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Metabolic correlates of selection on aerobic capacity in laboratory mice: a test of the model for the evolution of endothermy

Andrzej K. Gębczyński* and Marek Konarzewski
Institute of Biology, University of Białystok, Swierkowa 20B, 15-950 Białystok, Poland
*Author for correspondence (andgebcz@uwb.edu.pl)

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SUMMARY

According to the aerobic capacity model of the evolution of endothermy, high levels of basal/resting metabolic rate (BMR/RMR) underlying endothermy have evolved as a correlated response to selection for high rates of aerobic metabolism ($\dot{V}_{O2max}$). To test the model we studied metabolic, behavioural and morphological correlates of replicated selection on maximum body mass-corrected metabolism elicited by swimming ($\dot{V}_{O2swim}$) in male laboratory mice. While 10 generations of selection did not change body mass, it resulted in a 12% difference in $\dot{V}_{O2swim}$ between mice of selected and control line types and significant, correlated responses in maximum metabolic rates elicited by exposure to cold in a helium–oxygen atmosphere ($\dot{V}_{O2He}$), and during forced running on a motorized treadmill ($\dot{V}_{O2run}$). Selected and control lines also significantly differed with respect to duration of running (a measure of stamina, t_{run}), and the distance run to exhaustion (d_e). However, the selection protocol did not result in elevated BMR and voluntary activity. Higher $\dot{V}_{O2max}$ in selected animals was positively correlated with higher masses of gastrocnemius muscles and heart but not of other visceral organs (intestine, stomach, liver and kidneys). These findings provide a mechanistic explanation for the lack of correlation between basal and maximal metabolic rates in selected mice. Overall, our study does not support the assumptions of the aerobic capacity model for the evolution of endothermy.

Key words: evolution of endothermy, artificial selection, metabolic rate, aerobic capacity.

INTRODUCTION

Endothermy is a hallmark of the physiology of birds and mammals. It is therefore unsurprising that the evolution of endothermy has attracted much attention by palaeontologists, as well as ecologists, geneticists and physiologists (for reviews, see Ruben, 1995; Else et al., 2004; Hillenius and Ruben, 2004). Yet, the mechanisms of the evolution of endothermy are still a matter of debate. For the last three decades a central hypothesis in this debate has been the aerobic capacity model by Bennett and Ruben (Bennett and Ruben, 1979), who proposed that endothermy evolved as a correlated response to selection on the ability to maintain maximal levels of aerobic metabolism. According to their model, the high levels of compulsory heat production characteristic of endotherms (i.e. high basal or resting metabolic rates, BMR/RMR) arose as a consequence of the elevated cost of maintenance of the metabolic machinery necessary to handle high rates of aerobic metabolism.

One of the main reasons Bennett and Ruben's model has received so much attention is that despite its palaeontological connotations it can be tested with extant living animals. The model became particularly attractive once formalized in quantitative genetics terms by Hayes and Garland (Hayes and Garland, 1995), who proposed to test it through analysis of the genetic correlation between resting (basal) and maximal metabolic rates ($\dot{V}_{O2max}$). Hayes and Garland reasoned that selection on $\dot{V}_{O2max}$ should result in a correlated increase of BMR, leading to the acquisition of endothermy only if the two traits are heritable and share a significant proportion of additive genetic variance. Until recently, however, most of the published tests of the aerobic capacity model were carried out at a phenotypic, rather than genetic, level (for reviews, see Gomes et al., 2004; Rezende et al., 2004). Phenotypic variation is inadequate for testing quantitative genetics hypotheses, as both the sign and magnitude of phenotypic and genetic correlations may differ (Lynch and Walsh, 1998; Roff, 2002). To date, only two studies have analysed components of genetic variance pertaining to Bennett and Ruben's hypothesis. Dohm and colleagues (Dohm et al., 2001) analysed variance by means of the 'animal model' approach and reported a significant correlation between BMR and $\dot{V}_{O2max}$ in house mice, but only when they used the constrained model of the partitioning of genetic variance (e.g. with dominance variance set to zero). Likewise, Sadowska and colleagues (Sadowska et al., 2005) reported a significant genetic correlation between BMR and the $\dot{V}_{O2max}$ elicited by swimming in the bank vole (*Myodes glareolus*).

However, both analyses were carried out on non-manipulated populations. From a methodological perspective a much stronger test of the aerobic capacity model should be provided by artificial selection experiments, which allow one to manipulate the frequencies of genes directly related to the expected correlations (Garland, 2003). We are aware of the results of three such experiments, of which two do not support the predictions of the aerobic capacity model. A direct artificial selection on high levels of voluntary wheel running in house mice did not result in elevated BMR (Vaanholt et al., 2007; Kane et al., 2008), in spite of a significant elevation of $\dot{V}_{O2max}$ resulting from the selection (Swallow et al., 1998; Rezende et al., 2005). Książek and colleagues (Książek et al., 2004) demonstrated a negative, rather than a positive, relationship between basal and maximal metabolic rates (elicited by swimming) in laboratory mice divergently selected on BMR. Furthermore, we (Gębczyński and Konarzewski, 2009) demonstrated a lack of difference in $\dot{V}_{O2max}$ (elicited by running) between those strains of mice. In contrast, however, artificial selection on $\dot{V}_{O2max}$ elicited by swimming in 30°C water resulted in an elevated BMR in the bank vole (Sadowska, 2008).

