

Artificial selection for high activity favors mighty mini-muscles in house mice

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Houle-Leroy, Philippe, Helga Guderley, John G. Swallow, and Theodore Garland, Jr. Artificial selection for high activity favors mighty mini-muscles in house mice. *Am J Physiol Regul Integr Comp Physiol* 284: R433–R443, 2003; 10.1152/ajpregu.00179.2002.—After 14 generations of selection for voluntary wheel running, mice from the four replicate selected lines ran, on average, twice as many revolutions per day as those from the four unselected control lines. To examine whether the selected lines followed distinct strategies in the correlated responses of the size and metabolic capacities of the hindlimb muscles, we examined mice from selected lines, housed for 8 wk in cages with access to running wheels that were either free to rotate (“wheel access” group) or locked (“sedentary”). Thirteen of twenty individuals in one selected line (line 6) and two of twenty in another (line 3) showed a marked reduction (~50%) in total hindlimb muscle mass, consistent with the previously described expression of a small-muscle phenotype. Individuals with these “mini-muscles” were not significantly smaller in total body mass compared with line-mates with normal-sized muscles. Access to free wheels did not affect the relative mass of the mini-muscles, but did result in typical mammalian training effects for mitochondrial enzyme activities. Individuals with mini-muscles showed a higher mass-specific muscle aerobic capacity as revealed by the maximal *in vitro* rates of citrate synthase and cytochrome *c* oxidase. Moreover, these mice showed the highest activities of hexokinase and carnitine palmitoyl transferase. Females with mini-muscles showed the highest levels of phosphofructokinase, and males with mini-muscles the highest levels of pyruvate dehydrogenase. As shown by total muscle enzyme contents, the increase in mass-specific aerobic capacity almost completely compensated for the reduction caused by the “loss” of muscle mass. Moreover, the mini-muscle mice exhibited the lowest contents of lactate dehydrogenase and glycogen phosphorylase. Interestingly, metabolic capacities of mini-muscled mice resemble those of muscles after endurance training. Overall, our results demonstrate that during selection for voluntary wheel running, distinct adaptive paths that differentially exploit the genetic variation in morphological and physiological traits have been followed.

aerobic capacity; correlated response; exercise physiology; muscle metabolic capacities; wheel running behavior

SELECTIVE BREEDING has proven effective at altering many aspects of the phenotype, including behavioral,

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and physiological traits (2, 9, 10, 13). When selection is imposed on an organismal trait, such as behavior or body size, various lower-level traits may change as well. For example, selection for fast growth in pigs leads to more efficient feed conversion, less fat accumulation, and a greater number of muscle fibers, whereas selection for high back fat thickness leads pigs to grow more slowly and have fewer muscle fibers (27).

When replicate lines are subjected to the same selection regimen, they can show heterogeneous responses. Such heterogeneity is often observed for traits other than the one under direct selection, including traits at lower levels of biological organization that may be functionally related to the selected trait. The causes of heterogeneous responses in correlated traits include founder effects and genetic drift, variability of pleiotropic effects among loci, and multiple physiological responses to selection (1, 7, 21, 23, 25). Even if replicate selected lines show similar phenotypic responses, these may have evolved by different genetic mechanisms (e.g., Refs. 3, 15). Although selection experiments are becoming increasingly popular in comparative and evolutionary physiology (8–11, 13), few studies have explored the potential for heterogeneous responses of the morphological and physiological mechanisms underlying adaptation (but see Refs. 5, 6, 16, 33).

Swallow et al. (29) used selective breeding to create four replicate lines of mice that exhibit high levels of voluntary wheel running while maintaining four random-bred lines as a control. After 14 generations of selection, mice from the selected lines were running more than twice as far as, and at higher speeds than, mice from the control lines (31). As wheel running in these mice is presumed to be primarily aerobic (see Refs. 14, 18, 19, 30, 34), we reasoned that the selection protocol would favor mice with a high aerobic capacity in their hindlimb muscles. Although access to running wheels led to effects typical of endurance training, after 14 generations mice from selected lines did not have significantly higher muscle aerobic capacities than those from control lines (18; see also Ref. 34).

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A complementary response to selection for high wheel running could involve changes in muscle mass. Body mass of mice from the selected lines has become significantly lower than that in control lines (31). Although a loss of body fat explains much of this change (32; see also Ref. 4), decreased muscle mass could contribute. In humans, endurance runners are typically relatively small individuals who have reduced mass of some hindlimb muscles (e.g., see Ref. 20). In rats, voluntary wheel running can reduce muscle mass, especially in the hindlimbs (26). This suggests that selection for increased voluntary wheel running could favor decreases in the mass of muscles used for running.

Indeed, at generation 22, mice from the four selected lines show a significant reduction in mass of the triceps surae complex (corrected for variation in total body mass; 12), an important extensor of the ankle. Moreover, two of the selected lines exhibit a discrete polymorphism in the triceps surae that almost halves muscle mass and seems to be inherited as a simple Mendelian recessive (12). Hierarchical models that included effects of genetic drift and/or selection indicated that the small-muscle allele was present at low frequency (~7%) in the base population and has since experienced strong positive selection in the selected but not control lines (12). We hypothesized that the "mini-muscles" may possess functional characteristics that facilitate high levels of wheel running. For example, if the mini-muscles were primarily composed of small oxidative fibers, they could facilitate wheel running as this would increase muscle mass specific aerobic capacity as well as reduce diffusion distances for delivery of oxygen and blood-borne substrates (24).

To examine possible heterogeneity in the response of hindlimb muscles to selection for voluntary wheel running, we housed mice from the selected and control lines for 8 wk in cages with access to running wheels that were either free to rotate (wheel-access group) or locked (sedentary group). This design allowed us to examine whether effects of selection are only expressed in the presence of the selected behavior, i.e., wheel running.

To obtain an overview of the capacities of the major pathways of energy metabolism in the hindlimb muscles, we measured three enzymes that may limit flux in these pathways: phosphofructokinase [PFK (glycolysis)], pyruvate dehydrogenase [PDH (mitochondrial pyruvate oxidation)], and carnitine palmitoyl transferase [CPT (mitochondrial fatty acid oxidation)]. We also measured enzymes that deliver glucose to glycolysis, hexokinase (HK), and glycogen phosphorylase [both in the activated (GP α) and total forms (GP α + GP β)], as well as the terminal enzyme of anaerobic glycolysis, lactate dehydrogenase (LDH). Finally, we measured citrate synthase (CS) and cytochrome *c* oxidase (CCO) as markers of mitochondrial abundance, respectively, located in the matrix and inner mitochondrial membrane. By measuring enzymes in a wide range of pathways, we sought to establish whether putative modifications of muscle metabolic capacity

were generalized or whether they were pathway specific. Previously, we presented overall comparisons of muscle metabolic capacity in relation to selection and wheel access (18). Here we examine variation in enzyme activities in relation to the small-muscle polymorphism.

METHODS

The selection experiment. Mice used in this study were sampled from generation 14 (2nd litters) of our artificial selection experiment for increased voluntary activity on running wheels. Swallow et al. (29) provide full details of the selection experiment and only a brief overview will be given here.

The original progenitors for the selection experiment were outbred Hsd:ICR house mice (*Mus domesticus*) obtained from Harlan Sprague Dawley, Indianapolis, IN. In each generation, 10 pairs (families) of mice were used to propagate each line, four selected for high wheel running and four randomly bred as control lines. The selection criterion was the total number of revolutions run on days 5 and 6 of a 6-day period when the mice had access to running wheels (beginning at 5.5–8 wk of age): in the selected lines, the highest running male and female from each family were chosen as breeders. In the control lines, one male and one female from each family were chosen randomly. The 10 males and 10 females from each line were paired randomly within the line, but no matings occurred between siblings. Pups were weaned at 21 days of age, weighed, and toe clipped for identification. The pups were housed with three siblings until the following day, when they were housed individually with access to running wheels that were either free to rotate or locked.

Animal husbandry. Routine housing was as described by Swallow et al. (31). In the selection experiment and for the mice studied in this study, voluntary wheel running was measured on Wahman-type activity wheels as described in Swallow et al. (31) and Houle-Leroy et al. (18).

