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Contractile abilities of normal and "mini" triceps surae muscles from mice (*Mus domesticus*) selectively bred for high voluntary wheel running

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Syme, Douglas A., Kristin Evashuk, Benjamin Grintuch, Enrico L. Rezende, and Theodore Garland, Jr. Contractile abilities of normal and "mini" triceps surae muscles from mice (*Mus domesticus*) selectively bred for high voluntary wheel running. *J Appl Physiol* 99: 1308–1316, 2005. First published June 9, 2005; doi:10.1152/japplphysiol.00369.2005.—As reported previously, artificial selection of house mice caused a 2.7-fold increase in voluntary wheel running of four replicate selected lines compared with four random-bred control lines. Two of the selected lines developed a high incidence of a small-muscle phenotype ("mini muscles") in the plantar flexor group of the hindlimb, which apparently results from a simple Mendelian recessive allele. At *generations 36–38*, we measured wheel running and key contractile characteristics of soleus and medial gastrocnemius muscles from normal and mini muscles in mice from these selected lines. Mice with mini muscles ran faster and a greater distance per day than normal individuals but not longer. As expected, in mini-muscle mice the medial and lateral gastrocnemius muscles were ~54 and 45% the mass of normal muscles, respectively, but the plantaris muscles were not different in mass and soleus muscles were actually 30% larger. In spite of the increased mass, contractile characteristics of the soleus were unchanged in any notable way between mini and normal mice. However, medial gastrocnemius muscles in mini mice were changed markedly toward a slower phenotype, having slower twitches; demonstrated a more curved force-velocity relationship; produced about half the mass-specific isotonic power, 20–50% of the mass-specific cyclic work and power (only 10–25% the absolute power if the loss in mass is considered); and fatigued at about half the rate of normal muscles. These changes would promote increased, aerobically supported running activity but may compromise activities that require high power, such as sprinting.

experimental evolution; fatigue; muscle mechanics; power; selective breeding; work

SWALLOW ET AL. (34) describe an artificial selection experiment using a base population of outbred, Hsd:ICR house mice (*Mus domesticus*) in which four replicate lines were subject to selective breeding for high levels of voluntary wheel running whereas another four lines were random bred as controls. Compared with mice from the control lines, mice from the selected lines ran ~70% more revolutions/day after 10 generations of selection (34), 100% more after 14 generations (17), and 170% more revolutions and about double the average running speed after 23 generations (5, 11, 13). These mice have been the focus of anatomical, behavioral, physiological, and psychological investigations on the correlated effects of such selection (e.g., Refs. 7, 11, 23–25, 28, 29, 35–37).

More recently it has been noted that some individuals in both the selected and control lines express a small-muscle phenotype in which the plantar flexor muscle group (soleus, gastrocnemius, and plantaris) is 44–50% lighter than normal for the body mass (5, 12, 17). Evidence suggests that this small-muscle phenotype, coined "mini muscle" (17), is coded by an allele that was present in the base population at a frequency of ~7% and appears to be inherited as a single, autosomal recessive (12). Although the mini-muscle phenotype has fluctuated in frequency between 0 and 10% in the one random-bred control line in which it is observed, it has increased in frequency to ~50% by *generation 22* in the two selected lines that ever expressed the phenotype, and statistical analyses indicate that it has been favored by the selection protocol (12). The dramatic increase in its expression in these two lines of activity-selected mice leads to obvious questions about its functional significance.

Selective breeding may have favored mini muscles because they possess contractile abilities conducive to powering high levels of endurance running and/or because the underlying allele has pleiotropic effects, such as reduced body mass and increased ventricular mass, which may improve endurance running even if the muscles themselves are not adaptive (12). Individuals with mini muscles sometimes do (12) but sometimes do not (17) appear to have smaller body masses than their normal counterparts, and running did not differ significantly between normal and mini mice in the selected lines at *generations 22–24* (5, 12). On the other hand, mini muscles show increased expression of HSP72 independent of access to running wheels (5), and they have increased mass-specific aerobic capacities that almost fully compensate for the reduced muscle mass as well as metabolic enzyme profiles reminiscent of muscles in endurance-trained mammals (17). These observations have left unclear whether the possession of mini muscles or the allele responsible actually confers a selective advantage in the context of the selective breeding protocol.

The objectives of the present study were to compare the voluntary wheel-running behavior of mice with normal and mini muscles in the selected lines at *generations 36–38* and then to compare the contractile characteristics of these mini and normal muscles to understand how they may be different and whether such differences would promote high wheel running from a mechanical and energetic perspective. Such dif-

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ferences might include increased resistance to fatigue, perhaps accompanied by faster toward slower fiber-type transitions and increased oxidative capacity as has been observed in mouse muscle during endurance exercise training (1, 3). These changes would likely be accompanied by slowing of the contraction kinetics and reduced mass-specific power output, alterations that would neither promote nor hinder endurance running directly but might constitute an unavoidable "trade-off" for high-power activities such as sprinting. Although comparative studies of species differences have provided correlational evidence to suggest many muscle characteristics that are adaptive for various types of behavior, we know of no examples in which an experimental evolutionary approach (6, 7, 11, 12, 16, 17, 28, 38) has actually been used to identify such characteristics nor of a demonstration of the predicted physiological trade-off between endurance capacity and high power in locomotor muscles of a population under clearly defined selection.

MATERIALS AND METHODS

Experimental animals and muscle preparation. All procedures involving animals conformed to approved University of California, Riverside and University of Calgary/Canadian Council on Animal Care animal care guidelines. Male mice were randomly selected from *generations 36–38* of a selective breeding experiment for high voluntary wheel running (for complete details of the selection experiment, see Ref. 34). Selection was applied for 31 generations and then suspended for *generations 32–35* as the colony was moved from the University of Wisconsin-Madison to the University of California, Riverside. Mice used in the present study were from two of the lines selected for high voluntary wheel running, with laboratory designations *3* and *6*. Previous work shows that these are the only selected lines to exhibit the mini-muscle phenotype (12) (see also Refs. 5, 17). Sampling for the present study (see RESULTS) and from other generations since *generation 22* (Garland T Jr., unpublished observations) has revealed that selected *line 3* is now apparently 100% mini-muscle mice, whereas selected *line 6* is still segregating for the putative Mendelian recessive allele that causes the mini-muscle phenotype. Hence, mice included in the present study were of three types: *line 3* mini muscles; *line 6* mini muscles; *line 6* normal muscles. Because a primary objective was to understand why the high incidence of the mini-muscle phenotype in the selected lines is an adaptive response to the selection protocol, normal muscles from mice in selected *line 6* served as controls.