The inconsistency in analyses of genetic correlations between basal and maximal metabolic rates point to the limitations of inference based on the quantitative genetics approach. There are at least two major reasons why testing the aerobic capacity model by this approach may prove equivocal. First, the components of variance/covariance matrices not only differ between species but also may vary between populations of the same species (Lynch and Walsh, 1998; Simões et al., 2008). Thus, the results of quantitative genetic analyses may not be repeatable across species and populations. More importantly, however, artificial selection experiments that strictly emulate the evolutionary scenario envisaged by the aerobic capacity model may not be feasible, as their design and execution is always subject to compromise between the conflicting demands of statistics, logistics and the selection objective (Garland, 2003; Konarzewski et al., 2005). Nonetheless, selection experiments are the only way of experimentally testing the aerobic capacity model. Most importantly, when appropriately designed they allow one to study different evolutionary 'solutions' arising as a response to the applied selection regime (Garland, 2003; Houle-Leroy et al., 2003).

Here we used artificial selection to create replicated lines of laboratory mice selected for high levels of aerobic capacity elicited by 5 min swimming in 25°C water. Ten generations of selective breeding resulted in $\dot{V}_{O2max}$ averaging 12% higher in selected than in control lines. The objective of our study was threefold. First, we tested whether a selection-induced change in $\dot{V}_{O2max}$ elicited by swimming resulted in changes of maximum oxygen consumption during cold exposure, forced running and spontaneous locomotor activity. We thus tested whether the applied selection protocol resulted in a concomitant increase in thermogenic and locomotor capacity, the latter being directly related to Bennett and Ruben's (Bennett and Ruben, 1979) hypothesis. Second, we looked for correlated changes in BMR, as predicted by the aerobic capacity model (Hayes and Garland, 1995). Last, we analysed the selection-induced changes in the masses of internal organs (liver, kidneys, small intestines and heart) as well as musculature (gastrocnemius muscles) to detect possible mechanistic links between observed changes in metabolic capacities and organ sizes.

MATERIALS AND METHODS

Animal husbandry, breeding design and a sequence of trials

We used male laboratory mice from generation F10 for the control and selected line types, the latter subjected to artificial selection on maximal metabolic rate elicited by swimming (hereafter $\dot{V}_{O2swim}$), carried out in the Institute of Biology, University of Białystok, Poland. Briefly, the base population was derived from crossing outbred Swiss–Webster mice originating from two independent breeding colonies maintained at University Children's Hospital (Cracow, Poland) and the Centre for Breeding of Laboratory Animals (Warsaw, Poland). We established eight genetically isolated lines and in each of them maintained 10–15 families in each generation. In four of the lines, mice were selected for high levels of $\dot{V}_{O2swim}$, and the other four were randomly bred control lines. Animals with the highest mass-corrected $\dot{V}_{O2swim}$ were chosen as progenitors of the selected lines.

Litter size in our lines varied between 5 and 15. At weaning we separated the offspring by gender and randomly culled their numbers to 5. Whenever available, no fewer than three randomly chosen males and three females from each family were subjected to metabolic trials. Of these, no more than two (typically one) males and females were chosen as progenitors and mated outside their families. Thus, depending on the initial litter sizes, no less than the top 10% of the offspring in each of the lines were bred each generation. Exactly the same procedure was applied to the control lines, except that the mated individuals were picked at random, but still mated outside their families.

After weaning the animals were housed in same-sex and same-family groups of up to five per cage at 23°C. They were maintained on a 12 h:12 h light–dark cycle and had unlimited access to murine chow (Labofeed H, FPP, Kcynia, Poland) and water. $\dot{V}_{O2swim}$ was measured in 12–18 week old mice. A subset of males not qualifying as progenitors was tested for BMR and voluntary locomotor activity (the order of these measurements was random among individuals). We then measured the maximum metabolic rates elicited by running ($\dot{V}_{O2run}$) and cold exposure in Helox ($\dot{V}_{O2He}$). Finally, the animals were killed and dissected.

Measurement of $\dot{V}_{O2swim}$, $\dot{V}_{O2He}$ and BMR

For measurement of $\dot{V}_{O2swim}$, $\dot{V}_{O2He}$ and BMR we used two positive-pressure, open-circuit respirometry systems, differing only with respect to the oxygen analyser (S-3A/I Applied Electrochemistry, Pittsburgh, PA, USA or Sable Systems FC-1B, Henderson, NV, USA). Outside atmospheric air (or a mixture of 79% helium/21% oxygen – Helox) was pushed through a column of Drierite to remove water vapour and then forced through a copper coil submerged along with metabolic chamber(s) in a water bath to equalize and control the temperature. Depending on the type of measurement, the air stream was then divided into up to three independent streams, each fed to a separate mass flow controller (Sierra Instruments, Monterey, CA, USA or ERG-1000, Warsaw, Poland). In measurements of BMR we sequentially monitored three metabolic chambers (each 350 ml in volume) in each respirometry setup. The number of chambers was reduced to two (560 ml each) and one (350 ml) during measurements of $\dot{V}_{O2swim}$ and $\dot{V}_{O2He}$, respectively.

