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Locomotor trade-offs in mice selectively bred for high voluntary wheel running

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SUMMARY

We investigated sprint performance and running economy of a unique 'mini-muscle' phenotype that evolved in response to selection for high voluntary wheel running in laboratory mice (*Mus domesticus*). Mice from four replicate selected (S) lines run nearly three times as far per day as four control lines. The mini-muscle phenotype, resulting from an initially rare autosomal recessive allele, has been favoured by the selection protocol, becoming fixed in one of the two S lines in which it occurred. In homozygotes, hindlimb muscle mass is halved, mass-specific muscle oxidative capacity is doubled, and the medial gastrocnemius exhibits about half the mass-specific isotonic power, less than half the mass-specific cyclic work and power, but doubled fatigue resistance. We hypothesized that mini-muscle mice would have a lower whole-animal energy cost of transport (COT), resulting from lower costs of cycling their lighter limbs, and reduced sprint speed, from reduced maximal force production. We measured sprint speed on a racetrack and slopes (incremental COT, or iCOT) and intercepts of the metabolic rate *versus* speed relationship during voluntary wheel running in 10 mini-muscle and 20 normal S-line females. Mini-muscle mice ran faster and farther on wheels, but for less time per day. Mini-muscle mice had significantly lower sprint speeds, indicating a functional trade-off. However, contrary to predictions, mini-muscle mice had higher COT, mainly because of higher zero-speed intercepts and postural costs (intercept–resting metabolic rate). Thus, mice with altered limb morphology after intense selection for running long distances do not necessarily run more economically.

Key words: artificial selection, exercise, experimental evolution, maximum metabolic rate, oxygen consumption, sprint speed, trade-off.

INTRODUCTION

For most animals, the ability to move through the environment is fundamental to many fitness-critical functions, including defending territories, finding mates and food, migration and escaping from predators. Mechanistically, locomotion is a complex, highly integrative, whole-animal trait that incorporates numerous organ systems [e.g. circulatory, muscular, nervous, sensory, respiratory, skeletal (Swallow et al., 2009)]. From an ecological and evolutionary perspective, two very important elements of locomotion are energy costs and performance abilities (e.g. speed, stamina, agility). Locomotion may be energetically expensive, and whether a large or small portion of the daily energy budget is spent on activity, the expense of locomotion can affect behaviour (particularly in animals that travel extensively). Aside from energy costs, the limits to locomotor performance constrain an animal's ability to perform many behaviours. Accordingly, it is reasonable to assume that, in many circumstances, selection may favour an increase in both locomotor economy and certain aspects of performance.

A common measure used to assess the energetic costs of locomotion is the 'cost of transport' (COT), which is defined as the energy required to move a unit distance. When comparing animals that vary in body size, COT is commonly expressed on a mass-specific basis, thus indicating the energy required to move a unit mass a unit distance, and larger animals generally have lower mass-specific COT (e.g. Taylor et al., 1970; Taylor et al., 1982; John-Alder et al., 1986). In most terrestrial runners, transport costs include two components. One is the energy cost associated with movement *per se,* usually defined as the regression of metabolic power (e.g. oxygen consumption) on running speed. This is called the incremental cost of transport (iCOT). For many runners, the speed *versus* power relationship is linear, so the iCOT is independent of speed (Taylor et al., 1970; Taylor et al., 1982; John-Alder et al., 1986). In addition to iCOT, the second cost associated with locomotion is the 'postural cost', manifested as an elevation of the zero-speed intercept of the speed *versus* power relationship above resting metabolic rate (Taylor et al., 1970; Taylor et al., 1982; John-Alder et al., 1986). Selection to reduce COT could affect iCOT, postural costs, or both. All else being equal, a reduced COT will decrease the energy requirements of locomotion at a given speed, and, therefore, increase the maximal aerobic speed (the highest speed that can be powered by aerobic pathways) and hence endurance.

A long-term selection experiment (Swallow et al., 1998; Swallow et al., 2009) that includes four replicate lines of mice bred for high levels of voluntary wheel running and four non-selected control lines provides a good system to examine whether costs associated with running may change as a result of intense selection on running behaviour. On a daily basis, mice from the selected (S) lines run 2.5- to 3.0-fold farther than control mice, and the increased distance is mainly accomplished by higher running speeds (Koteja et al., 1999; Rhodes et al., 2000; Girard et al., 2001; Rezende et al., 2005; Rezende et al., 2009). As a group, the four replicate selected lines show a diverse suite of morphological, biochemical, physiological, and behavioural differences from the four non-selected control lines (e.g. Swallow et al., 1999; Girard et al., 2001; Garland and Freeman, 2005; Kelly et al., 2006; Bilodeau et al., 2009; Rezende et al., 2009; Swallow et al., 2009).

