am. Nat*. **104**, 1–24.

Garton, D. W., Hsu, M. J. and Harder, J. D. (1994). Environmental temperature and metabolic rates during gestation and lactation in golden hamsters (*Mesocricetus auratus*). *Physiol. Zool.* **67**, 496–514.

Genoud, M. (1988). Energetic strategies of shrews: ecological constraints and evolutionary implications. *Mammal. Rev.* **18**, 173–193.

Glazier, D. S. (1985a). Relationship between metabolic rate and energy expenditure for lactation in *Peromyscus. Comp. Biochem. Physiol*. **80**A, 587–590.

Glazier, D. S. (1985b). Energetics of litter size in five species of *Peromyscus* with generalisations for other mammals. *J. Mammal*. **66**, 629–642.

Hammond, K. A. and Diamond, J. (1992). An experimental test for a ceiling on sustained metabolic rate in lactating mice. *Physiol. Zool*. **65**, 952–977.

Hammond, K. A. and Diamond, J. (1994). Limits to dietary nutrient uptake and intestinal nutrient uptake in lactating mice. *Physiol. Zool*. **67**, 282–303.

Hammond, K. A. and Diamond, J. (1997). Maximal sustained energy budgets in humans and animals. *Nature* **386**, 457–462.

Hammond, K. A., Kent Lloyd, K. C. and Diamond, J. (1996). Is mammary output capacity limiting to lactational performance in mice? *J. Exp. Biol*. **199**, 337–349.

Hammond, K. A., Konarzewski, M., Torres, R. M. and Diamond, J. (1994). Metabolic ceilings under a combination of peak energy demands. *Physiol. Zool*. **67**, 1479–1506.

Harvey, P. H. (1990). Life-history variation: size and mortality patterns. In *Primate Life History and Evolution* (ed. T. H. Clutton-Brock and P. H. Harvey), pp. 81–88. New York: Wiley-Liss, Inc.

Harvey, P. H., Pagel, M. D. and Rees, J. A. (1991). Mammalian metabolism and life histories. *Am. Nat*. **137**, 556–566.

Harvey, P. H., Promislow, D. E. L. and Read, A. F. (1989). Causes and correlates of life history differences among mammals. In *Comparative Sociobiology* (ed. R. Foley and V. Standen), pp. 305–318. Oxford: Blackwell.

Hayes, J. P., Garland, T. and Dohm, M. R. (1992a). Individual variation in metabolism and reproduction of *Mus*: are energetics and life history linked. *Funct. Ecol*. **6**, 5–14.

Hayes, J. P., Speakman, J. R. and Racey, P. A. (1992b). Sampling bias in respirometry. *Physiol. Zool.* **65**, 604–619.

Haysson, V. and Lacy, R. C. (1985). Basal metabolic rates in mammals: Taxonomic differences in the allometry of BMR and body mass. *Comp. Biochem. Physiol*. **81**A, 741–754.

Henneman, W. H. (1983). Relationship among body mass, metabolic rate and the intrinsic rate of natural increase in mammals. *Oecologia* **56**, 104–108.

Johnson, M. S., Thomson, S. C. and Speakman, J. R. (2001). Limits to sustained energy intake. I. Lactation in the laboratory mouse *Mus musculus*. *J. Exp. Biol.* **204**, 1925–1935.

Joliffe, I. T. (1986). *Principal Component Analysis*. New York: Springer-Verlag.

Kenagy, G. J. (1987). Energy allocation for reproduction in the golden-mantled ground squirrel. *Symp. Zool. Soc. Lond*. **57**, 259–273.

Kleiber, M. (1961). *The Fire of Life: An Introduction to Animal Energetics*. New York: Wiley.

Konarzewski, M. and Diamond, J. (1995). Evolution of basal metabolic rate and organ masses in laboratory mice. *Evolution* **49**, 1239–1248.

Koteja, P. (1996). Limits to the energy budget in a rodent, *Peromyscus maniculatus*; does gut capacity set the limit? *Physiol. Zool*. **69**, 994–1020.

Koteja, P., Krol, E. and Stalinski, J. (1994). Maximum cold- and lactation-induced rate of energy assimilation in *Acomys cahirinus*. *Polish Ecol. Stud.* **20**, 369–374.

Krebs, H. A. (1950). Body size and tissue respiration. *Biochim. Biophys. Acta* **4**, 249–269.

Kunkele, J. and Kenagy, G. J. (1997). Inefficiency of lactation in primiparous rats: the costs of first reproduction. *Physiol. Zool*. **70**, 571–577.