Gas streams were forced through individual metabolic chambers at 700 ml min^{-1} and 400 ml min^{-1} during measurement of maximum metabolic rates and BMR, respectively. The streams were then directed to a computer-controlled channel multiplexer (Henderson, NV, USA). The analysed gas stream was sub-sampled at the rate of 75 ml min^{-1}, scrubbed of CO_2 (Carbosorb AS, BDH Laboratory Supplies, Poole, Dorset, UK), re-dried (Drierite), and then passed through O_2. The electrical signal from the analyser was filtered through a noise reduction system, interfaced to an analog-to-digital converter and fed to a computer that averaged readings every 0.5 s (maximum metabolic rate trials) or 1 s (BMR trial).

To measure $\dot{V}_{O2swim}$ we used a vertically positioned cylindrical Plexiglas metabolic chamber (250 mm high, 115 mm diameter), perfused with atmospheric air. The chamber was partly filled with water, leaving an air volume of 560 ml. Water temperature was maintained at 25±0.2°C. Each mouse was placed just above the water level on a movable platform, and allowed 10 min for adaptation. The platform was then abruptly submerged to force the animal to swim. $\dot{V}_{O2swim}$ was defined as the highest oxygen consumption averaged over 2 min of a 5 min swim. We used the 2 min period for calculation of $\dot{V}_{O2swim}$ because it has the highest repeatability of any interval tested in preliminary trials. For each individual we calculated $\dot{V}_{O2swim}$ residual from the regression of $\dot{V}_{O2swim}$ on body mass, date of measurement, time of day and metabolic chamber number (coded as a categorical variable). This residual was subsequently used as a criterion in the artificial selection protocol.

To measure $\dot{V}_{O2He}$ we placed the mouse in a metabolic chamber perfused with Helox and submerged in glycol-based coolant at –2.5±0.2°C. We defined $\dot{V}_{O2He}$ as the highest O_2 averaged over 2 min of the last 5 min of 15 min Helox exposure.

Measurements of BMR were taken at 31–32°C, a temperature within the thermoneutral zone of our mice (M.K., unpublished results). Before measurement 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.K., unpublished results). We defined BMR as the lowest readout recorded during last 2 h of the 3 h trial period that did not change over 4 min by more than 0.01% of oxygen concentration.

Measurement of $\dot{V}_{O2run}$

Measurements of $\dot{V}_{O2run}$ were carried out in a motorized treadmill enclosed within a metabolic chamber (700 ml in volume). We used a similar measurement protocol to that of Swallow et al. (Swallow et al., 1998). Animals were individually placed in the chamber while the treadmill was stopped, and 'resting' oxygen consumption was recorded for 2 min. The treadmill was then started at an initial speed of 1.5 km h^{-1}. Mice were induced to run by a mild electric current (200 V, 0.5–1.5 mA) provided through a horizontal grid of six 2 mm bars spaced 5 mm apart at the end of the moving belt. After 1.5 min, treadmill speed was increased to 2 km h^{-1} and subsequently speed was increased every 2 min by 0.5 km h^{-1}. Trials were ended when the mouse failed to keep pace with the treadmill. For each individual, $\dot{V}_{O2run}$ was analysed as the highest oxygen consumption averaged over 1–4 min sections of the whole trial. Duration of running (a measure of stamina) was defined as the time elapsing from starting to stopping the treadmill. The distance run to exhaustion (d_e) was defined as the sum of products of treadmill speed and time of running at each speed.

All metabolic trials were carried out between 08:00 and 20:00 h. Metabolic data were analysed with Sable System (1991) DATACAN V software. We calculated oxygen consumption rates using equation 4a of Withers (Withers, 1977), and attempted to correct instantaneous values of O_2 consumption for the chamber washout time by applying a *Z* transformation (Bartholomew et al., 1981) (implemented in DATACAN V software). However, as the magnitude of the correction was less than 1%, and caused an increase of the variance of the measurement, we elected not to apply the correction in our final analyses.

Measurement of core temperature

Rectal temperature was measured to the nearest 0.1°C using a thermocouple digital thermometer (model BAT-12, Physitemp Instruments, Clifton, NJ, USA) before and after measurement of $\dot{V}_{O2swim}$, and $\dot{V}_{O2He}$. The decrease in core temperature from baseline was expressed as the magnitude of hypothermia (ΔT_{He} and ΔT_{swim} for $\dot{V}_{O2He}$ and $\dot{V}_{O2swim}$, respectively).

