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RESTING METABOLIC RATE AND MORPHOLOGY IN MICE (*MUS MUSCULUS*) SELECTED FOR HIGH AND LOW FOOD INTAKE

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Summary

We investigated the relationship between resting metabolic rate (RMR) and various morphological parameters in non-breeding mice, selected for high and low food intake corrected for body mass. RMR was measured at 30 °C, and mice were subsequently killed and dissected into 19 body components. High-food-intake mice had significantly greater body masses and a significantly elevated RMR compared with the low-intake mice. Data pooled across strains indicated that body mass, sex and strain together explained over 56 % of the observed variation in RMR. The effects of strain and sex on RMR and tissue morphology were removed, and three separate statistical analyses to investigate the relationship between RMR and organ morphology were performed: (i) employing individual regression analysis with each tissue component as a separate predictor against RMR; (ii) individual regression analysis with residual organ mass against residual RMR (i.e. with strain, sex and body mass effects removed); and (iii) pooling of some organ masses into functional groupings to reduce the number of predictors. Liver mass was the most significant morphological trait linked to differences in RMR. Small intestine length was significantly greater in the high-intake line; however, no difference was observed between strains in the dry mass of this organ, and there was no evidence to associate variability in the mass of the alimentary tract with variability in RMR. The effects of strain on RMR independent of the effect on body mass were consistent with the anticipated effect from the strain differences in the size of the liver.

Key words: resting metabolic rate, body mass, morphology, selection, food intake, mouse, *Mus musculus*.

Introduction

An area of study that has generated considerable interest in recent years is the attempt to unravel factors that lead to variability in basal (or resting) metabolic rate. Previous investigations have approached this problem using comparisons both between (Hayssen and Lacy, 1985; Daan et al., 1989; Speakman, 2000) and within (Daan et al., 1990; Speakman et al., 1994; Konarzewski and Diamond, 1995; Meerlo et al., 1997) species, with attempts being made to understand whether, and how, the observed variation affects life-history strategies (e.g. Thompson, 1992).

Studies of the variation in resting and daily energy metabolism have generated the hypothesis that variability in resting metabolic rate (RMR) may be positively associated with maximum sustainable metabolic rates (Drent and Daan, 1980; Weiner, 1992; Peterson et al., 1990; Hammond and Diamond, 1997; but see Ricklefs et al., 1996; Speakman, 2000) or maximum aerobic capacity (Bennett and Ruben, 1979; Koteja, 2000). The presence of such an association may provide a mechanism by which variability in RMR could influence life history. The nature of any putative relationship between RMR and maximum sustainable metabolic rate is, however, obscure. It is often suggested that maximum sustained metabolic rate may be limited centrally by the capacity of the alimentary tract to process food or peripherally through the capability of tissues, such as skeletal muscle, to consume energy (for a review, see Speakman, 2000). The idea that sustained maximum metabolic rates may be limited by the capability of the alimentary tract to digest, assimilate and produce sufficient energy substrates has been supported by several studies (Kirkwood, 1983; Weiner, 1992; Hammond and Diamond, 1992; Speakman and McQueenie, 1996; Hammond and Diamond, 1997). This ‘central limitation’ hypothesis predicts that, as the mass of the alimentary system increases, its capacity to process energy also increases and that the sustained metabolic rate can increase as a direct result of the elevation in assimilated energy. However, an associated

cost is the increased expense of supporting the alimentary organs, which is reflected in an elevated RMR. Additional studies of tissue metabolism *in vitro* endorse the idea that the tissues associated with the digestive system may have relatively high levels of oxygen consumption (Field et al., 1939; Krebs, 1950).

Within species, animals vary considerably in their resting metabolic rates (Daan et al., 1989; Hayes et al., 1992; Speakman et al., 1994; Meerlo et al., 1997; Burness et al., 1998; McLean and Speakman, 2000) and, in general, not all this variation is due simply to body mass differences between individuals (Speakman, 1996). It has also been established that, *in vitro*, certain tissues and organs, such as the liver, brain, gastrointestinal tract and kidney, have mass-specific metabolic rates over 100 times higher than others, such as bone and adipose tissue (Krebs, 1950; Schmidt-Nielsen, 1985). Hence, although organs such as the kidney and liver may account for a small proportion of an individual's total body mass, their contribution to RMR may be disproportionately large (Daan et al., 1990; Ricklefs, 1996). A high level of phenotypic plasticity (Via et al., 1995) in metabolically active organs is also known to occur intra-specifically in reptiles (Secor et al., 1994), birds (Piersma and Lindstrom, 1997) and mammals (Hammond et al., 1994; Speakman and McQueenie, 1996).