One dramatic response to the selection regimen has been an increase in frequency of the 'mini-muscle' phenotype, characterized

by a 50% reduction in hindlimb muscle mass and inherited as a Mendelian recessive (Garland et al., 2002; Houle-Leroy et al., 2003; Hannon et al., 2008; Hartmann et al., 2008; Middleton et al., 2008). Within the S lines, one has become fixed for the mini-muscle phenotype, a second remains polymorphic, and the other two lines apparently lost the mini phenotype by random genetic drift during early generations of the experiment. Fitting with population genetic models indicates that mini-muscles have been favoured by the selection protocol (Garland et al., 2002), but the reason for this is as yet unclear.

Although mini-muscle individuals tend to run faster on wheels than those with normal muscles, they do not consistently run further on a daily basis (Garland et al., 2002; Kelly et al., 2006), but (see Syme et al., 2005; Hannon et al., 2008; Gomes et al., 2009). Nevertheless, the mini-muscle trait might provide an advantage in terms of endurance capacity during voluntary running. The reduction in limb mass in these mice is reminiscent of the thin, lightweight limb morphology seen in 'classical' cursorial mammals, such as deer and antelope [references in Garland and Freeman (Garland and Freeman, 2005)] (Kelly et al., 2006). Although much of the cost of cursorial locomotion seems to involve supporting body mass (e.g. Fedak et al., 1982; Heglund et al., 1982), the kinetic energy of limb motion can be a substantial fraction of total energy expenditures during running (e.g. Martin, 1985; Claremont and Hall, 1988). Accordingly, we hypothesized that the lighter limbs of mini-muscle individuals should reduce the energy cost of limb cycling during locomotion, and hence improve running economy. Moreover, studies of isolated medial gastrocnemius demonstrate increased fatigue resistance of mini-muscles (Syme et al., 2005), which may be related to altered enzyme activities, including twice the mass-specific aerobic capacity of normal mice in mixed hindlimb muscle (Houle-Leroy et al., 2003; Guderley et al., 2006), a shift toward slower myosin heavy chain isoforms (Guderley et al., 2006; Guderley et al., 2008; McGillivray et al., 2009), and increased capillarity (Wong et al., 2009). Beyond this, mini-muscle individuals have significantly longer and thinner femora and tibiafibulae (with no difference in bone masses), larger heart ventricles, and increased maximal oxygen consumption when measured in hypoxia (Garland et al., 2002; Swallow et al., 2005; Kelly et al., 2006; Rezende et al., 2006a).

In the present study, we measured energy costs of voluntary running and the maximal forced sprinting performance in S mice from three lines, one fixed for mini-muscles and two that do not exhibit the trait. We hypothesized that iCOT would be lower in mini-muscle mice than in normal mice because a smaller muscle (a reduction in hindlimb mass) would reduce the cost of cycling the leg. Reducing the energy required for contraction cycles may further contribute to increased resistance to fatigue (Syme et al., 2005), substantial energy savings, and enhanced sustained running ability in mini-muscle mice.

We also hypothesized that maximal sprint speeds would be lower in mini-muscle mice than in normal mice because the former have a smaller and slower medial gastrocnemius as well as thigh muscle reduced in mass by ~50%, which should result in reduced maximal power output and force production during sprinting. Compared with normal mice, mass-specific maximum power output of the medial gastrocnemius in mini mice is reduced by about half during isotonic shortening and by about 50–80% during cyclic contractions (Syme et al., 2005). Furthermore, considering the reduced mass of the gastrocnemius in mini-muscle mice, the absolute power from this muscle that is available for running is reduced to 10–20% of that in normal mice (Syme et al., 2005). We anticipated that this reduction in power would be more detrimental to sprinting ability than potential enhancements from possessing longer hindlimbs (Kelly et al., 2006).

MATERIALS AND METHODS

Animals and experimental protocol

We studied mice (*Mus domesticus* Schwarz and Schwarz 1943) from generation 46 of an ongoing artificial selection experiment for high voluntary wheel running, which includes four selected (S) lines (lab designations 3, 6, 7 and 8) and four control (C) lines (lab designations 1, 2, 4 and 5). The S lines include the highest-running males and females from each family as the breeders for the next generation (determined from highest number of revolutions run on days 5 and 6 of a 6-day period of wheel access). In the C lines, breeders are chosen at random from within each family (Swallow et al., 1998).

