

Anatomic and Energetic Correlates of Divergent Selection for Basal Metabolic Rate in Laboratory Mice

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ABSTRACT

The aerobic capacity model postulates that high basal metabolic rates (BMR) associated with endothermy evolved as a correlated response to the selection on maximum, peak metabolic rate $\dot{V}O_{2max}$. Furthermore, the model assumes that BMR and $\dot{V}O_{2max}$ are causally linked, and therefore, evolutionary changes in their levels cannot occur independently. To test this, we compared metabolic and anatomical correlates of selection for high and low body mass-corrected BMR in males of laboratory mice of F18 and F19 selected generations. Divergent selection resulted in between-line difference in BMR equivalent to 2.3 phenotypic standard deviation units. $\dot{V}O_{2max}$ elicited by forced swimming in 20°C water was higher in the low BMR than high BMR line and did not differ between the lines when elicited by exposure to heliox at -2.5°C. Moreover, the magnitude of swim- and heliox-induced hypothermia was significantly smaller in low BMR mice, whereas their interscapular brown adipose tissue was larger than in high BMR mice. Our results are therefore at variance with the predictions of aerobic capacity model. The selection also resulted in correlated response in food consumption (*C*) and masses of metabolically active internal organs: kidneys, liver, small intestine, and heart, which fuel maximum, sustained metabolic rate (SusMR) rather than $\dot{V}O_{2max}$. These correlated responses were strong enough to claim the existence of positive, genetic correlations between BMR and the mass of viscera as well as *C*. Thus, our findings support the suggestion that BMR evolved as a correlated response to selection for SusMR, not $\dot{V}O_{2max}$. In functional terms BMR should therefore be interpreted as a measure of energetic costs

of maintenance of metabolic machinery necessary to sustain high levels of energy assimilation rate.

Introduction

Basal metabolic rate (BMR) is measured under strictly defined laboratory conditions that are very much different from natural settings (Hulbert and Else 2004). Yet, factors underlying variation in BMR are of great interest not only to physiologists but also to ecologists and evolutionary biologists (for review, see McNab 2002). A key assumption of ecological and evolutionary considerations is that BMR is inexorably linked to other components of energy budgets. These links result in close associations of BMR with important ecological characteristics, such as food habits and prime life-history traits—population density, productivity, and longevity (McNab 2002; White and Seymour 2004). Most notably, the explanation of variation in BMR is a part of the aerobic capacity model of the evolution of endothermy (Benett and Ruben 1979; Hayes and Garland 1995). The model assumes that physiological capacities for high rates of aerobic metabolism, typical of homeotherms, are inescapably related to high levels of BMR; that is, the selection for high aerobic capacity results in increased BMR as a correlated evolutionary response.

There are several proximate mechanisms that are likely to explain the link between BMR and aerobic capacity. Several studies suggested that high levels of BMR are associated with increased mitochondria density (Else and Hulbert 1985), increased leakiness of plasma membranes (Wu et al. 2001; Else et al. 2004), and increased relative proportion of metabolically active internal organs (Daan et al. 1990; Konarzewski and Diamond 1995). Thus, from the functional point of view, one can hypothesize that BMR primarily reflects the minimum energetic costs of the running of metabolic machinery necessary to generate high aerobic capacity (Farmer 2000; Koteja 2000, 2004). However, studies on the contribution of metabolically active body components to BMR, as well as postulated relationships between maximal and basal metabolism, yielded conflicting results. For example, while some authors found strong correlations between mammalian resting metabolic rates and internal organ masses (e.g., Konarzewski and Diamond 1995), other studies failed to detect them or reported only weak associations (Koteja 1996a; Speakman and Johnson 2000; Selman et al. 2001; Speakman et al. 2004). The results of studies on the correlation

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between BMR and aerobic capacity are even more inconclusive (reviews in Hayes and Garland 1995; Ricklefs et al. 1996; Speakman 2000). Reasons for this seem threefold. First, the aerobic capacity model requires that there is a positive additive genetic correlation between resting and maximal metabolic rates (Hayes and Garland 1995). However, many earlier studies attempted to identify postulated correlates of BMR on an interspecific level (e.g., Ricklefs et al. 1996; Speakman 2000), which makes interpretation of genetic associations difficult. Second, repeatability of within-individual measurements of energy expenditures in free-ranging animals is low, which hampers their interpretation (e.g., Berteaux et al. 1996). Third, and most important, to our knowledge none of the earlier studies attempted to directly manipulate genetic architecture of BMR (or maximum metabolic rates) and then rigorously analyze the consequences of such manipulation for body composition and aerobic capacity. The major advantage of experimental manipulation of the levels of BMR is its explanatory power to distinguish noncausative correlations between BMR, aerobic capacity, and anatomy from biologically meaningful, inescapable links. Furthermore, an experimental approach is likely to shed light on the genetic architecture of postulated associations, which is essential for evolutionary inference (Hayes and Garland 1995; Garland 2003).

