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## RESEARCH ARTICLE

# Effects of oxygen availability on maximum aerobic performance in *Mus musculus* selected for basal metabolic rate or aerobic capacity

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**SUMMARY**

**Maximum aerobic metabolism cannot increase indefinitely in response to demands for ATP production and, therefore, must be constrained by one (or many) of the steps of the oxygen transport and utilization pathways. To elucidate those constraints we compared peak metabolic rate elicited by running ($\dot{V}_{O2,run}$) in hypoxia (14% $O_2$), normoxia (21% $O_2$) and hyperoxia (30% $O_2$) of laboratory mice divergently selected for low and high basal metabolic rate (L-BMR and H-BMR, respectively), mice selected for maximum metabolic rate elicited by swimming ($\dot{V}_{O2,swim}$) and mice from unselected lines. In all line types $\dot{V}_{O2,run}$ was lowest in hypoxia, intermediate in normoxia and highest in hyperoxia, which suggests a 'central' limitation of oxygen uptake or delivery instead of a limit set by cellular oxidative capacity. However, the existence of a common central limitation is not in agreement with our earlier studies showing that selection on high $\dot{V}_{O2,swim}$ (in contrast to selection on high BMR) resulted in considerably higher oxygen consumption during cold exposure in a He–$O_2$ atmosphere than $\dot{V}_{O2,run}$. Likewise, between-line-type differences in heart mass and blood parameters are inconsistent with the notion of central limitation. Although responses of $\dot{V}_{O2,run}$ to hypoxia were similar across different selection regimens, the selection lines showed contrasting responses under hyperoxic conditions. $\dot{V}_{O2,run}$ in the H-BMR line type was highest, suggesting that selection on high BMR led to increased cellular oxidative capacity. Overall, between-line-type differences in the effect of the oxygen partial pressure on $\dot{V}_{O2,run}$ and in the components of $O_2$ flux pathways are incompatible with the notion of symmorphosis. Our results suggest that constraints on $\dot{V}_{O2,max}$ are context dependent and determined by interactions between the central and peripheral organs and tissues involved in $O_2$ delivery.**

Key words: maximum oxygen consumption, constraints of aerobic capacity, basal metabolic rate.

## INTRODUCTION

Maximum aerobic metabolic rate ($\dot{V}_{O2,max}$) determined during running on a treadmill is a standard tool to study maximum sustainable metabolic power production, which depends on the integrated function of a wide variety of organ systems (e.g. Swallow et al., 1998; Koch and Britton, 2001; Rezende et al., 2005). Since it sets the upper limit to sustainable activity, $\dot{V}_{O2,max}$ is relevant ecologically, evolutionarily and physiologically, as well as clinically. As $\dot{V}_{O2,max}$ cannot increase indefinitely in response to increasing demands for ATP production, it is important to learn what kind of constraints limit $\dot{V}_{O2,max}$. Conceptually, $\dot{V}_{O2,max}$ could be set by the interactions between five sequential steps of $O_2$ transport and uptake: (1) ventilatory convection, (2) alveolar–capillary diffusion, (3) blood convection, (4) tissue capillary-to-cell diffusion and (5) mitochondrial capacity for $O_2$ uptake (Taylor and Weibel, 1981; Wagner, 1996). In principle, each of the sequential steps of $O_2$ delivery and uptake can be limiting and many earlier studies identified some of them as limiting, while often pointing to the existence of excess transport capacity at other steps. For example, several studies suggested that cardiac output (that is, blood convection) will limit $\dot{V}_{O2,max}$ (Bishop, 1997; Richardson et al., 1999a), and studies on human athletes suggested that their muscle tissue oxidative capacity is in excess of $O_2$ delivery capacity (e.g. Richardson et al., 1999b). Alternatively, it has been proposed that all steps in the $O_2$ cascade are optimized by natural selection, so their transport capacities match each other and none of them alone would set the limit for maximum organismal performance ['symmorphosis' (*sensu* Taylor and Weibel, 1981)].

Several decades of studies on $\dot{V}_{O2,max}$ have shown that the identification of limiting steps of $O_2$ delivery is not straightforward (Koteja, 1986; Henderson et al., 2002; Howlett et al., 2003; Seymour et al., 2004; Gonzales et al., 2006; Kirkton et al., 2009). A promising approach to identifying possible limits to $\dot{V}_{O2,max}$ is offered by artificial selection experiments on metabolic rates (Swallow et al., 2009) (for reviews, see Garland and Rose, 2009; Garland et al., 2010). Animals manipulated through artificial selection can be useful in two ways. First, one can consider artificial selection as a tool for 'engineering' animals that are close to the within-species boundaries of variation of traits related to metabolic rates. It is instructive to take advantage of that artificially increased variation and carry out comparative analysis in much the same way as at the interspecific level, but being freed from many confounding factors, such as between-species differences in anatomy, physiology, phylogeny, etc. Second, within-selection analyses can also be informative, because mechanisms limiting $\dot{V}_{O2,max}$ can be manifested as differences in within-selection responses to the applied metabolic stressor(s).