Sampling strategy. We determined activities of muscle enzymes (CCO, CS, CPT, PDH HK, GP, PFK, and LDH) in 10 mice per line with locked wheels (sedentary group) and in 10 mice per line with access to free wheels (wheel access group). Within each family and sex, one individual was assigned to a free wheel (wheel access group) and one was assigned to a locked wheel (sedentary group). Therefore, each line (4 selected and 4 control) was represented by five wheel access males, five wheel access females, five sedentary males, and five sedentary females. Within each line, one mouse in each of the four subgroups came from the same family to obtain a balanced design. When, as occurred twice in the control lines, an individual died, the corresponding same-sex sibling (in the opposite activity group) was omitted from analyses to retain a balanced design.

Muscle dissection, enzyme extraction, and assay. Mice were killed by cervical dislocation to avoid effects of pharmaceuticals. Within ~10 min of death, all the muscles of the left hindlimb (except the triceps surae, which includes the lateral and medial heads of the gastrocnemius, soleus, and plantaris) were weighed, frozen on dry ice, and placed at -80°C . The masses of the left and right triceps surae, which were used for other experiments, were also recorded (see Ref. 12). The muscle samples were transported in liquid nitrogen to Université Laval for enzymatic determinations and measurements of protein concentrations as described in Houle-Leroy et al. (18). Enzyme activities and protein concentrations were measured in a pool of all hindlimb muscles, except the triceps surae.

Statistical analysis. The general linear models procedure in JMP (SAS Institute) was used to apply analysis of covariance (ANCOVA) models to our data. Inspection of graphs of muscle mass in relation to body mass revealed the presence of individuals with extremely small muscles (see Fig. 1). These individuals only occurred in selected lines 3 and 6 but were present in both mice with and without access to freely rotating wheels and in both sexes. Our statistical analysis examined whether this mini-muscle phenotype was responsible for the heterogeneity of muscle metabolic capacities apparent among the selected lines (18). Given that males and females differ in many traits, including wheel running, body mass, and muscle enzyme activities (18, 19, 29, 31), we analyzed them separately. We used two complementary approaches.

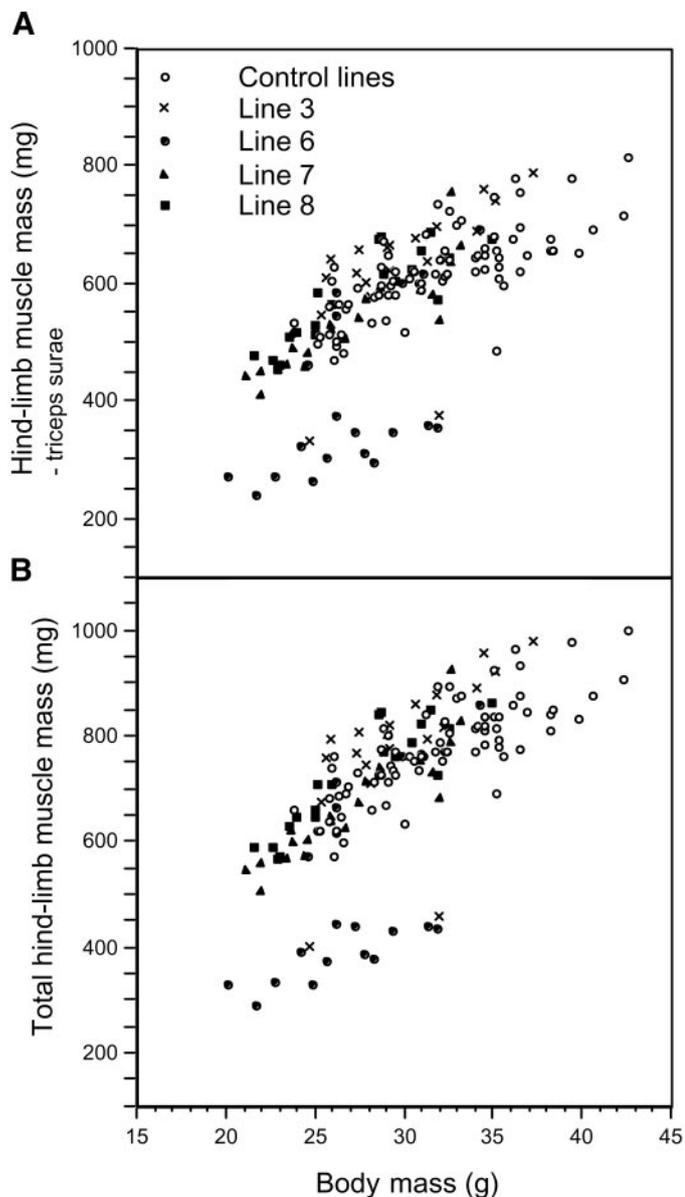


Fig. 1. Relationship between body mass and mass of left hindlimb muscles [excluding (A) and including (B) triceps surae mass] for 156 mice. Experimental design was balanced between control and selected mice as well as between sexes and activity states. Normal size muscled mice: $r^2 = 0.62$ (A), $r^2 = 0.69$ (B); mini-muscled mice: $r^2 = 0.58$ (A), $r^2 = 0.66$ (B).

First, we performed cross-nested two-way ANCOVAs to test the effects of line type (selected vs. control mice) and activity group (sedentary vs. wheel access mice) on our 156 experimental mice (see also Ref. 18) adding “mini” as a factor to code for mini- vs. regular-sized muscles. In these models, the main grouping factors, line type and activity group, were considered fixed effects. Replicate line ($n = 8$ total), nested within line type, was a random effect. Family, nested within line, was also included as a random effect. In these mixed models (i.e., with both random and fixed effects), we tested the effects over appropriate error terms as follows. Effects of line type were tested over the mean squares of line, and effects of line were tested over the mean squares of family. Effects of activity and the activity-line type interaction were tested over the mean squares of the line-activity interaction. Effects of mini were tested over the mean square of the error term. This analysis served to evaluate whether the occurrence of the mini-muscle phenotype in two of the four selected lines was responsible for the heterogeneity of muscle metabolic capacities among selected lines.

In models examining quantitative effects of wheel running (i.e., restricting analysis to mice with access to free wheels), we performed one-way ANCOVAs to test the effects of line type. We included replicate line (nested within line type) as a random effect and mini as a factor coding for muscle size.

Second, to examine specifically whether muscle metabolic characteristics differed between the normal and mini-muscle phenotypes, we focused on the two selected lines (3 and 6) in which the mini-muscle phenotype occurred. We compared the properties of the mice from these lines using mini, line, activity group, and family (nested within line) as factors; one sibling was sedentary, whereas the other had access to a free wheel.

Several covariates were used in all of the ANCOVA models. Body mass, age, time of death, and z-transformed time of death² were included as covariates in all models of muscle and body masses, enzyme activities, and protein fractions. Because body mass differed among the selected lines, all the ANCOVA models analyzing the enzyme activities, expressed both in units per gram muscle and as total hindlimb contents, were tested with and without body mass as a covariate. In models of wheel running amounts, covariates used were body mass, age, wheel freeness, and number of toes cut for identification.

RESULTS

Body and hindlimb muscle masses. As expected, body mass and the mass of the hindlimb muscles we studied were positively correlated (Fig. 1). However, two distinct relationships were apparent: 141 of the experimental mice were described by one relationship. A group of mice (7 females and 8 males) from two selected lines showed a completely distinct relationship between body and hindlimb muscle mass (Fig. 1); 13 of the 15 mice were from line 6 (7 males, 6 females), the other two were from line 3 (one male, one female). For a given range of body mass, mice from this mini-muscle group had masses of their hindlimb muscles (all muscles except the triceps surae) that were approximately one-half of those in the other group (240–376 vs. 411–748 mg; Fig. 1A). When we included the mass of the triceps surae to obtain the total hindlimb muscle mass, the same individuals composed the mini-muscle group. The total hindlimb muscle mass in the mini-

Table 1. *Body and hindlimb muscle masses for selected lines*

Variable	Male						Female					
	Line 3		Line 6		Line 7	Line 8	Line 3		Line 6		Line 7	Line 8
	Mini	Normal	Mini	Normal			Mini	Normal	Mini	Normal		
Body, g												
Sedentary	32.0(1)	34.4 ± 1.4(4)	29.6 ± 1.3(4)	29.9(1)	30.8 ± 1.1	32.2 ± 0.7	ND	28.4 ± 1	23.1 ± 0.9(3)	26.1 ± 0(2)	24.8 ± 0.6	24.8 ± 0.5
Wheel access	ND	31.1 ± 1.1	27.7 ± 0.3(3)	32.6 ± 1.6(2)	29.5 ± 1.1	29.2 ± 0.4	24.7(1)	26.8 ± 0.6(4)	23.3 ± 1.7(3)	25.3 ± 0.8(2)	22.9 ± 0.9	22.9 ± 0.4
Hindlimb muscles, mg												
Sedentary	376(1)	741 ± 23(4)	359 ± 6(4)	602(1)	595 ± 26	643 ± 20	ND	616 ± 20	259 ± 10(3)	564 ± 19(2)	499 ± 12	532 ± 21
Wheel access	ND	658 ± 17	319 ± 16(3)	655 ± 36(2)	592 ± 48	648 ± 15	332(1)	622 ± 18(4)	299 ± 15(3)	482 ± 19(2)	464 ± 19	487 ± 12

Values are means ± SE. Sample size per cell was $n = 5$, except where it was indicated in parentheses. Hindlimb muscle mass excludes the mass of the triceps surae. ND, no data available.

muscle group was 289–458 mg, whereas that of the other mice was 506–910 mg (Fig. 1B). Neither sex nor wheel access appeared to affect expression of the mini-muscle phenotype. In line 6, of the seven males with mini-muscles, three had access to free wheels; of the six females, three had access to free wheels. For the mice with mini-muscles in line 3, the female had access

to a free wheel, whereas the male was sedentary. Means for each subgroup are reported in Table 1.