One of the most promising experimental approaches to study functional significance of variation in traits related to fitness is artificial selection (Garland 2003). It allows an analysis of directional changes in phenotypic and genetic architecture and their correlates under controlled and reproducible conditions. Correlated responses to artificial selection pressure may thus reveal physiological and biochemical mechanisms underlying postulated associations. Here we report the results of divergent artificial selection for BMR in laboratory mice. By means of this selection, we created two lines of mice whose body mass-corrected BMRs at generation F19 are consistently separated by 2.3 phenotypic standard deviations (SDs). The objective of our study was threefold. First, we asked whether a decrease or an increase in BMR results in respective changes in masses of internal organs: small intestine, liver, heart, kidneys, and interscapular brown adipose tissue (IBAT), as well as metabolically inert fat stores, estimated here by means of total body electrical conductivity (TOBEC) technique (Walsberg 1988; Koteja 1996b). By doing so we aimed to test whether BMR mainly reflects metabolic costs of maintenance of energetically expensive components of metabolic machinery, fueling high aerobic capacity. The second question we addressed was whether changes in BMR are correlated with the changes in peak metabolic rate, as predicted by the aerobic capacity model. We analyzed between-line differences in two measures of aerobic capacity: maximum oxygen consumption elicited (1) by cold exposure to heliox atmosphere and (2) by forced swimming in 20°C water. Finally, we tested between-line differences in the rate of energy assimilation, because it is likely that BMR is

coupled with energy expenditures sustained over long periods of time rather than peak metabolic rates (Farmer 2000; Koteja 2000, 2004). To our knowledge, this is the first attempt to test the functional significance of variation in BMR by means of artificial selection.

Material and Methods

Animals

We used males of outbred Swiss-Webster laboratory mice of the lines subjected to divergent artificial selection toward high (H-BMR) and low (L-BMR) basal metabolic rate, carried out in the Institute of Biology, University of Białystok, Poland. Briefly, depending on the breeding success, in subsequent generations we maintained 15–20 families in each selected line. Whenever possible, no less than three randomly chosen males and three females from each family were subjected to metabolic trials. Animals characterized by highest and lowest residual values of BMR were chosen as progenitors of the high and the low line, respectively, and mated outside their families. A similar procedure was repeated in subsequent offspring generations, applying directional individual selection design (Falconer and Mackay 1996). BMR was measured in 12–18-wk-old mice. Mass-independent BMRs of individual mice were calculated as residuals from the regression of BMR on metabolic chamber number as categorical predictor, body mass, date of measurement, and time of day.

The animals were housed in same-sex and same-family groups of four to five per cage at 23°C. They were maintained on a 12 : 12 light-dark cycle and had unlimited access to murine chow and water.

Measurements of Oxygen Consumption

For all metabolic measurements, we used a positive-pressure, open-circuit respirometry system. Outside atmospheric air (or a mixture of 79% helium/21% oxygen: heliox) was pushed through a column of Drierite to remove water vapor and forced through a copper coil submerged along with metabolic chambers in a water bath to equalize and control the temperature. Depending on the type of measurement, the airstream was then divided to up to three independent streams (plus control airstream), each fed to a separate mass flow controller (Sierra Instruments, Monterey, Calif., or ERG-1000, Warsaw). In measurements of BMR, we sequentially monitored three metabolic chambers (each 350 cm³ in volume). To achieve the greatest possible measurement precision, the number of chambers was reduced to two (560 cm³ each) during measurements of the maximum metabolic rate elicited by swimming ($\dot{V}O_{2\text{swim}}$) and reduced to one (350 cm³) for the estimation of maximum metabolic rate elicited by exposure to heliox ($\dot{V}O_{2\text{heliox}}$).

The gas streams were forced through individual metabolic chambers at the rate of 400 mL/min or 700 mL/min during

measurements of BMR and maximum metabolic rates, respectively. The streams were then directed to a computer-controlled channel multiplexer, part of a Sable Systems TR-1 oxygen analyzer setup (Henderson, Nev.). The analyzed gas stream was scrubbed of CO₂ (Carboabsorb AS, BDH Laboratory Supplies, U.K.), redried (Drierite), subsampled at the rate of 75 mL/min with a subsampler, and then passed through the sensor of an S-3A/I Applied Electrochemistry (Pittsburgh, Pa.) analyzer. The electrical signal from the analyzer was filtered through a baselining system, interfaced to an analog-to-digital converter, and fed to a computer that averaged readings every 1 s (BMR trials) or 0.5 s ($\dot{V}O_{2\max}$ trials).

All metabolic trials were carried out between 0800 and 2000 hours. Before measurements of BMR, mice were fasted for 6 h. We elected not to fast them for a longer period because longer fasts resulted in increased locomotor activity (M. Konarzewski, A. Książek, and I. B. Łapo, unpublished results). Measurements were taken during the last 2 h of the 3-h trial period at 31–32°C, a temperature within the thermoneutral zone of our mice (M. Konarzewski et al., unpublished results). During the 2-h measurement period, each metabolic chamber was sequentially monitored for about 20 min. We defined BMR as the lowest readout that did not change during 4 min by more than 0.01% of oxygen concentration. If the readout for a given animal did not meet these criteria, the animal was subjected to another metabolic trial 1 wk later. Animals whose metabolic rates failed to meet the criteria the second time were excluded from further procedures.