Despite this, associations between resting metabolic rate and organ morphology in non-breeding animals have proved difficult to establish. A low metabolic rate was associated with a low lean mass of the heart and kidney in kestrels (*Falco tinnunculus*) fed a low-maintenance diet (Daan et al., 1990), although these individuals also had significantly larger brain and leg muscle masses compared to individuals fed a high maintenance diet. European starlings (*Sturnus vulgaris*) maintained on a low-quality diet increased their gastrointestinal, gizzard and liver mass, whilst individuals maintained on a high-quality diet did not, but these changes in morphology were not correlated with differences in basal metabolic rate (BMR) between the groups (Geluso and Hayes, 1999).

In mammals, the relationships between metabolic rate and morphology have been reported to be similarly poor. Meerlo et al. (Meerlo et al., 1997), for example, found that a high residual BMR was associated only with a high lean heart dry mass in the short-tailed field vole *Microtus agrestis*, and other studies have failed to find any significant correlations between resting metabolic rates and residual organ masses (Koteja, 1996a; Speakman and Johnson, 2000). Konarzewski and Diamond (Konarzewski and Diamond, 1995) suggested that, in six inbred strains of mice, those strains with 'exceptionally high (or low) BMRs tended to have disproportionately large or small organs'. However, in this latter study, the masses of only four organs were measured (heart, kidney, liver and small intestine) and, hence, correlations between the sizes of these organs and other variables, e.g. total muscle mass, cannot be discounted.

In the present study, we sought to examine the association between resting metabolic rate and tissue masses of non-breeding animals using male and female mice from two mouse (*Mus musculus*) strains (M lines), selected for 38 generations from a common background for high and low food intake corrected for body mass (Hastings et al., 1997). Previous studies of the metabolic rates of these lines using calorimetry have indicated a substantial difference in their 24-h energy expenditures (Bünger et al., 1998), perhaps indicative of differences in their RMR. Such an approach, using an artificially selected substrate from the laboratory to tease apart questions of functionality, is being increasingly recognized as valuable in the field of ecophysiology, where natural variation in traits may be insufficient to reveal their functional associations (Garland and Carter, 1994).

Materials and methods

Study animals and their maintenance

The strains of mouse (*Mus musculus*) used during this experiment were divergently selected for high and low food intake at the Institute of Cell, Animal and Population Biology, University of Edinburgh, UK, and are described elsewhere in detail (Hastings et al., 1997; Bünger et al., 1998). For reference, in previous publications, these strains have been referred to as the M-lines (MH and ML for high- and low-maintenance requirements respectively). Here, we will refer to them simply as the high- and low-intake-selected strains. The M lines were derived from a common background population generated by a three-way cross [two inbred (CBA, JU) and one outbred (CFLP) line] (Sharp et al., 1984) and were developed by divergent selection over 38 generations using a phenotypic index of voluntary food intake between 8 and 10 weeks of age and mean body mass at 8 and 10 weeks intended to minimise correlated changes in body mass. Initially, three independent lines were selected in each direction. The three replicates were intercrossed at generation 20, and a single line was subsequently maintained. After generation 38, selection was suspended. At the start of generation 43, full sibling matings were used to reproduce the lines to develop resources for future mapping studies. Mice used during this study were samples from generation 47, and a total of 20 males (11 high-intake and 9 low-intake) and 19 females (9 high-intake and 10 low-intake) were used. Mice were housed individually at 22±3 °C in shoebox cages (48 cm×15 cm×13 cm) under a photoperiod of 14 h:10 h L:D (lights on at 07:00 h) at the University of Aberdeen, UK. All individuals had access to water and food *ad libitum* [CRM (P) Pelleted Rat and Breeder and Grower Diet, Special Diets Services, BP Nutrition, UK], with sawdust provided for bedding.