Voluntary wheel running was measured over a 6-day period, exactly as in the routine testing protocol of the selection experiment (34). Mice were 48 ± 0.49 (SE) days old (range 39–55 days) at the beginning of the wheel tests. Mice were housed individually in cages with access to 1.12-m-circumference activity wheels. A computer monitored the total number of wheel revolutions per day, the number of 1-min intervals that the mice were active on the wheels each day (i.e., intervals that showed at least one revolution), and the maximum revolutions per minute observed in any 1-min interval during each day. An approximate average revolutions per minute during daily activity was attained by dividing the total number of wheel revolutions per day by the number of minutes that the mouse was active on the wheel. Values reported are averages of *days 5* and *6*, as is used to choose breeders in the selected lines (on the basis of total revolutions). Mice were then air shipped from University of California, Riverside to Calgary, where they were housed as littermates in groups of four in filter-top cages with food and water ad libitum, 12:12-h light-dark cycle, at room temperature until being killed for measurements of muscle performance.

Muscles were isolated and mounted in experimental chambers, and initial preparations for measurements were made as described previously (e.g., Refs. 40, 42). Briefly, mice were weighed and then killed by cervical dislocation, and the left leg was removed, skinned, and placed in a dish containing physiological saline (composition in mM: 137 NaCl, 3 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 1 NaH_2PO_4, 24 $NaHCO_3$, and 10 glucose, pH 7.4) bubbled with 94:6 O_2-CO_2. The dissection stage was cooled to ~15°C, and the saline was changed frequently during dissections to inhibit proteases and limit muscle deterioration. The soleus muscle and medial gastrocnemius muscle (henceforth termed the gastrocnemius unless specified otherwise) were isolated for measures of mechanical performance. The Achilles tendon was split longitudinally so that a section of tendon remained attached to the distal end of each muscle. The proximal tendon of the soleus was cut from the tibia, whereas a small fragment of bone was left attached to the short, proximal tendon of the gastrocnemius. Segments of 5-0 silk suture were tied to the tendons and bone at the end of each muscle. The muscles were then transferred to chambers filled with circulating physiological saline, bubbled with 94:6 O_2,CO_2, and maintained at 27°C. It was not necessary to maintain normal body temperatures because only relative comparisons between normal and mini muscles were required for purposes of this study; a cooler temperature was selected to sustain viability of the preparations.

Two apparatuses were used to accommodate the smaller soleus and larger gastrocnemius. The tendon on one end of the soleus was tied to the tip of the arm of a model 350 servomotor (Cambridge Technology), and the tendon on the other end was tied to a stainless steel pin attached to a BG-50G force transducer (Kulite Semiconductor Products). The tendon on one end of the gastrocnemius was tied to the arm of a model 305B-LR servomotor (Aurora Scientific), and the tendon on the other end was tied to a stainless steel pin attached to an ELG-V-500G load cell (Entran Sensors and Electronics). The servomotors were used to control and measure the length of the muscles and the force transducers to measure force. Platinum stimulating electrodes were placed alongside the muscles and connected to custom-made, low-impedance current sources gated by Grass SD9 stimulators that were in turn gated by a command signal from a computer. The servomotor and stimulator were controlled by custom software written in LabView 6.1 through PCI-MI0-16-E4 data-acquisition and control cards (National Instruments). Signals from the servomotor, force transducer, and stimulator were collected at 5 kHz on the computer by using custom software written in LabView.

The stimulus voltage was adjusted to 150% of that required to elicit maximum twitch force (1-ms stimulus pulse duration). The muscle length was systematically varied until the length giving maximal isometric twitch force was found. Muscle fiber length was then measured by using a calibrated ocular micrometer, and this length was used for the remainder of the experiments.

Measures of mechanical performance of muscles. Several measurements were made to assess contractile characteristics relevant to the capacity for power running, including the ability to produce force, rate of contraction and relaxation, ability to shorten under isotonic load, ability to do work as might occur during locomotion, and fatigue resistance.

Isometric twitches were recorded from which measures of the rates of activation and relaxation were made. These included the time for force to increase from 10 to 90% of maximal during contraction (T_{10-90}), the time for force to fall from 90 to 10% of maximal during relaxation (T_{90-10}), and the duration of the twitch when force is at or above half-maximal amplitude (T_{50-50}). Maximum, isometric tetanic force was elicited with a train of stimulus pulses lasting 500 ms for the soleus and 100 ms for the gastrocnemius. These stimulus durations were long enough to ensure that force reached or closely approached a plateau. A stimulus frequency of 125 Hz was used for the soleus and 175 Hz for the gastrocnemius. Force was expressed relative to the cross-sectional area of the muscle, which was calculated from the muscle length, mass, and assuming a density of 1,050 kg/m^3.

Force-velocity characteristics were measured in fully activated muscle and thus reflect the inherent rate functions of the cross bridges and their impact on the ability of the muscle to shorten under load and hence to generate force and power. The muscle was first stimulated tetanically until force reached a plateau. Muscle length was then shortened rapidly by 0.1–0.5% depending on the shortening velocity under study, an amount sufficient to cause force to drop close to the level it would attain during subsequent isovelocity shortening. The muscle was then shortened at a constant velocity, and the associated stable force was measured. This process was repeated over a series of shortening velocities so that forces ranging from isometric to near zero were obtained. The force-velocity data were then fitted to the Hill equation (15) from which the maximum shortening velocity (V_{max}) and the curvature of the force-velocity relationship were obtained. V_{max} varies as a function of the detachment rate of the cross bridges and was obtained by extrapolating the Hill equation to zero force. Although extrapolation of a Hill curve tends to slightly overestimate the intercepts (26), visual comparison of our data sets to the mathematically derived V_{max} confirmed that the result was legitimate and faithfully represented the characteristics of the muscle. The curvature of the force-velocity relationship was described by the ratio a/P_o where a is a constant from the Hill equation and P_o is isometric force. This ratio reflects the inherent ability of the cross bridges to produce power, where faster, more powerful muscles have straighter relationships and higher a/P_o ratios.

