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Limits to sustained energy intake XII: is the poor relation between resting metabolic rate and reproductive performance because resting metabolism is not a repeatable trait?

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SUMMARY

Many studies have investigated the consequences of individual variation in resting metabolic rate at thermoneutrality (RMRt) on reproductive performance. Despite strong theoretical reasons for expecting such an association, results have generally been disappointing. A fundamental assumption of these studies is that RMRt is a repeatable trait. We examined repeatability of RMRt in female MF1 mice over short (15 days apart; *N*=238) and long intervals (110 days apart; *N*=33). In the long-term experiment, after the first RMRt measurement, females were separated in two groups: the first was kept virgin (*N*=16); the second was allowed to breed (*N*=17) and measured 15 days after they had weaned their pups. We also examined the association between RMRt and reproduction. We used Pearson's correlation (*r*) and intraclass correlation coefficients (ρ) to estimate repeatability. There was a strong effect of body mass on RMRt for all measurements. Over the short interval, repeatability was significant for body mass (*r*=0.86; ρ=0.86), RMRt (*r*=0.68; ρ=0.68,) and residual-RMRt (*r*=0.58; ρ=0.58). Over long intervals, repeatability of residual-RMRt was high in virgin females (*r*=0.59; ρ=0.60), but not in the breeders (*r*=0.38; ρ=0.39); body mass was repeatable only for non-breeders measured by *r* (*r*=0.55). There was no significant correlation between RMRt or residual-RMRt and litter size or litter mass. In conclusion, RMRt and residual-RMRt are highly repeatable traits in virgin MF1 female mice. The lack of association between non-reproductive RMRt and reproductive performance in MF1 mice does not come about because of its poor repeatability.

Key words: *Mus musculus*, repetability, resting metabolic rate, mouse, reproductive performance.

INTRODUCTION

Basal metabolic rate (BMR) is the amount of energy expended by a non-reproductive and non-growing animal at rest, within the thermoneutral zone, regulating its body temperature at euthermic levels in a post-absorptive state (Kleiber, 1961). A slightly less rigorously defined measure, resting metabolic rate in the thermoneutral zone (RMRt), relaxes the need for the animals to be non-reproductive and post-absorptive (Speakman et al., 2004). This latter definition overcomes the semantic difficulty of being unable to measure the BMR of an animal when it is reproducing, and also circumvents the problem of animals suppressing their metabolic rate when they are food deprived to make them post-absorptive (Speakman et al., 2004). Although BMR, and RMRt, were defined with the objective of establishing a standardised measurement for metabolism, they are highly variable between different species, even of the same body mass (e.g. Hemmingsen, 1960; Dawson and Hulbert, 1970; McNab, 1980; McNab, 2002; Henneman, 1983; Hayssen, 1984; Hayssen and Lacy, 1985; Ricklefs et al., 1996; Lovegrove, 2000).

In addition to high variation between species, it has become apparent that BMR and RMRt are also highly variable between individuals within species (e.g. Bech et al., 1999; Jackson et al., 2001; Johnson et al., 2001a; Weyer et al., 2000; Speakman et al., 2003; Rønning et al., 2005; Johnston et al., 2007; Sadowska et al., 2007). Everything else being equal, higher levels of BMR or RMRt would demand greater levels of food intake. Hence, if one considers two individuals, one with a high, and one with a low BMR, the individual with the high BMR would need to consume more energy each day to support its higher metabolic rate. Alternatively, if the two animals ingested the same energy, the one with the lower BMR would have more energy available to allocate to other activities such as reproduction (Gadgil and Bossert, 1980). Attempts to understand the inter-specific and inter-individual variability in BMR have therefore focussed on trying to discern advantages that might accrue to animals with high BMR levels (e.g. Labocha et al., 2004; Speakman, 2008).

The sustained maximum metabolic rate (SusMR) is the maximum rate of energy expenditure that an animal can sustain for a protracted period of time, without dependence on stored energy reserves. In many situations it is equivalent to the maximal sustained rate at which energy can be ingested and assimilated (maximal sustained energy intake SusEI) (Speakman and Krol, 2005). Like BMR and RMRt, SusMR and SusEI are highly variable between species and between individuals within species. SusEI may be limited by the extrinsic supply of energy from the environment (e.g. Speakman et al., 2003). However, animals may often be limited by aspects of their intrinsic physiology. An idea that emerged in the 1980s was that the maximum rates at which animals can ingest or expend energy might be linked to their BMRs (Drent and Daan, 1980). The basis of this idea was that SusMR and SusEI are ultimately limited by the capacity of the alimentary tract to absorb and process nutrients from the environment (Kirkwood, 1983; Tolkamp et al., 2002). An alimentary tract and associated organs (such as the liver) that can process energy faster will make more energy available to support SusMR. However, these larger and/or more metabolically active tissues will be reflected in a higher BMR. Hence, greater capacities for SusMR and SusEI would necessitate a bigger alimentary system that would require a higher BMR (Drent and Daan, 1980; Weiner,

1989; Weiner, 1992; Peterson et al., 1990; Hammond and Diamond, 1992; Hammond and Diamond, 1997; Lovegrove, 2000). Although the first ideas were that SusMR and BMR might be linked *via* the energy acquisition system, it was later recognized that links between the two might also come about because of commonality in the demands of the tissues where energy is ultimately utilized. If the tissues involved in energy utilisation have high rates of energy demand in the basal state, then a similar functional linkage between tissues, BMR (RMRt), SusMR and SusEI would emerge.