To measure $\dot{V}O_{2\text{heliox}}$, we placed the mouse in a metabolic chamber vented with heliox and submerged in a glycol-based coolant at $-2.5^\circ\text{C} \pm 0.2^\circ\text{C}$. We defined $\dot{V}O_{2\text{heliox}}$ as the highest O₂ averaged over 2 min of the last 5 min of 15 min of heliox exposure.

To measure $\dot{V}O_{2\text{swim}}$, we used a vertically positioned cylindrical Plexiglas metabolic chamber (250 mm high, 115 mm diameter), vented with atmospheric air. The chamber was partly filled with water, leaving an air volume of 560 mL above the water level. The temperature of water within the chamber was maintained at $20^\circ\text{C} \pm 0.2^\circ\text{C}$. Each mouse was placed just above the water level on a movable platform and was allowed 10 min for adaptation. The platform was then abruptly submerged to force the animal to swim. $\dot{V}O_{2\text{swim}}$ was defined as the highest oxygen consumption averaged over 2 min of 5 min of swimming.

Metabolic data were analyzed with a Sable System DATACAN V software. We calculated oxygen consumption rates using equation (4a) of Withers (1977).

Food Intake and Digestibility

Food intake and digestibility were measured in individual mice separately housed at 23°C in cages equipped with plastic grids. Food remains (orts) and feces dropping to the bottom of the

cage were separated from each other, dried in an oven at 70°C, and weighed to the nearest 0.001 g. Daily food intake was calculated individually for each mouse during six consecutive days as the mass of food disappearing from the food dispenser that day minus orts. Apparent digestibility was calculated as the difference between food intake and fecal output divided by food intake.

Measurements of Body Mass, Core Temperature, and TOBEC

Before each trial, the animals were weighed to the nearest 0.1 g. Colonic temperature was measured to the nearest 0.1°C with a thermocouple thermometer (BAT-12, Physitemp Instruments, Clifton, N.J.) immediately before each estimation of lean body mass (LBM) with a TOBEC analyzer (ACAN-2, Jagmar, Poland), and before and after each measurement of $\dot{V}O_{2\text{heliox}}$ and $\dot{V}O_{2\text{swim}}$. The difference between pre- and posttrial core temperatures was taken as the magnitude of postheliox (ΔT_{heliox}) and postswim (ΔT_{swim}) hypothermia.

We used readings obtained from the ACAN-2 analyzer as estimates of LBM. We did not attempt to convert them to the absolute values of LBM, which would have required the calibration of the readings against independent measures of LBM. However, the readings are a strict linear function of LBM and body temperature (Koteja 1996b), so we are confident that they reflect between-line differences in LBM.

Morphometrics

Following metabolic trials, mice were killed by cervical dislocation. Metabolically active internal organs (small intestine, liver, kidneys, heart, and IBAT) were excised, cleared of blood, foodstuffs, and adherent fat. Next, they were weighed with an accuracy of 0.001 g, dried to a constant mass at 70°C, and reweighed.

Sequence of Trials

It took us 6 wk to complete BMR measurements on 189 males and 126 females of generation F19 as a routine part of our selection experiment. Next, a subset of 40 males was subjected to the second BMR trial to estimate its repeatability. We then carried out $\dot{V}O_{2\text{swim}}$ trials on one to three randomly chosen males of each of the family of both lines (total of 112 animals). A week later, the same individual animals were subjected to $\dot{V}O_{2\text{heliox}}$ trial, followed by the estimates of LBM by means of TOBEC technique. Finally, the males not qualified as progenitors were killed and were subjected to anatomic analyses.

Food consumption and digestibility were estimated in 23 males randomly chosen from different families of both lines of generation F18. These estimates were a part of another experiment (A. Książek, M. Konarzewski, and I. B. Łapo, unpublished results).

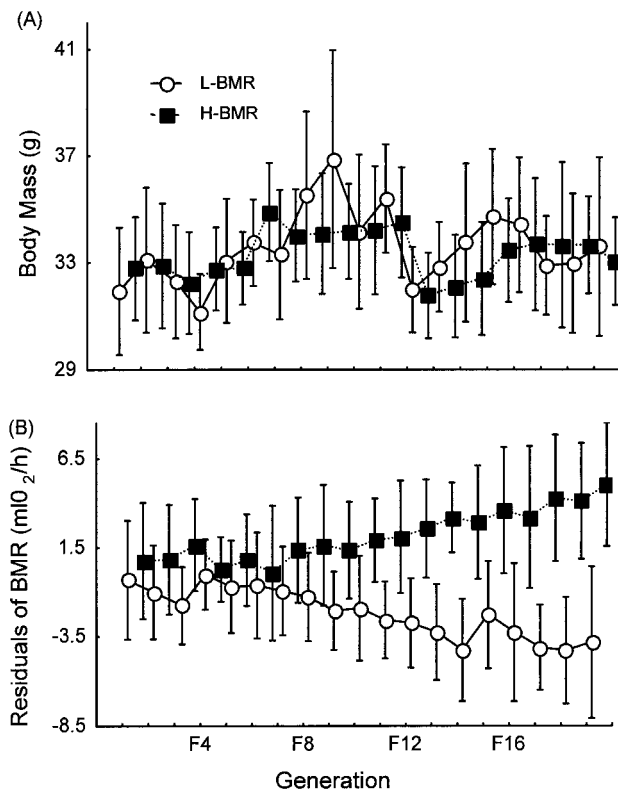


Figure 1. Changes in body mass (A) and body mass-corrected (B) basal metabolic rate (BMR) across generations of the low basal metabolic rate (L-BMR) and high basal metabolic rate (H-BMR) mouse lines.