In recent years, much of the progress in understanding complex interactions between subsequent components of the $O_2$ cascade has been made by studying $\dot{V}_{O2,max}$ in artificially selected rodents subjected to hypoxic and/or hyperoxic conditions, in comparison to normoxic conditions (Howlett et al., 2003; Howlett et al., 2009; Rezende et al., 2006a; Rezende et al., 2006b). Metabolic responses

to different partial pressures of $O_2$ ($P_{O_2}$) can give insights into which of these steps is limiting.

We hypothesise that under moderately hypoxic conditions one can expect a reduction in $\dot{V}_{O_2,max}$ (so-called central limitation) unless there exists an excess capacity for $O_2$ uptake and delivery at steps 1–3 that can offset the reduction of $P_{O_2}$ relative to normoxia. The existence of limitations at steps 1–3 would be further supported by a significant increase of $\dot{V}_{O_2,max}$ in response to hyperoxia as a result of facilitated pulmonary diffusion and/or blood convection. However, an increase in $\dot{V}_{O_2,max}$ under hyperoxia would be only possible if the extra $O_2$ can be consumed at capillary-to-cell and mitochondrial level. Otherwise, the lack of such an increase would point to potential constraints at the tissue level (so-called peripheral limitation). The consistency (or the lack thereof) of the above responses across different artificial selection regimens applied within species can inform us about the 'design constraint' related to the symmorphosis principle.

Our laboratory has been running two experiments on lines of mice: selection for high maximum metabolic rate elicited by swimming ($\dot{V}_{O_2,swim}$) and divergent selection on basal metabolic rate (BMR). These experiments provide us with a unique opportunity to examine interactions between aerobic limits elicited by different ambient partial pressure of $O_2$ and selection-generated differences in metabolism. We have already demonstrated that selection on $\dot{V}_{O_2,swim}$ resulted in correlated increases of both normoxic $\dot{V}_{O_2,max}$ elicited by running ($\dot{V}_{O_2,run}$) and heart mass, which we assume is a proxy of cardiac output (Bishop, 1997; Gębczyński and Konarzewski, 2009b). In both line types (selected and randomly bred), maximum metabolic rates elicited by cold exposure in a He–$O_2$ atmosphere (hereafter referred to as $\dot{V}_{O_2,cold}$) were significantly higher than $\dot{V}_{O_2,run}$ (Gębczyński and Konarzewski, 2009b). When compared with mice selected for high BMR, mice selected for low BMR differed in spontaneous locomotor activity and heart mass, but did not differ with respect to $\dot{V}_{O_2,run}$ in normoxia or $\dot{V}_{O_2,cold}$ (Książek et al., 2004; Brzęk et al., 2007; Gębczyński, 2008; Gębczyński and Konarzewski, 2009a). Moreover, in the BMR-selected lines, values of $\dot{V}_{O_2,run}$ and $\dot{V}_{O_2,cold}$ were similar (unlike in the $\dot{V}_{O_2,swim}$ line type).

In the current study our objective was to use different levels of $O_2$ availability to help identify possible limits to $\dot{V}_{O_2,max}$ elicited by forced exercise in mice from both selection experiments. We compared the responses to hypoxia and hyperoxia in mice of both line types in the context of the central and peripheral components of the $O_2$ cascade and their relevance to the idea of 'symmorphotic' design.

## MATERIALS AND METHODS

### Animals

#### Selection on BMR

We used 12-week-old males of outbred Swiss-Webster laboratory mice (*Mus musculus* Linnaeus 1758) subjected to divergent non-replicated artificial selection toward high (H-BMR) and low (L-BMR) BMR corrected for body mass [for details see Książek et al. (Książek et al., 2004)]. Lines are maintained in animal facilities at the Institute of Biology, University of Bialystok, Poland. Briefly, depending on breeding success, in subsequent generations we maintain 30–35 families in each selected line type. Whenever possible, no less than three randomly chosen males and three females from each family were tested for BMR at 31–32°C, a temperature within the thermoneutral zone of our mice (M.K., unpublished data). Mice are fasted for 6 h before measurements. We defined BMR as the lowest 4 min readout during the last 2 h of the 3 h trial period, within which oxygen concentration does not change by more than 0.01%. Animals characterized by the highest (in H-BMR line type) or lowest (L-BMR line type) body-mass-corrected BMR are chosen as progenitors and always mated outside their families.

In the study described here we used 38 H-BMR males and 39 L-BMR males of generation F34. In H-BMR and L-BMR males the BMR averaged 71.0±2.3 and 44.5±2.1 ml $O_2$ $h^{-1}$, respectively, a 60% between-line-type difference. This difference is sufficiently large to be confidently attributed to the results of selection, rather than to genetic drift (Gębczyński and Konarzewski, 2009a). Body mass did not differ between line types (H-BMR line type: 36.4±0.7 g, L-BMR line type: 36.5±0.6 g).

#### Selection on $\dot{V}_{O_2,swim}$

We used 205 12-week-old males from the 12th generation. Body-mass-corrected $\dot{V}_{O_2,swim}$ differed considerably between selected and random bred (control) individuals entering the experiment (282.5±4.2 ml $O_2$ $h^{-1}$ and 253.2±4.4 ml $O_2$ $h^{-1}$, respectively; ANCOVA, $F_{3,6}$=23.1, $P$<0.01).