Voluntary activity

Activity was measured using passive infrared sensors (TL-xpress, Crow Electronics Engineering, Fort Lee, NJ, USA) installed over each cage and monitored every 1 s by computer (PCL-711 analog–digital interface, Advantech, Cincinnati, OH, USA). Movement of the animals switched the sensors on for 2–3 s and the computer registered the on–off state. We used logical sum of signals (for each channel separately) calculated over 3 s periods (that is, any 'on' state registered during a 3 s period was enough to score the entire period as 'active'). We estimated each animal's activity as a daily sum of all 3 s activity periods during 2 consecutive days (48 h), but each day was analysed separately.

Morphometrics

Following metabolic trials, mice were killed by cervical dislocation. Internal organs (small intestine, stomach, liver, kidneys, heart), interscapular brown adipose tissue (IBAT), and gastrocnemius muscles (from both hind legs) were excised, cleared of blood, foodstuffs and adherent fat. The cleaned organs were weighed ±0.001 g, dried to a constant mass at 65°C over 48 h, and reweighed.

Statistics

In most analyses we used a mixed model extension of a general linear model (GLM) implemented in procedure MIXED (SAS Institute 1990, www.sas.com), with line type (selected *vs* control) as a fixed factor, line (replication) nested within line type and family affiliation nested within line type and line as random factors. Depending on the analysis, body mass and time of day were incorporated as covariates and tested for possible interactions. Data on voluntary activity were analysed using repeated-measures

Table 1. Summary of results of breeding for maximum body mass-corrected metabolism elicited by swimming

	Line type *P*	Line *P*	Body mass *P*	Adjusted means ± s.e.m.	
				Control	Selected
Body mass	0.85	0.19		37.8±0.2	37.9±0.2
$\dot{V}_{O2swim}$ (F10)	0.003	0.13	<0.0001	267.2±1.7	299.6±1.4
$\dot{V}_{O2swim}$ (trial)	0.003	0.15	<0.0001	266.9±2.2	302.1±2.1
ΔT_{swim}	0.04	0.83[a]	0.13	7.8±0.2	6.9±0.2
$\dot{V}_{O2He}$	0.04	0.09	<0.0001	360.3±7.2	386.6±7.0
ΔT_{He}	0.23	0.20	0.0002	3.8±0.2	3.2±0.2
$\dot{V}_{O2run}$	0.006	0.26[a]	<0.0001	288.5±3.2	306.8±3.2
t_{run}	0.011	0.46[a]	0.18	6.3±0.2	7.1±0.2
d_e	0.014	0.49	0.18	228.8±6.3	272.9±6.4
BMR	0.52	0.09	<0.0001	59.7±1.7	61.3±1.7
Activity	0.42	0.64[a]	0.52	245.1±7.3	257.1±8.2

Measured effects: body mass (g), maximum metabolic rate elicited by swimming for all mice in F10 generation [$\dot{V}_{O2swim}$ (F10), ml O_2 h^{-1}], animals used in experimental trials [$\dot{V}_{O2swim}$ (trial), ml O_2 h^{-1}], hypothermia elicited by swimming (ΔT_{swim}, °C), basal metabolic rate (BMR, ml O_2 h^{-1}), run-elicited maximum metabolic rate averaged over 4 min of maximum oxygen consumption ($\dot{V}_{O2run}$, ml O_2 h^{-1}), duration of running (stamina, t_{run}, min), average distance run to exhaustion (d_e, m), maximum metabolic rate ($\dot{V}_{O2He}$, ml O_2 h^{-1}), hypothermia elicited by Helox atmosphere and T_a of –2.5°C (ΔT_{He}, °C) and duration of daily voluntary activity (min day^{-1}).

Line type affiliation, replicate lines, family affiliation (nested within line type and line, not presented) and channel affiliation (in activity and BMR analysis, not specified here) were main factors, whereas body mass was a covariate. All *P*-values are for 2-tailed tests.

[a]In SAS PROC MIXED restricted likelihood was maximized by setting the variance of replicate lines factor equal to 0. For this reason *P*-value was obtained from SAS PROC GLM.

ANOVA, with channel number (i.e. activity sensor) and line type as fixed factors, line affiliation nested within line type, and family affiliation nested within line as random factors. Metabolic chamber number was not significant in analyses (P>0.1). Before performing statistical analyses, assumptions of parametric tests were assured (Sokal and Rohlf, 1995).

RESULTS

Body mass of mice of control and selected line types did not differ statistically (Table 1). In contrast, the between-line type difference in $\dot{V}_{O_2swim}$ was highly significant (Table 1) and averaged 31.7 ml O_2 h^{-1}, or more than 10% (Table 1). Likewise, the between-line type difference in $\dot{V}_{O_2swim}$ of the subset of animals subject to the remaining experimental trials was also highly significant (Fig. 1A). The much higher $\dot{V}_{O_2swim}$ of selected lines was associated with less severe swim-elicited hypothermia (ΔT_{swim}) than in the control mice (Table 1), which nevertheless averaged 6.9±0.2°C and was highly statistically different from 0°C (P<0.001).