The product of shortening velocity and the associated force yields isotonic power, which rises with increasing shortening velocity, reaches a maximum, and then falls with further increases in velocity. Maximum isotonic power was obtained by rearranging the Hill equation to solve for power, taking the first derivative with respect to shortening velocity, equating this to zero, solving for force (which is the force at which power is maximal), and then substituting this force back into the original Hill equation to calculate the associated shortening velocity from which maximal power can then be calculated. This is the power the muscle produces when shortening at a constant, optimal velocity and when the muscle is fully and continuously activated. See Josephson (19) for further details and discussion of analysis of force-velocity characteristics and power output of muscle.

Although maximum isotonic power as calculated above provides useful information about muscle, animals do not use their muscles in this fashion during locomotion (19). Thus cyclic work and power were measured by the work-loop technique, which provides a more realistic estimate of the ability of muscles to produce repetitive movements such as running (e.g., Refs. 21, 41). Briefly, the servomotor was used to impose sinusoidal changes in muscle length that mimic the cyclic lengthening and shortening that muscles experience during locomotion. The amplitude of the length change cycle (strain) was fixed at ±5% of the muscle resting length, and the frequency of the oscillation was set to 2 and 4 Hz for the soleus and 4 and 8 Hz for the gastrocnemius; these frequencies and amplitudes were selected to encompass a range over which the muscles were expected to produce near-maximal and also submaximal work and power output and which is realistically experienced during running by mice and rats (e.g., Refs. 18, 39). Although absolute power and the cycle frequency at which it is maximized would be less at the experimental temperature of 27°C than at body temperature, we only required a relative comparison between power from normal and mini muscles. Thus selection of frequencies and strains that maximized power or precisely mimicked conditions in a running mouse were not essential.

The muscle was stimulated phasically during the length change cycle, where phase is the timing of the onset of stimulation expressed relative to the length change cycle. The stimulus phase and duration were altered systematically until the net work done by the muscle was maximal. Net work was measured as the integral of force with respect to muscle length during a complete length cycle; it is the difference between the work done by the muscle when it shortens and the work required to lengthen the muscle and is the net mechanical energy that the muscle contributes during the cycle. Cyclic power was obtained by multiplying the net work done per cycle by the cycle frequency. Work and power were quantified relative to muscle mass. Cyclic power is conceptually different from maximum isotonic power calculated from force-velocity data because it accounts for both the work done by the muscle while it shortens and the energy and time required to lengthen the muscle. Also it is measured while the muscle shortens and lengthens over unsteady and realistic length trajectories and velocities, and it accounts for the dynamic and constraining natures of muscle activation and relaxation during each cycle (e.g., Refs. 8, 20).

The rate that muscles fatigue can be used as an indication of their suitability for powering sustained activities such as endurance (wheel) running. Normal and mini muscles were thus subjected to a fatiguing protocol to compare the rates at which they fatigued. In these protocols the soleus underwent 500-ms-duration, isometric tetanic contractions every 15 s, whereas the gastrocnemius underwent 100-ms-duration, isometric tetanic contractions every 15 s. These were continued until force declined to 70% of the prefatigue level. Because the ability to produce work and power is more severely hampered during fatigue than the ability to produce isometric force, and because the ability to produce work and power is most relevant to movement (33, 42), cyclic work was also recorded during the fatigue protocol at the times when isometric force had decreased by 10, 20, and 30%. Work during fatigue was recorded at a 2-Hz cycle frequency in soleus muscle and at 4 Hz in gastrocnemius muscle by using the same stimulus parameters that resulted in maximum work during the previous measures. At the conclusion of the fatigue protocol, the muscles were allowed to recover until it was evident that the majority of recovery was complete (typically 30–60 min), and preparations that did not show force recovery of at least 85% were discarded. Force and work were then standardized to the initial, unfatigued value, and plotted as a function of time during fatiguing stimulation. The slopes of the least-squares linear regressions relating relative force or work to time were used as measures of the rates of fatigue.

After the conclusion of the experiment the muscle was removed from the chamber, trimmed of external tendon and any fat or other loose connective tissue, blotted on filter paper, sealed in a 0.5-ml centrifuge tube to prevent desiccation, and weighed on an analytical balance. In some animals the lateral gastrocnemius and plantaris muscles were also isolated and weighed to obtain a complete record of how the masses of the components of the entire plantar flexor muscle group have changed in mini mice.

Statistical analysis. Wheel-running data were obtained from 40 normal and 39 mini mice, as classified postmortem after direct observation of the muscle phenotype; a marked reduction in mass of the gastrocnemius muscle was used to identify the mini phenotype. A subset of these same mice was used for muscle contractile measures. Contractile experiments were performed on six soleus and gastrocnemius muscles from both normal and mini mice in *line 6*, and six soleus and gastrocnemius muscles from mini mice in *line 3*. Measurements from *line 6* normal, *line 6* mini, and *line 3* mini muscles were compared by ANOVA and analysis of covariance. Covariates in the analyses of covariance included body mass, age, and wheel resistance, depending on the trait. Body masses before and after the wheel running experiments were compared by paired *t*-tests within groups. Data that did not satisfy assumptions of normality or variance equality were transformed before statistical analysis as indicated in RESULTS. Tests are two-tailed, and *P* values should be interpreted accordingly. All data are presented as means and SE.

In an attempt to avoid false positive results stemming from repeated comparisons of data from the same individual mice, we employed the False Discovery Rate procedure described by Curran-Everett (10). On the basis of *1*) a desired false discovery rate of 0.05, *2*) a total of ~50 statistical comparisons in the study, and *3*) the statistical *P* values of all the comparisons from the study, the procedure establishes that comparisons with $P < 0.03$ should be considered statistically significant, and we emphasize discussion of those results.