Statistics

In most analyses we used the general linear models (GLM) of ANCOVA, with the effect of line as a fixed factor and family affiliation nested within line as a random factor controlling for possible effect of animal's relatedness (SAS Institute 1990). Depending on the analysis, body mass, day of measurement (with the first day set as day 0), and time of day were incorporated as covariates and tested for possible interactions. The effect of metabolic chamber number, controlling for the possible systematic biases resulting from the use of a multichannel system, was not significant ($P > 0.1$ in all series of measurements). However, to achieve greater precision of the estimates used in the artificial selection procedure, it was retained in the statistical models of BMR. Before performing statistical analyses, assumptions of parametric tests were assured (Sokal and Rohlf 1995).

Data on food consumption and digestibility were analyzed in two ways. (1) Their values averaged over six consecutive days of the feeding trial were subjected to ANCOVA/ANOVA as described above, with the initial body mass as covariate. We then used the residuals from these analyses to calculate SDs,

necessary to estimate genetic correlations. (2) Repeated measures ANOVA were undertaken, with line as a fixed factor and individual mice as a random factor nested within line. To account for the effect of body mass in repeated measures ANOVA, daily food consumption was first regressed on body mass measured at the beginning of a given day, and the obtained residuals were then analyzed.

In each generation, it took us 4–6 wk to complete the whole set of BMR measurements. It was therefore essential to test whether measurements collected over such a long period of time are repeatable. We estimated BMR repeatability as the Pearson product-moment correlation coefficient between the residuals from the two multiple regressions of BMR on body mass, date of measurement, and time of day, as well as the effect of line affiliation and metabolic chamber number, coded as “dummy variables” (Draper and Smith 1981). The first regression was calculated taking the random subset of BMR measurements on 40 males (part of the selection protocol). The second regression was calculated on BMRs of the same individual animals measured 5 wk later. We did not estimate the repeatability as the intraclass correlation coefficient (Lessells and Boag 1987) because body mass of individual mice increased significantly between the consecutive BMR measurements.

At the very beginning of our selection experiment, it became clear that we were unable to precisely measure BMR in more than eight to nine animals a day, which forced us to resign from the maintenance of replicated sublines within the selected lines. The lack of replications created a serious interpretation problem, because one can argue that highly significant, between-line differences observed in our experiment were likely to arise due to genetic drift rather than genuine inescapable associations between analyzed traits (Henderson 1989, 1997; Garland 2003). We attempted to bypass this problem by maintaining 15–20 families per selected line, which effectively reduced inbreeding coefficient and thus the effect of genetic drift (Falconer and Mackay 1996). Furthermore, to interpret our results, we relied on not only ANOVA/ANCOVA models described above but also analyzed them according to the guidelines suggested by Henderson (1989, 1997). We first expressed values of a particular trait for individual animals as residuals from multiple regression of this trait on body mass and, where appropriate, metabolic chamber number, date of measurement, and time of day. Thus, the residuals containing the effect of line and family affiliation were corrected for the effect of confounding variables. We then calculated within-line means of residual values (computed from family means) and the respective within-line SDs (hereafter called phenotypic SDs, interpreted as the products of the square root of the heritabilities and genetic SDs; Henderson 1997). Finally, we calculated standardized between-line difference (d) as the difference between within-line residual mean values divided by averaged within-line phenotypic SD (Henderson 1989, 1997).

In the absence of exact knowledge of narrow sense heritability

Table 1: Summary of results of ANCOVA or ANOVA

	Line Affiliation			Family Affiliation			Body Mass			Date			Time		
	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>
BMR	151.3	1, 143	<.0001	3.06	38, 143	<.0001	63.39	1, 143	<.0001	14.04	1, 143	.0003	6.36	1, 143	.01
$\dot{V}O_{2\text{swim}}$	7.87	1, 67	.006	2.29	38, 67	.001	14.51	1, 67	.0003	5.83	1, 67	.02
ΔT_{swim}	55.42	1, 67	<.0001	2.76	38, 67	.0001	.39	1, 67	.5	1.32	1, 67	.2
$\dot{V}O_{2\text{heliox}}$	2.41	1, 67	.1	2.6	38, 67	.0003	8.0	1, 67	.0062	1, 67	.6
ΔT_{heliox}	10.86	1, 67	.001	3.68	38, 67	<.0001	.61	1, 67	.401	1, 67	.9
<i>C</i>	15.47	1, 20	.0008	7.49	1, 20	.01
<i>D</i>	.15	1, 21	.7

Note. ANCOVA or ANOVA of basal metabolic rate (BMR), swim-elicited ($\dot{V}O_{2\text{swim}}$), heliox-elicited ($\dot{V}O_{2\text{heliox}}$) maximum metabolic rates, the respective magnitudes of elicited hypothermia (ΔT_{swim} and ΔT_{heliox}), as well as food consumption (*C*) and its apparent digestibility (*D*). Line affiliation and family affiliation (nested within line) were main factors, whereas body mass, date of the measurement, and time of day were covariates.