In each generation, mice are housed four per cage from weaning (21 days of age) until the period of selection (6–8 weeks of age), at which time they are housed with wheel (circumference=1.12 m) access for 6 days. *Ad libitum* food and water were provided. Animals were held on a 12 h:12 h L:D photoperiod (light from 07:00–19:00 h). Daily wheel activity was recorded with a computer. The thirty females used in this experiment were sampled from S lines 3, 7 and 8. All line 3 mice show the mini-muscle phenotype, but it is absent from lines 7 and 8.

Following the routine 6-day wheel test as part of the regular selection protocol, mice were housed four per cage and later allowed access to running wheels (the same wheels used for the selection protocol) for 5 days before being measured in respirometry wheels enclosed in metabolic chambers [design shown in fig. 1 in Chappell et al. (Chappell et al., 2004)]. Age at the start of these respirometry trials averaged 137 days (range 123–147). Briefly, the wheel chamber consisted of a Plexiglas© enclosure containing a running wheel (1.12 m circumference) attached to a standard housing cage supplied with bedding, a food hopper containing rodent chow, and a drinking tube. Each wheel enclosure was equipped with an internal fan to circulate air and a small generator that served as a tachometer and transduced wheel speed and direction into electrical signals. Mice were placed in wheel chambers at about 11:30 h (i.e. the middle of the normally resting phase of the daily activity cycle). Oxygen and carbon dioxide concentration, flow rate, temperature and wheel speed were measured over a 23.5 h period and recorded on a Macintosh computer equipped with LabHelper software (Warthog, http://www.warthog.ucr.edu). Air flow was maintained at 2,500 ml min^{-1} with Porter Instruments mass flow controllers (Hatfield, PA, USA) and 2.5 min gas reference readings were obtained every 45 min to control for any baseline drift in the gas analysers. Excurrent air was subsampled at about 100 ml min^{-1}, dried with magnesium perchlorate, and directed to an oxygen analyzer (Oxilla, Sable Systems, Henderson, NV, USA) and carbon dioxide analyzer (CA-2A, Sable Systems).

After the wheel test, mice were chased along a photocell-lined racetrack to determine apparent maximal sprint speed, following standard procedures for small rodents (Djawdan and Garland, 1988; Garland et al., 1988; Friedman et al., 1992; Dohm et al., 1994; Garland et al., 1995; Dohm et al., 1996). Wheel measurements and sprint speed were, on average, 11.3 days apart. The track was 6 m in length with 12 photocells spaced at 0.5 m intervals, and a width of 7.5 cm. The substratum was a textured rubber conveyor belt material that provided excellent traction. Sprint speed was computed from time elapsed between successive photocell stations, and the fastest 1-m interval (three adjacent photocells) was recorded for each run. A subjective behavioural score (five categories from 'poor' to 'excellent') of running effort was also recorded for each mouse. This was used to

assess each mouse's motivation to run. Five trials were done on each of two consecutive days to assess repeatability. The single fastest 1-m interval for each individual was used as its maximum speed.

Calculation of O_2 and CO_2

We assumed a constant respiratory quotient (RQ) of 0.85 [based on measurements from Chappell et al. (Chappell et al., 2004)] and calculated oxygen consumption ($\dot{V}_{O_2}$) as:

$$\dot{V}_{O_2} = \dot{V}\,(F_{iO_2} - F_{eO_2}) / [1 - F_{eO_2}\,(1 - \mathrm{RQ})]\,, \quad (1)$$

where $\dot{V}$ is the flow rate, F_{iO_2} and F_{eO_2} are fractional incurrent and excurrent oxygen concentrations, respectively. In order to avoid either frequent CO_2 scrubber changes or long lag times because of the large scrubber volumes, CO_2 was not removed before oxygen measurements. Carbon dioxide production ($\dot{V}_{CO_2}$) was used to validate the RQ assumptions; it was computed as:

$$\dot{V}_{CO_2} = \dot{V}\,(F_{eCO_2} - F_{iCO_2}) / \{1 - F_{eCO_2}\,[1 - (1 / \mathrm{RQ})]\}\,, \quad (2)$$

where F_{iCO_2} and F_{eCO_2} are fractional incurrent and excurrent CO_2 concentrations, respectively.