Lactation is one of the most energetically demanding periods in the lives of small rodents (reviewed by Speakman, 2008; Naya et al., 2008). Many studies have been performed comparing RMRt of lactating animals to non-breeding individuals (Hammond and Diamond, 1992; Hammond and Diamond, 1994; Hammond et al., 1994; Hammond et al., 1996; Konarzewski and Diamond, 1995; Rogowitz and McClure, 1995; Rogowitz, 1998; Speakman and McQueenie, 1996; Johnson and Speakman, 2001; Johnson et al., 2001a; Johnson et al., 2001b; Johnson et al., 2001c; Speakman et al., 2001; Król and Speakman, 2003a; Król and Speakman, 2003b; Król et al., 2003; Król et al., 2007). These studies are consistent with the idea that BMR (RMRt) and SusMR (SusEI) should be closely associated. Moreover, this association is also consistent with changes in aspects of morphology that may limit either uptake of energy or its utilisation. Lactating mice have bigger alimentary tracts, bigger livers and bigger mammary glands than non-breeding mice. They also have greater RMRt, SusEI and SusMR. Such associations are also apparent between BMR and reproductive performance when comparisons are made across species (Glazier, 1985a; Glazier, 1985b; Genoud, 1988).

If the hypothesis associating high BMR (or RMRt) to high SusMR (and SusEI) is correct, we would expect the same associations to be found between individuals that vary in their BMR. However, at this inter-individual level the data are far more equivocal. Daan et al. (Daan et al., 1989; Daan et al., 1990a) indicated that residual variability in BMR was associated with variations in the sizes of organs that would be expected to be the most energy demanding. Many other studies, however, have failed to find such links (Koteja, 1996; Corp et al., 1997; Burness et al., 1998; Geluso and Hayes, 1999). At peak lactation there is a very weak correlation between RMRt and SusEI, but this disappears when the shared variation due to body mass is removed (Johnson et al., 2001a). Moreover, many studies have looked for, but failed to establish any significant associations between individual variation in RMRt and reproductive performance (Derting and McClure, 1989; Earle and Lavigne, 1990; Hayes et al., 1992a; Stephenson and Racey, 1993a; Stephenson and Racey, 1993b; Johnston et al., 2001a; Król et al., 2003c).

One potential reason why our previous studies of sustained energy intake using the MF1 (and C57BL/6) mice have failed to establish significant links between RMRt, SusEI and reproductive performance is that RMRt might not be repeatable in this mouse strain. Repeatability is clearly a key aspect of any trait that we consider has been a target for selection, as it places an upper limit on the possible heritability (Falconer and Mackay, 1996). Previous studies have suggested that BMR is a repeatable trait in different species of birds and mammals (Bech et al., 1999; Labocha et al., 2004; Sadowska et al., 2005; Vézina and Williams, 2005; Versteegh et al., 2008) (reviewed by Nespolo and Franco, 2007; Versteegh et al., 2008; Boratynski and Koteja, 2008; Broggi et al., 2009). However, other studies have failed to detect significant repeatability for BMR in some species (Russell and Chappell, 2007; Bozinovic, 2007) pointing to the necessity of experimental verification. In the current paper we first explored short-term repeatability of RMRt in MF1 mice by measuring RMRt twice with a 15 day interval between measurements. To assess the effect of reproduction, we compared repeatability of RMRt over the longer term (110 days between measurements) of two groups of mice; in one group all females were kept virgins (non-breeding group) and in the other, the first and the second measurements of RMRt were separated by one reproductive event (breeding group). We used RMRt rather than BMR because this is the trait previous studies have used to try and identify associations between resting metabolism and reproductive performance.

MATERIALS AND METHODS

Virgin female mice (*Mus musculus* L.: outbred strain MF1) were purchased from Harlan UK Ltd, Oxon, UK at 6 weeks of age in seven separate batches. After 1 week of acclimation to their new surroundings following transport, they were housed individually in shoebox cages with sawdust and paper bedding. The lights were maintained on a 12 h:12 h L:D photoperiod (lights on at 07:00 h) and the ambient temperature was regulated at 21±1°C. Animals received pelleted rodent food [CRM(P), Special Diet Services, BP Nutrition, UK] and water *ad libitum*.

Protocol evaluation

Protocols for the measurement of BMR and RMRt are similar across all studies but vary in their exact details. Individuals are generally placed in a respirometry chamber (of variable volume) for a period of some hours, with (BMR) or without (RMRt) a variable prior period of food (and occasionally water) deprivation. Measurements while the animals are in the chamber may be almost continuous, or, if the gas analyser alternates between several chambers may be intermittent. In this latter case the cycle of measurement generally depends on how many chambers are in the system. The final BMR or RMRt measurement is defined as the lowest metabolism during the entire measurement over some pre-defined period, normally of several minutes. In our laboratory we do not deprive the mice of food prior to measurement and continuously measure them for 3 h (without food or water in the chamber) at 30°C, taking a measurement every 30 s. We use the lowest 10 consecutive measures (5 min) as the estimate of RMRt (e.g. Selman et al., 2001). Hayes et al. (Hayes et al., 1992b) showed that even if animals are at rest, if metabolism is a normally distributed trait with a fixed mean and standard deviation, the measured BMR or RMRt will depend on the exact protocol parameters (total measurement duration, sampling interval and integrated duration for measurement). Differences in repeatability between studies may in part reflect the exact protocols for measurement. There is, however, an additional complication. Careau et al. (Careau et al., 2008) suggested that there are links between behaviour of individuals in respirometry chambers and their personalities. Some individuals may have ‘restless personalities’ and never settle in the chamber, whereas others may calm down relatively quickly. These personality differences might precipitate an illusion of repeatability in the measurement because a restless mouse would be restless in all repeated measures and a calm one would be calm in all measures.

To remotely monitor the behaviour of mice during our standard protocols, sixteen 6-month-old MF1 mice (eight male and eight females) were implanted intraperitoneally with temperature transmitters (PDT-4000 E-Mitter, Mini Mitter Company Inc., USA) under general anaesthesia (a mixture of isoflorane and oxygen). Mice were allowed at least 3 weeks to recover from the surgery before measuring their metabolism. Transponder energizers (ER-4000 Receiver, Mini Mitter Company Inc., USA)

were placed under the respirometry chambers allowing us to non-invasively monitor the body temperature and general activity throughout the respirometry measurements using the VitalView® Data Acquisition System [Mini Mitter Company, Inc., Bend, OR, USA; see Harkin, for detailed description (Harkin, 2002)]. Instrumented animals were placed in respirometry chambers and oxygen consumption, body temperature and general activity were measured using our standard protocol (details below).