(h^2) of BMR and other traits, we conservatively assumed that their h^2 is close to 0.1. The coefficient of inbreeding (*F*; Falconer and Mackay 1996) of our selected lines was 0.25 (M. Konarzewski, A. Książek, and I. B. Łapo, unpublished results), and the total number of families in generation F19 equaled 40. We applied this information to interpolate values by Henderson (1997) presented in Table 3 to estimate 95% confidence intervals (CIs) of d , which may result from genetic drift and sampling error alone, that is, in the absence of genetic correlation between BMR and other analyzed traits. Thus, values of d laying beyond CIs should be indicative of genuine genetic correlations because between-line differences due to genetic drift should be smaller than those resulting from correlated response to selection for BMR (Henderson 1997). We concluded that even if between-line difference in a trait under consideration was statistically significant, there was little justification to suggest that BMR and a given trait are genetically correlated unless d was >1.0 . This is because values of $d < 1$ can be ascribed to genetic drift alone (Henderson 1997).

Results

Body Mass, BMR, and Its Repeatability

Body mass (BM) of mice of the H-BMR and L-BMR lines of generation F19 did not differ statistically (ANOVA, $F_{1,146} = 1.73$, $P = 0.2$). Furthermore, throughout the whole experiment, BM did not diverge between the lines (Fig. 1A). In contrast, between-line difference in mass-corrected BMR systematically increased starting from generation F7 (Fig. 1B). At generation F19, the difference was highly statistically significant (Table 1) and averaged 8.9 mL O_2 /h, which was equivalent to 2.3 phenotypic SDs (Table 2). BMR was also significantly affected by family affiliation, date of measurement, and not surprisingly, BM (Table 1).

Repeatability of BMR expressed as a correlation coefficient among residuals from regressions of BMR on BM, date of measurement, and time of day was highly significant ($r = 0.72$,

$P < 0.0001$) and reflected a good separation of the H-BMR from the L-BMR line, obtained on the basis of the measurements collected over the period of 5 wk (Fig. 2A). However, the effect of line affiliation evident in Figure 2A significantly elevates among-individual variation, and therefore correlation depicted in Figure 2A may upwardly bias within-individual repeatability. We therefore recalculated the residuals after removing the effect of line affiliation. The resulting correlation coefficient was much lower, albeit still highly significant ($r = 0.44$, $P < 0.005$; Fig. 2B).

Food Consumption, Digestibility, and Aerobic Capacity

Directional selection on BMR resulted in a significant correlated response in food consumption estimated in males of generation F18. Daily food consumption of mice of the H-BMR line was significantly higher than that of the L-BMR line when analyzed by either means of repeated measures ANOVA ($F_{1,21} = 12.95$, $P = 0.002$) or ANCOVA (Tables 1, 2). When expressed in phenotypic SD units, between-line difference in *C* was 1.5, which suggests positive genetic correlation between BMR and *C* (Table 2). On the other hand, neither repeated measures ANOVA ($F_{1,21} = 0.15$, $P > 0.7$) nor ANCOVA indicated significant between-line differences in digestibility (Tables 1, 2). This strongly indicates that mice of H-BMR line were not only characterized by higher *C* but also higher energy assimilation rate.

Maximum metabolic rate elicited by swimming ($\dot{V}O_{2\text{swim}}$) was significantly higher in L-BMR than in H-BMR mice (Table 1), but standardized between-line difference d was much below 1.0 when expressed in phenotypic SD units (Table 2). Family relatedness was significant as a random factor, whereas body mass and time of day were significant as covariates (Table 1). High metabolic capacity of L-BMR mice was corroborated by their substantially lower postswim hypothermia (ΔT_{swim}), as compared with H-BMR line (Tables 1, 2). When standardized in SD units, between-line difference was sufficiently large to claim

Table 2: Least square mean values (\pm SE) and residual mean (\pm SD) values calculated for L-BMR and H-BMR lines

	L-BMR		H-BMR		<i>d</i>
	Least Square Mean \pm SE	Residual Mean \pm SD	Least Square Mean \pm SE	Residual Mean \pm SD	
BMR	50.12 \pm .5	-3.82 \pm 4.2	59.04 \pm .5	5.05 \pm 3.5	2.29
$\dot{V}O_{2\text{swim}}$	260.1 \pm 2.8	8.4 \pm 22.4	249.1 \pm 2.9	-3.9 \pm 19.0	.59
ΔT_{swim}	9.1 \pm .2	-1.2 \pm 1.3	11.4 \pm .2	1.2 \pm 1.7	1.56
$\dot{V}O_{2\text{heliox}}$	291.5 \pm 3.4	-9 \pm 29.8	299.2 \pm 3.4	6.3 \pm 20.4	.29
ΔT_{heliox}	5.6 \pm .2	-.4 \pm 1.4	6.4 \pm .2	.3 \pm 1.1	.57
<i>C</i>	5.2 \pm .1	-.3 \pm .3	5.8 \pm .1	.3 \pm .5	1.50
<i>D</i>	.81 \pm .004	.8 \pm .009	.81 \pm .004	.8 \pm .016	0

Note. Metabolic rates (mL O₂/h), magnitudes of hypothermia ($^{\circ}$ C), food consumption (*C*, g/d) and its apparent digestibility (*D*, unitless), calculated for low basal metabolic rate (L-BMR) and high basal metabolic rate (H-BMR) lines from statistical models summarized in Table 1. Mean residual values of these traits (\pm phenotypic SD) were calculated from multiple regressions and averaged over family means within each line. Standardized between-line differences (*d*) are expressed in averaged phenotypic SD units.

the existence of the negative genetic correlation between BMR and ΔT_{swim} (Table 2).