Resting metabolic rate measurements

All measurements of resting metabolic rate (RMR) were undertaken on individual mice between 18 and 20 weeks of age. Immediately prior to a measurement of RMR, body mass was recorded (Sartorius, to ±0.01 g) and rectal body temperature was measured (Digitron, 2751-K, to ±0.1 °C). Mice were not denied access to food or water before respirometry measurements, but most food intake occurred nocturnally (C. C. Velten and L. Bünger, unpublished

observations), and measurements were made at least 4 h after lights on. Resting metabolic rate (RMR) was quantified as the rate of oxygen consumption at 30 °C (within the thermoneutral zone) during the light phase (between 10:00 h and 17:00 h) using an open-flow respirometry system and employing the protocol previously described elsewhere (Speakman and McQueenie, 1996; Speakman and Johnson, 2000). In brief, mice were placed individually in a sealed Perspex chamber contained within an incubator (INL-401N-010, Gallenkamp), and air, dried using silica gel (BDH, UK), was drawn through the system (Charles Austin Pumps Ltd) at a rate of 600–800 ml min^{-1} (DM3A, Alexander Wright flowmeter). Subsequently, excurrent air was dried, and a sample was passed through the oxygen analyser (Servomex plc, Crowburgh, UK) at 150 ml min^{-1}. To maximize accuracy in the derived estimate of energy expenditure, carbon dioxide was not absorbed prior to determination of oxygen content (Koteja, 1996b). The measurements from the oxygen analyser were recorded directly on a microcomputer at intervals of 30 s, and the 10 lowest consecutive readings (equivalent to 5 min within the respirometry chamber) were used to estimate RMR, employing the appropriate equation from Hill (Hill, 1972) and corrected for temperature and pressure. Each individual was generally in the respirometry chamber for a period of 3 h and always for a minimum of 2 h. Previous studies of the repeatability of RMR measurements using this protocol in our laboratory on mice suggest that the coefficient of variation (CV) for day-to-day replicates is 8 % (Krol and Speakman, 1999).

Organ morphometrics

Following RMR measurements, mice were killed, and immediately after death we dissected and weighed 19 organs; the liver, kidneys, heart, lung, brain, thyroid, spleen, pancreas, stomach, large intestine, small intestine, gonads, brown adipose tissue, abdominal fat, mesenteric fat, subcutaneous fat, tail and pelage to an accuracy of 0.0001 g (Ohaus Analytical Plus) and the carcass to an accuracy of 0.01 g (Sartorius, 0.01 g). The carcass included both skeletal muscle and bone. The lengths of the extended, but not stretched, small and large intestines were measured (to an accuracy of 1 mm) using a ruler. Any residual gut contents within the small and large intestines were removed before weighing. All tissues were dried to a constant mass at 60 °C in an oven (Gallenkamp) over a minimum period of 14 days, and their dry masses were recorded.

Statistical analyses

Statistical analysis was undertaken using the MINITAB (Version 10) statistical package (Minitab Inc., State College, PA 16801-3008, USA). Distributions of all variables were tested for normality using the Anderson–Darling test (Sokal and Rohlf, 1968). The masses of several of the morphological components were not normally distributed, so all the variables were transformed to natural logarithms to normalize them. Because these two strains of mice had been selected for high and low food intake, while attempting to keep body mass constant, it is important to distinguish two levels at which associations might be apparent between RMR and tissue morphology. First, there is the within-strain effect and, second, there is the between-strain effect. This latter association is probably a consequence of the correlated responses of both RMR and tissue morphology to the selected trait (food intake corrected for body mass), although, with single high-intake and low-intake lines, we cannot discount the possibility that the responses reflect genetic drift (Hill, 1980).

To explore these potentially different effects, we initially examined the effects of body mass, sex and strain on the variation in the resting metabolic rate and tissue masses using generalised linear modelling (GLM). To explore the within-strain effects, we examined the association between RMR and the individual organ masses with the shared variation in these traits due to strain and sex effects eliminated and with the shared variation due to strain, sex effects and body mass eliminated (using residuals). Stepwise regression was used to identify the most significant predictors from the organ masses.

To examine whether the between-strain effects were consistent with the within-strain effects or whether the effects of selection had produced different responses, we used the model derived from the within-strain effects to predict the extent of difference in RMR that might be anticipated given the correlated responses in morphology to selection. We then compared the actual response in RMR with this prediction.