Resting metabolic rate (RMRt)

Resting metabolic rate (RMRt) was measured using four open-flow respiratory systems as described previously (Hayes et al., 1992b; Speakman and McQueenie, 1996). All measurements were made during the day between 09:00 and 17:00 h (2 h after normal lights on and before lights off) and started either in the morning around 09:00 h or in the afternoon around 13:30 h. In this system, fresh air was pumped (Charles Austin Pumps Ltd, Byfleet, UK) through a sealed Perspex chamber (volume 1155 ml) within an incubator (INL-401N010; Gallenkamp, Loughborough, UK) set at 30°C [within the thermal neutral zone for these mice (Speakman and Rossi, 1999)]. Mass-flow controllers (MKS Instruments UK, Cheshire, UK) provided 500–700 ml min^{-1} which was monitored using a wet type laboratory gas flow meter (Model DM3A; G. H. Zeal Ltd, Alexander Wright Division, London, UK). Air leaving the animal chamber was dried using silica gel and 150 ml min^{-1} was passed through a gas analyser (Servomex 1100A or Servomex Xentra, Servomex Ltd, Crowborough, UK). CO_2 was not absorbed prior to gas analysis as this maximizes the accuracy of energy expenditure measures when RQ is unknown (Koteja, 1996). Oxygen concentrations were measured continuously, and averaged values were stored every 30 s for 180 min. RMRt was quantified as the oxygen consumption over the lowest 10 consecutive values (5 min), excluding periods that included transient drops in the measurements (see below). Volumes were corrected for temperature and pressure, using the appropriate equation (Hill, 1972). The RMRt data (ml O_2 min^{-1}) were converted to energy equivalents using an oxycalorific value of 21.1 J ml^{-1} O_2 derived from the Weir equation (Weir, 1949). Body mass was measured before and after each run and the mean value was used in further analysis.

Repeatability measurements

The short-term repeatability of RMRt was estimated by making two measurements of 238 animals, split in seven batches. The first measurement was carried out at an age of 70±10 days and the second measurement was made 15±5 days later. In the long-term repeatability experiment, a group of 33 individual mice was used, on which two measurements of RMRt were carried out; one at 70±10 days of age and the second measurement at 180±3 days of age. To explore the effects of reproduction on repeatability of RMRt, mice from the long-term experiment were divided into two different groups. The first group (N=16) was never bred (the non-breeding group) and the second group (N=17) was bred at 115 days of age (i.e. after the first RMRt measurement; the breeding group). Females from the breeding group were paired polygamously (one male to two females). Pregnancy was detected by an increase in body mass. The sexes were kept together for about 10 days, depending on whether or not females gained weight significantly. After removing males, the females were housed in individual cages and checked twice a day to determine the day of parturition. Numbers of pups and their mass were recorded daily until weaning (21 days after parturition). After weaning females were housed in groups of three or four until the second measurement of RMRt was carried out, about 14 days later.

Reproductive output

We measured litter size and litter mass at weaning for the 17 mice that were bred in the long-term repeatability experiment.

Data analysis

The body mass and RMRt variables were log-transformed before analysis to normalize them. The same statistical analysis was applied to the three sets of repeatability data, i.e. the short-term analysis and the long-term analysis for both breeding and non-breeding females. Repeatability of RMRt was assessed by Pearson's product moment correlation coefficient r, and the coefficient of intraclass correlation (ρ) (Sokal and Rohlf, 1981; Lessells and Boag, 1987). In the intraclass correlation the observed mean squares (MS) from an analysis of variance are decomposed to estimate the within and among individual variance. The intraclass correlation coefficient (ρ) is given by:

$$\rho = \frac{S_A^2}{S^2 + S_A^2},$$

where S_A^2 is the among group variance component and S^2 is the within group variance component. These variance components are calculated from the mean squares within (MS_W) and mean squares among (MS_A) individuals in the analysis of variance; $S^2=MS_W$ and $S_A^2=(MS_A–MS_W)/N_0$ (Sokal and Rohlf, 1981; Lessells and Boag, 1987) where N_0 is related to sample size. When N_0=2, the intra-class correlation varies between –1, when the trait is completely unrepeatable and all the variance resides within individuals (MS_A=0) and +1 when the trait is perfectly repeatable and all the variance resides among individuals (MS_W=0). At higher values of N_0 the upper boundary, when MS_W=0, remains at +1, but the lower boundary when MS_A=0 increases above –1, but is still always less than 0 [see figure 1 in Lessells and Boag (Lessells and Boag, 1987)]. Both coefficients (ρ and r) were used to estimate repeatability of residual RMRt obtained from a linear regression of log RMRt on log body mass. In an initial analysis we included time of day (morning or afternoon) and measurement machine (n=1–4) as random factors, but neither were significant and were eliminated from further analyses. A repeated measures general linear model (GLM) with body mass as covariate was used to compare trials. Least squares linear regression was used to determine if there were relationships between litter mass and litter size at weaning, and both RMRt and residual RMRt. Repeated measures analysis was carried out using SPSS-17.0; coefficient of intraclass correlation (ρ) was calculated following procedures described by Sokal and Rohlf (Sokal and Rohlf, 1981) and Lessells and Boag (Lessells and Boag, 1987); all other analyses were performed using MINITAB®15.0.