Heliox-elicited maximum metabolic rate ($\dot{V}O_{2\text{heliox}}$) did not differ between the lines (Tables 1, 2). Across both lines, $\dot{V}O_{2\text{heliox}}$ and $\dot{V}O_{2\text{swim}}$ were significantly positively correlated ($r = 0.46$, $df = 38$, $P < 0.005$). Postheliox hypothermia was significantly lower in the L-BMR than in the H-BMR line (Tables 1, 2). However, this difference was too small to suggest the negative genetic correlation with BMR (Table 2). Between-line differences in postswim and postheliox hypothermia were largely due to low posttrial core temperatures in the H-BMR line, since pretrial temperatures did not differ between the lines (ANCOVA, $P > 0.05$).

Body Composition

BM-corrected masses of four visceral organs (small intestine, liver, kidneys, and heart) were consistently and substantially higher in H-BMR than in L-BMR mice (Tables 3, 4). In all cases, between-line differences were large enough to claim the existence of positive genetic correlations between BMR and the masses of examined viscera (Table 4). Likewise, larger internal organs of H-BMR mice were consistent with their much higher BM-corrected ACAN readings, which reflected considerably higher proportions of their lean body mass (Tables 3, 4). On the other hand, between-line difference in the mass of IBAT was opposite to what was observed in visceral organs. It was highly statistically significant and indicative of the negative genetic correlation with BMR (Tables 3, 4).

All between-line differences in internal organ masses were also highly significant when the absolute, rather than BM-corrected values, were analyzed ($P < 0.0001$ in all analyses). Furthermore, ANCOVAs on fresh organ masses corrected for their

dry masses did not reveal statistically significant between-line differences in organ water content ($P > 0.1$ in all cases). Thus, it is very unlikely that differences in organ masses presented in Tables 3 and 4 can be attributed to between-line differences in the content of metabolically inert water.

Discussion

Are BMR and Its Correlates Heritable?

All students of adaptive and evolutionary significance of variation in metabolic rates explicitly or tacitly assume that this variation is heritable; that is, the ratio of additive genetic variance to total phenotypic variance (h^2) is greater than 0 (Falconer and Mackay 1996). This assumption is critical, because only traits with nonzero heritabilities can be targets of both natural and artificial selection (Fisher 1958; Falconer and Mackay 1996). On the other hand, strong selection pressures on traits that have been closely and consistently related to fitness result in a reduced additive genetic variance and, therefore, low heritabilities (Fisher 1958; Lynch and Walsh 1998). Clearly, this reasoning should apply to BMR and its correlates if they were of selective importance. Indeed, in their recent study, Dohm et al. (2001) found little support for the existence of additive genetic variance of $\dot{V}O_{2\text{max}}$ in laboratory mice. Other studies, however, yielded estimates of heritability of $\dot{V}O_{2\text{max}}$ that significantly differed from 0 in humans (Bouchard et al. 1999) and garter snakes (*Thamnophis sirtalis*; Garland et al. 1990). Also, selection for voluntary wheel running in mice resulted in a small but significantly correlated response in $\dot{V}O_{2\text{max}}$ (Swallow et al. 1998). This finding is strongly supported by a significant response of $\dot{V}O_{2\text{max}}$ in deer mice (*Peromyscus maniculatus*) subjected to natural directional selection (Hayes and O'Connor 1999). Thus, it is safe to conclude that there is low but sufficient

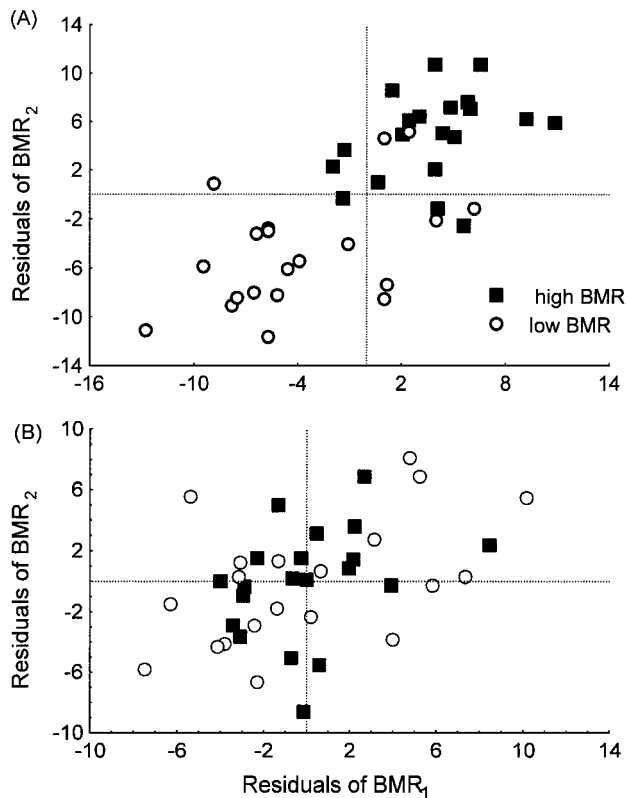


Figure 2. Repeatability of basal metabolic rate (BMR) estimated as correlations between measurements taken 5 wk apart on the same individual mice. Values are residuals from multiple regressions of BMR on body mass, date of measurement, and time of day (A), and line affiliation (B).

additive genetic variance underlying aerobic capacity to test their genetic associations with other traits.