As suggested by our inspection of the data, for both male and female mice, the mass of the total hindlimb muscles was significantly ($P < 0.01$) lower in mice with the mini-muscle phenotype, for both ANCOVA models (Table 2). Exclusion of body mass as a covariate did not

Table 2. *Statistical effects (F test) from 2-way ANCOVAs for mini-muscle factor*

	Male		Female	
	All individuals ($n = 78$)	Line 3 and 6 ($n = 20$)	All individuals ($n = 78$)	Line 3 and 6 ($n = 20$)
Body mass, g	$F_{1,27} = 0.97$	$F_{1,5} = 0.27$	$F_{1,27} = 0.70$	$F_{1,5} = 0.55$
Hindlimb muscle mass, mg				
All covariates	$F_{1,26} = 27.07\$$	$F_{1,4} = 424.51\$$	$F_{1,26} = 53.10\$$	$F_{1,4} = 36.84†$
Without body mass	$F_{1,27} = 27.18\$$	$F_{1,5} = 135.59\$$	$F_{1,27} = 40.0\$$	$F_{1,5} = 34.11†$
Enzyme activities, U/g muscle				
CCO	$F_{1,26} = 35.14\$$	$F_{1,4} = 13.99*$	$F_{1,26} = 16.81‡$	$F_{1,4} = 13.39*$
CS	$F_{1,26} = 29.83\$$	$F_{1,4} = 24.34†$	$F_{1,26} = 87.94\$$	$F_{1,4} = 8.97*$
HK	$F_{1,26} = 19.68\$$	$F_{1,4} = 27.26†$	$F_{1,26} = 21.74\$$	$F_{1,4} = 6.68$
CPT	$F_{1,26} = 5.66*$	$F_{1,4} = 0.91$	$F_{1,26} = 14.19‡$	$F_{1,4} = 12.19*$
PDH	$F_{1,26} = 11.52†$	$F_{1,4} = 2.32$	$F_{1,26} = 0.69$	$F_{1,4} = 0.18$
GP _a	$F_{1,26} = 1.90$	$F_{1,4} = 4.03$	$F_{1,26} = 1.36$	$F_{1,4} = 1.71$
GP _{tot}	$F_{1,26} = 2.09$	$F_{1,4} = 0.12$	$F_{1,26} = 0.01$	$F_{1,4} = 0.01$
PFK	$F_{1,26} = 0.79$	$F_{1,4} = 3.40$	$F_{1,25} = 12.68†$	$F_{1,3} = 97.08†$
LDH	$F_{1,26} = 0.47$	$F_{1,4} = 1.43$	$F_{1,26} = 0.28$	$F_{1,4} = 0.48$
Enzyme contents, U				
CCO	$F_{1,26} = 0.15$	$F_{1,4} = 0.001$	$F_{1,26} = 0.26$	$F_{1,4} = 1.59$
CS	$F_{1,26} = 0.16$	$F_{1,4} = 0.02$	$F_{1,26} = 0.07$	$F_{1,4} = 0.30$
HK	$F_{1,26} = 0.72$	$F_{1,4} = 0.02$	$F_{1,26} = 1.63$	$F_{1,4} = 5.90$
CPT	$F_{1,26} = 0.10$	$F_{1,4} = 0.06$	$F_{1,26} = 1.59$	$F_{1,4} = 1.87$
PDH	$F_{1,26} = 0.50$	$F_{1,4} = 0.01$	$F_{1,26} = 3.75$	$F_{1,4} = 3.11$
GP _a	$F_{1,26} = 4.77*$	$F_{1,4} = 51.58†$	$F_{1,26} = 7.57*$	$F_{1,4} = 8.94*$
GP _{tot}	$F_{1,26} = 7.31*$	$F_{1,4} = 2.43$	$F_{1,26} = 20.61\$$	$F_{1,4} = 17.37*$
PFK	$F_{1,26} = 1.08$	$F_{1,4} = 0.38$	$F_{1,25} = 1.43$	$F_{1,3} = 174.63‡$
LDH	$F_{1,26} = 4.49*$	$F_{1,4} = 22.69†$	$F_{1,26} = 10.85†$	$F_{1,4} = 11.32*$
Protein concentrations, mg/g muscle				
Sarcoplasmic	$F_{1,26} = 0.28$	$F_{1,4} = 0.49$	$F_{1,26} = 0.21$	$F_{1,4} = 2.10$
Myofibrillar	$F_{1,26} = 8.00†$	$F_{1,4} = 2.63$	$F_{1,26} = 25.88\$$	$F_{1,4} = 16.90*$
Total	$F_{1,26} = 4.22*$	$F_{1,4} = 1.93$	$F_{1,26} = 6.58*$	$F_{1,4} = 12.49*$
Protein contents, mg				
Sarcoplasmic	$F_{1,26} = 10.92†$	$F_{1,4} = 66.65†$	$F_{1,26} = 14.71‡$	$F_{1,4} = 91.65‡$
Myofibrillar	$F_{1,26} = 19.26‡$	$F_{1,4} = 54.24†$	$F_{1,26} = 71.82\$$	$F_{1,4} = 30.77†$
Total	$F_{1,26} = 23.42\$$	$F_{1,4} = 93.96‡$	$F_{1,26} = 48.11\$$	$F_{1,4} = 24.04†$

Contents were expressed per hindlimb muscle mass (excluding triceps surae mass). Covariates used in these models were body mass, age, time of death, and (z-transformed time of death)². * $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$; § $P \leq 0.0001$. When F values are not followed by a symbol, the result was not statistically significant. See Fig. 2 for definitions.

alter the statistical conclusions. The same results were found for mass of the triceps surae alone (data not shown). Hindlimb muscle mass increased with body mass for both the normal and mini-muscle mice (Fig. 1). Nonetheless, body mass did not differ significantly between mice with mini and normal muscles ($P > 0.05$; Table 2). In females, some heterogeneity in body mass was apparent among replicate lines ($F_{6,31} = 4.30$; $P < 0.005$), especially for the selected lines. Female mice from line 3 were larger by almost 13% than females in the other selected lines (Table 1).

Wheel running. As was true for mice from generation 22 (10), wheel running did not differ between mice with mini and normal muscles for either sex, whether expressed as distance run (total revolutions/day), average speed (rpm), or active time on the wheel (1-min intervals with any revolutions/day) (Table 3).

Specific enzyme activities. Considerable heterogeneity in enzyme activities (unit/g muscle) was found among the replicate lines within line type (control vs. selected) for both sexes, especially for CCO, CS, HK, glycogen phosphorylase [total form (GPtot)], and PFK (18). We therefore focused on the enzyme activities in the selected lines, separating the mini and normal mice in lines 3 and 6 [Supplemental Materials Tables 1 and 2; available online at <http://ajpregu.physiology.org/cgi/content/full/284/2/R443/DC1>], to explore if this heterogeneity was related to the mini-muscle phenotype. The relationships between muscle mass and enzyme activities for all the selected and control mice are shown in Fig. 2.