RESULTS

As soon as mice were placed into the chamber, they showed a period of elevated metabolism, physical activity and high body temperature (Fig. 1A–D). This period lasted for about 60–90 min. The mice then settled down and became inactive. These periods of inactivity corresponded to periods of stable low oxygen consumption. Generally, the mice would be inactive for a period, and then prior to being removed would show some additional periods of activity when oxygen consumption rose and body temperature was also elevated. This pattern was true for all the mice in the study. An unexpected pattern, however, was that transient reductions in metabolic rate could be observed in some (but not all) animals (see

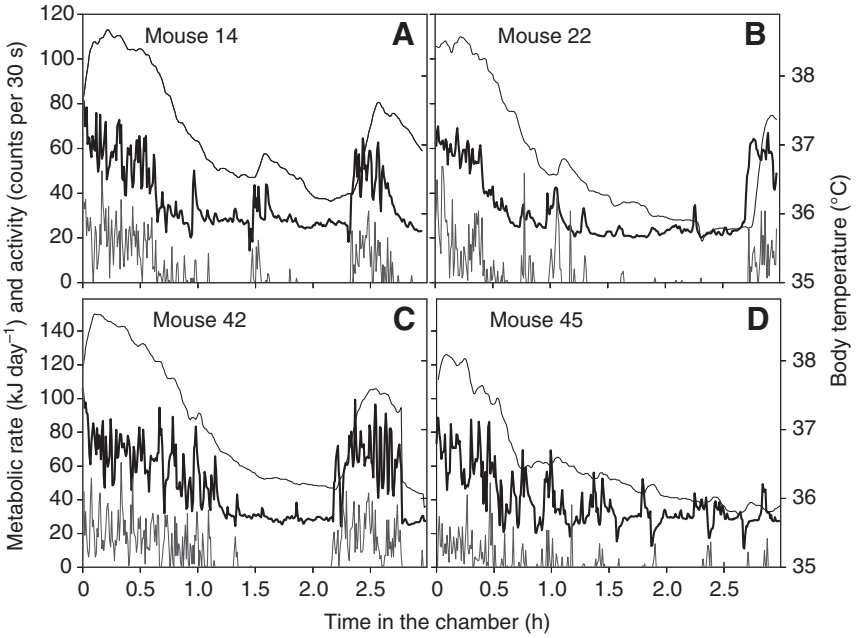

Fig. 1. Simultaneous recordings of metabolic rate (bold black line), general activity (grey line) and body temperature (black line) for four representative mice made at 30 s intervals over a period of approximately 3 h.

Fig. 1A,D, mice 14 and 45). It is unclear what causes these reductions, but our measurements show that they did not consistently correspond to changes in general activity or body temperature, although often a short bout of activity followed the end of a depression. From direct visual observations of the mice during some of these declines it is clear that the mice were resting and were not blocking the inlet or outlet ventilation tubes of the chamber. The drops were never observed during the periods at the start and end of runs when the chambers were empty, and they were recorded using multiple machines.

These transient decreases in oxygen consumption generally lasted between 1 and 4 min and involved drops of up to 50% relative to the adjacent metabolism. Because our analysis to define RMRt automatically selects the period from the entire measurement with the lowest mean calculated across 10 consecutive readings, the estimated RMRt inevitably coincided with the period of a transient drop. This could potentially cause a problem. Either because animals might consistently show the drops and this would generate a spurious increase in the repeatability, or because they might only occasionally show them, in which case repeatability would be diminished. In the light of these data, we therefore analysed the data for short-term repeatability in two ways. First, we used our standard analysis protocol to define the RMRt. This would include the transient drops in metabolism when they occurred. Second, we analysed the data selecting the 5 min period during which the variation in the consecutive 30 s measurements was lowest. This corresponded to a period when the metabolism exhibited a stable low rate not including the transient declines in metabolism.

The mean RMRt based on the lowest readings over 5 min (which could include a transient drop) was slightly lower than the mean based on the lowest readings over 5 min at minimum variation (which excluded the drops; Table 1). Also, a slightly higher repeatability was found for RMRt including the drops (Table 2). For the long-term repeatability analysis, we used the data excluding transient drops.

Table 1. Longitudinal measurements of mean body mass and resting metabolic rate over short- and long-term intervals

Group/trait	First measurement	Second measurement
Short-term repeatability		
Mean BM (g)	30.78±0.18	31.88±0.20
Mean RMRt (kJ day^{-1}, including drop)	22.14±0.19	22.19±0.18
Mean RMRt (kJ day^{-1}, excluding drop)	22.52±0.17	22.49±0.16
Long term repeatability: non-breeding		
Mean BM (g)	30.0±0.42	38.11±1.12
Mean RMRt (kJ day^{-1})	20.36±0.37	21.78±0.50
Long term repeatability: breeding		
Mean BM (g)	31.76±0.74	38.23±0.50
Mean RMRt (kJ day^{-1})	22.35±0.74	25.59±0.55

Mean (±standard error) body mass (BM) and resting metabolic rate (RMRt) were measured in two cohorts of female mice. First, for the short-term repeatability (N=238) the first and second measurement were about 15 days apart and RMRt is expressed in two ways; based on lowest metabolic rate (including transient drop) and based on the metabolic rate at minimal variation (excluding transient drop, see text for details). Over the long term (about 110 days), RMRt is expressed as the metabolic rate at minimum variation and is shown for females that were not bred (N=16) and those that were bred (N=17) between measurements.

Table 2. Repeatability of body mass, resting metabolic rate and residual RMRt over the short term

Trait	Statistics	Based on lowest RMRt	Based on lowest RMRt at minimal variation
BM	*r*	0.86	0.86
	ρ	0.79	0.79
RMRt	CV	5.7%	5.0%
	ρ	0.69	0.68
	r	0.69	0.68
Residual RMRt	ρ	0.61	0.58
	r	0.61	0.58

BM, body mass; RMRt, resting metabolic rate.

Shown are coefficient of variation (CV) for RMRt and repeatability measured by Pearson's correlation coefficient (*r*) and intraclass correlation coefficient (ρ) for body mass, RMRt or residual RMRt measured on two occasions 15 days apart (age ~70 days). Calculations are shown for RMRt calculated as the lowest metabolic rate over ten consecutive readings (i.e. 5 min, first column) or as the metabolic rate over ten consecutive readings at minimal variation (excluding transient drop, column 2, see text for details). All values for *r* and ρ were significant at $P<0.0001$.