Results

Between-strain differences

Body mass and resting metabolic rate

Males from the high-intake strain of mice were significantly heavier than males from the low-intake strain (t_{21}=3.290, P=0.004: Table 1).

For female mice, similar patterns were evident, with the high-intake mice being significantly heavier (t_{20}=2.53, P=0.01). On average, RMR was also significantly higher (t_{21}=3.70, P<0.001) in high-intake males compared with low-intake males and significantly higher (t_{20}=3.72, P<0.001) in high intake females compared with low-intake females (Table 1).

Pooling the data across both strains, there were significant independent effects of strain (t=1.99, P=0.05), sex (t=–2.67, P=0.011) and body mass (t=3.20, P=0.003) on metabolic rate (Fig. 1). None of the two-way or the three-way interaction terms was significant (all P>0.05). The least-squares fit regression:

$$\log_e \mathrm{RMR} = -3.84 + 1.042 \log_e M_b + 0.118\text{‘strain’} - 0.145\text{‘sex’}, \quad (1)$$

where RMR is in ml min^{-1} and body mass, M_b, is in g, explained 56.8 % of the individual variation in RMR (F=15.33, P<0.001), where ‘strain’ was a dummy variable coded 1 for the high-intake line and 0 for the low-intake line, and ‘sex’ was a dummy variable coded 1 for males and 0 for females.

Table 1. *Gross aspects of the morphology and metabolic rate of male and female mice drawn from two strains selected for high and low food intake*

	High-intake line		Low-intake line	
	Males	Females	Males	Females
Body mass (g)	33.5±0.46	34.2±0.89	31.0±0.62	31.2±0.62
RMR (ml O_2 min^{-1})	0.763±0.02	0.897±0.07	0.654±0.02	0.595±0.03
Small intestine length (cm)	51.1±0.87	53.3±0.97	43.9±1.10	46.2±1.20
Large intestine length (cm)	11.4±0.26	12.4±0.49	10.9±0.32	10.1±0.18

Values are means ± S.E.M. (*N*=39).
RMR, resting metabolic rate.

Table 2. *Mean dry mass of 19 tissues dissected from male and female mice drawn from two strains selected over 38 generations for high and low food intake*

	Organ mass (g)			
Organ	High males (*N*=11)	Low males (*N*=9)	High females (*N*=9)	Low females (*N*=10)
Carcass	5.38±0.158	4.82±0.124	5.33±0.181	4.42±0.077
Pelage	2.768±0.110	2.634±0.064	2.038±0.067	2.169±0.100
Tail	0.550±0.017	0.426±0.009	0.481±0.014	0.354±0.008
Large intestine	0.127±0.005	0.132±0.005	0.129±0.010	0.133±0.007
Small intestine	0.229±0.010	0.219±0.011	0.159±0.009	0.144±0.008
Pancreas	0.118±0.016	0.112±0.010	0.143±0.014	0.133±0.026
Stomach	0.057±0.003	0.063±0.107	0.052±0.002	0.053±0.003
Liver	0.531±0.016	0.445±0.014	0.665±0.027	0.453±0.016
Brown adipose tissue	0.128±0.014	0.157±0.012	0.056±0.003	0.068±0.009
Abdominal fat	0.841±0.097	0.810±0.078	0.691±0.129	0.983±0.085
Mesenteric fat	0.244±0.034	0.255±0.033	0.187±0.031	0.240±0.041
Subcutaneous fat	0.721±0.088	0.729±0.085	0.415±0.045	0.801±0.130
Spleen	0.024±0.001	0.023±0.002	0.034±0.002	0.025±0.002
Thyroid	0.104±0.006	0.102±0.007	0.064±0.004	0.104±0.010
Gonads	0.364±0.028	0.304±0.023	0.194±0.018	0.125±0.012
Kidneys	0.155±0.007	0.139±0.010	0.109±0.004	0.097±0.004
Heart	0.052±0.002	0.041±0.003	0.050±0.003	0.034±0.002
Lungs	0.172±0.025	0.115±0.008	0.118±0.013	0.110±0.016
Brain	0.100±0.022	0.095±0.002	0.112±0.003	0.095±0.003

Values are means ± S.E.M.
High, mice selected for high food intake; Low, mice selected for low food intake.