Table 1. *Physical characteristics and running behavior of 79 mice from the wheel activity experiments*

	Mini *Line 3*	Mini *Line 6*	Normal *Line 6*	3-Group Comparison	
				F_{df}	P
Body mass start, g	29.4±0.5	27.9±0.7	30.0±0.4	$F_{2,75} = 3.16$	0.0481
Body mass end, g	28.3±0.5†	28.1±0.7‡	30.4±0.4§*	$F_{2,75} = 7.41$	0.0012
Revolutions per day	10,181±650	12,751±1,002	9,298±539*	$F_{2,74} = 4.57$	0.0135
Minutes run per day	396±17	431±26	420±14	$F_{2,74} = 0.88$	0.4173
Average RPM	25.5±0.9	28.7±1.4	21.5±0.8*	$F_{2,74} = 11.49$	<0.0001
Maximum RPM	41.1±1.2	44.8±1.8	35.7±1.0*	$F_{2,74} = 11.62$	<0.0001

Values are least-squared adjusted means ± SE from SAS Proc Mixed with age and wheel resistance as covariates. All wheel running data are means from *days 5 + 6* of the 6-day tests, as is used in the routine selective breeding protocol. Body mass start and end refer to these 6-day wheel tests. Statistical effects from ANOVA comparing the 3 groups: F_{df} statistic and degrees of freedom, P = significance level. *Significantly ($P < 0.01$) different from other groups; †$t_{21} = 2.65$, $P = 0.015$ compared to mass at start (paired t-test); ‡$t_9 = -0.73$, $P = 0.49$ compared to mass at start (paired t-test); §$t_{37} = -1.63$, $P = 0.11$ compared to mass at start (paired t-test).

Furthermore, we report test statistics, degrees of freedom, and P values for all statistical tests; independent conclusions may be drawn from these as desired.

RESULTS

Body mass and wheel running. At the start of wheel-running trials, when mice averaged 7 wk old, body masses differed marginally among groups, and the differences became more pronounced by the end of the 6-day trials (Table 1). In particular, body masses of the normal and mini mice from *line 6* increased slightly, as would be expected because mice are still growing somewhat at this age, but mini mice from *line 3* actually lost mass (~4%, $P = 0.015$; Table 1). Thus, at the end of wheel trials, mini mice were ~7% lighter on average than normal mice (Table 1).

Mini mice from both *lines 3* and *6* ran substantially more revolutions per day than did normal mice from *line 6* (Table 1). The three groups did not differ in the number of 1-min intervals that the mice were active on the wheels each day, but mini mice from both lines ran faster than normal mice (Table 1).

Muscle masses. Of the *line 3* mice, 100% of the 22 individuals examined showed the mini muscle phenotype. Of the *line 6* mice, 37% of the 27 individuals examined showed the mini muscle phenotype. After adjustment for variation in body mass, the mass of the combined plantar flexor muscle group (soleus + medial and lateral gastrocnemius + plantaris) did not differ between the mini mice in *lines 3* and *6* but was only ~54% the mass of these muscles in normal mice (Table 2), similar to the original report (12). However, the differences between mini and normal mice were not uniform across the four muscles within this group; rather, the soleus muscles of mini mice were actually ~30% heavier, the medial gastrocnemius muscles were ~54% of the mass in normal mice, the lateral gastrocnemius muscles were ~45% of the mass in normal mice, and the two strains showed no statistical difference in plantaris mass (Fig. 1, Tables 2 and 3).

Muscle mechanics. At the time of death, the body mass did not vary significantly among groups (Table 3). The fiber lengths of the soleus muscles at which force was maximal were not different between the groups (Table 3). There was a marginally significant difference between fiber lengths in mini gastrocnemius muscles of mice from *lines 6* vs. *3* (Table 3); however, the trend is in the same direction as body mass and is likely just a scaling effect. As with the complete set of mice examined (Table 2), the subset of mice used for the muscle experiments had larger soleus muscles and smaller medial gastrocnemius muscles in the mini mice compared with normal, with no statistical differences between the mini mice of *lines 3* and *6* (Table 3). Maximal, isometric tetanic force per unit cross-sectional area produced by the soleus tended to be higher in normal mice, and for the gastrocnemius it was significantly greater in *line 6* normal mice compared with *line 6* mini mice (Table 3).

For the soleus, T_{10-90} was longer in normal than mini muscles from mice in *line 6* but not *line 3*, and it was not different between mini muscles from mice in *lines 3* and *6* (Fig. 2); when the data for mini muscles from *lines 3* and *6* were pooled, there was also a highly significant difference between mini and normal muscles (Fig. 2). However, the absolute difference was only ~3 ms (20%), which does not constitute a

Table 2. *Muscle masses (mg) log_{10} transformed and analyzed with log body mass and age as covariates.*

	N M3,M6,N6	Mini *Line 3*	Mini *Line 6*	Normal *Line 6*	3-Group Comparison	
					F_{df}	P
Body mass at death, g	25, 12, 19	1.55±0.01	1.55±0.01	1.59±0.01*	$F_{2,52} = 3.47$	0.0385
Muscle mass	18, 6, 12	2.03±0.01	2.04±0.02	2.30±0.01*	$F_{2,31} = 116$	<0.0001
Soleus mass	25, 12, 19	1.09±0.01	1.10±0.02	0.98±0.01*	$F_{2,51} = 17.5$	<0.0001
Medial gastrocnemius mass	25, 12, 19	1.54±0.01	1.56±0.02	1.83±0.01*	$F_{2,51} = 145$	<0.0001
Lateral gastrocnemius mass	18, 6, 12	1.63±0.01	1.61±0.02	1.99±0.02*	$F_{2,31} = 183$	<0.0001
Plantaris mass	18, 6, 12	1.24±0.02	1.27±0.03	1.30±0.02	$F_{2,31} = 2.03$	0.1480

Values are least-squares adjusted means ± SE (on log_{10} scale) from SAS Proc Mixed with log body mass and age as covariates. Body mass at death is for measurement of muscle masses and determination of mini vs. normal classification (only age is a covariate). Muscle mass is the combined soleus, medial + lateral gastrocnemius, and plantaris. Statistical effects from ANOVA comparing the 3 groups: F_{df} statistic and degrees of freedom, P = significance level. *Significantly ($P < 0.05$) different from other groups.