Maximum metabolic rate elicited by exposure to Helox at –2.5°C ($\dot{V}_{O_2He}$) was significantly higher in selected than in control lines (Table 1; Fig. 1B). Mice of both control and selected line types became significantly hypothermic (P<0.001). However, the magnitude of hypothermia (ΔT_{He}) did not differ between the two line types (Table 1).

Maximum oxygen consumption elicited by running ($\dot{V}_{O_2run}$) differed significantly between selected and control line types when averaged over 1–4 min of the highest parts of readings (P=0.04, 0.03, 0.02 and 0.007, respectively; Table 1; Fig. 1C). The total duration of running (t_{run}) and the average distance run to exhaustion (d_e) (Fig. 1D) were significantly longer in selected than in control animals (Table 1).

In contrast to the significant between-line type differences in the selected trait – $\dot{V}_{O_2swim}$, as well as correlated measures of maximal aerobic capacity, there were no between-line type differences in BMR or voluntary activity (Table 1; Fig. 2A,B).

Both wet and dry masses of visceral organs and tissues did not differ between selected and control line types, except for the heart and gastrocnemius muscles, which were significantly heavier in selected than in control mice (Table 2).

DISCUSSION

The primary goal of our study was to test the aerobic capacity model (Bennett and Ruben, 1979), as formalized by Hayes and Garland (Hayes and Garland, 1995). We first discuss the suitability of our selection experiment for testing the model and then present our results in a broader physiological and evolutionary context.

Recall that the cornerstone of Hayes and Garland's (Hayes and Garland, 1995) reasoning is the existence of a genetic correlation between resting and maximal metabolic rates. Any selection experiment aiming to test the correlation should therefore result in a successful manipulation of one of the two metabolic rates, and a subsequent analysis of the correlated response of the other one

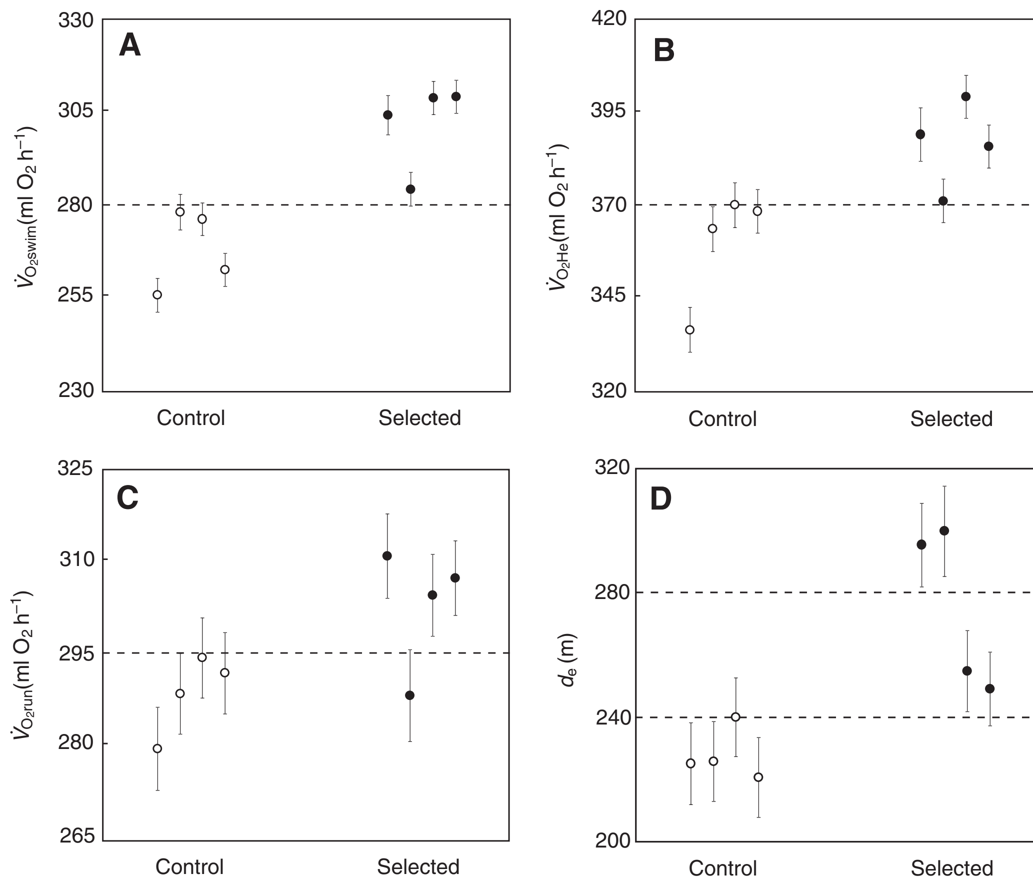

Fig. 1. (A) maximum metabolic rate elicited by swimming ($\dot{V}_{O_2swim}$), (B) exposure to cold ($\dot{V}_{O_2He}$), (C) running (averaged over 4 min of maximum oxygen consumption, $\dot{V}_{O_2run}$) and (D) distance run to exhaustion (d_e) in lines of mice selected for maximum body mass-corrected metabolism elicited by swimming (filled circles) and random-bred control lines (open circles). Symbols depict adjusted means (±s.e.m.) from statistical models presented in Table 1.