Organ morphology

The mean dry masses of each tissue for mice of both sexes from the high-intake and low-intake lines are presented in Table 2. For 10 of these tissues, there were significant relationships between the individual variations in tissue mass and total live body mass. For several tissues, however, notably the small intestine, pancreas, brown adipose tissue, subcutaneous fat, gonads, kidneys, heart, lungs and brain, individual variations in tissue mass were not significantly linked with the overall variations in total body mass. As some of the tissue masses were correlated with body mass, and there were differences in body mass between strains and sexes, these associations might confound comparisons of the effects of strain on tissue morphology. We therefore explored the effects of strain, sex and body mass together by entering them as independent predictors in GLM analyses for each tissue. These analyses (Table 3) revealed that, for all tissues, there were no significant effects of the interactions between strain and body mass, between sex and strain, or between sex and body mass, indicating that the gradients of the 'within-strain' and 'within-sex' mass effects were not significantly different between strains or sexes. Independent strain effects on the masses of the pelage, tail, large intestine, stomach, liver, brown adipose tissue, abdominal, mesenteric and subcutaneous fat deposits, thyroid, heart and brain were significant (Table 3), while sex effects were apparent in the pelage, tail, small intestine, pancreas, liver, brown adipose tissue, spleen, gonads and kidneys.

Despite the absence of clear effects of strain on the masses of the alimentary tract, there were very obvious differences in the lengths of the small and large intestines for both males and females. The small intestine was significantly longer (t_{36}=5.12,

Table 3. *Effects of strain (high or low food intake), sex and body mass on the dry masses of 19 separate tissues in mice, using generalized linear modelling*

Tissue	Mass *F*	Strain *F*		Sex *F*	
Carcass	25.7***	0.8		2.4	
Pelage	19.3***	10.5***	L	11.8***	M
Tail	10.7**	31.8***	H	9.2**	M
Large intestine	8.6**	7.1*	L	3.4?	
Small intestine	0.6	0.1		33.6***	M
Pancreas	0.9	0.7		5.3*	F
Stomach	12.7***	11.6**	L	0.0	
Liver	9.4**	9.5**	H	19.8**	F
Brown adipose tissue	1.0	4.8*	L	34.2***	M
Abdominal Fat	8.0**	8.3**	L	1.4	
Mesenteric Fat	6.2*	6.6*	L	0.2	
Subcutaneous Fat	3.3	8.4**	L	0.1	
Spleen	4.3*	0.18		15.6***	F
Thyroid	4.7*	12.0***	L	1.5	
Gonads	1.6	1.8		25.6***	M
Kidneys	2.9	0.2		21.4***	M
Heart	0.9	11.4**	H	1.9	
Lungs	1.3	0.1		0.4	
Brain	0.1	6.5*	H	3.6?	F

*0.01<*P*<0.05; **0.001<*P*<0.01; ***P<0.001; ?0.05<*P*<0.1. In all cases, the two-way interactions between mass and strain, strain and sex, and mass and sex, and the three-way interaction were not significant (P>0.05).

The effect of body mass on organ mass was always positive.

Against the column labelled Strain, H indicates that the high-strain mice and L the low-strain mice, respectively, had larger organs once other effects had been controlled for. Against the column labelled Sex, M indicates that males and F indicates that females, respectively, had larger organs once other factors had been controlled for.

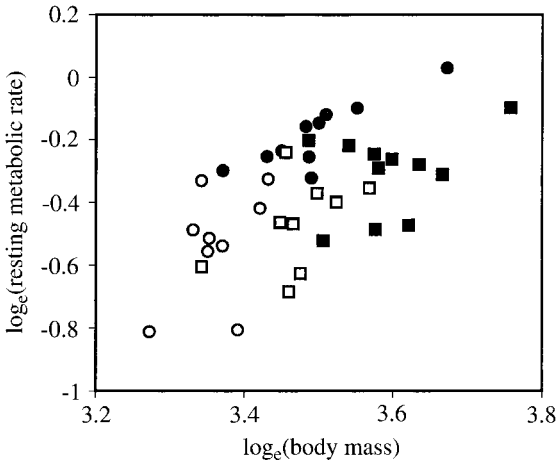

Fig. 1. Overall relationship between log$_e$(body mass) (g) and log$_e$(resting metabolic rate) (ml O_2 min^{-1}). Filled symbols represent mice from the lines selected for high food intake and open symbols represent mice selected for low food intake. Circles represent females and squares represent males.