Short-term repeatability

The mean coefficient of variation (CV) across individuals between the RMRt measurements was 5.0%. Absolute mean RMRt for the first trial (22.5 kJ day^{-1}) was not different from the second trial measured 15 days later (22.5 kJ day^{-1}; Table 1). There was a highly significant effect of body mass on RMRt for both measurements (both traits log converted: $F_{1,237}$=96.2, $P<0.0001$ for the first measurement and $F_{1,237}$=86.1, $P<0.0001$ for the second; Fig. 2). The slope of the allometric relationship for the first measurement was b=0.68 and for the second measurement was b=0.62 (Fig. 2). Repeatability measured by Pearson's r and intraclass correlation coefficient (ρ) over the short interval was highly significant for body mass, RMRt and residual RMRt (Table 2, Fig. 3).

Long-term repeatability in non-breeding mice

Linear regression in the non-breeding group, using log-transformed data, revealed there was a significant relationship between RMRt and body mass for mice at 70 days old (R^2=0.25; $F_{1,15}$=4.68, P=0.048; Fig. 4A) as well as for mice aged 180 days (R^2=0.61, $F_{1,15}$=22.1, $P<0.001$; Fig. 4A). We calculated the CV for each pair of measurements and then took the mean of these CVs across all individuals. This mean CV was 5.1% and values of Pearson's correlation r between the two measurements was significant for absolute RMRt and residual RMRt (Table 3). Repeatability measured by intraclass correlation coefficient (ρ) was significant for both RMR and residual RMRt (Table 3, Fig. 5A). Repeatability for body mass was significant when estimated by Pearson's coefficient (r=0.55; Table 3). However, the large increase in body mass between 70 to 180 days of age resulted in non-significant value for repeatability of body mass measured by ρ (Table 3)

Long-term repeatability in breeding mice

In breeding animals, there was a significant relationship between log RMRt and log body mass prior to breeding (R^2=0.84, $F_{1,16}$=79.4, $P<0.0001$; Fig. 4B). In the post-lactation period the amount of variation in RMRt explained by variation in body mass was lower than that before breeding, but was still significant (R^2=0.28, $F_{1,16}$=7.1, P=0.02; Fig. 4B). Mean CV between the two measurements was 11.3%. Values of r or repeatability, ρ, between the two measurements were not significant for body mass, RMRt or residual RMRt (Table 3, Fig. 5B).

RMRt and reproductive output

At weaning the average litter size was 7.5 (ranging from three to 12) and average litter mass was 93 g (ranging from 50.4 to 134.3 g). There was no significant correlation between log RMRt or residual RMRt and log litter mass or log litter size using either pre-breeding or post-lactation measurements (Table 4).

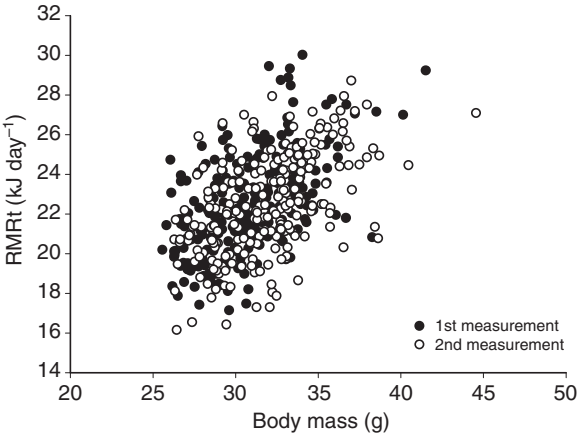

Fig. 2. Relationship between resting metabolic rate (RMRt) and body mass at two time points about 15 days apart (short term). Raw data are plotted.

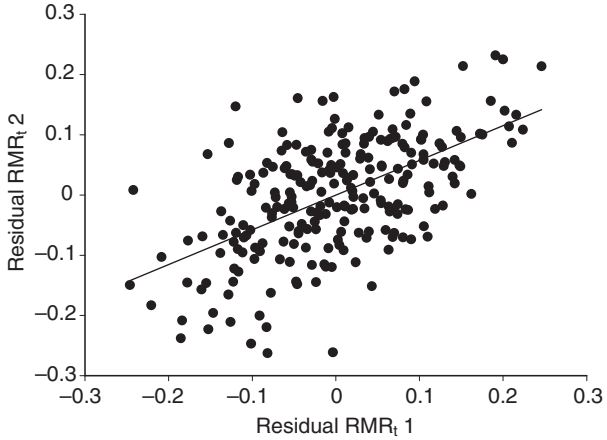

Fig. 3. Relationship between residual RMRt relative to body mass for the two measurements of RMRt measured over a short time interval (about 15 days apart). Residuals were calculated from the relationship between $\log_e$ RMRt and $\log_e$ body mass for both measurements.

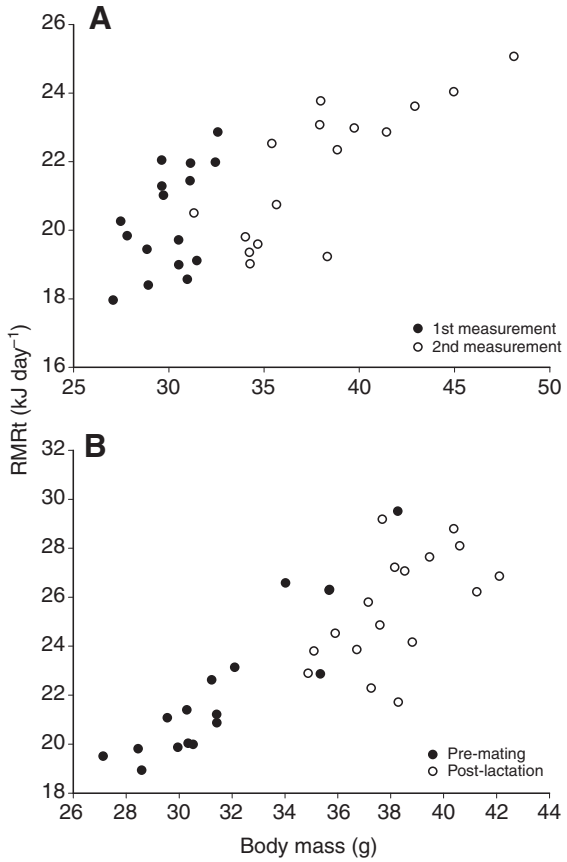

Fig. 4. Resting metabolic rate (RMRt) plotted against body mass for two time points measured about 110 days apart (long interval). (A) The relationship in non-breeding females. (B) The relationship between measurements for animals that were bred between the measurements (pre- and post-lactation).