'LabAnalyst' software was used to smooth metabolic data *via* a nearest-neighbour algorithm and the 'instantaneous' transformation was used to resolve short-term events (Bartholomew et al., 1981). LabAnalyst was also used to subtract baselines, interpolate through references, correct lag times, and compute O_2 and CO_2 (e.g. Fig. 1).

We calculated slopes (iCOT) and intercepts of the speed *versus* O_2 relationship using least-squares linear regression of speed and O_2 for each individual (e.g. Fig. 2). Data were obtained with the LabAnalyst stepped sampling procedure (1-min means separated by 3 min; the initial 1-min block was the midpoint of the entire 23.5 h of recording) to eliminate autocorrelation, as sequential samples are not independent (Chappell et al., 2004; Rezende et al., 2006b). Speeds less than 0.5 m min^{-1} were discarded to eliminate any effects of electrical noise in the tachometer. Outliers were removed by visual inspection. Resting metabolic rate was measured as described below. The *Y*-intercept was used to calculate the postural cost (intercept – resting metabolic rate), which is generally thought to be the cost associated with holding a body in an upright position (Taylor et al., 1970; Schmidt-Nielsen, 1972; Bennett, 1985). Traits calculated for the wheel metabolic trials were as follows:

Distance run = total distance run over 23.5 h recording period (m).

Run time = total time spent running (wheel rotating) over 23.5 h recording period (min).

Maximum wheel speed (V_{max}) = highest speed in a 1.5 s sample interval (m min^{-1}).

Maximum 1-min wheel speed ($V_{max}1$) = maximum voluntary speed averaged over 1 min (m min^{-1}).

Maximum voluntary O_2 ($O_2 1$) = Maximum voluntary O_2 averaged over 1 min (ml min^{-1}).

Daily energy expenditure (DEE) = O_2 averaged over 23.5 h recording period (ml min^{-1}).

Resting metabolic rate (RMR_{10}) = Minimum O_2 averaged over 10 min (ml min^{-1}).

Incremental cost of transport (iCOT) = Slope of speed *versus* O_2 regression (ml O_2 m^{-1}), using 1-min means separated by 3 min.

Intercept = Intercept of speed *versus* O_2 regression (ml O_2 min^{-1}).

Postural cost = Intercept – RMR_{10} (ml O_2 min^{-1}).

Absolute cost of transport = [(iCOT × distance) + (postural cost × run time)] (ml O_2).

Ecological cost of transport (% DEE) = [100 × (distance × slope) / DEE] (Garland, 1983).

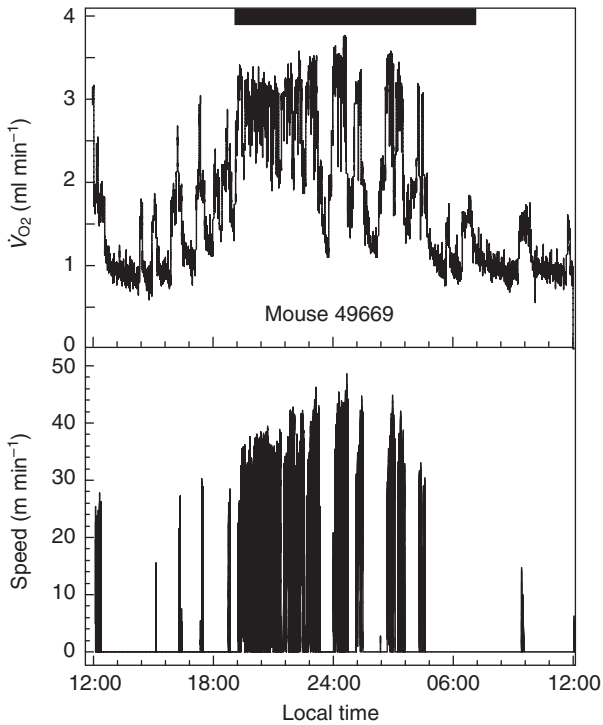

Fig. 1. Example of voluntary wheel running recorded over 23.5 h, showing speed and oxygen consumption. Shaded bar indicates lights out.

Statistics

We compared mini-muscle mice (line 3) with normal mice (lines 7 and 8) using analysis of variance (ANOVA, for wheel-running traits) or covariance (ANCOVA, for metabolic traits and sprint speed) with body mass as a covariate, and a planned contrast (SAS PROC MIXED, version 9.1). Repeatability of sprint speed between days 1 and 2 was assessed using a paired *t*-test and a Pearson product-moment correlation. Significance was judged at α=0.05, and we report two-tailed significance levels unless we had specific directional predictions.