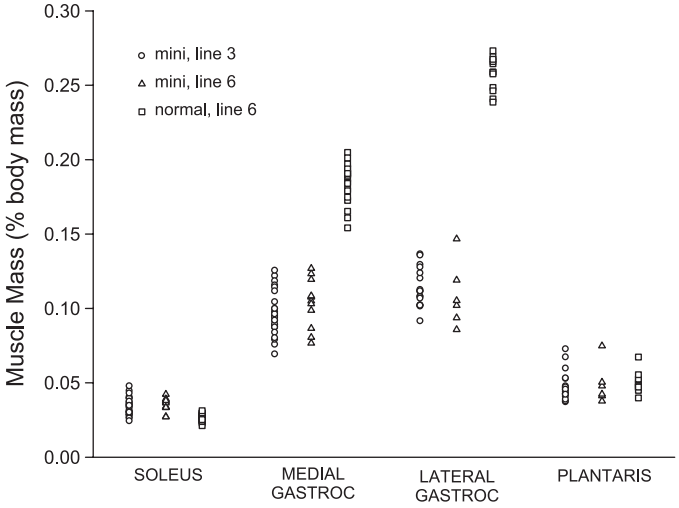

Fig. 1. Masses of individual soleus, lateral, and medial gastrocnemius (gastroc), and plantaris muscles from normal and mini-muscle mice. See Table 2 for statistical analysis. As in previous studies of these lines (5, 12, 17), mice were categorized as normal or mini on the basis of the relative mass of their gastrocnemius muscles.

marked change in overall twitch speed. T_{50-50} and T_{90-10} of soleus muscles were significantly faster in *line 6* mini mice compared with either the *line 6* normal or *line 3* mini mice (Fig. 2); these differences were on the order of 20–25% and result in a twitch that is considerably faster from a functional perspective. In gastrocnemius muscle, T_{10-90} was not different between mini and normal muscle or lines (Fig. 2). T_{50-50} was marginally nonsignificantly different between the groups, but it was significantly different in normal compared with the combined mini muscle groups and was highly nonsignificantly different between mini muscles from *lines 3* and *6* ($P = 0.74$) (Fig. 2). T_{90-10} was significantly slower in the gastrocnemius of both mini groups compared with normal muscles (Fig. 2).

The curvature of the force-velocity relationship was not different between mini or normal muscles for the soleus; however, the curvature for mini gastrocnemius muscles was significantly greater (i.e., smaller a/P_o) than for normal muscles (Fig. 3). V_{max} did not vary significantly between mini or normal muscles in either the soleus or gastrocnemius (Fig. 3). The similarity of V_{max} between the soleus and gastrocnemius likely reflects the pinnate architecture of the intact gastrocnemius muscle.

In soleus, maximal isotonic power was not different between the mini or normal muscles (Fig. 3). In the gastrocnemius, however, the mini muscles produced half or less the mass-specific isotonic power of normal muscles, and the mini muscles of *line 6* produced less power than those of *line 3* (Fig. 3). The particular shortening velocity (V) at which power is maximal can be quantified as the dimensionless ratio V/V_{max} and is characteristically 0.15–0.40 for most vertebrate skeletal muscles (30). This ratio averaged 0.263 ± 0.003 for soleus and was not significantly different between mini or normal muscles ($F_{2,14} = 2.138$, $P = 0.15$). For gastrocnemius, the ratio was not different between mini lines ($t_{10} = 1.12$, $P = 0.29$) but was smaller in mini (0.317 ± 0.012, pooled data) compared with normal muscles (0.387 ± 0.012) ($F_{2,14} = 7.84$, $P = 0.005$), indicating that mini muscles produced their maximal power at slightly slower relative shortening velocities.

Cyclic work and power produced by the muscles were measured at two different cycling frequencies, analogous to different stride frequencies during running (Fig. 4). The net work done per cycle decreased with increasing cycle frequency in both soleus and gastrocnemius muscle, as is typical (e.g., Ref. 18). The power outputs remained relatively unchanged with changes in cycle frequency, suggesting the muscles were working at or near their maximum power at the cycle frequencies chosen for study. For soleus muscle, the only difference in work or power between mini or normal muscles was at 4 Hz, where *line 6* mini mice were marginally greater (Fig. 4). For the gastrocnemius, mini muscles of the two lines did not differ, but mini muscles in general produced only 16–40% the mass-specific work and power of normal muscles, the discrepancy being greater at the higher cycle frequency (Fig. 4).

In the soleus, the rates of fatigue of both isometric force and net work during cyclic contractions were not significantly different between mini or normal muscles (Fig. 5). In contrast, the rates of fatigue of isometric force and cyclic net work were significantly slower in mini vs. normal gastrocnemius muscle (Fig. 5) but were not different between the mini lines. Of note, the reduction in the rate of fatigue of cyclic work in mini gastrocnemius muscles was particularly marked compared with the relative rate of fatigue of isometric force.

Table 3. *Physical characteristics of the 18 mice and their muscles from the mechanics experiments*

				3-Group Comparison	
	Mini *Line 3*	Mini *Line 6*	Normal *Line 6*	F_{df}	P
Soleus					
Body mass, g	34.9±1.0	32.7±1.8	34.6±2.0	$F_{2,14} = 0.535$	0.597
Fiber length, mm	13.9±0.5	13.3±0.3	13.3±0.2	$F_{2,14} = 1.14$	0.346
Soleus mass, mg	12.6±0.3	12.4±1.0	9.12±0.54*	$F_{2,14} = 8.91$	0.003
Soleus mass, % body mass	0.0363±.0014	0.0378±.0012	0.0264±.0027*	$F_{2,14} = 25.4$	<0.001
Isometric force, kN/m^2	333±12	344±4	381±18	$F_{2,14} = 3.83$	0.045
Medial gastrocnemius					
Body mass, g	35.4±1.1	31.1±1.6	35.1±1.9	$F_{2,14} = 2.34$	0.131
Fiber length, mm	16.0±0.3†	14.5±0.4	15.8±0.4	$F_{2,14} = 5.35$	0.018
Gastroc mass, mg	36.6±1.9	33.2±3.3	67.5±3.7*	$F_{2,14} = 38.5$	<0.001
Gastrocnemius mass, % body mass	0.104±0.007	0.106±0.005	0.192±0.003*	$F_{2,14} = 88.1$	<0.001
Isometric force, kN/m^2	222±13	167±18	295±44†	$F_{2,14} = 5.20$	0.019

Values are simple means ± SE, $n = 6$ for each group. Statistical effects from ANOVA comparing the 3 groups: F_{df} statistic and degrees of freedom, P = significance level. *Significantly different from other groups; †significantly different from *line 6* mini.