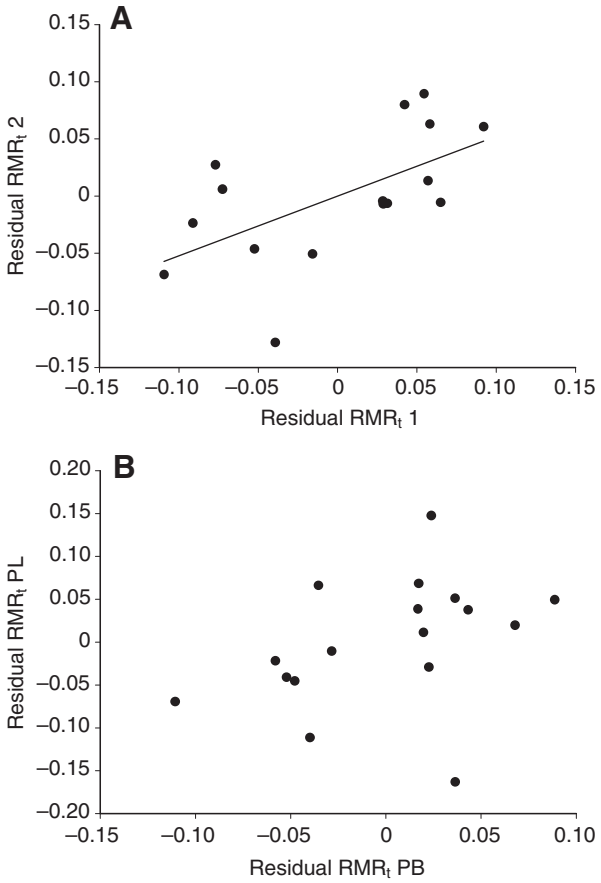

Fig. 5. Relationship between residual RMRt derived from a regression of $\log_e$ RMRt on $\log_e$ body mass, for two measurements taken about 110 days apart (long interval). (A) The relationship in the non-breeding-group and (B) the relationship for the breeding group.

DISCUSSION

Time course of metabolism in instrumented animals

Detailed analysis of the respirometry traces of instrumented animals revealed that almost all individuals rested at some stage during the 3 h measurements. High values of RMRt reported previously (Johnson et al., 2001b; Król et al., 2003) were therefore probably correct estimates of RMRt, and not a consequence of individuals failing to settle down during the 3 h measurement period. Our detailed analysis of body temperature and behaviour did, however, highlight a potentially more serious issue: transient reductions in metabolism in some individuals (see Fig. 1A,D; mice 14 and 45). The nature of these decreases in metabolism is not clear, but they have also been observed by others using similar high resolution systems, and consequently they are not unusual to our equipment, study animals and protocols. Moreover, they were never observed when the chamber was empty, suggesting they were not equipment

Table 3. Repeatability of body mass, resting metabolic rate and residual RMRt over the long term

Trait	Statistics	Non-breeding females	Breeding females
BM	*r*	0.55 (*P*=0.028)	0.39 (*P*=0.12)
	ρ	–0.46 (*P*=1.0)	–0.46 (*P*=1.0)
RMRt	CV	5.1%	11.3%
	r	0.64 (*P*=0.007)	0.47 (*P*=0.06)
	ρ	0.41 (*P*=0.06)	0.05 (*P*=0.97)
Residual RMRt	*r*	0.59 (*P*=0.017)	0.38 (*P*=0.13)
	ρ	0.6 (*P*=0.005)	0.39 (*P*=0.42)

BM, body mass; RMRt, resting metabolic rate.

Shown are the coefficient of variation (CV) for RMRt and repeatability measured by Pearson's correlation coefficient (*r*) and intraclass correlation coefficient (ρ) for BM, RMRt and residual RMRt between measurements at the age of 70 days and 180 days. RMRt is defined as the metabolic rate over 10 consecutive readings at minimum variation. Data is shown for non-breeding females (*N*=16), and for females that were bred in-between the repeated measurements (*N*=17, pre-breeding *vs* 14 days post-lactation).

Table 4. Correlation between reproductive output (litter size and litter mass at weaning), body mass, resting metabolic rate and residual RMRt

	Pre-breeding			Post-lactation		
	Body mass	RMRt	Res-RMRt	Body mass	RMRt	Res-RMRt
Litter size	r=–0.42 (P=0.10)	r=–0.44 (P=0.08)	r=–0.14 (P=0.58)	r=–0.1 (P=0.7)	r=–0.34 (P=0.19)	r=–0.34 (P=0.18)
Litter mass	r=–0.23 (P=0.37)	r=–0.34 (P=0.19)	r=–0.31 (P=0.23)	r=–0.07 (P=0.8)	r=–0.37 (P=0.14)	r=–0.41 (P=0.10)

RMRt, resting metabolic rate; Res-RMRt, residual resting metabolic rate.

artefacts. The reason they are probably not more frequently reported is that many researchers use multi-channel systems where the measurement period for each chamber is intermittent and averaged over much longer periods (generally 10–15 min), compared with our high resolution systems where we obtain almost continuous measurements for each individual at 30 s intervals.