RESULTS

Routine wheel testing

Results from days 5 and 6 of the 6-day wheel exposure used to identify breeders in the selection experiment (Table 1) indicate that mini-muscle mice did not differ from normal selected-line mice in distance run or in maximum speed attained in any 1-min interval. However, on average, mini-muscle mice spent less time running (two-tailed, P=0.0017) and ran at higher mean speeds (P=0.0018) than normal mice (Fig. 3).

Costs of voluntary locomotion

Two mice were tested twice for voluntary running costs because of poor performance or equipment problems during the initial measurement. During the metabolic trials, neither distance run nor time spent running (Table 2) was significantly different between mini-muscle and normal mice. However, V_{max} and $V_{max}1$ were significantly higher in mini-muscle mice (P=0.0009 and P=0.0010, respectively). No body mass effects were found, and therefore, body mass was not used as a covariate in analyses of running behaviour (Table 3 reports the mean of masses from before and after respirometry measurements, and on both days of sprint speed tests).

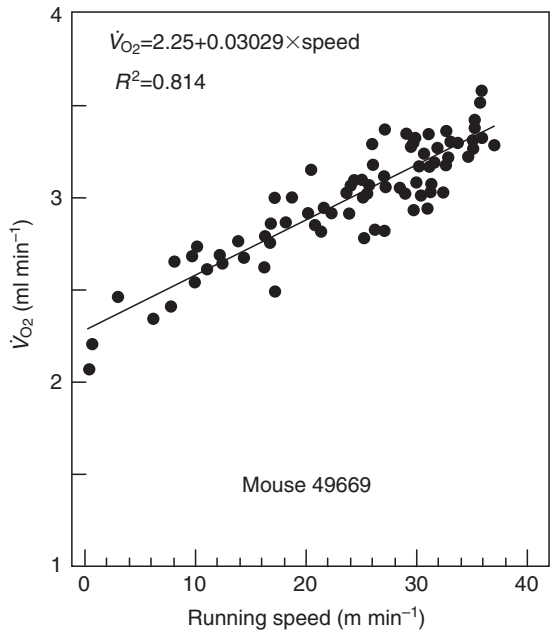

Fig. 2. Example of typical relationship between running speed and oxygen consumption during 23.5 h of voluntary wheel running. Cost of transport (slope), intercept, resting metabolic rate, and postural cost were obtained for 30 female mice. ANCOVA results accounting for body mass are listed in Table 3.

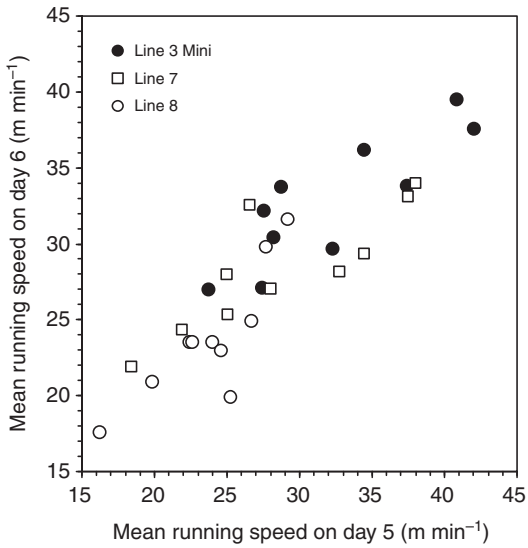

Fig. 3. Mean running speed on days 5 and 6 of the routine 6-day wheel exposure as used to pick breeders in the selection experiment. Mini-muscle mice ran significantly faster than mice with normal muscles (see Table 1).

As might be expected from the elevated maximum voluntary running speeds of mini-mice, the highest voluntary oxygen consumption during any 1-min interval ($\dot{V}_{O_2}1$) was also significantly higher in mini-muscle *versus* normal mice (P=0.0122; Table 3). DEE was significantly higher in mini-muscle mice than in normal mice (P=0.0442), but RMR_{10} did not differ (Table 3). We found no statistical differences in RER between mini-muscle and normal mice, either during the highest single minute of oxygen consumption or during the 10-min RMR measurement (Table 3; results were similar when body mass was not included in the model).