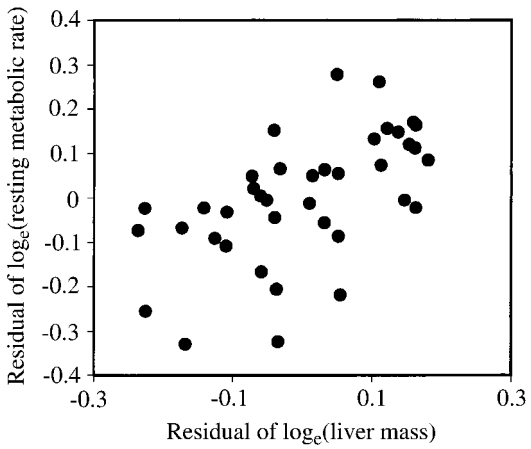

Fig. 2. Relationship between the residual of log$_e$(liver mass) (g) and the residual of log$_e$(resting metabolic rate) (ml O_2 min^{-1}) across 39 mice drawn from two lines selected for high and low food intake, with the effects of strain and sex on both traits removed.

P<0.001) in high-intake mice compared with low-intake mice (Table 1), and similar effects were apparent in the large intestine (t_{36}=1.29, P=0.12; Table 1).

Within-strain effects

To explore the associations between RMR and tissue morphology, within strains, we first removed the strain and sex effects on both RMR and tissue masses using the exponents derived from the individual GLM analyses which had included total body mass and strain and sex (dummy variables) as predictors (excluding the non-significant interaction terms).

We examined the relationships between individual variations in RMR and the mass of each tissue component separately by entering each tissue mass as a separate predictor in individual regression analyses (Table 4). This analysis revealed that the masses of five organs were significantly positively linked with individual variation in RMR (once the strain and sex effects had been removed). These were the carcass, tail, liver, spleen and heart. The strongest relationship was between the mass of the liver and RMR (Fig. 2). We entered the individual masses collectively as independent predictors in a stepwise regression analysis (backward deletion: F to enter or remove=4.0). The only variable that entered the equation as a significant predictor was liver mass. These analyses indicate that the major factor influencing variation in the RMR of these animals was variability in the mass of the liver. However, these analyses are questionable for several reasons. First, we already know that several of the organ masses were significantly associated with overall body mass (independent of the strain effect), and overall body mass was also correlated with RMR (independent of strain); hence, at least some of the explained variation in RMR caused by individual organ masses must reflect this shared variation. Second, the individual organ masses are inter-correlated with one another independently of their relationships to overall mass, further compromising the relationship between any particular organ mass and RMR. Finally, the

Table 4. *Relationships between resting metabolic rate and tissue morphology*

	RMR			Residual RMR		
Tissue	CD	*F*	Sign	CD	*F*	Sign
Carcass	**13.6**	**5.8***	+	0.8	0.3	
Pelage	0.3	0.1		**10.6**	**4.4***	–
Tail	**20.9**	**9.8*****	+	8.7	3.5?	+
LI	6.2	2.4?	+	0.2	0.1	
SI	0.0	0.0		0.8	0.3	
Pancreas	1.0	0.4		0.1	0.0	
Stomach	2.9	1.1		1.0	0.4	
Liver	**33.5**	**18.6*****	+	**21.2**	**10.0****	+
BAT	0.4	0.2		2.8	1.1	
Abdominal WAT	0.0	0.0		5.4	2.1	
Mesenteric WAT	0.4	0.1		2.2	0.8	
Subcutaneous WAT	1.4	0.5		9.3	3.8?	–
Spleen	**18.4**	**8.3****	+	**10.7**	**4.4***	+
Thyroid	0.8	0.3		0.8	0.3	
Gonads	3.7	1.4		1.1	0.4	
Kidneys	8.2	3.3?		3.4	1.3	
Heart	**11.8**	**4.9***	+	9.6	3.9?	–
Lungs	6.1	2.4		3.3	1.3	
Brain	5.9	2.3		6.1	2.4	

Results for individual regressions are presented in which the dependent variable was resting metabolic rate (RMR) and the predictor variables were the masses of the individual tissues (with the effects of strain and sex differences on both traits removed) and where the dependent variable was residual RMR and the predictor variables were also residual organ masses (i.e. with the variation due to differences in overall body mass and both sex and strain differences removed).