Briefly, we established eight genetically isolated Swiss-Webster laboratory mice lines and in each of them maintained 10–15 families. In four of the lines, mice were selected for high $\dot{V}_{O_2,swim}$, and the other four were randomly bred (hereafter referred to as RB), control lines. To measure $\dot{V}_{O_2,swim}$ we used a vertically positioned cylindrical Plexiglas® metabolic chamber supplied with atmospheric air (700 ml $min^{-1}$). The chamber was partly filled with water maintained at 25±0.2°C, leaving a dry volume of 560 ml. 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. $\dot{V}_{O_2,swim}$ was defined as the highest body-mass-corrected oxygen consumption averaged over 2 min of a 5 min swim. Animals with the highest $\dot{V}_{O_2,swim}$ were chosen as progenitors of the selected lines. 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. Exactly the same procedure was applied to the RB lines, except that the mated individuals were picked at random, but still mated outside their families.

### Husbandry

After weaning, animals were housed in same-sex and same-family groups of up to five individuals 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.

### Measurements of $\dot{V}_{O_2,run}$

Upon completion of measurements of BMR and $\dot{V}_{O_2,swim}$ as part of the routine selection protocols, randomly selected males (up to three individuals from each family within line type, not qualified as progenitors) were randomly assigned to three $P_{O_2}$ trials and subject to measurements of $\dot{V}_{O_2,run}$ carried out using a motorized treadmill enclosed in a metabolic chamber (700 ml in volume) that was fitted to the above-described respirometry system. We elected to carry out measurements of $\dot{V}_{O_2,run}$ sequentially because of logistical constraints – first, within selection on BMR, then within selection on $\dot{V}_{O_2,swim}$. This was a compromise between statistical and logistical demands, which allowed us to minimize the overall duration of measurements, and therefore to minimize the confounding effect of aging on measured traits (P. Brzęk, A. Książek, A. Dobrezyń and M.K., unpublished data), albeit at the expense of the desirable random block design of the experiment.

We used a measurement protocol similar to earlier published studies (Swallow et al., 1998; Rezende et al., 2006a), with an

enclosed treadmill functioning as a metabolic chamber. Outside atmospheric air, or mixtures of 70% nitrogen/30% oxygen or 86% nitrogen/14% oxygen, was dried and forced through a copper coil submerged along with the treadmill in a water bath to equalize and control temperature (23±1.0°C). The flow rate was 700 ml $min^{-1}$ (mass flow meter, Sierra Instruments, Monterey, CA, USA). Animals were individually placed in the chamber while the treadmill was stopped and the system was sealed and submerged in the water bath. 'Resting' oxygen consumption was recorded for 2 min. The treadmill was then started at an initial speed of 0.7 km $h^{-1}$. Mice were induced to run by a mild electric current (200 V, 0.5–1.5 mA) through a horizontal grid of six 2 mm bars spaced 5 mm apart at the end of the moving belt. 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, on average after 7.9 min of running (from 6.7 min in hypoxia to 8.7 min in hyperoxia). Oxygen concentration was recorded every 0.5 s by a computer. We used the highest oxygen consumption averaged over 1 min as $\dot{V}_{O_2,run}$.

Each mouse was measured only once, in hypoxia, normoxia or hyperoxia. Three animals failed to run on the treadmill and were subsequently removed from the experiment (two individuals and one individual from the BMR and $\dot{V}_{O_2,swim}$ selection experiments, respectively). For this reason within-line-type sample sizes varied between experimental groups (see Table 1).

We carried out measurements changing the $P_{O_2}$ every half a day, whereas the $P_{O_2}$ of the very first set of measurements on the very first morning was selected at random. The next $P_{O_2}$ value was randomly drawn from the two remaining options. The next day, we started with measurements at the same $P_{O_2}$ as on the previous afternoon and continued with the third (yet not applied) $P_{O_2}$, and so on. This sequence assured an equal distribution of $P_{O_2}$ values between morning and afternoon measurements.

All metabolic trials were carried out between 08:00 and 20:00 h. Metabolic data were collected and analysed with Sable System (Sable System, Salt Lake City, UT, USA) 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. (Bartholomew et al., 1981) implemented in DATACAN V software]. However, as the magnitude of the correction was less than 1%, and caused an increase in variance, we elected not to apply the correction in our final analyses. We used the highest oxygen consumption averaged over 1 min as $\dot{V}_{O_2,run}$.

### Measurements of red blood cells and haemoglobin in mice selected on their $\dot{V}_{O_2,swim}$

Immediately after measurements of $\dot{V}_{O_2,run}$, $\dot{V}_{O_2,swim}$-selected and RB mice were killed by cervical dislocation. Blood for measurements was taken directly from the heart. To determine red blood cell (RBC) concentrations, 5 µl of blood was diluted with 1 ml of Hayem reagent solution and the cells were counted under a microscope using a Bürker counting chamber (Carl Roth, Karlsruhe, Germany). Haemoglobin concentration was estimated, in duplicate, by adding 20 µl of blood to a test tube containing 5 ml of Drabkin's reagent and the mixture was incubated for 30 min at room temperature in the light. Absorbance was then measured at 540 nm using a UV-1201V spectrophotometer (Shimadzu Corp., Kyoto, Japan). Concentrations were determined from calibration curves obtained with standard haemoglobin solutions diluted in Drabkin's reagent at the following concentrations: 0.0, 5.0, 10.0, 15.0 and 20.0 g 100 $ml^{-1}$.