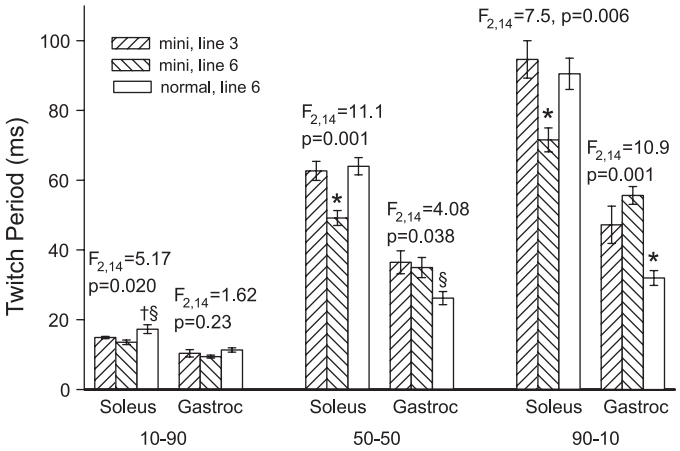

Fig. 2. Duration of isometric twitches in soleus and medial gastrocnemius muscles from mini and normal mice. 10–90, Time required for force to rise from 10 to 90% of maximum during contraction; 90–10, time required for force to fall from 90 to 10% of maximum during relaxation; 50–50, duration of the twitch measured at half amplitude. Values are means ± SE. F_{df} statistics from ANOVA and associated P values for each comparison of the 3 groups of muscle are shown. *Significantly different from other groups. †Significantly different compared with *line 6* mini. §Significantly different from mini muscles of *lines 3* and *6* combined ($F_{1,15} = 8.83$, $P = 0.009$ for soleus 10–90; $F_{1,15} = 8.47$, $P = 0.01$ for gastrocnemius 50–50).

DISCUSSION

These experiments addressed why the high incidence of a mini-muscle phenotype in replicate lines of mice selectively bred for high voluntary wheel running appears to be adaptive (12). We conclude that the changes observed in both the soleus (increased mass) and gastrocnemius (reduced mass and slowed contractile mechanics) would favor enhanced endurance wheel running. Furthermore, these changes predict that selection for elevated levels of endurance running and the associated high incidence of the mini phenotype are incompatible with maintenance of high levels of power. Although such divergence in form and function appears reasonable and is observed in comparisons among muscles and species, such changes have never been demonstrated in the context of a significant trade-off that can occur in muscles after a short number of generations of defined selection in a vertebrate population.

At *generation 22*, mice from the selected *line 3* exhibited a 70% occurrence of the mini-muscle phenotype, whereas those of selected *line 6* exhibited a 40% occurrence (12). We found that, at *generations 36–38*, all of the mice in *line 3* expressed the mini-muscle phenotype, suggesting that the allele responsible has become fixed in this line, whereas in *line 6* only 37% of the mice expressed the phenotype, similar to the level at *generation 22*. The line difference with respect to cross-generational changes in frequency of the mini-muscle allele could be attributable to random genetic drift and/or the interaction between the selection protocol and the "gene pool" of the two lines. In any case, within *line 6*, mini mice ran 37% more revolutions per day than did their counterparts with normal-sized muscles, which is consistent with the analysis indicating that the mini-muscle allele has been favored by the selective breeding (see Ref. 12). The significantly enhanced running performance in mini mice appears to be a recent phenomenon, or recently augmented, as such differences were not observed after *generations 14*, *22*, and *23* in an analysis of all eight lines (4 selected and 4 control) (5, 12, 17).

Line 3 mini mice lost body mass during the standard 6-day wheel test, whereas those of *line 6* (both normal and mini) showed a slight increase in body mass (Table 1). This is particularly interesting in light of observations that the mini mice of both lines ran significantly farther and faster but not longer than normal mice (Table 1). We do not have measures of cage activity during wheel tests (see discussion in Ref. 28), and this may also influence changes in body mass. Similar to the mini vs. normal running patterns observed in selected mice in the present study (Table 1), Koteja et al. (24) noted at *generation 10* that selected mice spent about the same time running but ran faster than control mice, yet their daily rates of food consumption were only slightly higher (the normal/mini phenotypes were not known for their animals). They concluded

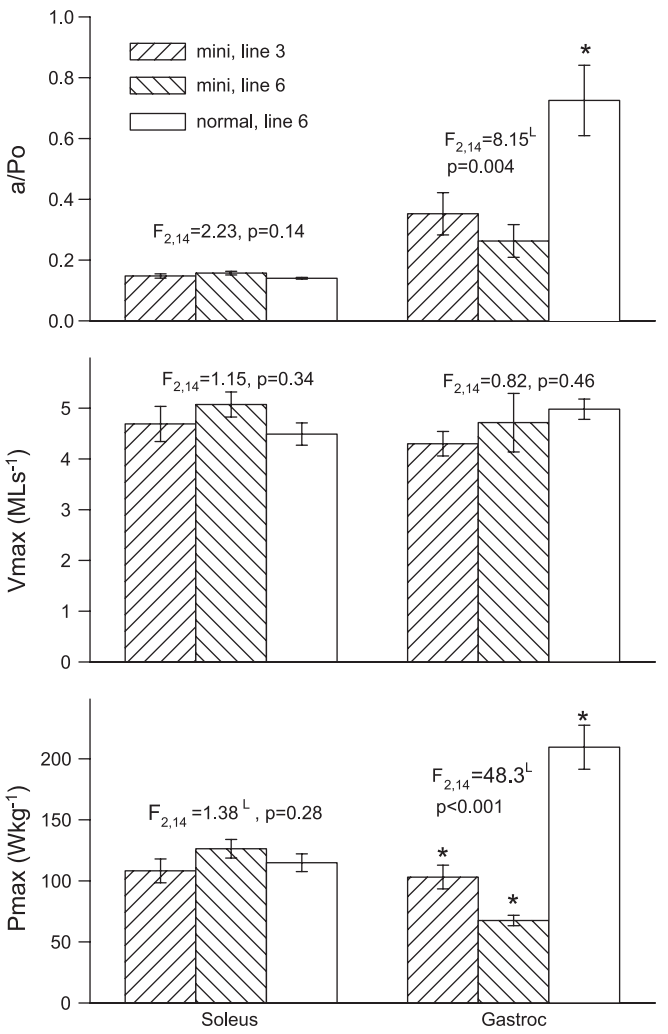

Fig. 3. Force-velocity characteristics of soleus and medial gastrocnemius muscles from normal and mini mice. *Top*: curvature of the Hill force-velocity relationship (the constant a divided by isometric tetanic force P_o). *Middle*: maximum shortening velocity (V_{max}) extrapolated from the Hill force-velocity equation. *Bottom*: maximum isotonic power (Pmax) calculated from force-velocity data as described in MATERIALS AND METHODS. Values are means ± SE. F_{df} statistics from ANOVA and associated P values for each comparison of the 3 groups of muscle are shown. [L]Data were natural log transformed for statistical analysis to satisfy normality and variance assumptions. *Significantly different from other groups.