Inspection of Fig. 2A, left, indicates that mice with the mini-muscle phenotype exhibited considerably higher activities (U/g muscle) of CCO, CS, and HK than their counterparts with normally sized muscles. That these high activities are attributable to the small muscle phenotype per se and not to line origin is suggested by the fact that the two individuals with mini-muscles from line 3 always were positioned with the mini-muscle individuals from line 6. Both when all individuals were analyzed and when only lines 3 and 6 were analyzed, mini-muscles from male and female mice consistently showed significantly higher CCO,

CS, and HK activities than normal sized muscles (Table 2). The inclusion of body mass did not change the statistical conclusions, except for HK in females. In this case, when body mass was included in the model of line 3 and 6, the mini-factor was not significant ($F_{1,4} = 6.68$; $P = 0.061$; Table 2), but when body mass was removed, mini-muscled females showed significantly higher levels of HK ($F_{1,5} = 8.19$; $P = 0.035$).

The two other mitochondrial enzymes, CPT and PDH, followed similar trends as CCO, CS, and HK, with mini-muscled mice having higher activities (Fig. 2B, left; supplemental materials Table 1). Statistical analyses revealed that mini-muscles had significantly higher activities of CPT in both ANCOVA models for female mice, whereas, in males, the effect was significant ($F_{1,26} = 5.66$; $P = 0.025$) only when we tested all individuals (Table 2). For PDH activities in males, mini-muscles had higher activities only when all mice were tested ($F_{1,26} = 11.52$; $P = 0.002$), whereas no significant differences were found for females (Table 2). All these differences remained apparent when body mass was not present as a covariate in the statistical analysis.

The activities of glycolytic enzymes were not systematically enhanced in the mini-muscles. For PFK, inspection of Fig. 2A, left, suggested that mini-muscled mice have higher specific activities. However, ANCOVA models revealed that only mini-muscles from females had significantly ($P < 0.01$ for both models) higher specific activities of PFK and only when one outlying value (the highest, Fig. 2) was eliminated, whereas, in males, PFK did not differ ($P > 0.05$) between the two phenotypes (Table 2). On the other hand, the mini-muscle phenotype did not significantly ($P > 0.05$) affect the specific activities of GP (GPa and Gptot) or LDH in male or female mice (Table 2). Again, for these enzymes (PFK, LDH, and GP), the inclusion of body mass in the models did not change the conclusions, except for PFK in females from line 3 and 6, where PFK level was higher in mini-muscles only when body mass was included in the model ($F_{1,3} = 97.08$; $P = 0.002$; Table 2). This effect became only

Table 3. Wheel running data for selected lines during the final week of access to wheels and statistical effects (F test) from 1-way ANCOVA for mini-muscle factor

	Line 3		Line 6		Line 7	Line 8	Statistical effects
	Mini	Normal	Mini	Normal			
Total revolutions/day							
Male	ND	9,715 ± 1,547	9,646 ± 1,155	7,771 ± 1,964	9,003 ± 327	5,923 ± 439	$F_{1,23} = 0.19$; $P = 0.67$
Female	16,063	14,072 ± 2,079	15,441 ± 2,667	14,103 ± 1,340	10,120 ± 972	12,188 ± 1,002	$F_{1,23} = 0.81$; $P = 0.38$
Average rpm							
Male	ND	24.4 ± 3.4	23.0 ± 2.0	17.0 ± 2.0	19.6 ± 4.2	16.0 ± 1.1	$F_{1,23} = 1.58$; $P = 0.22$
Female	29.0	29.2 ± 2.3	31.3 ± 2.9	30.5 ± 3.5	24.4 ± 2.0	22.4 ± 1.7	$F_{1,23} = 0.004$; $P = 0.95$
1-Min intervals/day							
Male	ND	407 ± 45	417 ± 13	441 ± 63	407 ± 59	378 ± 22	$F_{1,23} = 0.09$; $P = 0.77$
Female	556	475 ± 51	490 ± 43	464 ± 6	412 ± 24	541 ± 25	$F_{1,23} = 0.57$; $P = 0.46$

Values are means ± SE. Sample size per cell was as given in Table 1. One revolution = 1.12 m · 1-min intervals day⁻¹ is the number of 1-min intervals during which any wheel revolutions occurred. Covariates used were body mass, age, wheel freeness, and number of toes cut for identification. ND, no data available.

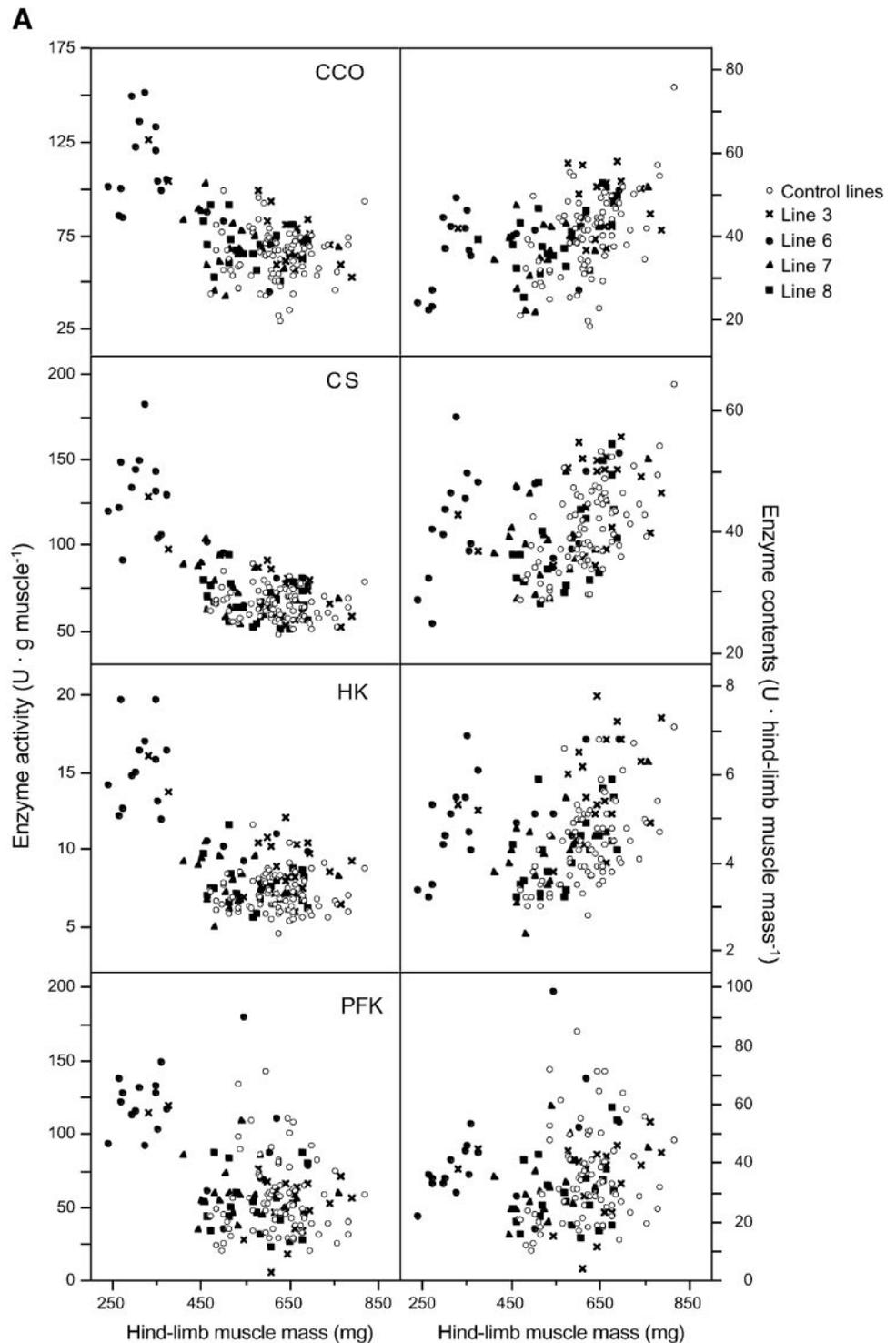


Fig. 2. Relationship between enzyme activity, expressed as specific activity (*left*) or hindlimb muscle contents (*right*) and hindlimb muscle mass (excluding triceps surae) for 156 mice. Experimental design was balanced between control and selected mice as well as between sexes and activity states. CCO, cytochrome *c* oxidase; CS, citrate synthase; HK, hexokinase; PFK, phosphofructokinase (*A*); PDH, pyruvate dehydrogenase; CPT, carnitine palmitoyl transferase; GP α , glycogen phosphorylase (activated); GP tot , glycogen phosphorylase (total); LDH, lactate dehydrogenase (*B*).

marginally significant when body mass was omitted ($F_{1,4} = 7.01$; $P = 0.057$).