CD is the coefficient of determination ($r^2 \times 100$).

BAT, brown adipose tissue; WAT, white adipose tissue; LI, large intestine; SI, small intestine.

Sign indicates the direction of any significant association.

Values in bold type are significant: *$0.01<P<0.05$; **$0.001<P<0.01$; ***$P<0.001$; ?$0.05<P<0.1$.

use of stepwise multiple regression is questionable in this circumstance, not only because the predictors are not orthogonal but also because the ratio of predictors (N=19) to observations (N=39 mice) was not very high (approximately 2, when 6 is desirable).

To eliminate the first of these problems, we repeated the analyses but this time utilised the residual RMR as the dependent variable (taking into account the effects of strain, sex and body mass) and sought associations between this trait and the residual masses of the individual organs (again removing the effects of strain, sex and mass) – thus removing any shared variability due to mass, sex and strain effects (Table 4). In this revised analysis, three residual organ masses had significant associations with residual RMR: the residual masses of the liver and spleen were positively associated, while the residual mass of the pelage was negatively associated. The most significant correlate was the residual liver mass (Fig. 3). In a stepwise multiple regression analysis,

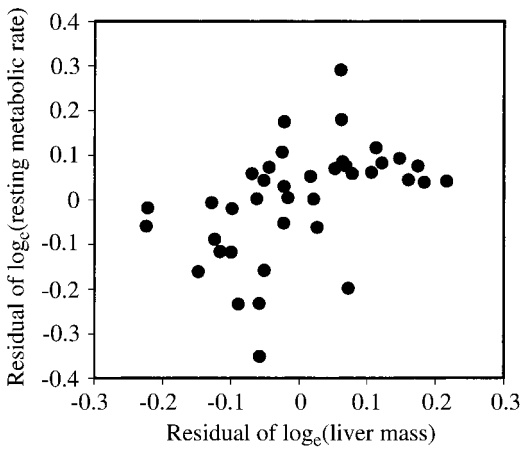

Fig. 3. Relationship between the residual of log$_e$(liver mass) (g) and the residual of log$_e$(resting metabolic rate) (ml O_2 min^{-1}) with the shared variation due to correlations between both traits and body mass as well as the effects of sex and strain removed.

the only two variables that remained significant were the residual masses of the liver (positive association) and the pelage (negative association), which together explained 33.4 % of the variation in residual RMR. This analysis also points to a dominant effect of the liver even when the shared variation due to body mass is eliminated (Fig. 3), but the stepwise regression analysis is flawed by the lack of orthogonality in the predictor variables and the poor ratio of sample size to the number of predictors.

To overcome this latter problem, we pooled the masses of several organs into functional groupings, thus reducing the number of predictor variables from 19 to 5. The functional groupings were the 'carcass', consisting of the carcass, tail and pelage, the alimentary system, consisting of the stomach, large intestine, small intestine and pancreas, the 'white adipose tissue', consisting of the subcutaneous, mesenteric and abdominal fat stores, the 'other organs', consisting of the brown adipose tissue, spleen, thyroid, gonads, kidneys, heart, lungs and brain, and, finally, we separated the liver as the fifth variable, since the previous analyses had indicated its importance and we did not want its effects to mask other potentially important variables. We eliminated the independent effects of strain and body mass on these variables and then sought relationships between the residual masses of these five predictors and residual RMR in a stepwise regression. The only factor that emerged as a significant predictor of residual RMR in this analysis was the residual mass of the liver.

Between-strain effects of morphology on RMR

The effects of strain on RMR amounted to 0.118 log units (equation 1: S.D.=0.057). Since the dominant morphological variable influencing the RMR in the within-strain analysis was the mass of the liver, we addressed the question of whether the effect of strain on RMR could be explained solely by the correlated response to selection of (or drift in) the mass of the liver, or whether other factors might be involved – such as an independent correlated response or genetic drift sampling in

RMR or an effect mediated *via* other tissues. The effect of the residual log(liver mass) on residual logRMR had a gradient of 0.557. Given that the effect of selection strain on liver mass amounted to 0.157 log units, the anticipated effect of the changed liver mass on RMR was 0.557×0.157=0.087 log units. This prediction did not differ from the observed effect of strain on RMR (t=0.50, P>0.05). Hence, the effect of strain on RMR could be interpreted as entirely a consequence of the strain differences in the mass of the liver.