Published estimates of heritability of BMR suggest that it is even lower than that of $\dot{V}O_{2\max}$ and perhaps close to 0 (Lacy and Lynch 1979; Dohm et al. 2001; Nespolo et al. 2003; Bacigalupe et al. 2004). Furthermore, heritabilities of some traits closely related to BMR, such as body temperature and mass of IBAT, are also barely different from 0 (Lacy and Lynch 1979; Lynch and Sulzbach 1984; Bacigalupe et al. 2004). However, there is strong evidence that makes generality of nonsignificance of h^2 of BMR questionable. First, heritabilities of masses of metabolically active internal organs, such as liver, kidneys, and components of the central nervous system, are high and fall between 0.4 and 0.8 (Schlager 1968; Roderick et al. 1973; Jones et al. 1992; Bacigalupe et al. 2004). Many studies repeatedly reported strong phenotypic correlations between masses of these organs and BMR (e.g., Daan et al. 1990; Konarzewski and Diamond 1995; Speakman et al. 2004). Although phenotypic correlations are not necessary proxies for genetic correlations (Roff 1997), their consistency across numerous independent analyses are pervasive of genetic correlations, which would not

exist without sufficient additive genetic variance of BMR. Second, BMR differs by about 30% among strains of laboratory mice (Konarzewski and Diamond 1995), which again suggests the presence of additive genetic variance (Falconer and Mackay 1996). Finally, our experiment provides compelling evidences for the existence of heritable variation of BMR in laboratory mice.

We showed that BMR measurements collected on the same individuals over long periods of 5 wk are repeatable (Fig. 2). The feature inherent to any heritable trait is its statistically significant repeatability, because nonreproducible characters cannot be heritable (Falconer and Mackay 1996). Under most circumstances, repeatability sets an upper limit to heritability (Dohm 2002). In our study, within-individual repeatability was 0.44 (Fig. 2B), which is in good agreement with recent estimates of repeatability of BMR in the bank vole (*Clethrionomys glareolus*; Labocha et al. 2004). This suggests that the selection progress evident in our experiment (Fig. 1B) can be mostly attributed to genetic variance.

Repeatability is also an important determinant of effectiveness of selection (Falconer and Mackay 1996). Figure 2A clearly shows that a high cross-line repeatability of BMR allowed us to correctly assign progenitors of the L-BMR and H-BMR lines, which is an essential condition for the progress of any selection. Nevertheless, separation of the lines at generation F19 was small in magnitude and reached 2.3 when expressed as phenotypic SDs. It is much smaller as compared with the results of selections on traits physiologically related to metabolic rates, for example, voluntary wheel running (Swallow et al. 1998) or swim-stress induced analgesia (Konarzewski et al. 1997; Łapo et al. 2003). Thus, although our experiment showed that it is possible to select for BMR, slow selection progress is clearly indicative of it being significantly different from 0, albeit with low heritability. This supposition is further corroborated by the estimates of realized heritability in our selection experiment (M. Konarzewski and A. Książek, in preparation), as well as estimates of h^2 of BMR in the bank vole (P. Koteja, personal communication).

Test of the Aerobic Capacity Model

From the quantitative genetics perspective, an implicit assumption of aerobic capacity model is the existence of a positive additive genetic correlation between BMR and maximal metabolic rates (Hayes and Garland 1995). Clearly, our results are at variance with the predictions of original formulation of the model. Instead of a postulated positive association between BMR and aerobic capacity, we observed higher values of $\dot{V}O_{2\text{swim}}$ in mice of the L-BMR than in the H-BMR line and no between-line difference in $\dot{V}O_{2\text{heliox}}$ (Tables 1, 2). These observations are strongly supported by lower swim- and heliox-induced levels of hypothermia in L-BMR than in H-BMR mice (Tables 1, 2). Higher aerobic capacity of L-BMR mice is further

Table 3: Summary of results of ANCOVA of internal organ and IBAT mass along with TOBEC readings estimating lean fresh body mass

	Line Affiliation			Family Affiliation			Body Mass		
	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>
Small intestine	27.79	1, 67	<.0001	2.26	38, 67	.001	11.83	1, 67	.001
Liver	88.38	1, 67	<.0001	2.5	38, 67	.0005	35.68	1, 67	<.0001
Kidneys	122.6	1, 67	<.0001	5.09	38, 67	<.0001	27.3	1, 67	<.0001
Heart	32.33	1, 67	<.0001	1.64	38, 67	.03	10.2	1, 67	.002
IBAT	24.78	1, 67	<.0001	1.89	38, 67	.01	19.97	1, 67	<.0001
TOBEC readings	141.75	1, 65	<.0001	2.75	38, 65	.0002	7.75	1, 65	.007

Note. Line affiliation and family affiliation (nested within line) were main factors, whereas body mass was a covariate. IBAT = interscapular brown adipose tissue, TOBEC = total body electrical conductivity.

corroborated by their larger masses of IBAT (Tables 3, 4). Most importantly, the magnitude of between-line differences in ΔT_{swim} and IBAT was large enough to claim the existence of the negative genetic correlations between these traits and BMR. Hence, our results do not support the existence of an inexorable positive correlation between BMR and maximum metabolic rates, as postulated by Benett and Ruben's (1979) model.