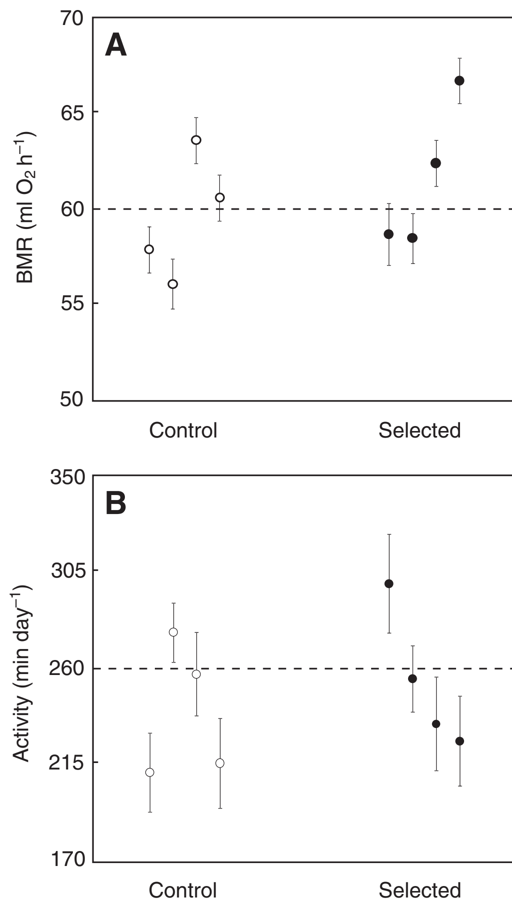

Fig. 2. (A) basal metabolic rate (BMR) and (B) voluntary cage activity in lines of mice selected for maximum body mass-corrected metabolism elicited by swimming (filled circles) and random-bred control lines (open circles). Symbols depict adjusted means (±s.e.m.) from statistical models presented in Table 1.

(Garland, 2003). Our selection resulted in over 10% difference in the primary trait – $\dot{V}_{O2swim}$ – between selected and control line types, which was not associated with a significant between-line type difference in BMR. Thus, a simple conclusion is that, at least in our study system, we found no support for the existence of a genetic correlation between BMR and $\dot{V}_{O2swim}$, which is inconsistent with the aerobic capacity model as formalized by Hayes and Garland (Hayes and Garland, 1995). However, this conclusion is valid only if $\dot{V}_{O2swim}$ can be used as a proxy of the 'maximal aerobic capacity' that was targeted by natural selection, as proposed by Bennett and Ruben (Bennett and Ruben, 1979) and Hayes and Garland (Hayes and Garland, 1995), and if the magnitude of the between-line type difference in $\dot{V}_{O2swim}$ was sufficient to reveal a correlated response in BMR.

Swimming in cold water elicits both thermogenic and locomotory responses in rodents (Konarzewski et al., 1997; Sadowska et al., 2005; Gębczyński and Konarzewski, 2009), of which only maximal aerobic capacity related to locomotory activity is directly relevant to the original formulation of the aerobic capacity model. Our selection resulted in a significant increase of cold-elicited maximal aerobic capacity ($\dot{V}_{O2He}$), which strongly suggests that a between-line type difference in $\dot{V}_{O2swim}$ itself cannot be used to test Bennett and Ruben's (Bennett and Ruben, 1979) hypothesis. However, the selection also resulted in a statistically significant increase in $\dot{V}_{O2run}$ by about 5%. The magnitude of this increase is comparable to that reported at the same stage of selection (10th generation) in mice selected for voluntary wheel running (Swallow et al., 1998). On the other hand, the $\dot{V}_{O2run}$ of our selected and control mice was, respectively, 15% and 5% lower than the comparable $\dot{V}_{O2run}$ reported by Swallow and colleagues (Swallow et al., 1998). This was most probably due to differences in the genetic backgrounds of the base populations giving rise to Swallow and colleagues' and our lines. Nevertheless, even though the increase in $\dot{V}_{O2run}$ in our study was relatively small, it was consistent with between- line type differences in gastrocnemius and heart mass. That finding provides strong support for the existence of a functional correlation between $\dot{V}_{O2run}$ and underlying anatomy. Furthermore, our selected mice had much higher stamina quantified by both time and distance run to exhaustion (Table 1). Interestingly, the relative magnitude of the between-line type differences in t_{run} and d_e is greater than that of the selected trait – $\dot{V}_{O2swim}$.