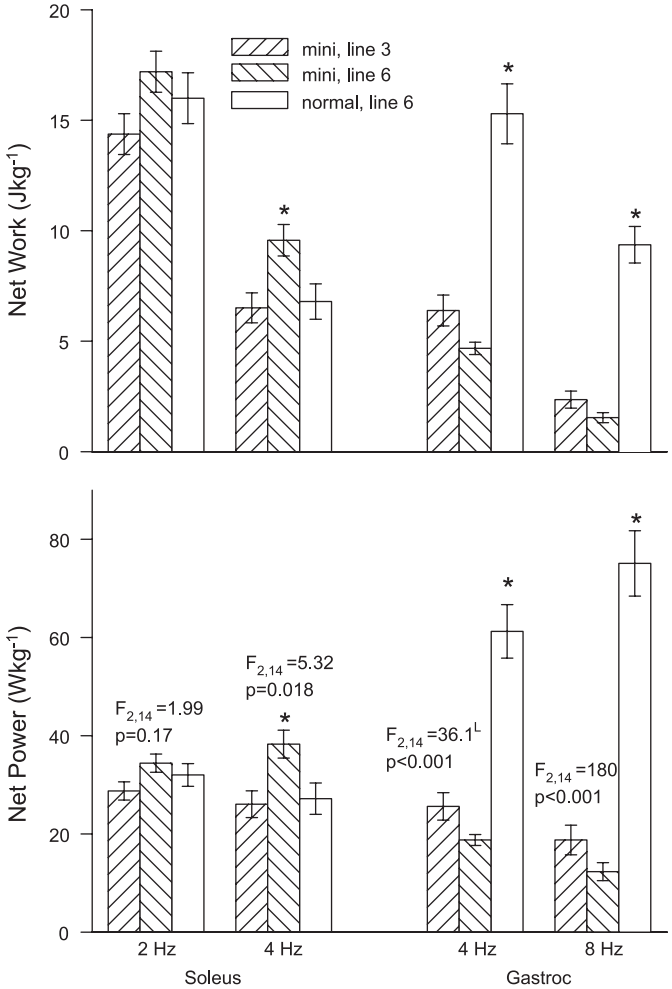

Fig. 4. Net work and power of soleus and medial gastrocnemius muscles from normal and mini mice during cyclic contractions measured by work loop analysis. Strain amplitude was 10% muscle length. The cycle frequency was 2 and 4 Hz for the soleus and 4 and 8 Hz for the gastrocnemius. Stimulation parameters were adjusted to maximize work and power. Values are means ± SE. F_{df} statistics from ANOVA and associated P values for each comparison of the 3 groups of muscle are shown; the statistical results for work (not shown) are identical to those for power. [L]Data were natural log transformed for statistical analysis to satisfy normality and variance assumptions. *Significantly different from other groups.

that increased running speed as opposed to increasing the time spent running may confer an ability to increase daily movement distances without incurring a substantially greater metabolic cost. Although this mechanism could explain the lack of body mass loss in mini mice from *line 6*, it would be at odds with the observation that mini mice from *line 3* lost mass even though they ran less than mini mice from *line 6*.

Reports based on mice from *generations 14* and *22* agree with our results that the plantar flexor muscle group is ~50% smaller in mini than normal mice (12, 17) (Table 2). Furthermore, here we report for the first time a comparison of the masses of the individual muscles in normal and mini mice and note that they do not all change in the same fashion (Fig. 1, Tables 2 and 3). The lateral gastrocnemius, with the largest change in mass in mini mice, would provide the most unequivocal metric for classification of mice as normal or mini. Clearly, the mini phenotype is not expressed as just a uniform reduction in the mass of the ankle extensors but is a specific and differential effect on the individual components. This suggests that the selection protocol may be resulting in unique and functionally relevant changes in each muscle, changes that are borne out to some extent on analysis of the physiology and contractile mechanics of individual muscles, as discussed next.

Sustained wheel running appears to be an aerobic activity in these mice, and there is a substantial effect of voluntary and forced wheel running on aerobic capacity in muscles of rodents (17, 27, 43); thus selection for this behavior might be accompanied by associated changes in the muscles that promote sustained, aerobic locomotion. Houle-Leroy et al. (17) note that the hindlimb muscles of mini mice have higher mass-specific aerobic capacities than their normal counterparts and that these almost fully compensate for any reduced capacity as a result of the reduced muscle mass. Furthermore, only aerobic capacities are compensated in the muscles studied, suggesting that aerobic but not anaerobic capacity is important to support the high levels of voluntary wheel running (note that the soleus, plantaris, and gastrocnemius were excluded from their analyses). It is thus reasonable to expect that changes in the physiology and mechanical characteristics of hindlimb muscles of mice from the selectively bred lines should be in a direction that promotes sustained, aerobic, and economical endurance running, and that mini muscles may exhibit such changes.

As a global observation, the contractile characteristics of the soleus muscles were not markedly different between normal and mini mice. Interestingly, there were some line effects, such that soleus muscles from mini mice in *line 6* were slightly faster in their twitch characteristics (Fig. 2), perhaps explaining their ability to do slightly more work and produce more power at the higher operating frequency than the *line 3* mini or *line 6* normal muscles (Fig. 4). These increases in twitch speed would not translate directly into changes in any of the force-velocity characteristics of the muscle, and they do not appear to have done so (Fig. 3). Thus the contractile physiology of the soleus muscle, being already relatively slow and aerobic, does not appear to be altered substantially in the "renovation" of the plantar flexor group of mini mice. Whether it has remained generally unchanged in response to selection for increased

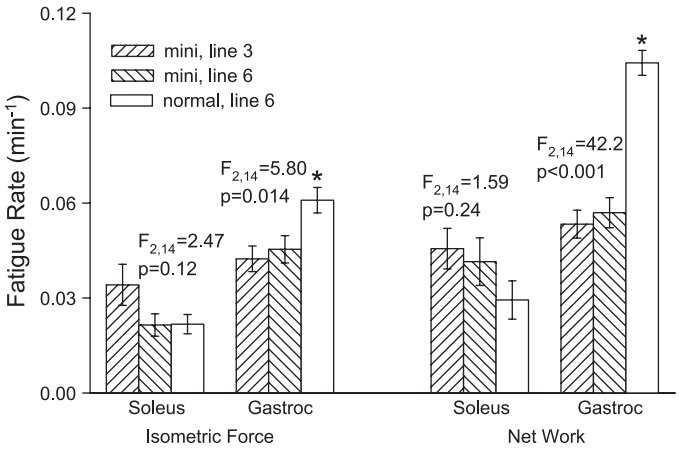

Fig. 5. Rates of fatigue of isometric tetanic force and cyclic work for soleus and medial gastrocnemius muscles from normal and mini mice. The fatigue protocol is described in MATERIALS AND METHODS. Values are means ± SE. F_{df} statistics from ANOVA and associated P values for each comparison of the 3 groups of muscle are shown. *Significantly different from other groups.

wheel running would require comparisons with the contractile performance of soleus muscles from control lines of mice.