Inability to detect and eliminate these periods of decreased metabolism from analysis could be a serious problem in multichannel systems because it is impossible to establish in such systems whether a low measurement is a truly low metabolic rate – or a measurement during which there was a transient decline in metabolism. Multi-channel systems have several problems, as was highlighted by Labocha et al. (Labocha et al., 2004), together with some ideas of how to reduce these problems, but the problem identified here cannot be resolved by these approaches. On average, the estimated metabolism including these declines was 2% lower than when they were eliminated by taking the least variable 5 min. However, individual estimates were up to 25% lower. Hence, in a large population this effect may be relatively trivial. However, in a smaller sample, and when individual variation is an important aspect of the study, these transient drops may become much more significant. We suggest that wherever possible estimates of BMR and RMRt should be made using quasi-continuous monitoring systems with high frequency measurements which allow resolution of these events, and allow them to be eliminated from the analysis. Using the absolute lowest values for RMRt in the analysis of repeatability (including the drops) yielded a slightly greater estimate of repeatability than using the RMRt measured at lowest variation (i.e. excluding the transient drops).

Short-term repeatability of RMRt

The RMRt measured here was highly repeatable in non-breeding MF1 mice over short intervals. These results agree with estimates of repeatability between 0.6 and 0.7 for RMR that have been observed in various other mammalian species: wild-caught *Dipodomys merriami* (Hayes et al., 1998), bank voles, *Myodes* (*Clethrionomys*) *glareolus* (Labocha et al., 2004), free-living populations of North American red squirrels, *Tamiasciurus hudsonicus* (Larivee et al., 2010), and in a wild population of weasels, *Mustela nivalis*, during the summer (Szafranska et al., 2007). High (0.6–0.7) short-term repeatability in RMRt has also been shown in several species of birds, such as laboratory housed zebra finches, *Taeniopygia guttata* (Vézina and Williams, 2005), and in European stonechats, *Saxicola torquata rubicola* (Versteegh et al., 2008). Much lower repeatability has been observed in other studies. For instance, in a wild population of bank voles a ρ of 0.34 was found (Boratynski and Koteja, 2008). Moreover, no significant repeatability was observed in deer mice, *Peromyscus maniculatus sonorensis* (Russell and Chappell, 2007), and in leaf-eared mice (Bozinovic, 2007). The reasons behind the large differences in repeatability between studies are unclear. Perhaps it is caused by subtle differences in protocols between laboratories that are known to affect the RMR estimates (Hayes et al., 1992b). Alternatively it may reflect differences in the selection history of different species. Whatever the cause, a comparison of these various studies suggests that RMRt and BMR cannot be assumed to be repeatable without verification when studying new species.

Long-term repeatability of RMRt

In our results, repeatability values of residual RMRt over a short interval (averaging 15 days) measured by both Pearson's correlation and intraclass correlation, was very similar to the repeatability by the same measures over a long interval (about 110 days) in non-breeding females (Tables 2 and 3). Other studies of repeatability of RMRt comparing long with short intervals in both birds and mammals have generated contrasting results about the effect of time (between measurements) on repeatability.

Greenfinches (*Carduelis chloris*) captured in the field and kept in captivity showed high repeatability of mass-specific RMR over the short term (4 days: r=0.89; 8 days: r=0.84) and lower, but significant, repeatability over the long term [130 days: r=0.65 (Hõrak et al., 2002)], which to some extent reflects the repeatability in body mass. A study on kittiwakes (*Rissa tridactyla*) showed a significant relationship between residual RMR during the incubation and the chick-rearing periods [r=0.64 (Bech et al., 1999)]. They also found significant repeatability of BMR by intraclass correlation (ρ=0.52) for comparisons during incubation, chick-rearing and pre-breeding periods, from different years. High repeatability for BMR using intraclass correlation was found in male and non-reproductive female zebra finches, *Taeniopygia guttata*, but declined considerably in males from values of 0.63 (over an 8-day interval) to 0.29 [over intervals of 127–249 days (Vézina and Williams, 2005)]. Although these results suggest an effect of time on repeatability of RMR, contradictory results have been found, even for the same species and between sexes. In reproductive female zebra finches, Vézina and Williams (Vézina and Williams, 2005) also found high repeatability of RMR for egg-laying and chick-rearing stages, with no time effect over a period of 8–10 months. Another study on zebra finches showed very little change in repeatability of residual RMR in males using measurements separated by 1.5 months and 2.5 years: from r=0.46 to r=0.52, but in females there was a higher change in repeatability from r=0.41 to r=0.52 (Rϕnning et al., 2005). This conflicting result for the same species was attributed to the differences in the time interval used to measure repeatability in both studies as well as to the number of measurements they averaged to calculate each repeated measurement (Vézina and Williams, 2005). This difference in a single species reveals the difficulty in finding a pattern to describe the effect of time on repeatability, if the time intervals being compared are not the same.

In mammals, studies comparing the effect of time on repeatability are sometimes restricted to one sex and/or to a single reproductive state. For example, in a free-ranging population of male weasels, repeatability of RMR corrected for body mass was high in summer (ρ=0.55, r=0.54; N=22) and in summer and winter combined, when corrected for the effect of season (ρ=0.63, r=0.62), suggesting that

repeatability was independent of time scale (Szafranska et al., 2007). In a wild population of North American red squirrels, repeatability of RMR, measured by Pearson's correlation coefficient was high (r=0.77) over the short term (45-day interval) in reproductively active males and in non-reproductive females combined. However, over the long term (192-days interval) repeatability was significant (r=0.72) only for non-reproductive females (Larivée et al., 2010). In a wild population of bank voles, repeatability of mass-independent BMR over long intervals (ρ=0.23; 54 days) declined 32% in comparison with repeatability over short intervals (ρ=0.34; 5 days); repeatability of absolute BMR showed a 47% decline over long intervals (Boratynski and Koteja, 2008). Our results show that residual RMR in non-breeding females over long intervals decreased only marginally, by 1–2%, in comparison to short intervals. Together, these data reveal no clear patterns. The extent to which the time interval between measurements affects repeatability depends on the length of the interval between repeated measurements, on the method used to measure repeatability, and on the species involved.