Taken together the differences in $\dot{V}_{O2run}$, t_{run} and d_e show that our selection protocol affected traits related to endurance more than those related to maximum metabolic rate. It is important to note that running endurance is largely a factor of aerobic capacity (Lambert and Noakes, 1990) and, therefore, is directly relevant to Bennett and Ruben's hypothesis of selection on high and sustained activity. Following Hayes and Garland (Hayes and Garland, 1995), most subsequent studies testing the model used short-term metabolic rates ($\dot{V}_{O2max}$ elicited by running or acute cold exposure) as a proxy for sustainable exercise capacity (e.g. Bishop, 1999; Sadowska et al., 2005; Rezende et al., 2005). We conclude that between-line type differences in $\dot{V}_{O2run}$, t_{run}, d_e, and gastrocnemius and heart masses in our mice make them a valid model for testing the aerobic capacity model, at least in terms of the ability to sustain high aerobic metabolism over short periods [i.e. minutes, rather than hours or days (see Peterson et al., 1990)].

The question then arises of why our selection experiment did not result in a correlated response in BMR. One possible explanation is that BMR could have responded to indirect selection much slower than the trait under selection – $\dot{V}_{O2swim}$. A trait is unlikely to respond to selection when its heritability is low (Falconer and Mackay, 1996). Indeed two earlier studies suggested that additive genetic variation for BMR may be very low in laboratory mice (Dohm et al., 2001) and in a wild rodent, the leaf eared mouse [*Phylottis darwini* (Nespolo et al., 2005)]. However, Sadowska and colleagues (Sadowska et al., 2005) reported relatively high values of narrow-sense heritability (h^2) of about 0.4 for mass-independent BMR in the bank vole (*Myodes glareolus*). Sadowska and colleagues' (Sadowska et al., 2005) findings are in good agreement with estimates of BMR heritability in Swiss–Webster mice (Konarzewski et al., 2005), from which the mice used in the present study were derived. Most importantly, in a concurrent experiment carried out in our laboratory we were able to directly select for BMR in Swiss–Webster mice, obtaining lines differing by about 40% in BMR (Książek et al., 2004; Gębczyński and Konarzewski, 2009). It seems therefore unlikely that the lack of response in BMR in mice selected for $\dot{V}_{O2swim}$ can be attributed to insufficient additive genetic variation.

The simplest explanation is that no genetic correlation between maximal metabolic rates and BMR currently exists in our mice, because they do not share enough biochemical or physiological pathways. To test this, we analysed selection responses of the masses of organs tightly linked to aerobic performance. We assumed that the mass of a particular organ can serve as a proxy for its metabolic activity, which seems to be a valid assumption at least in the case

Table 2. Summary of results of breeding for maximum body mass-corrected metabolism elicited by swimming on fresh and dry mass of internal organs, interscapular brown adipose tissue (IBAT) and gastrocnemius muscles

	Line type	Line	Body mass	Adjusted means ± s.e.m.	
	P	*P*	*P*	Control	Selected
Fresh mass					
Small intestine	0.98	0.16	<0.0001	1.723±0.033	1.721±0.032
Liver	0.87	0.33	<0.0001	2.265±0.030	2.272±0.029
Kidneys	0.30	0.14	<0.0001	0.630±0.015	0.654±0.015
Stomach	0.62	0.26[a]	0.0006	0.296±0.006	0.292±0.005
Heart	0.03	0.40	<0.0001	0.161±0.004	0.175±0.003
IBAT	0.07	0.19	<0.0001	0.158±0.007	0.181±0.007
Gastrocnemius muscles	0.03	0.60[a]	<0.0001	0.342±0.004	0.358±0.004
Dry mass					
Small intestine	0.47	0.29	<0.0001	0.427±0.007	0.435±0.007
Liver	0.67	0.40[a]	<0.0001	0.693±0.008	0.698±0.007
Kidneys	0.63	0.46[a]	<0.0001	0.177±0.004	0.180±0.004
Stomach	0.63	0.29	0.008	0.074±0.002	0.075±0.002
Heart	0.02	0.53	<0.0001	0.040±0.001	0.043±0.001
IBAT	0.15	0.15	<0.0001	0.100±0.006	0.114±0.006
Gastrocnemius muscles	0.02	0.66[a]	<0.0001	0.090±0.001	0.094±0.001

Line type affiliation, replicate lines, family affiliation (nested within line type and line, not presented) were main factors, whereas body mass was a covariate.
[a]In SAS PROC MIXED restricted likelihood was maximized by setting the variance of replicate lines factor equal to 0. For this reason the *P*-value was obtained from SAS PROC GLM.

of skeletal muscles and internal organs, such as liver, kidneys and the heart (Even et al., 2001; Legerlotz et al., 2008; Książek et al., 2009). Furthermore, the masses of internal organs are heritable (Książek et al., 2004 and references therein) and, therefore, are likely to respond to selection. $\dot{V}_{O2run}$ is largely dependent on the mass and function of muscles and the circulatory system (Goldspink, 1999; Bishop, 1999; Bishop, 2005; Weibel et al., 2004; Weibel and Hoppeler, 2005), whereas BMR is mainly affected by visceral organs (Konarzewski and Diamond, 1995; Even et al., 2001; Książek et al., 2004; Brzęk et al., 2007; Gębczyński, 2008). We found between-line type differences in the masses of gastrocnemius muscles and the heart. These differences, however, were not paralleled by significant differences in the masses of intestine, stomach, liver and kidneys. This finding is consistent with the lack of correlation between basal and maximal metabolic rates in our mice.