### Statistics

$\dot{V}_{O_2,run}$ of mice selected on $\dot{V}_{O_2,swim}$ was analysed with a mixed model extension of a general linear model (proc MIXED, version 9.1, SAS Institute, Cary, NC, USA, 1996) with line type (selected *vs* random bred) and partial oxygen pressure (hypoxia, normoxia and hyperoxia) as fixed factors, line (replication) nested within line type and family affiliation (nested within line) as random factors. Body mass measured immediately prior to the trial was used as a covariate. The effects of $P_{O_2}$, line type and $P_{O_2}$ × line-type interaction were tested by means of an *F*-test against variation of a random $P_{O_2}$ × line-within-line-type interaction as the error term. We also applied a similarly structured statistical model (but without $P_{O_2}$ as a fixed factor, body mass as covariate and line nested within line type as the error term) to analyse between-line-type differences in the number of RBCs and haemoglobin concentration.

Our selection on BMR was not replicated. To directly compare responses of mice from the BMR-selected lines with the $\dot{V}_{O_2,swim}$-selected and RB lines we pooled $\dot{V}_{O_2,run}$ for all line types and then analysed the between-line-type variation by means of the proc MIXED procedure with the model structure as described above (which we refer to as the 'common' model). Because the line type × $P_{O_2}$ interaction was statistically significant in a common model, we carried out separate analyses for all line types within each level of $P_{O_2}$ (with a random effect of line nested within line type as an error term).

To further elucidate the effect of $P_{O_2}$ we separately compared $\dot{V}_{O_2,run}$ of mice from our two artificial selection regimes with $\dot{V}_{O_2,run}$ of four RB lines. We also analysed $\dot{V}_{O_2,run}$ of mice divergently selected on BMR (H-BMR *vs* L-BMR) by means of the mixed model as described above for selection on $\dot{V}_{O_2,swim}$, with family affiliation (nested within line type) as the error term.

Because mice used in this experiment were drawn from the pool of animals not qualified as progenitors, they cannot be considered as a random unbiased sample. For all analyses, assumptions of parametric tests were met (Sokal and Rohlf, 1995). All *P*-values shown are from two-tailed tests.

Table 1. Unadjusted body mass and peak metabolic rate of the mouse lines used in different partial pressures of oxygen

| | | $\dot{V}_{O_2,run}$ (ml $O_2$ $h^{-1}$)* | | |
|---|---|---|---|---|
| Mouse line | Body mass (g) | Hypoxia (14% $O_2$) | Normoxia (21% $O_2$)x | Hyperoxia (30% $O_2$) |
| H-BMR | 36.4±0.7 (32) | 237.3±8.2 (9) | 304.0±7.5 (11) | 345.5±6.8 (13) |
| L-BMR | 36.5±0.6 (39) | 227.3±7.1 (12) | 291.1±6.9 (12) | 317.1±6.6 (14) |
| $\dot{V}_{O_2,swim}$ | 36.4±0.5 (110) | 235.3±3.6 (37) | 304.1±3.8 (35) | 321.4±4.0 (38) |
| Random bred (control) | 35.6±0.5 (95) | 228.3±3.8 (35) | 286.5±4.1 (29) | 303.6±4.0 (31) |

*$\dot{V}_{O_2,run}$, peak metabolic rate elicited by running; H-BMR, high basal metabolic rate; L-BMR, low basal metabolic rate; $\dot{V}_{O_2,swim}$, line type selected for high level of maximum oxygen consumption elicited by swimming.
Values are means ± s.e.m. (*N*).

## RESULTS

### Common model

Mean body mass did not vary between lines and $\dot{V}_{O2,run}$ was strongly affected by $P_{O2}$ in all line types (Table 1).

A mixed 'common' model with line type (H-BMR, L-BMR, $\dot{V}_{O2,swim}$ and RB) revealed a weak line type × $P_{O2}$ interaction ($F_{6,12}$=2.89, $P$=0.05, Fig. 1). To pinpoint the source of this interaction we repeated the analysis of $\dot{V}_{O2,run}$ within each level of $P_{O2}$. These analyses did not detect an effect of line type at 14% and 21% $O_2$ ($F_{3,6}$=1.68, $P$=0.27 and $F_{3,6}$=3.39, $P$=0.09, respectively), but revealed a significant line-type effect at 30% $O_2$ ($F_{3,6}$=6.58, $P$=0.02). A Tukey's test indicated that this effect was due to significant differences between H-BMR and randomly selected line types ($P$=0.05).