Repeatability of body mass

Repeatability of body mass has been shown in several species of small mammals either by Pearson or intraclass correlation in wild populations (Szafranska et al., 2007; Boratynski and Koteja, 2008) (Larivee et al., unpublished data) and in captivity (Labocha et al., 2004). The negative values for repeatability of body mass over the long term in the present study, measured by the intraclass correlation ρ, resulted from the way ρ is calculated (see Materials and Methods). In theory, ρ should vary from a negative value (somewhere between minus one and zero), indicating that all variation is within individuals, to plus one, where all the variation is among individuals (i.e. every time an individual is measured the same value is obtained). The variance component among groups is calculated by the difference between the mean square among and within groups (Sokal and Rohlf, 1981; Lessells and Boag, 1987). When the mean square within groups is higher than that among groups, the denominator of the equation, and hence the resultant value of ρ, is negative (Lessells and Boag, 1987). This means that ρ is sensitive to changes in the means of the traits in question as well as their variances. If the population mean of the first measurement differs from the population mean of the second measurement, this difference will result in an increased within-individual variance, yielding a decreased ρ (Hayes and Jenkins, 1997; Hayes et al., 1998). The fact that repeatability of whole RMRt and body mass estimated by ρ was non-significant over the long term was caused by the increase in body mass over this interval in our mice. Pearson's product-moment correlation coefficient, r, on the other hand, assesses the consistency of a trait relative to the population mean, and therefore gives a more realistic estimate of repeatability when the population mean changes. The high repeatability of body mass over the long term (measured by Pearson's r correlation) only for the non-breeding group (Table 3) suggests a change of body composition after reproduction, despite the fact that mean body mass increased in both breeding and non-breeding groups (Table 1).

Association of non-reproductive RMRt with reproductive parameters

The non-significant association between both litter size and litter mass with RMRt measured in the pre-breeding and post-lactation periods was consistent with our previous results in the same strain of mice, which was based on measurements of RMRt before reproduction (Johnson et al., 2001) or at peak lactation (Speakman and Król, 2003). Similar results were found for measurements in different strains of laboratory mice (Hayes et al., 1992a; Johnston et al., 2007), and in other species (Derting and McClure, 1989; Stephenson and Racey, 1993a; Stephenson and Racey, 1993b). In combination with the high repeatability of RMRt over short and long intervals in virgin females, these data indicate that the lack of association between RMRt and reproductive performance in mice (Johnson et al., 2001; Johnston et al., 2007) (our results) is not caused by poor repeatability of RMRt. The widespread idea that repeatability sets an upper limit to heritability (Falconer and Mackey, 1996) has been contested, and estimating repeatability may be problematic for highly plastic or strongly context-dependent traits (Dohm, 2002). Here we showed high repeatability of RMRt for non-reproductive mice, however, the fact that RMRt is not repeatable after a reproductive event suggests plasticity of RMRt, which may or may not be reversible.

When these changes in phenotype are reversible, it is named flexibility, which is a category of plasticity (Piersma and Drent, 2003). Flexibility of RMRt is a widespread phenomenon in birds that involves reversible changes in response to environmental factors such as temperature and seasonality (review in McKechine, 2008), as well as adjustments to the events during the animal's life, such as reproduction. In zebra finches, RMRt is highly repeatable for females in a given breeding season (between laying eggs and chick-hearing) and in non-breeding state (Vézina and Williams, 2005), and when RMRt before and after a breeding event are compared (Rønning et al., 2005).

Plasticity of RMRt has been observed in different species of mammals exposed to different conditions, such as cold acclimation (e.g. Hart, 1957; Russell and Chappell, 2007; McDevitt and Speakman, 1995), caloric restriction (e.g. Ferguson et al., 2007; Martin et al., 2007; Hambly et al., 2005; Selman et al., 2005) and reproduction, with a significant increase of RMRt during lactation (e.g. Johnson et al., 2001b; Naya, 2008). However, we are not aware of any study showing flexibility of RMRt (i.e. where RMRt returns to the previous level), since no measurements of RMRt were taken again when animals were returned to the original conditions. Rather, the very few examples of studies on repeatability over long intervals on wild population of mammals, especially where reproduction must be involved, pointed in the opposite direction; suggesting no repeatability of RMR after reproduction. For example, the fact that Szafranzka et al. (Szafranzka et al., 2007) only found repeatability after controlling for the seasonal effect and that Larivée et al. (Larivée et al., 2010) did find repeatability over long intervals, but only for non-reproductive females in American red squirrels, may be due to a lack of flexibility of RMR after a reproductive event. The increase in RMRt we found here in post-lactating mice may be attributed in part to the incomplete reversal of the hypertrophy in the alimentary tract and associated organs such as the liver, heart and mammary glands, which is known to occur during lactation (Konarzewski and Diamond, 1995; Speakman and McQueenie, 1996; but see Johnson et al., 2001a). During the post-lactation period, adipose tissue replaces all glandular structures in the mammary gland in a process called involution, which occurs during the first 2 to 3 days after weaning. Mice return to oestrus cycling within 4 to 5 days of the end of lactation (Monks et al., 2008). Although the process of involution of the mammary glands is completed soon after weaning, the organs associated with increase in energy expenditure during lactation, such as the liver and heart, do not reach the normal values of virgin females of the same age, even 42 days after weaning (Bergman et al., 1994). This might explain the higher values of RMRt we found in the post-lactation period (about 14 days after

weaning) compared with the pre-breeding period. The fact that a reproductive event prevents repeatability suggests plasticity of RMRt and may explain the absence of association between RMRt and reproductive output.

In conclusion, our results show high repeatability of RMRt and of residual RMRt in non-breeding mice over both short and long intervals, measured by both Pearson's correlation, r, and intraclass correlation, ρ. However, repeatability was lost when mice bred between measurements, and we found no relationship between reproductive output and RMRt. Lack of repeatability is thus not responsible for the absence of correlation between RMRt and reproductive output. The fact that this trait is not repeatable after a reproductive event is consistent with the idea of plasticity of RMRt in response to lactation demands.

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