Only 29 mice were included in the RMR_{10} and postural cost analyses because one mouse was very active even when not running and did not provide a valid RMR. Contrary to predictions, the slope of the speed *versus* O_2 regression, or iCOT, was not significantly different between mini-muscle and normal selected lines (Table 3). Also unexpected, was the significant difference in intercept between mini-muscle and normal mice, with the former having a 7.6% higher value (P=0.0017). Because intercepts were different between normal and mini phenotypes, but RMR was not different, postural cost was also significantly higher in mini-muscle than in normal mice (P=0.0187).

Table 1. Wheel running on days 5 and 6 of the routine test

		Least squares means ± s.e.m.		
Trait	*N*	Mini-muscle (line 3)	Normal (line 7)	Normal (line 8)
Mean distance (m)	30	15,526±960	17,856±960	13,921±960
Mean time (min)	30	473.3±33.0	639.7±33.0	588.6±33.0
Mean speed (m min^{-1})	30	32.49±1.49	28.57±1.49	23.84±1.49
Max speed (m min^{-1})	30	49.39±2.47	48.66±2.47	39.93±2.47

Sprint performance

No mice were excluded from sprint speed analyses because of low behavioural scores. In the pooled sample of 30 mice, maximum 1-m sprint speed was significantly repeatable between day 1 and 2, as indicated by a Pearson product-moment correlation (r=0.787 for log-transformed values; P<0.0001). On average, mice ran faster on day 2 (two-tailed P=0.0173 for log-transformed values). Using the higher of the two daily values for each mouse (no transformation necessary), maximum sprint speed averaged 23.5% higher in normal than in mini-muscle mice (one-tailed P=0.0481; Table 3).

DISCUSSION

Cursoriality in mammalian runners is often associated with specific morphological features, including relatively long limbs, high metatarsal/femur ratios, and more proximal musculature (Garland and Janis, 1993; Steudel and Beattie, 1993; Carrano, 1999), which in turn are correlated with increased locomotor abilities (Garland and Janis, 1993) and more extensive movement in nature (Kelly et al., 2006). Limb morphology is also thought to have a substantial influence on COT in mammalian runners (Hildebrand, 1962; Myers and Steudel, 1985), based on the assumption that the work performed to cycle the limbs during a stride constitutes a substantial part of the total COT (Martin, 1985; Claremont and Hall, 1988). Therefore, a smaller and/or more proximally distributed limb mass would require less energy to cycle and hence should lower the iCOT. In support of this idea, Myers and Steudel (Myers and Steudel, 1985) found that artificial alterations in human limb mass that alter kinetic energy of the limb can result in significant changes in COT. Yet Taylor et al. (Taylor et al., 1974) suggest no difference in COT or iCOT in a comparison of cheetahs, goats and gazelles that were similar in size but differed markedly in limb morphology, although statistical analysis was not performed on the results. Phylogenetic non-independence may also confound interpretation of these and other tests of 'cursorial morphology' (e.g. see Garland and Janis, 1993; Autumn et al., 1999; Barbosa and Moreno, 1999; Kelly et al., 2006).

Here, we used an experimental evolution approach (Garland and Rose, 2009) and studied mice selectively bred for high voluntary

Table 2. Wheel-running during metabolic measurements

Trait	*N*	Least squares means ± s.e.m. Mini-muscle (line 3)	Normal (line 7)	Normal (line 8)	Mini *vs* normal
Distance run (m)	30	7,967±912	6,688±912	7,566±912	0.4590
Run time (min)	30	301.8±34.4	286.4±34.4	352.7±34.4	0.6758
V_{max} (m min^{-1}; 1.5 s)	30	52.69±1.92	46.22±1.92	41.65±1.92	0.0009
$V_{max}1$ (m min^{-1}; 1 min)	30	46.63±1.99	40.56±1.99	34.76±1.99	0.0010

wheel running to examine how limb morphology may affect sprinting performance and costs of transport during voluntary wheel running. In particular, we tested the performance and cost impact of the mini-muscle phenotype that has increased in frequency in two of the selected lines and has become fixed in one of these lines. The reduced hindlimb mass and longer, thinner hindlimb bones of mini-muscle mice give them a more 'cursorial' morphology than normal mice (Kelly et al., 2006), and this phenotype has been favoured by selection for high voluntary wheel running (Garland et al., 2002). We hypothesized that voluntary running costs would be lower in mini-muscle mice *versus* normal mice, due to the reduction in hindlimb mass in mini-muscle animals. This prediction seems reasonable, although the expected effect of their longer hindlimbs (Kelly et al., 2006) is not entirely clear (e.g. see Steudel-Numbers et al., 2007), and we do not know their effective hindlimb length (Pontzer, 2007) while running on wheels. Additionally, because of a smaller hindlimb muscle mass and altered contractile properties (Syme et al., 2005), we expected a reduction in maximum contractile force and hence slower sprint speed in mini-muscle mice.