The lack of a correlated response in BMR in our study agrees with the results of extensive experimental selection on voluntary wheel running in mice (Swallow et al., 1998). That selection regime also did not result in elevated BMR, despite a significant increase in maximal metabolic rates elicited by running (Kane et al., 2008). Even though Sadowska (Sadowska, 2008) demonstrated that selection on $\dot{V}_{O2max}$ in the bank vole simultaneously increased BMR, our results suggest that ‘constructing’ an animal having higher aerobic capacity is not ineluctably linked to an elevated BMR. Although our inference is based on the lack of statistical significance of between-line type differences in BMR, it is supported by the lack of change in internal organ masses quantitatively contributing to BMR (Konarzewski and Diamond, 1995). As these two tests are independent, we believe that our ‘negative’ conclusions are justified. Furthermore, the lack of consistency of a correlated response of BMR to selection on $\dot{V}_{O2max}$ in several studies questions the evolutionary persistence of a genetic correlation between the two and undermines the notion that it was one of the basic metabolic mechanisms operating in the ancestors of extant homeotherms.

However, the inconsistency of the results of these studies questions the validity of the aerobic capacity model as formulated by Hayes and Garland (Hayes and Garland, 1995), rather than the evolutionary mechanism originally proposed by Bennett and Ruben (Bennett and Ruben, 1979). Bennett and Ruben (Bennett and Ruben, 1979) did not specify whether they meant natural selection on maximal aerobic capacity sustained over short or extended periods. They repeatedly referred to selection on ‘sustained (locomotor) activity’ as the main factor driving the evolution of endothermy. Later Farmer (Farmer, 2000; Farmer, 2003) put forward a thermoregulatory model, which suggests that high resting metabolic rates in birds and mammals evolved as a means of endothermic thermoregulation. According to the model, an increased thermogenic capacity served for the incubation of eggs and offspring (that is, long-term energy expenditures), rather than for stabilizing the body temperature of adults *per se*. Thus, the cost of high metabolic rates paid by adults has been returned as an increased growth rate and an improved developmental stability, both of which would contribute to decreased mortality and better quality of young. However, Gębczyński (Gębczyński, 2008) found no correlation between BMR and maximum cold-induced oxygen consumption and total non-shivering thermogenesis in mice divergently selected for BMR. The present study also did not support the existence of a genetic correlation between cold-induced maximal metabolic rate (here quantified as $\dot{V}_{O2He}$) and BMR. Furthermore, there was no genetic correlation between cold-elicited metabolic rate and BMR in the bank vole (Sadowska et al., 2005). All these findings are inconsistent with Farmer’s model.

Unlike Farmer (Farmer, 2000), Koteja (Koteja, 2000) proposed that the evolution of endothermy should be specifically linked with selection on sustained, long-term metabolic expenditure (*sensu* Hammond and Diamond, 1997) elicited by locomotion, rather than short-term metabolic rates stressed by Hayes and Garland (Hayes and Garland, 1995). In particular Koteja (Koteja, 2000) stressed that high levels of sustained energy expenditure, incurred by parental care, would require an increased capacity of visceral organs, especially the alimentary tract, for processing large amounts of food. High levels of metabolic activity of those organs would then result in elevation of BMR, leading to endothermy. In other studies from our laboratory we demonstrated that selection on increased BMR results in increased relative masses of internal organs fuelling

sustained energy expenditure (Książek et al., 2004; Brzęk et al., 2007; Gębczyński, 2008). Recently, we showed that high levels of BMR are genetically correlated with high levels of voluntary locomotory activity (Gębczyński and Konarzewski, 2009), as predicted by Koteja (Koteja, 2000).

The results presented here cross-validate and complement our earlier findings: unlike selection for increased BMR, the selection on $\dot{V}_{O2max}$ did not result in elevated levels of voluntary activity (Table 1) and increased masses of internal organs underlying energy assimilation (Table 2). Conversely, selection for high BMR did not result in an increased $\dot{V}_{O2run}$ (Gębczyński and Konarzewski, 2009). Taken together our results support the validity of the evolutionary scenario highlighting the significance of the selection on long-term energy expenditure as a driving force behind the evolution of endothermy.

LIST OF ABBREVIATIONS

BMR	basal metabolic rate
d_e	distance run to exhaustion
RMR	resting metabolic rate
t_{run}	duration of running
$\dot{V}_{O2He}$	maximal metabolic rate elicited by cold exposure in Helox
$\dot{V}_{O2max}$	maximal metabolic rate
$\dot{V}_{O2run}$	maximal metabolic rate elicited by running
$\dot{V}_{O2swim}$	maximal metabolic rate elicited by swimming
ΔT_{He}	hypothermia elicited by Helox
ΔT_{swim}	hypothermia elicited by swimming

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