### Divergent selection on BMR

A mixed model of ANCOVA of $\dot{V}_{O2,run}$ with line type (H-BMR, L-BMR and RB) revealed a significant effect of $P_{O2}$ ($F_{2,6}$=191.6, $P$<0.001) and line type ($F_{2,6}$=19.1, $P$=0.002) with a nonsignificant line type × $P_{O2}$ interaction ($F_{4,6}$=3.7, $P$=0.07). $\dot{V}_{O2,run}$ differed significantly between all three levels of $P_{O2}$ (pair-wise Tukey's test, $P$=0.01). The Tukey's test also revealed significant differences between H-BMR and the other two line types ($P$=0.05).

Within-selection ANCOVA with $P_{O2}$ and line type (L-BMR and H-BMR only) as fixed effects did not reveal a significant line type × $P_{O2}$ interaction ($F_{2,47}$=0.77, $P$=0.47), but detected strong effects of $P_{O2}$ ($F_{2,47}$=36.8, $P$<0.001) and line type ($F_{1,47}$=11.5, $P$=0.001), with significant differences between all three levels of $P_{O2}$ (pair-wise Tukey's test, $P$<0.001; Fig. 2).

### Selection on $\dot{V}_{O2,swim}$

$\dot{V}_{O2,run}$ was significantly affected by $P_{O2}$ ($F_{2,12}$=453.1, $P$<0.0001) with values significantly different between all three $P_{O2}$ levels (pair-wise Tukey's test, $P$<0.001; Fig. 3). $\dot{V}_{O2,run}$ was also affected by the line type ($F_{1,12}$=21.8, $P$=0.0005), with values consistently higher in lines selected for $\dot{V}_{O2,swim}$ across all $P_{O2}$ levels (Fig. 3). The $P_{O2}$ × line type interaction was not significant ($F_{2,12}$=2.6, $P$=0.1).

Comparison of the number of RBCs of selected *vs* RB lines did not reveal statistically significant differences ($F_{1,6}$=1.6, $P$=0.22).

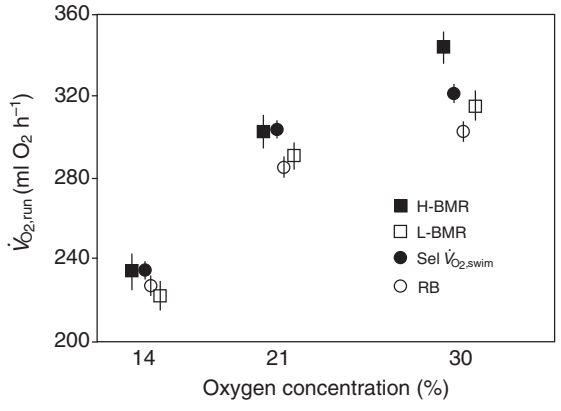


Fig. 1. Maximum metabolic rate elicited by running ($\dot{V}_{O2,run}$) at different $O_2$ partial pressures in mice experimentally selected for high basal metabolic rate (H-BMR, solid squares), low BMR (L-BMR, open squares), high maximum metabolic rate elicited by swimming (Sel $\dot{V}_{O2,swim}$, solid circles), and random-bred (RB, open circles) line types. Symbols indicate adjusted means (± s.e.m.) from the 'common' model (see text).

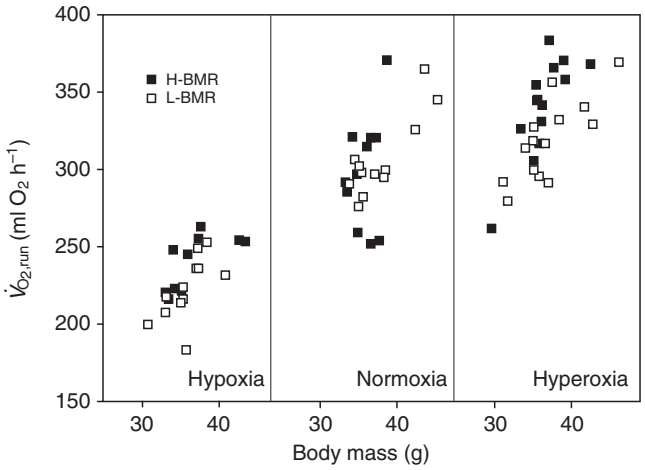


Fig. 2. Individual variation of maximum metabolic rate elicited by running at different $O_2$ partial pressures in mice divergently selected for high (H-BMR, solid squares) and low (L-BMR, open squares) BMR.

Likewise, haemoglobin concentration did not differ significantly between line types ($F_{1,6}$=1.9, $P$=0.26).

## DISCUSSION

### Possible limitations to $\dot{V}_{O2,max}$

Recall that $\dot{V}_{O2,max}$ can be considered to be set by interactions between five major processes of $O_2$ transport and uptake: (1) ventilator convection, (2) alveolar–capillary diffusion, (3) blood convection, (4) tissue capillary-to-cell diffusion (Taylor and Weibel, 1981; Wagner, 1996) and possibly (5) mitochondrial oxidative capacity (Weibel et al., 1991). What can our results tell us about possible limiting steps?