Discussion

In previous studies, the heart and kidney (Daan et al., 1990) and the alimentary tract (Speakman and McQueenie, 1996; Konarzewski and Diamond, 1995) were the dominant variables correlated with differences in resting metabolic rate (RMR). Other studies have showed no positive dominant variables (Koteja, 1996a; Burness et al., 1998; Geluso and Hayes, 1999; Speakman and Johnson, 2000). In contrast, the indication from the various analyses performed in the present study was that the most significant morphological trait linked to changes in RMR was the mass of the liver. Despite the fact that the high-intake strain had a longer small intestine, by approximately 15 %, this length difference was not mirrored by an increased dry tissue mass of the small intestine (Table 2). In addition, there was no evidence associating variability in the mass of the alimentary tract with differences in RMR.

These observations suggest that mice possess considerable flexibility in their gut morphology correlated with the capability to process an increased food intake. Most importantly, however, this variability can be achieved without necessarily involving an increased amount of tissue or an increased RMR. Our findings do not support the suggestion that the correlation between sustained daily intake and resting metabolic rate stems from the effects of changes in the masses of the alimentary tract that are permissive of greater food intake but require elevated maintenance costs (Weiner, 1992; Hammond and Diamond, 1997). However, our study does indicate that increases in metabolic rate may be contingent on changes in wider aspects of the 'alimentary system' as a whole – most particularly in the size of the liver, which may also be enlarged in response to differences in food intake and, hence, the processing requirement for this food.

An effect of liver mass on residual RMR is understandable because it is the heaviest organ in the body, being 2–3 times heavier than, for example, the small intestine and 8–10 times heavier than the heart, and makes up approximately 5 % of the total tissue mass (Table 2). Moreover, *in vitro* studies indicate it has one of the highest rates of tissue oxygen consumption (Field et al., 1939; Krebs, 1950). In combination, the large tissue mass expending energy at high rates explains why the liver contributes significantly to the RMR.

These effects of liver mass are consistent with our previous study in reproducing mice (Speakman and McQueenie, 1996) in which the dominant variable associated with changes in RMR between pre-breeding and lactating females was the mass of the total alimentary system, since this included the mass of the liver. In addition, the between-strain effects on RMR measured here could be accounted for completely by the correlated response to selection (or drift) in the size of the liver. However, the general importance of liver mass as a key factor influencing individual variation in metabolic rates is unclear, since it has not emerged as a significant factor in many other studies (Daan et al., 1990; Konarzewski and Diamond, 1995; Koteja, 1996a; Burness et al., 1998; Geluso and Hayes, 1999), including studies in our laboratory (Speakman and Johnson, 2000). This may be because in these previous studies variability in liver mass has been relatively small compared with the differences generated by comparing animals within and between the high- and low-intake strains used here or the differences between pre-breeding and lactating mice reported previously (Speakman and McQueenie, 1996). The impact of this low variability in liver mass on RMR may therefore have been masked by the impacts of other factors – either effects of other morphological factors or the roles of environmental effects on individual variability in RMR.

Our study highlights the value of using artificial selection experiments to generate unique resources that can be employed to test ecophysiological hypotheses concerning the associations of traits (as suggested by Garland and Carter, 1994). These data provide support for the idea that sustained daily energy expenditure and basal (resting) metabolic rate may be linked in a causal manner by the effects of sustained demands on the sizes of organs that are expensive to maintain in the resting state (Drent and Daan, 1980; Weiner, 1992; Peterson et al., 1990; Hammond and Diamond, 1997; but see Ricklefs et al., 1996; Speakman, 2000). In the broadest sense, our studies implicate the alimentary system in this association, but it appears that the liver is the most significant organ in this respect rather than the alimentary tract itself, as has been assumed previously (e.g. Weiner, 1992; Konarzewski and Diamond, 1995; Hammond and Diamond, 1997).

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