Training effects on enzyme specific activities. Houle-Leroy et al. (18) demonstrate pronounced effects of voluntary wheel running for enzymes involved in aerobic carbohydrate metabolism in these mice. Such effects remained apparent when the normal and mini-muscle phenotypes were separated. To examine

whether training effects were significant in mice with the mini-muscle phenotype, we carried out one-factor ANCOVAs, focusing on the mini-muscled mice in line 6 ($n = 13$). For CCO, CS, HK, and PDH, voluntary wheel running significantly ($P < 0.03$) increased specific activities in the hindlimb muscles, indicating that a response to training still occurs in the mini-muscled phenotype.

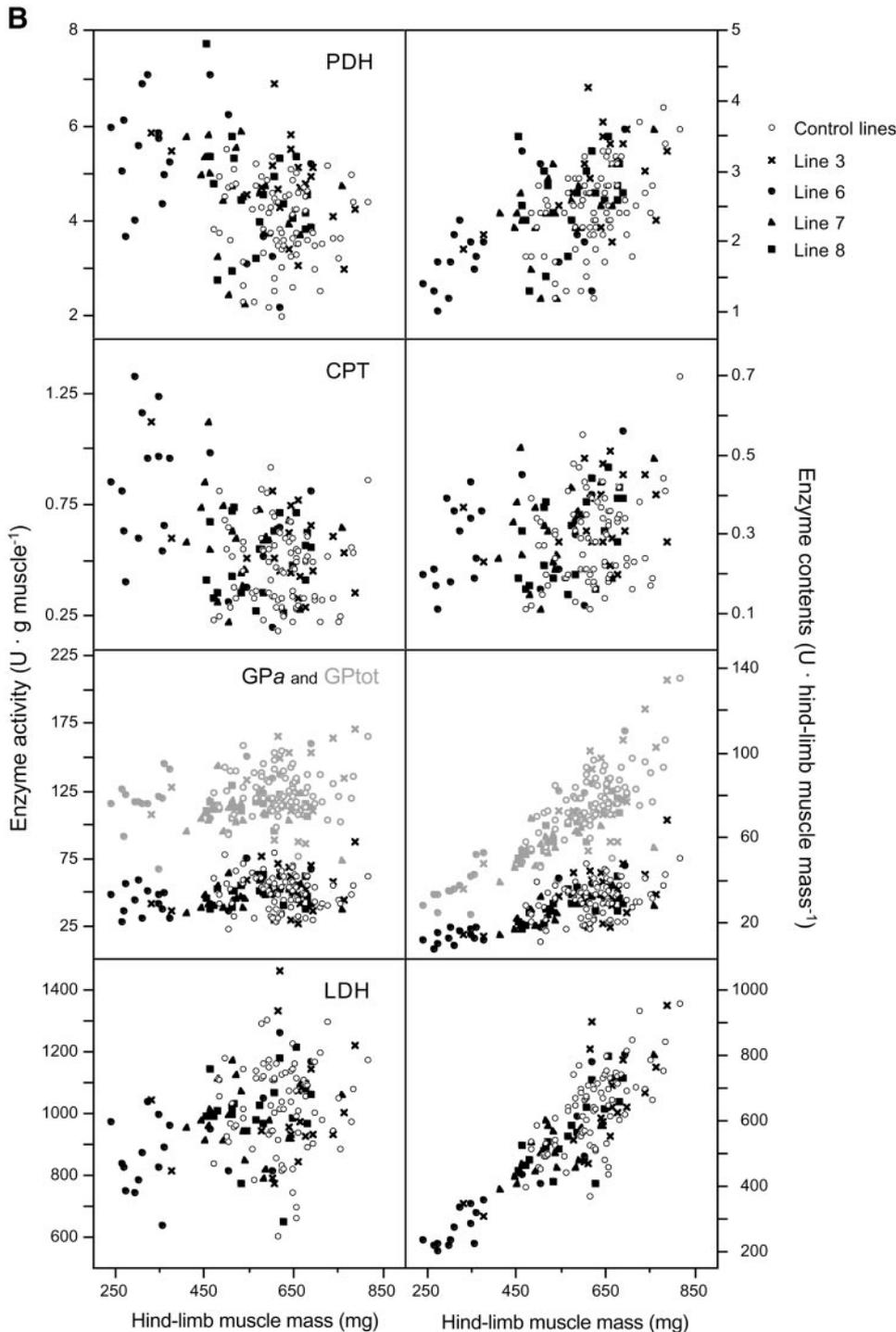


Fig. 2.—Continued

Total enzyme contents. The increased mass-specific activities of the mitochondrial enzymes (especially CS, CCO, and CPT) and HK in the mini-muscles led the total contents of these enzymes to be similar to those in the normal muscles (Fig. 2, *A–B*, *right*; Supplemental Materials Table 2). This was true for both male and female mice. Thus the increase in the specific activity of the mitochondrial enzymes completely compensated for the decrease in muscle size. Whereas the specific activities of PDH were not significantly increased in

mini-muscles, except in males, hindlimb muscle PDH contents did not differ ($P > 0.05$) between mice with the mini- and normal muscle phenotypes for either sex (Table 2).

The mice with the mini-muscle phenotype had the lowest contents of $GP\alpha$, GP_{tot} , and LDH (Fig. 2*B*, *right*). Again, the two mini-muscle individuals from line 3 exhibited the same pattern as those from line 6. Statistical analyses confirmed the significance ($P < 0.05$) of this pattern for both sexes using both ANCOVA

models, except that the GPTot contents did not differ between mini- and normally muscled males in the comparison of the mice in lines 3 and 6 (Table 2).

Generally, PFK was not affected by the mini-muscle phenotype. However, when the ANCOVA focused on the females in lines 3 and 6, PFK contents were higher in mini- than in normally sized muscles only when one outlying value was eliminated ($F_{1,3} = 174.63$; $P < 0.001$; Table 2). Overall, for all enzyme contents, the same statistical conclusions were obtained with and without body mass as a covariate in the models.

Protein concentrations and contents. Whereas myofibrillar (i.e., structural) and total protein concentrations (mg/g muscle) were lower in mini-muscles than in normal muscles, the concentrations of sarcoplasmic (e.g., enzymes and other soluble proteins) proteins varied less (Fig. 3, left; Supplemental Materials Table 3). For sarcoplasmic proteins, neither ANCOVA model showed significant effects of the mini-muscle phenotype for either sex (Table 2). By contrast, both ANCOVA models revealed that, in females, myofibrillar

and total protein levels were significantly lower ($P < 0.05$) in mini-muscles, whereas in males, the effect of the mini-muscle phenotype was only apparent when we analyzed all individuals (Table 2).

When protein levels were expressed as the total content per hindlimb (Fig. 3, right; Supplemental Materials Table 4), both ANCOVA models revealed that sarcoplasmic, myofibrillar, and total protein contents were significantly ($P < 0.01$) lower in mice with the mini-muscle phenotype in both sexes (Table 2).

For all categories of proteins (sarcoplasmic, myofibrillar, and total), for both mass-specific protein levels (mg/g muscle) and total hindlimb contents (mg/hindlimb muscles) as well as for both sexes, the same statistical patterns were found with and without body mass as a covariate. The only exception was that the total protein concentration was significantly lower in mini-muscles of females in lines 3 and 6, only when body mass was included ($F_{1,4} = 12.49$; $P = 0.024$; Table 2), whereas the effect was only marginally significant when body mass was excluded ($F_{1,5} = 6.02$; $P = 0.058$).