Maximum oxygen consumption of mice from both selection experiments was significantly affected by gas composition, and relative to normoxia, significantly decreased in response to hypoxia (Fig. 1). This pattern was also reported in rats selected for increased endurance capacity (Henderson et al., 2002; Howlett et al., 2003)

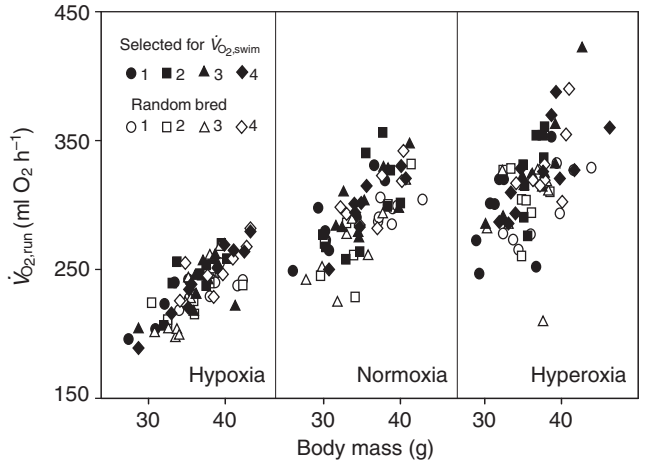


Fig. 3. Individual variation of maximum metabolic rate elicited by running at different $O_2$ partial pressures in mice experimentally selected for high maximum metabolic rate elicited by swimming ($\dot{V}_{O2,swim}$; four lines, close symbols) and their random-bred control (four lines, open symbols) line types.

and mice selected for high voluntary activity (Rezende et al., 2006a; Rezende et al., 2006b). The most straightforward interpretation of such an outcome is that decreased $\dot{V}_{O_2,run}$ in hypoxia is due to limitations in $O_2$ delivery in steps 1–3, which is further supported by an increase of $\dot{V}_{O_2,run}$ in hyperoxia. However, although in our experiment $\dot{V}_{O_2,run}$ consistently increased in hyperoxia in both selected and randomly bred lines, there was no difference between normoxia and hyperoxia in the control lines of the selection on voluntary wheel running (Rezende et al., 2006a). Thus, responses to hypoxia were similar across different selection regimens, but differed in mice exposed to hyperoxic conditions. Interestingly, significant between-line differences at hyperoxia reported in our study suggest that peripheral oxidative capacity can substantially increase through selection on high BMR (Fig. 1).

To identify which of the specific steps in the oxygen transport cascade are limiting oxygen delivery it is worth noting that in a previous study we demonstrated that in mice selected for increased $\dot{V}_{O_2,swim}$ the level of $\dot{V}_{O_2,cold}$ was at least 25% higher that $\dot{V}_{O_2,run}$ (25% for RB and 26.7% for selected lines) (Gębczyński and Konarzewski, 2009b). Likewise, it has been demonstrated that in lines of mice selected for high voluntary activity the $\dot{V}_{O_2,max}$ elicited by cold exposure was approximately 32% higher than that on the treadmill, with a similar mean increase for both selected (31.6% increase) and RB (32.4% increase) lines (Rezende et al., 2005). In contrast, in mice selected for divergent BMR, $\dot{V}_{O_2,max}$ elicited by cold (Książek et al., 2004) and noradrenaline injection (Gębczyński, 2008) did not differ from $\dot{V}_{O_2,run}$ in normoxic conditions (Gębczyński and Konarzewski, 2009a) (this study).

The main difference between oxygen delivery in cold ($\dot{V}_{O_2,cold}$) and exercise is the tissues where the $O_2$ is used. In exercise most of the $O_2$ is consumed in skeletal muscle (Mathieu et al., 1981), whereas during cold exposure $\dot{V}_{O_2,cold}$ largely reflects the extraction of $O_2$ by brown adipose tissue (Zaninovich et al., 2003). That is, steps 1–3 are common to both exercise and thermogenesis, and yet, mice selected on $\dot{V}_{O_2,swim}$ (Gębczyński and Konarzewski, 2009b) and voluntary activity (Rezende et al., 2005) are capable of delivering more oxygen under cold exposure. In other rodent species, $\dot{V}_{O_2,cold}$ is also higher than $\dot{V}_{O_2,run}$ [e.g. deer mice (Chappell, 1984; Hayes and Chappell, 1986); ground squirrels (Chappell and Bachman, 1995)]. This points to step 4 (tissue capillary-to-cell diffusion) as limiting $\dot{V}_{O_2,run}$ in mice selected on $\dot{V}_{O_2,swim}$ and voluntary running (but not in mice selected on BMR, as their $\dot{V}_{O_2,max}$ elicited by exercise and thermogenesis did not differ). This view is further supported by results of studies on rats selected for divergent low- and high-endurance exercise capacity (Henderson et al., 2002; Howlet et al., 2003; Howlet et al., 2009; Gonzales et al., 2006). These studies demonstrated that the first structural and functional responses to selection enabling greater $O_2$ utilization in the high capacity runner line type were muscle adaptations that improved oxygen extraction, with little difference in $O_2$ delivery capacity at the level of heart and lungs (Henderson et al., 2002; Howlett et al., 2003). In particular, at generation seven of selection (G7), increased muscle extraction was coupled with 33% between-line-type difference in skeletal muscle oxygen conductance (Howlett et al., 2003).