It is important to note, however, that selection for BMR resulted in a substantial increase in energy assimilation rate accompanied by an increase of internal organs associated with processing metabolic substrates. In particular, our study suggests the existence of positive genetic correlations between BMR and body mass-corrected sizes of small intestine, liver, kidneys, and heart, which (except for the heart) are unlikely to contribute to high levels of $\dot{V}O_{2\text{max}}$ and primarily serve to fuel high levels of sustained maximum energy expenditures (SusMR; Peterson et al. 1990; Hammond and Diamond 1997). Thus, our findings strongly support the suggestion that the evolution of endothermy and therefore high levels of BMR was a side effect of selection for SusMR, not $\dot{V}O_{2\text{max}}$ (Farmer 2000; Koteja 2000,

2004). The proximate mechanism of the link between BMR and SusMR lies in the increased size of internal organs, which permits high rates of energy assimilation but inescapably incurs elevated maintenance costs, reflected in BMR (Kersten and Piersma 1987; Daan et al. 1990; Weiner 1992; Hammond and Diamond 1997).

Functional Variation in Organ Masses and BMR

The magnitude of indirect responses of internal organ masses to selection for BMR suggests that BMR is inexorably linked and most probably genetically correlated to changes in their size. Internal organs have disproportionately high mass-specific metabolic rates (Krebs 1950) so that they most likely contribute disproportionately to BMR. However, several studies did not find correlations between BMR and particular organs, such as small intestine or kidneys, or the correlations became nonsignificant when organ water content was taken into account (e.g., Koteja 1996a; Selman et al. 2001). Our results showed that observed changes in organ masses cannot be attributed to sig-

Table 4: Least square mean values (\pm SE) of body mass, internal organ mass, and IBAT mass (g) along with TOBEC readings

	L-BMR		H-BMR		<i>d</i>
	Least Square Mean \pm SE	Residual Mean \pm SD	Least Square Mean \pm SE	Residual Mean \pm SD	
Body mass	33.6 \pm .3	33.6 \pm 3.3	33.1 \pm .3	33.0 \pm 1.6	.22
Small intestine	1.092 \pm .02	-.063 \pm .11	1.231 \pm .02	.063 \pm .12	1.11
Liver	2.077 \pm .03	-.158 \pm .21	2.435 \pm .03	.189 \pm .15	1.91
Kidneys	.506 \pm .006	-.043 \pm .061	.596 \pm .006	.050 \pm .051	1.64
Heart	.211 \pm .004	-.014 \pm .018	.241 \pm .004	.016 \pm .022	1.52
IBAT	.229 \pm .005	.020 \pm .022	.192 \pm .005	-.017 \pm .034	1.35
TOBEC readings	219.1 \pm 2.4	-20.5 \pm 12.6	260.8 \pm 2.4	19.7 \pm 21.5	2.35

Note. Calculated from statistical models summarized in Table 3. Mean residual values of these traits (\pm phenotypic SD) were calculated from multiple regressions and averaged over family means within each line. Standardized between-line differences (*d*) are expressed in averaged phenotypic SD units. L-BMR = line with low basal metabolic rate, H-BMR = line with high basal metabolic rate, IBAT = interscapular brown adipose tissue, TOBEC = total body electrical conductivity.

nificant between-line differences in their hydration. Also, the lack of between-line difference in body mass (Table 4), along with conspicuous differences in absolute masses of internal organs, suggests that between-line difference in TOBEC readings (i.e., lean fresh body mass) was principally due to the relative increase/decrease of the size of metabolically active internal organs rather than divergent changes in metabolically inert fat. This is an important observation, because a conspicuous between-line difference in fat accumulation may by itself create spurious between-line differences in body mass-corrected metabolic rates.

The masses of internal organs correlate with each other (Konarzewski and Diamond 1995). However, it remains an open question to what extent the organ mass is a good approximation of its metabolic costs of maintenance contributing to BMR (Speakman et al. 2004). Heritabilities of aerobic enzyme activities are low (Garland et al. 1990). Likewise, brush-border activities of intestinal transporters do not change appreciably with the size of intestines (Fan et al. 1996). We can therefore hypothesize that changes in internal organ size rather than changes in their mass-specific metabolic rates are primary contributors to BMR.

Even though the strength of our inference is weakened by the lack of replications, our results demonstrate that artificial selection experiments have much to offer in physiological and evolutionary ecology. Most importantly, they call attention to the necessity of integration of the analyses of genetic variation into rigorous and conclusive tests of the hypotheses related to the evolution of metabolic rates.

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