The mini-muscle mice in our study ran at higher mean voluntary speeds than normal mice, which is consistent with previous results that mini-muscle mice run faster on wheels and sometimes run more revolutions per day than normal mice (Garland et al., 2002; Syme et al., 2005; Kelly et al., 2006; Hannon et al., 2008; Gomes et al., 2009). However, contrary to our predictions, one of the two main components of transport costs, iCOT – the slope of the speed *versus* metabolic rate relationship (Fig. 2) – was not significantly different between mini-muscle and normal mice. The other major component of running costs is the so-called 'postural cost' (Taylor et al., 1970; Taylor et al., 1982): the elevation of the zero-speed intercept above resting metabolism. An unexpected result was that mini-muscle mice have a higher intercept than normal mice, which contributes to a higher postural cost in mini-muscle mice. The combination of similar iCOT and higher postural costs means that running costs at any speed are higher in mini-muscle mice than in normal mice.

In large part our prediction of reduced COT in mini-muscle mice was based on the assumption that lighter limbs would be less costly to cycle during running. Previous studies have shown that the cost of leg cycling significantly contributes to running costs (Martin, 1985; Claremont and Hall, 1988). In some circumstances, the cost of leg cycling may be more substantial than the cost of supporting body mass during locomotion. Moreover, there is a greater increase in metabolic rate when an animal is carrying a given load on the feet rather than more proximally on the leg (Martin, 1985), which also suggests the thinner, lighter limbs of mini-muscle mice should provide an energetic savings during running. However, reduced limb muscle mass leads to increased muscle stress and increased costs associated with supporting the body during locomotion (Reilly et al., 2007).

Stride frequency may also be important in determining COT. In smaller animals, higher stride frequencies are often associated with higher costs (Heglund and Taylor, 1988). Because stride frequency is in part a function of limb dimension, it is reasonable to assume that limb morphology could have a significant impact on locomotor performance, including both sprint speed (e.g. Bonine and Garland, 1999) and COT. More proximal muscle distributions may lead to higher stride frequencies and, therefore, higher cost of muscular work per unit time as a result of more rapid leg cycling (Heglund and Taylor, 1988; Raichlen, 2006). However, as speed = stride frequency × stride length, energy costs per unit distance covered should be the same or lower for cycling a thin 'cursorial' leg than for 'normal' limb configurations, unless the cursorial limb has reduced stride length. At present we do not have kinematic data for voluntary running in mini-muscle *versus* normal mice. If limb cycling is in part a resonant property of limb structure (Ahlborn et

Table 3. Body mass (mean), cost of transport and maximal sprint speed

Trait	*N*	Least squares means ± s.e.m. Mini-muscle (line 3)	Normal (line 7)	Normal (line 8)	Body mass	Mini (line 3) *vs* normal (lines 7 and 8)
Body mass (g)	30	29.75±0.80	30.33±0.80	30.96±0.80	–	0.3679
Maximum voluntary O_2 over 1 min (ml O_2 min^{-1})	30	4.565±0.200	3.967±0.198	4.018±0.201	0.0369	0.0284
Respiratory exchange ratio at maximum voluntary O_2 over 1 min	30	0.980±0.039	1.083±0.037	1.011±0.037	0.2461	0.1690
Daily energy expenditure O_2 (ml O_2 min^{-1})	30	1.896±0.061	1.743±0.061	1.730±0.061	0.0045	0.0442
Resting metabolic rate over 10 min (ml O_2 min^{-1})	29	0.760±0.027	0.730±0.251	0.727±0.253	0.0003	0.3347
Respiratory exchange ratio at RMR over 10 min	29	0.892±0.023	0.890±0.021	0.909±0.0225	0.0027	0.7759
iCOT (ml O_2 m^{-1})	30	0.0339±0.0022	0.0336±0.0022	0.0365±0.0022	0.1511	0.6839
Intercept (ml O_2 min^{-1})	30	2.452±0.044	2.272±0.043	2.258±0.044	0.3169	0.0017
Postural cost (ml O_2 min^{-1})	29	1.674±0.045	1.544±0.043	1.531±0.043	0.2974	0.0187
Ecological cost of transport (% DEE)	30	0.1003±0.0101	0.0870±0.0100	0.1022±0.0101	0.0326	0.6505
Absolute cost of transport (ml O_2 day^{-1})	29	823.9±79.3	667.2±75.0	801.3±75.5	0.1909	0.3575
Maximum sprint speed (m s^{-1})	30	0.9226±0.1332	1.3450±0.1318	1.0663±0.1333	0.3010*	0.0481*

*One-tailed *t*-test.

al., 2006), then lighter limbs might cycle faster, with reduced stride length. That could be part of the explanation for the higher running costs we observed in the mini-muscle animals.