However, further selection on low- and high-endurance exercise capacity resulted in substantial differences in pulmonary function (Kirkton et al., 2009) and cardiac output (Gonzales et al., 2006). Howlett et al. therefore hypothesized that central factors (cardiovascular and/or pulmonary changes) could also become limiting during $\dot{V}_{O_2,run}$ (Howlett et al., 2009). In our mice, divergently selected on BMR, the probable role of lungs as the central limitation in oxygen delivery is supported by the lack of differences between $\dot{V}_{O_2,max}$ elicited by cold, noradrenaline injection and exercise (Książek et al., 2004; Gębczyński, 2008; Gębczyński and Konarzewski, 2009a). Pulmonary limits are particularly well documented in thoroughbred racehorses, in which maximal exercise results in hypercapnia, hypoxemia and, sometimes fatal, exercise-induced pulmonary haemorrhaging (West and Mathieu-Costello, 1995). However, studies on humans indicate that their ventilatory capacity is largely excessive and fully exploited only in the most elite endurance athletes (Lindstedt and Conley, 2001). Likewise, results of interspecific comparisons led Weibel and coworkers (Weibel et al., 1991) to the conclusion that the capacity of mammalian lung for oxygen uptake exceeds the normal demand and that they have a 'reserve capacity'. The excess capacity of lungs suggested by interspecific comparisons is difficult to reconcile with our results on mice divergently selected for BMR (and the above mentioned studies on rats), which indicate that limitations on ventilator capacity are context dependent.

Another possible limiting step of the oxygen delivery cascade is cardiac output (Mitchell et al., 1958; Liguzinski and Korzeniewski, 2007). Heart size/mass has often been used as a predictor of cardiac output and ultimately $\dot{V}_{O_2,max}$ (Bishop, 1997; Richardson et al., 1999a; Chen et al., 2001; Hussain et al., 2001). All else being equal, heart mass should be closely correlated with stroke volume (Karas et al., 1987; Bishop, 1997) and therefore, can be considered as a significant predictor of the heart's pumping capacity. Moreover, the highly significant positive correlation between $\dot{V}_{O_2,max}$ of mice selected for high voluntary activity and ventricle mass at different oxygen concentrations (Rezende et al., 2006b) suggest that cardiac output is indeed an important factor in determining individual differences in $\dot{V}_{O_2,max}$.

However, the possibility of cardiac output acting as a limiting step does not square with our earlier studies of divergent selection on BMR that resulted in a significantly increased heart mass in the H-BMR line type (Książek et al., 2004; Gębczyński, 2008; Gębczyński and

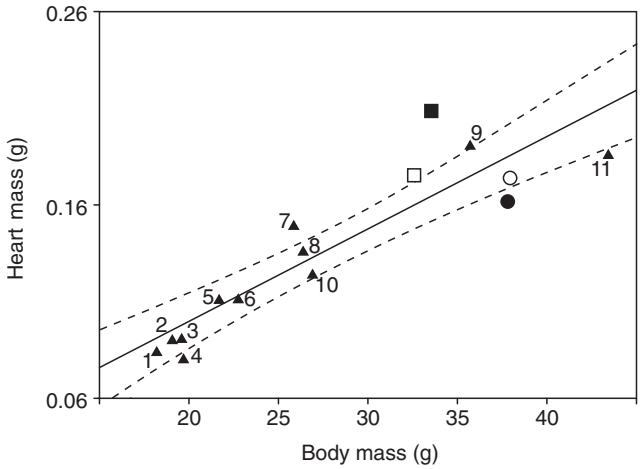


Fig. 4. The relationship between heart and body mass in strains of mice subjected to artificial selection experiments: closed square, H-BMR; open square, L-BMR [means of values from Książek et al., Brzęk et al. and Gębczyński (Książek et al., 2004; Brzęk et al., 2007; Gębczyński, 2008)]; open circle, mice selected for $\dot{V}_{O_2,swim}$; closed circle, randomly bred (Gębczyński and Konarzewski, 2009b); closed triangles, mice of SWR/J strain (1), C57BL/6 strain (2), HRS/J strain (3), A/J strain (4), DBA/2 strain (5), AKR/J (6) (Konarzewski and Diamond, 1995), DBA/2 strain (7), C57BL/6 strain (8), C57BL/6(WW) strain (9) (Vaanholt et al., 2009), wild-type mice (10) (Luptak et al., 2007), SW strain (11) (Zhao and Cao, 2009). Dashed lines indicate 0.95 confidence limits.

Konarzewski, 2009b), but this did not provide any compensation compared with the L-BMR line type and RB lines under hypoxic conditions (Fig. 1). To put this finding in an even broader context, we plotted literature data on heart masses *vs* body masses of mice subjected to different selection regimens (Fig. 4), which confirms an exceptionally high heart mass of H-BMR mice. The question then rises of why selection on high BMR resulted in increased cardiac output, which after all, may indicate central limitation on $\dot{V}_{O2,max}$. We suggest that the increased heart mass of H-BMR mice is part of an indirect response to selection resulting in enlarged metabolically active internal organs (liver, small intestine and kidneys in addition to the heart), whose metabolism accounts for a significant proportion of BMR (Konarzewski and Diamond, 1995; Książek et al., 2004; Gębczyński and Konarzewski, 2009a). However, an increase of mass of those organs did not result in a correlated increase in $\dot{V}_{O2,max}$ elicited by cold or exercise (Gębczyński and Konarzewski, 2009a). Thus, it is unsurprising that in this study we did not observe any compensation in $\dot{V}_{O2,run}$ of H-BMR mice in hypoxia.