Despite the lack of notable differences in the physiology of the soleus muscles between normal and mini mice, they were significantly larger in mini mice (Tables 2 and 3, Fig. 1). This may reflect an increased contribution to powering sustained running with the increased demands of the higher running speeds and greater distances run by mini mice (Table 1) or with the loss of mass in their agonist, the gastrocnemius muscle. Hypertrophy and an increased contribution of slow muscles such as the soleus, being aerobic and more economical at generating force and power than faster muscles (e.g., Refs. 4, 9), would benefit animals that perform high levels of endurance running. Likewise, a decreased mass and contribution from faster muscle such as the gastrocnemius would confer similar advantages.

In marked contrast to the soleus, the contractile characteristics of the medial gastrocnemius muscles in mini mice became slower in nearly every attribute measured, and substantially so. Slowing of the twitch (Fig. 2) suggests, in part, a slowing of the rate of calcium sequestration in the muscle cells (e.g., Ref. 31). An increase in curvature of the force-velocity relationship and subsequent reductions in the isotonic power output (Fig. 3) suggest a change in the averaged cross-bridge rate constants and perhaps an increase in the thermodynamic efficiency of the muscle (44), as might be expected with a transition toward a slower phenotype and an increased fiber-type heterogeneity (22) in the mini muscles. The lack of an associated reduction of V_{max} in mini gastrocnemius muscles was unexpected but would suggest that the muscles still possess a relatively high proportion of fast fiber types and may reflect in part the difficulties of estimating and interpreting V_{max} from force-velocity data obtained from muscles with a heterogeneous fiber distribution (22).

Functionally, these changes resulted in the mass-specific mechanical work and power output of the gastrocnemius muscles of mini mice being reduced by one-half to one-sixth, whether measured as isotonic power (Fig. 3) or during cyclic activity (Fig. 4). This is consistent with observations that work and power are reduced in slow relative to fast muscles (e.g., Refs. 18, 39). More strikingly, the reduced mass-specific power in concert with the approximate halving of the mass of the gastrocnemius would result in the absolute power-producing potential of mini gastrocnemius muscles being reduced to only 10–25% of that of normal muscles, depending on the operating conditions. The small increase in mass of the soleus would plainly not be adequate to fully compensate for this loss in power. However, clearly the loss of potential power in the ankle extensors of mini mice did not impede their routine wheel-running abilities (Table 1). Muscle recruitment patterns at different locomotor speeds in rats and cats (reviewed briefly in Ref. 18) suggest that routine wheel running likely utilizes only a fraction of the full power-producing potential of the gastrocnemius. Yet, when high power output is required from these muscles, as when sprinting, a reduced performance in mini mice would be expected. Potentially they can "afford" to sacrifice power because traits such as maximal sprint speed are not a target of selection in this experimental protocol. Likely the primary advantage to having slower and smaller gastrocnemius muscles in mini mice is based in the economy of running or fatigue resistance.

Rates of fatigue of soleus muscles from mini mice were, like most aspects of their mechanical performance, not different from normal mice (Fig. 5). Rates of fatigue of medial gastrocnemius muscles from mini mice were reduced (Fig. 5) and reflective of a slower phenotype with increased aerobic capacity. As has been noted previously (33, 42), the rate of fatigue of work was greater than the rate of fatigue of isometric force (Fig. 5), the difference being attributed primarily to the marked reduction in the ability of fatigued muscle to relax. This suggests that the mini gastrocnemius muscles have acquired a substantial resistance to the fatigue of relaxation (and thus work), allowing them to power dynamic contractions involving muscle shortening and extension (e.g., running) for longer periods. Any reductions in the rate of fatigue of relaxation would also afford a substantial energetic savings to running mice by reducing the energy required to extend their muscles (42).

The higher average and maximal routine wheel-running speeds of mini mice (Table 1) indicate that they may be calling more on the gastrocnemius for power than normal mice, an act they may now afford because of the reduced fatigability of this muscle in mini mice, presumably a result of a slower phenotype and/or increased aerobic capacity. The rate of fatigue is dependent on a complex interaction between power output and cycling frequency and is not related in a simple way to the amount of work done by the muscle or its power output (2, 32). Thus the particular limb cycling frequency chosen by mice while running will impact both running speed and the rate of fatigue, and likely then the magnitude of any benefit incurred by expressing the mini-muscle phenotype. In turn, mini mice may be expected to display a different running style than either normal selected or control mice, much like the more intermittent running behavior exhibited by selected vs. control mice (13), which quite likely has a basis in avoidance of fatigue.

In conclusion, the differences in the masses and contractile physiology of the mini compared with normal muscles are of a nature that should support higher levels of endurance wheel running, as are the differences in aerobic capacity (17) and glycogen depots (14) in muscles of these mice. All of these differences are consistent with the observation that the selective breeding protocol has favored an increase in frequency, to fixation in one line, of the putative Mendelian recessive allele that causes the mini phenotype (12). Moreover, in our sample of mice, mini individuals ran significantly further and faster than their linemates with normal muscles, a difference that was not apparent in a larger sample from *generation 22* that was not broken down for within-line analysis (12). Beyond differences in muscle performance per se, we do not yet know how the mini phenotype might impact the energetic costs of running, nor do we know whether mini mice may exhibit trade-offs associated with having smaller, less powerful muscles, such as a reduced maximal sprint speed. An analysis of the fiber-type composition of these animals would also provide valuable information to further understand why this particular muscle allele has been favored by selective breeding for high endurance running.

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