Another potential complication is that the effects of body size on locomotor costs may differ for walking *vs* running (Rubenson et al., 2007). Changes in COT at different gaits have been reported in at least one rodent (Kenagy and Hoyt, 1989). If the mini-muscle mice had different gait *versus* speed preferences than normal mice, perhaps because of different limb muscle characteristics, then their running costs might differ. However, we saw no inflection point in the speed *versus* metabolic rate relationship that might indicate cost variation due to gait changes (Fig. 2).

Speculatively, one possible explanation for the apparent selective advantage of the mini-muscle allele stems from the higher voluntary running speeds of mini-muscle mice (Table 1) (see also Syme et al., 2005; Kelly et al., 2006; Gomes et al., 2009). At any given speed, the mini-muscle phenotype provides no energy savings over the normal limb phenotype, but because absolute COT (including both iCOT and postural costs) decreases with increasing running speed (Taylor et al., 1970), the economy of running may be improved in faster-running mini-muscle individuals. We did not find a significant difference in overall running economy in this small sample of animals (Table 3), but a savings at high speeds might be apparent with a larger sample size.

It is also possible that the mini-muscle phenotype has another benefit that would have been difficult to discern through our measurements, such as reduced energy use by the slower and smaller mini muscles and thus reduced reliance on anaerobic metabolism during exercise (Barclay and Weber, 2004) (see also Gomes et al., 2009). Although measurements of work and oxygen consumption in individual muscles revealed that the mini muscles are not more efficient than their normal counterparts, the mini-muscle phenotype may allow mice to run faster without significant accumulation of anaerobic by-products associated with muscle fatigue which may decrease motivation to run (McGillivray et al., 2009). The significantly faster voluntary running speeds of mini-muscle mice (Tables 1 and 2) lend support to this hypothesis. By this means, an improvement of some aspects of running performance could be achieved within particular muscles, even if that improvement is not reflected in whole-animal running energetics.

From a broader perspective, it is important to keep in mind that limbs may be optimized for more than speed or running economy [e.g. manoeuvrability or grasping strength, both of which may be relevant to wheel running: see video (http://www.biology.ucr.edu/people/faculty/Garland/Girard01.mov) that accompanies Girard et al. (Girard et al., 2001)]. Also, from the perspective of the evolution of mini-muscle in our experiment, the selection protocol emphasizes distance run with unlimited access to food, so there may be little selection for the energy savings associated with reduced COT.

Our second main prediction was that mini-muscle mice would have reduced sprinting performance, i.e. lower maximal sprint speeds. Maximum running speed is largely a function of the ability of muscles to generate force, so higher muscle power output *via* differences in muscle volume, architecture or type of contractile fibres should lead to greater sprint speeds (Kumagai et al., 2000; Abe et al., 2001). Strong selection for sprinting ability would be expected to be associated with changes in muscle that enhance power output for a short, fast burst of anaerobic power, such as increased muscle mass and the proportion of fast-twitch fibres. Conversely, a smaller muscle mass with slower muscle fibres, as in the mini-muscle phenotype (Syme et al., 2005; Guderley et al., 2006; Guderley et al., 2008; Bilodeau et al., 2009; McGillivray et al., 2009), would lead to reduced power output (Syme et al., 2005) and hence impaired sprinting ability. As expected, we found that mini-muscle mice had significantly reduced maximal sprint speeds (Table 3). This may be an unavoidable trade-off between endurance capacity and high-power output, as predicted by Syme et al. (Syme et al., 2005). In voluntary wheel running, all of the mice from these lines typically run at speeds within their aerobic performance capacity, and much more slowly than maximal sprint speed (Rezende et al., 2005; Rezende et al., 2009). Therefore, such traits as muscle fatigue-resistance, endurance capacity, and reduced costs of transport may be favoured by the selection protocol even at the expense of sprint performance.

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