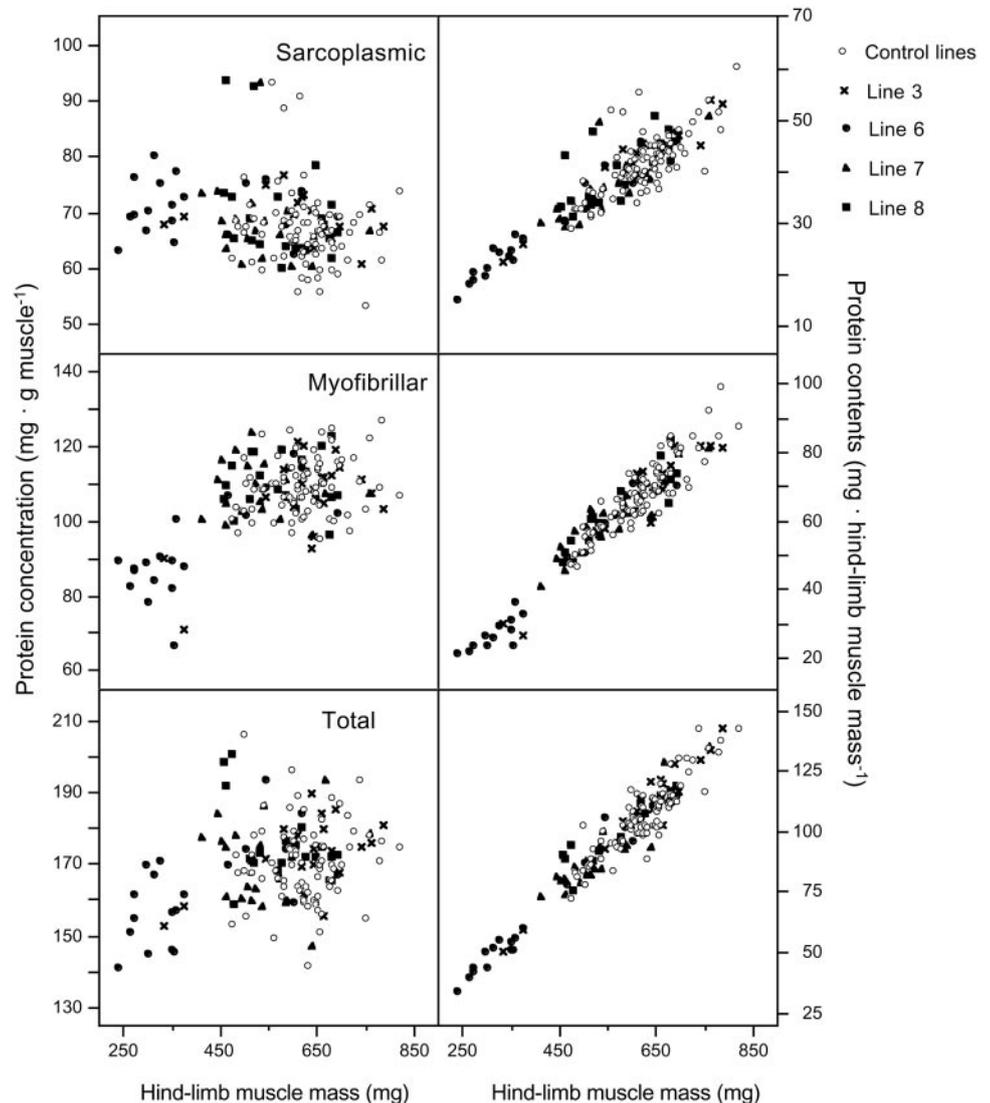


Fig. 3. Relationship between protein fractions, expressed as muscular concentration (left) or hindlimb muscle contents (right), and hindlimb muscle mass (excluding triceps surae) for 156 mice. Experimental design was balanced between control and selected mice as well as between sexes and activity states.

DISCUSSION

Replicated selection for high voluntary wheel running in house mice led to marked heterogeneity among the four selected lines in total hindlimb muscle mass, in the mass of the triceps surae complex (12), and in muscle metabolic capacities. In contrast, no statistically significant heterogeneity in muscle mass and little heterogeneity in muscle metabolic capacities were observed among the four replicate random-bred control lines (18). Comparison of the hindlimb muscles of the selected lines revealed that in one of the selected lines (line 6), 13 of the 20 mice showed the significant, almost 50% reduction in mass typical of the mini-muscle phenotype, along with two mice from line 3 (Fig. 1). The metabolic capacities of these mini-muscles differed markedly from those of normal muscles, particularly with respect to the enzymes of glucose oxidation. The virtually twofold increases in the specific activities (activity/g muscle) of these enzymes led their total contents (activity/hindlimb muscles) to be equivalent in the mini- and normal muscles (Fig. 2).

During voluntary wheel running, the highest levels of wheel running, expressed in revolutions run per day and in mean rpm, were attained in the selected lines in which the mini-muscle phenotype occurred. However, individuals with mini- vs. normal-sized muscles showed no significant difference in wheel running (Table 3). Thus mice with the mini-muscle phenotype ran as much as their counterparts with normal muscles, as was true for mice from generation 22 (12). Body mass, a trait that has responded negatively to selection (31, 32) and that was significantly lower in mini-muscled individuals at generation 22 (12), was not affected by the mini-muscle phenotype at generation 14. That this phenotype is not dependent on wheel running for its expression was shown by its approximately equal occurrence among wheel access and sedentary mice.

Within the selected lines, individuals with mini-muscles for both sexes showed the highest mass-specific activities (U/g muscle) for mitochondrial (CCO, CS, and CPT) and glycolytic (HK) enzymes (Fig. 2). In female mice, the mini phenotype apparently increased specific activities of PFK (but see RESULTS), whereas specific activities of PDH were elevated in mini-muscles only in males. The total contents of these enzymes in the hindlimb muscles were equivalent in the mice with mini- and normal muscles. On the other hand, mini-muscled mice had the lowest contents of LDH in their hindlimb muscles (Fig. 2). The combination of the lower LDH contents and the 1.5- to 2-fold higher activities (U/g muscle) of HK and PFK (in females) suggests that mini-muscles have an enhanced capacity for the oxidation of blood glucose. The reduced total contents of GP (both in activated and total forms) in mini-muscles (except in the comparison of males in lines 3 and 6) suggest sparing of muscle glycogen. Overall, the enzymatic "compensation" for the reduced muscle mass in the mini-phenotype was limited to aerobic pathways, a response that is similar to enzymatic adaptations to voluntary wheel running (18 and references

therein; 22, 26, 34) and to endurance training (17, referenced therein).

The increased frequency in the selected lines of small, highly aerobic muscles suggests that a marked reduction in muscle size, coupled with maintenance of aerobic capacity in the total hindlimb mass, facilitates high rates of aerobic activity (see also Ref. 12). The capacity for mechanical power development "lost" in the reduction of hindlimb muscle mass was apparently counterbalanced by the increased mass-specific capacity for oxidative ATP generation. As the high activities of mitochondrial markers (CS and CCO) suggest an increased mitochondrial abundance, the myofibrillar volume fraction (i.e., contractile proteins) may be reduced in these muscles, compounding the loss of contractile capacity caused by the reduction of overall muscle mass. In fact, mice with the small-muscle phenotype maintained similar concentrations of sarcoplasmic proteins (mg/g muscle) in the hindlimb muscles relative to the other selected mice but showed significantly reduced concentrations of structural (myofibrillar) proteins (Fig. 3). Nonetheless, as for mice from generation 22 (12), mice with mini-muscles ran as much as those with normally sized muscles.

Why did selection for voluntary wheel running not simply favor increases in muscle oxidative capacity as can be attained via training effects? Among selected mice, only those with mini-muscles exhibit enhanced oxidative capacities, as is shown by the tight grouping of the oxidative enzyme levels of the control and selected mice with normally sized muscles (Fig. 2). High mass-specific rates of sustained aerobic ATP production may be difficult to maintain in larger muscle fibers, perhaps because of difficulties of substrate delivery. Accordingly, we suggest that a reduction of large, fast-twitch glycolytic fibers in favor of fast-twitch oxidative fibers underlay the development of the mini-muscle phenotype. Muscles with smaller fibers are likely to have greater contact between fibers and capillaries, facilitating delivery of blood glucose and oxygen during exercise. On the other hand, such a change in fiber-type distribution would compromise the capacity for power development. Training effects during voluntary wheel running may lead to remodeling of fiber ultrastructure, but these changes are not as extensive as the postulated loss of large, fast-twitch glycolytic fibers. Possibly, skeletal muscle fibers may always maintain an oxidative "reserve" that can be added in response to training. Exploitation of this reserve may only be possible on a temporary basis for a given fiber type, making it difficult for selection to lead to higher oxidative capacities while maintaining a given profile of fiber types in a muscle. The cost of maintenance of mitochondrial membrane potential with its concomitant generation of reactive oxygen species may account for this response.