McNab, B. K. (1980). Food habits, energetics and the population biology of mammals. *Am. Nat*. **116**, 106–124.

McNab, B. K. (1994). Energy-conservation and the evolution of flightlessness in birds. *Am. Nat*. **144**, 628–642.

Pennycuick, P. R. (1967). A comparison of the effects of a variety of factors on the metabolic rate of the mouse. *Aust. J. Exp. Biol. Med. Sci*. **45**, 331–346.

Peterson, C. C., Nagy, K. A. and Diamond, J. (1990). Sustained metabolic scope. *Proc. Natl. Acad. Sci. USA* **87**, 2324–2328.

Piers, L. S., Diggavi, S. N., Thangam, S., van Raaij, J. M. A., Shelty, P. S. and Hautvast, J. G. A. J. (1995). Changes in energy expenditure, anthropometry and energy intake during the course of pregnancy and lactation in well-nourished Indian women. *Am. J. Clin. Nutr*. **61**, 501–513.

Read, A. F. and Harvey, P. H. (1989). Life history differences among the eutherian radiations. *J. Zool., Lond.* **219**, 329–353.

Ryan, B. F., Joiner, B. L. and Ryan, T. A. (1985). *Minitab Handbook*. Boston, MA: PWS-Kent. 385pp.

Spaaij, C. J. K., van Raaij, J. M. A., de Groot, L. G. P. G. M., van der Heijden, L. J. M., Boekholt, H. A. and Hautvast, J. G. A. J. (1994). Effect of lactation on resting metabolic rate and on diet and work induced thermogenesis. *Am. J. Clin. Nutr*. **59**, 42–47.

Speakman, J. R. (2000). The cost of living: Field metabolic rates of small mammals. *Adv. Ecol. Res*. **30**, 177–297.

Speakman, J. R. and McQueenie, J. (1996). Limits to sustained metabolic rate: The link between food intake, basal metabolic rate and morphology in reproducing mice, *Mus musculus*. *Physiol. Zool*. **69**, 746–769.

Speakman, J. R. and Racey, P. A. (1987). The energetics of pregnancy and lactation in the brown long-eared bat, *Plecotus auritus*. In *Recent Advances in the Study of Bats*, chapter 21 (ed. M. B. Fenton, P. A. Racey and J. M. V. Rayner), pp. 367–393. Cambridge: Cambridge University Press.

Speakman, J. R. and Rossi, F. (1999). No support for socio-physiological suppression effect on metabolism of paired white mice (*Mus* sp.) *Funct. Ecol*. **13**, 373–382.

Stearns, S. C. (1983). The influence of size and phylogeny on patterns of covariation among life-history traits in the mammals. *Oikos* **41**, 173–187.

Stephenson, P. J. and Racey, P. A. (1993a). Reproductive energetics of the Tenrecidae (Mammalia: Insectivora). I. The large-eared tenrec, *Geogale aurita*. *Physiol. Zool*. **66**, 643–663.

Stephenson, P. J. and Racey, P. A. (1993b). Reproductive energetics of the Tenrecidae (Mammalia: Insectivora). II. The shrew-tenrecs, *Microgale* spp. *Physiol. Zool*. **66**, 664–685.

Stephenson, P. J. and Racey, P. A. (1995). Resting metabolic rate and reproduction in the Insectivora. *Comp. Biochem. Physiol*. **112**A, 215–223.

Thompson, S. D. (1992). Gestation and lactation in small mammals: Basal metabolic rate and the limits of energy use. In *Mammalian Energetics. Interdisciplinary Views of Metabolism and Reproduction*, chapter 10 (ed. T. E. Tomasi and T. H. Horton), pp. 213–259. Ithaca: Comstock.

Thompson, S. D. and Nicoll, M. E. (1986). Basal metabolic rate and energetics of reproduction in therian mammals. *Nature* **321**, 690–693.

Trevelyan, R., Harvey, P. H. and Pagel, M. D. (1990). Metabolic rates and life histories in birds. *Funct. Ecol*. **4**, 135–141.

Weiner, J. (1989). Metabolic constraints to mammalian energy budgets. *Acta Theriol*. **34**, 3–35.

Weiner, J. (1992). Physiological limits to sustainable energy budgets in birds and mammals: Ecological implications. *Trends Ecol. Evol.* **7**, 384–388.

Western, D. (1979). Size, life history and ecology in mammals. *Afr. J. Ecol*. **17**, 185–204.

Western, D. and Ssemakula, J. (1982). Life history patterns in birds and mammals and their evolutionary interpretation. *Oecologia* **54**, 281–290.