Selection for high $\dot{V}_{O2,swim}$ also resulted in an increased heart mass, albeit not that conspicuous as in the case of H-BMR mice (Fig. 4) (Gębczyński, 2008; Gębczyński and Konarzewski, 2009b). Larger cardiac output delivered by the heavier hearts of mice selected on $\dot{V}_{O2,swim}$ is in agreement with their high $\dot{V}_{O2,cold}$, but does not necessarily result in the compensation of $\dot{V}_{O2,run}$ in hypoxia (Fig. 1) if the latter is limited peripherally in this line type. However, the lack of compensation of $\dot{V}_{O2,run}$ of H-BMR mice in hypoxia may be due to pulmonary limits suggested by comparable $\dot{V}_{O2,max}$ elicited by cold, noradrenaline injection and $\dot{V}_{O2,run}$ (Książek et al., 2004; Gębczyński, 2008; Gębczyński and Konarzewski, 2009a).

Apart from the pumping capacity of the heart, the key step in oxygen transport is convection through blood, which depends on both cardiac output and blood oxygen capacity. Recently, Kolb et al. showed that administration of an erythropoietin (EPO) analogue significantly increased $\dot{V}_{O2,max}$ by ~5% in mice selected on voluntary wheel running, with no dose × line type interaction (Kolb et al., 2010). This increase was presumably the result of higher blood haemoglobin content after EPO treatment. It is important to note in this context, that the line type selected on high BMR studied here is characterized by 30% smaller RBCs than low BMR mice. Nevertheless, both line types have a similar haematocrit (E. Bonda-Ostaszewska and S. Maciak, personal communication). Smaller and more numerous RBC should result in higher erythrocyte total surface area, which suggests more effective $O_2$ blood convection in H-BMR mice. Despite this, $\dot{V}_{O2,run}$ of H- and L-BMR line types did not differ. In contrast, $\dot{V}_{O2,run}$ of mice selected on high $\dot{V}_{O2,swim}$ is consistently higher than that of RB mice (Fig. 1), despite the lack of between-line-type differences in RBC and haemoglobin concentration. Clearly, variation of blood parameters in mice of our two selections is not consistently associated with differences in $\dot{V}_{O2,run}$.

### Is a response to selection on metabolic capacities symmorphothic

If responses to artificial selection on traits related to $\dot{V}_{O2,max}$ are symmorphotic, one would expect effects of $P_{O2}$ on $\dot{V}_{O2,max}$ to be independent of selection regimens, and differences between line types would remain constant across different air compositions. This is because according to the principle of symmorphosis, all traits related to the $\dot{V}_{O2,max}$ should evolve in concert, so that none of them would become a limiting (or excessive) step in oxygen delivery and utilization cascades. In the case of our study, this would manifest itself as the lack of line type × $P_{O2}$ interaction and was rejected in a 'common' model. Overall, our results suggest that the effect of $P_{O2}$ on $\dot{V}_{O2,run}$ was selection-history-dependent only for hyperoxia (Fig. 1), as in mice selected for high voluntary wheel running (Rezende et al., 2005). Interestingly, unlike in our selection on $\dot{V}_{O2,swim}$, in which hyperoxia elicited increased $\dot{V}_{O2,run}$ in RB lines (Fig. 1), $\dot{V}_{O2,run}$ elicited by normoxia and hyperoxia in the RB lines of Rezende et al.'s (Rezende et al., 2005) study did not differ. These contrasting responses of RB lines are also not in agreement with the notion of common symmorphotic design, as one would expect that increased $P_{O2}$ would not affect $\dot{V}_{O2,max}$ if cellular oxidative capacity was symmorphotic with normoxic delivery rates.

In summary, the apparently 'symmorphotic' consistent response of mice of all three selections to hypoxia and normoxia contrasts with their responses to hyperoxia and higher $\dot{V}_{O2,max}$ elicited by cold or after administration of EPO (Kolb et al., 2010). Thus, even though whole-organism $\dot{V}_{O2,max}$ must be physiologically constrained, the limitations are clearly context dependent (and probably species dependent as well), which makes the experimental search for generalizations about a possible symmorphotic nature of oxygen transport mechanisms a questionable effort.

## LIST OF ABBREVIATIONS

| | |
|---|---|
| BMR | basal metabolic rate |
| H-BMR, L-BMR | mice selected for high and low BMR, respectively |
| He–$O_2$ | helium–oxygen atmosphere |
| $P_{O2}$ | oxygen partial pressure |
| $\dot{V}_{O2,max}$ | maximum oxygen consumption |
| $\dot{V}_{O2,swim}$, $\dot{V}_{O2,run}$ | $\dot{V}_{O2,max}$ elicited by swimming or running, respectively |

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