In designing our experiment, we measured enzymes as representatives of metabolic pathways and mitochondrial markers not as indicators of flux through these pathways. Effectively, nonequilibrium enzymes such as HK, PFK, and GP only work near their maxi-

mal capacities (and reflect maximal pathway flux) in so-called high-flux muscles, such as found in bees, hummingbirds, and hagfish tongue (28). The characteristics that allow some systems to exploit their enzymatic capacities more fully than others are unknown. Clearly, the marked training response elicited by voluntary wheel running in rodents (18, 22, 26, 34) indicates that a high aerobic capacity is favorable for the sustained wheel running they voluntarily carry out. However, in our experiment, selection for high wheel running primarily increased muscle aerobic capacity by favoring the mini-muscle phenotype. Although we do not know whether the mini-muscles function as high-flux muscles, the marked shift in metabolic capacities between these muscles and the normal phenotype suggests such a difference. Thus voluntary wheel running by normal-muscled mice may be limited by the difficulty of shifting from a normal flux mode to a high flux mode. This difficulty is at least partially overcome by the training response that increases muscle aerobic capacity (18). Interestingly, even the mice with the mini-muscle phenotype showed a training response to voluntary wheel running. If the mini-muscle phenotype is so advantageous during selection for voluntary wheel running, then why is it not present in all selected lines? Presumably, the other selected lines lost the rare allele either at founding or, more likely, subsequently via random genetic drift (12).

In summary, our results demonstrate that selection for a behavioral trait can engender a variety of adaptive changes among subordinate traits that support the behavior. Considering the four selected lines, selective breeding for high wheel running showed only a weak tendency to enhance muscle aerobic capacity (18). However, in two lines (lines 3 and 6), selection has resulted in a high frequency (~50% at generation 22; 12) of a small hindlimb muscle phenotype that exhibits an enhanced mass-specific aerobic capacity. The increase in mass-specific aerobic capacity in these mini-muscles almost completely counteracts the reduction in capacity caused by the "loss" of mass. Individuals with mini-muscles run at the same intensity as their counterparts with normal-sized muscles. The observed heterogeneity of the response in muscle mass and aerobic capacity to selection suggests that wild animals exhibiting high rates of aerobic activity may vary in the underlying morphological, physiological, and biochemical traits that permit high activity.

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Supplemental Materials (for Web site)

Table 1. Enzyme activities of hind-limb muscles ($U \cdot g \text{ muscle}^{-1}$) for selected lines

Enzymes	Male						Female					
	Line 3		Line 6		Line 7	Line 8	Line 3		Line 6		Line 7	Line 8
	Mini	Normal	Mini	Normal			Mini	Normal	Mini	Normal		
CCO												
Sedentary	104.1	61.2 ± 3.5	107 ± 5	45.4	69.5 ± 3.9	61.6 ± 3.6	N.D.	62.9 ± 2.7	90.2 ± 5.4	66.1 ± 1.2	56.3 ± 5.0	66.7 ± 1.8
Wheel access	N.D.	80.7 ± 1.4	140 ± 5	74.1 ± 0.2	76.8 ± 6.8	73.9 ± 1.9	126.5	84.5 ± 7.8	125 ± 14	85.4 ± 2.7	81.3 ± 5.1	78.4 ± 7.2
CS												
Sedentary	98.2	59.5 ± 2.9	118 ± 7	63.4	60.9 ± 2.3	57.2 ± 4.1	N.D.	60.7 ± 3.1	111 ± 10	64.5 ± 0.8	67.8 ± 3.7	61.7 ± 3.3
Wheel access	N.D.	80.4 ± 3.0	142 ± 5	79.0 ± 2.1	77.2 ± 8.2	73.0 ± 3.1	128.9	82.7 ± 2.5	159 ± 12	99.0 ± 3.5	87.7 ± 3.2	79.0 ± 4.4
CPT												
Sedentary	0.60	0.44 ± 0.07	0.85 ± 0.16	0.20	0.48 ± 0.07	0.45 ± 0.05	N.D.	0.51 ± 0.06	0.69 ± 0.14	0.45 ± 0.07	0.43 ± 0.07	0.41 ± 0.07
Wheel access	N.D.	0.62 ± 0.08	1.15 ± 0.10	0.73 ± 0.08	0.74 ± 0.10	0.65 ± 0.03	1.12	0.57 ± 0.07	0.73 ± 0.11	0.65 ± 0.33	0.70 ± 0.05	0.51 ± 0.09
PDH												
Sedentary	5.5	4.0 ± 0.4	5.1 ± 0.3	3.3	3.6 ± 0.4	4.0 ± 0.1	N.D.	4.0 ± 0.3	4.9 ± 0.7	3.4 ± 0.3	4.3 ± 0.6	4.1 ± 0.4
Wheel access	N.D.	5.1 ± 0.1	5.6 ± 0.8	3.7 ± 1.5	4.7 ± 0.3	4.9 ± 0.2	5.9	5.6 ± 0.5	6.3 ± 0.4	6.6 ± 0.4	5.2 ± 0.2	5.3 ± 0.8
HK												
Sedentary	13.8	7.9 ± 0.6	14.3 ± 1.0	8.4	7.4 ± 0.3	6.5 ± 0.2	N.D.	7.4 ± 0.5	13.0 ± 0.6	8.6 ± 0.7	6.4 ± 0.4	6.6 ± 0.3
Wheel access	N.D.	10.7 ± 0.4	17.0 ± 1.5	10.4 ± 0.6	8.6 ± 0.6	8.2 ± 0.2	16.1	9.3 ± 0.6	17.3 ± 1.3	10.4 ± 0.2	9.1 ± 0.3	8.9 ± 0.8
GPα												
Sedentary	36.3	60.3 ± 9.3	39.8 ± 3.8	48.9	49.7 ± 3.1	48.3 ± 3.9	N.D.	62.5 ± 4.2	44.4 ± 8.2	67.2 ± 8.7	50.6 ± 4.3	53.7 ± 4.7
Wheel access	N.D.	51.7 ± 6.2	41.0 ± 5.4	64.9 ± 2.8	47.0 ± 3.7	44.8 ± 4.5	42.1	40.7 ± 11.8	48.3 ± 6.7	39.0 ± 3.1	41.3 ± 2.5	45.5 ± 2.9
GP tot												
Sedentary	128	139 ± 19	119 ± 18	135	103 ± 8	115 ± 2	N.D.	145 ± 7	122 ± 3	137 ± 13	122 ± 6	118 ± 3
Wheel access	N.D.	122 ± 8	118 ± 2	149 ± 11	103 ± 8	115 ± 6	108	103 ± 9	108 ± 8	99.2 ± 5.6	101 ± 3	111 ± 2
PFK												
Sedentary	120	53.4 ± 7.5	124 ± 10	87.0	69 ± 11	44.6 ± 9.5	N.D.	48.4 ± 5.9	120 ± 14	125 ± 55	55.6 ± 6.4	46.9 ± 4.8
Wheel access	N.D.	62.7 ± 3.7	126 ± 6	95 ± 16	61.0 ± 6.8	53 ± 10	114	34.0 ± 15.4	110 ± 9	49 ± 13	55.7 ± 8.5	62 ± 10
LDH												
Sedentary	815	1019 ± 68	871 ± 80	814	958 ± 41	963 ± 80	N.D.	1152 ± 104	850 ± 65	996 ± 52	1 072 ± 31	975 ± 59
Wheel access	N.D.	952 ± 56	813 ± 39	1214 ± 46	931 ± 54	1 101 ± 44	1043	868 ± 38	882 ± 79	881 ± 67	976 ± 39	1 002 ± 8

Values are means ± SE. The sample size per cell was as given in Table 1. N.D: no data available.

Table 2. Total enzyme contents (U) per hind-limb muscle mass for selected lines

Enzymes	Male						Female					
	Line 3		Line 6		Line 7	Line 8	Line 3		Line 6		Line 7	Line 8
	Mini	Normal	Mini	Normal			Mini	Normal	Mini	Normal		
CCO												
Sedentary	39.1	45.2 ± 2.3	38.4 ± 1.5	27.3	41.3 ± 2.0	39.8 ± 3.4	N.D.	38.7 ± 1.6	23.3 ± 0.5	37.3 ± 2.0	28.2 ± 2.9	35.5 ± 1.7
Wheel access	N.D.	53.1 ± 1.3	44.4 ± 1.1	48.5 ± 2.5	44.5 ± 2.4	47.8 ± 1.7	42.0	52.2 ± 3.5	37.9 ± 6.4	41.2 ± 0.4	37.5 ± 2.2	38.2 ± 3.6
CS												
Sedentary	36.9	44.1 ± 2.2	42.2 ± 2.8	38.1	36.4 ± 2.6	36.9 ± 3.4	N.D.	37.3 ± 1.8	28.7 ± 2.1	36.4 ± 0.8	33.8 ± 2.0	32.7 ± 1.8
Wheel access	N.D.	52.7 ± 1.1	45.3 ± 3.1	51.7 ± 1.4	44.7 ± 3.4	47.3 ± 2.5	42.8	51.3 ± 0.4	47.7 ± 5.7	47.7 ± 0.3	40.6 ± 1.6	38.6 ± 2.7
CPT												
Sedentary	0.23	0.33 ± 0.06	0.30 ± 0.05	0.12	0.29 ± 0.05	0.29 ± 0.04	N.D.	0.31 ± 0.03	0.18 ± 0.03	0.25 ± 0.05	0.21 ± 0.04	0.21 ± 0.03
Wheel access	N.D.	0.40 ± 0.04	0.36 ± 0.02	0.48 ± 0.08	0.43 ± 0.03	0.42 ± 0.02	0.37	0.35 ± 0.05	0.22 ± 0.04	0.30 ± 0.15	0.33 ± 0.02	0.25 ± 0.05
PDH												
Sedentary	2.1	3.0 ± 0.2	1.8 ± 0.1	2.0	2.2 ± 0.2	2.6 ± 0.1	N.D.	2.4 ± 0.1	1.2 ± 0.2	1.9 ± 0.2	2.1 ± 0.3	2.2 ± 0.2
Wheel access	N.D.	3.3 ± 0.1	1.8 ± 0.3	2.5 ± 1.1	2.8 ± 0.2	3.1 ± 0.1	1.9	3.5 ± 0.3	1.9 ± 0.2	3.2 ± 0.1	2.4 ± 0.1	2.6 ± 0.4
HK												
Sedentary	5.2	5.9 ± 0.6	5.1 ± 0.4	5.0	4.4 ± 0.2	4.2 ± 0.2	N.D.	4.5 ± 0.3	3.4 ± 0.1	4.8 ± 0.2	3.2 ± 0.2	3.5 ± 0.2
Wheel access	N.D.	7.0 ± 0.2	5.5 ± 0.7	6.8 ± 0	5.1 ± 0.4	5.3 ± 0.2	5.3	5.7 ± 0.2	5.1 ± 0.3	5.0 ± 0.1	4.2 ± 0.2	4.3 ± 0.4
GP_a												
Sedentary	13.6	45.0 ± 8.0	14.2 ± 1.3	29.4	29.9 ± 3.0	31.1 ± 2.8	N.D.	38.5 ± 2.7	11.5 ± 2.3	37.8 ± 3.6	25.3 ± 2.3	29.0 ± 3.6
Wheel access	N.D.	34.0 ± 4.3	13.1 ± 2.1	42.6 ± 4.1	27.6 ± 2.4	29.1 ± 3.0	14.0	24.8 ± 6.4	14.6 ± 2.5	18.8 ± 0.7	19.2 ± 1.7	22.2 ± 1.6
GP tot												
Sedentary	48.0	104 ± 16	42.8 ± 6.9	81.2	61.9 ± 6.8	73.8 ± 1.5	N.D.	89.3 ± 5.3	31.6 ± 1.8	77.3 ± 4.6	60.6 ± 2.5	62.6 ± 2.7
Wheel access	N.D.	80.7 ± 6.6	37.7 ± 2.3	98 ± 13	59.9 ± 3.8	74.3 ± 4.7	35.8	64.1 ± 4.8	32.6 ± 3.9	47.7 ± 0.8	47.0 ± 2.3	54.0 ± 1.3
PFK												
Sedentary	45.1	40.1 ± 6.4	44.6 ± 3.5	52.4	40.5 ± 5.1	28.9 ± 6.8	N.D.	30.2 ± 4.3	31.2 ± 4.5	70 ± 29	27.7 ± 3.2	24.8 ± 2.7
Wheel access	N.D.	41.1 ± 2.1	40.3 ± 3.7	61.5 ± 7.4	36.3 ± 5.1	34.7 ± 7.1	37.9	20.7 ± 8.8	32.7 ± 1.5	23.3 ± 5.4	25.5 ± 3.2	30.3 ± 5.2
LDH												
Sedentary	306	759 ± 72	313 ± 30	490	573 ± 46	621 ± 61	N.D.	711 ± 70	219 ± 8	563 ± 49	535 ± 24	516 ± 27
Wheel access	N.D.	630 ± 50	260 ± 21	793 ± 14	554 ± 67	712 ± 28	346	540 ± 25	265 ± 36	424 ± 15	454 ± 34	488 ± 15

Values are means ± SE. Sample size per cell was as given in Table 1. Hind-limb muscle mass exclude mass of triceps surae. N.D: no data available.

Table 3. Protein concentrations of hind-limb muscles ($\text{mg} \cdot \text{g muscle}^{-1}$) for selected lines

Protein fractions	Male						Female					
	Line 3		Line 6		Line 7	Line 8	Line 3		Line 6		Line 7	Line 8
	Mini	Normal	Mini	Normal			Mini	Normal	Mini	Normal		
Sarcoplasmic												
Sedentary	69.3	66.2 ± 2.1	71.0 ± 2.7	62.6	63.8 ± 1.7	66.8 ± 3.1	N.D.	71.8 ± 1.3	67.6 ± 2.0	72.1 ± 4.1	72.7 ± 5.3	72.1 ± 5.7
Wheel access	N.D.	67.1 ± 1.0	72.9 ± 3.9	71.7 ± 2.2	67.6 ± 0.8	66.0 ± 1.8	67.9	70.3 ± 2.5	74.1 ± 1.8	70.8 ± 4.4	68.8 ± 2.4	74.7 ± 4.7
Myofibrillar												
Sedentary	71.2	109 ± 2	84.3 ± 7.1	118	106 ± 3	108 ± 4	N.D.	107 ± 4	86.7 ± 2.0	108 ± 1	114 ± 4	111 ± 2
Wheel access	N.D.	109 ± 4	87.6 ± 1.7	108 ± 6	107 ± 3	114 ± 4	90.3	114 ± 3	85.3 ± 3.6	104 ± 3	108 ± 3	109 ± 3
Total												
Sedentary	158.1	176 ± 2	153 ± 4	160	172 ± 9	171 ± 1	N.D.	169 ± 6	149 ± 4	184 ± 10	168 ± 4	176 ± 4
Wheel access	N.D.	176 ± 3	165 ± 4	176 ± 9	168 ± 4	174 ± 2	153	179 ± 2	159 ± 8	172 ± 2	172 ± 5	180 ± 8

Values are means ± SE. Sample size per cell was as given in Table 1. N.D: no data available.

Table 4. Protein contents (mg) per hind-limb muscle mass for selected lines

Protein fractions	Male						Female					
	Line 3		Line 6		Line 7	Line 8	Line 3		Line 6		Line 7	Line 8
	Mini	Normal	Mini	Normal			Mini	Normal	Mini	Normal		
Sarcoplasmic												
Sedentary	26.1	49.1 ± 2.6	25.5 ± 1.2	37.7	37.9 ± 1.9	43.0 ± 2.8	N.D.	44.2 ± 0.8	17.5 ± 1.2	40.6 ± 0.9	36.5 ± 3.5	38.0 ± 1.9
Wheel access	N.D.	44.2 ± 1.2	23.3 ± 1.7	46.9 ± 1.1	40.1 ± 3.2	42.8 ± 1.8	22.5	43.6 ± 0.4	22.1 ± 1.2	34.2 ± 3.5	31.8 ± 0.9	36.5 ± 3.0
Myofibrillar												
Sedentary	26.8	80.5 ± 1.4	30.3 ± 2.7	71.0	62.7 ± 2.6	69.4 ± 1.4	N.D.	65.8 ± 3.1	22.4 ± 0.7	60.8 ± 1.4	56.8 ± 2.4	59.1 ± 2.1
Wheel access	N.D.	71.9 ± 4.3	28.0 ± 1.6	70.7 ± 0	63.4 ± 5.9	73.9 ± 3.5	30.0	70.7 ± 1.9	25.5 ± 1.9	50.3 ± 0.8	50.3 ± 2.6	53.2 ± 2.5
Total												
Sedentary	59.4	131 ± 5	54.8 ± 2.3	96.1	102 ± 8	110 ± 3	N.D.	104 ± 5	38.8 ± 2.5	104 ± 2	83.7 ± 3.1	93.5 ± 2.8
Wheel access	N.D.	116 ± 4	52.4 ± 1.3	115 ± 1	99.6 ± 9.3	112 ± 2	50.8	112 ± 4	47.7 ± 3.9	83.1 ± 4.5	79.7 ± 2.0	87.5 ± 3.1

Values are means ± SE. Sample size per cell was as given in Table 1. Hind-limb muscle mass exclude mass of