Physical performance and soleus muscle fiber composition in wild-derived and laboratory inbred mouse strains

Yoshikazu Totsuka,1 Yasumitsu Nagao,2 Takuro Horii,2 Hiromichi Yonekawa,3 Hiroshi Imai,2 Hideo Hatta,4 Yoshiaki Izaike,5 Tomoyuki Tokunaga,5 and Yoriko Atomi4

1YS New Technology Institute, Tochigi 329-0512; 2Laboratory of Reproductive Physiology, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502; 3Department of Laboratory Animal Science, The Tokyo Metropolitan Institute of Medical Science, Tokyo 113-8613; 4Department of Life Sciences, The Graduate School of Arts and Sciences, The University of Tokyo, Tokyo 153-8902; and 5Developmental Biology Department, Development and Differentiation Laboratory, National Institute of Agrobiological Sciences, Ibaraki 305-8602, Japan

Submitted 15 October 2002; accepted in final form 7 April 2003

Totsuka, Yoshikazu, Yasumitsu Nagao, Takuro Horii, Hiromichi Yonekawa, Hiroshi Imai, Hideo Hatta, Yoshiaki Izaike, Tomoyuki Tokunaga, and Yoriko Atomi. Physical performance and soleus muscle fiber composition in wild-derived and laboratory inbred mouse strains. J Appl Physiol 95: 720–727, 2003; 10.1152/japplphysiol.00946.2002.—We compared four inbred mouse strains in their physical performance, measured as a maximal treadmill running time, characteristics of soleus muscle, anatomic character, and growth. The strains used were Mus musculus domesticus (C57BL/6 (B6) and BALB/c), Mus musculus molossinus (MSM/Ms), and Mus spretus. Maximal running time was significantly different among these four mouse strains. Running time until exhaustion was highest in MSM/Ms and lowest in M. spretus. Maximal times for the laboratory mouse strains were nearly identical. Soleus muscle fiber type and cross-sectional area also differed significantly among the species. In particular, M. spretus was significantly different from the other inbred mouse strains. Growth in the wild-derived inbred mice appeared to be complete earlier than in the laboratory mice, and the body size of the wild strains was about half that of the laboratory strains. From these results, we propose that wild-derived inbred mouse strains are useful models for enhancing phenotypic variation in physical performance and adaptability.

Address for reprint requests and other correspondence: The Graduate School of Arts and Sciences, Dept. of Life Sciences, The Univ. of Tokyo, Tokyo 153-8902, Japan (E-mail: atomi@idaten.c.u-tokyo.ac.jp).

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IT IS WIDELY ACCEPTED that an individual’s traits depend on both genetic background and environmental factors (9, 19). The laboratory mouse is a good tool as a model animal for understanding genetic influences on differences in phenotypes (1, 23). C57BL/6 (B6) is widely used as a biomedical model (22). However, genetic variations of the laboratory mouse are small, and the phenotypic variation that they show is small compared with those of the wild mouse. Recently, inbred mouse strains have been established from naturally occurring mice to expand the genetic pool (20). In particular, Mus spretus has been known as a powerful genetic research tool. M. spretus is closely related to Mus musculus, the source of most classical inbred laboratory strains (13, 21, 27). The specific wild-mouse genetic characteristics and valuable phenotypes are made available for biological research.

It is possible to evaluate a mouse’s physical performance by using an exercise test on a treadmill (7, 28). Such physical performance tests are an effective measure of physiological ability. However, the tests have had difficulty in demonstrating large strain differences between classical laboratory inbred mice. Recently, Lerman et al. (17) reported that the forced endurance exercise performance of FVB/NJ (FVB) is very high in the classical laboratory mouse strains. Lerman’s report is a worthy contribution to experimental animal models of exercise physiology science. However, the main genetic background of old inbred strains was derived from a European subspecies of M. musculus, Mus musculus domesticus (34, 35, 37). Therefore, we tried to use newly established strains from wild mice, as reported by Koide et al. (14). In general, physical performance could be influenced by various physiological (respiratory-circulatory function, energy metabolism, musculoskeletal function, and neural transmission) and psychological factors (emotion, etc.). Moreover, the relative contributions of those functions to physical performance could be changed by the introduction of a different genetic background. Garland et al. (8) addressed such a question by an intraspecific comparison of the laboratory mouse and the wild mouse (M. m. domesticus). They examined whether differences in sprint speed in an individual mouse are correlated with gastrocnemius muscle mass or the percentage of cross-sectional area occupied by each major skeletal muscle fiber type. Their results suggested a lack of correlation between sprint speed ability and skeletal muscle size.

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or fiber composition. Use of wild-derived inbred mice of subspecies or species closely related to the classical laboratory mouse strains might reveal genetic factors underlying sprint speed ability. Moreover, skeletal muscle, which is a major organ for locomotor activity, has a species-specific fiber type (26, 33). Therefore, we compared skeletal muscle traits in wild-derived inbred mice with those in an existing inbred strain. Previous reports regarding not only skeletal muscle but also respiratory-circulatory function have shown evidence of genetic influences. For example, Bouchard et al. (3) showed a significant genetic effect on physical performance in a human twin study.

In the present study, we compared basic data on growth, tissue weights, maximal running time, and features of the soleus muscle in M. m. domesticus (B6 and BALB), Mus musculus molossinus (MSM/Ms), and M. spretus. Our results demonstrate that the wild-derived inbred mouse strains are an effective model for enlarging the variability in physical performance phenotypes and for studying the relationship between such phenotypic characteristics and genetic factors.

**METHODS**

**Experimental animals.** The nomenclature of the mouse strains is based on the report of Bonhomme and Guénet (2). They classify M. musculus as a polytypic species, which comprises four major subspecies (M. m. domesticus, Mus musculus bactrianus, Mus musculus castaneus, and M. m. musculus). Mus musculus molossinus is an Asian hybrid population between M. m. musculus and M. m. castaneus genomes (36). M. spretus is a closely related species to M. musculus. We used males of the classical laboratory mouse strains [B6 and BALB/cA (BALB)] as M. m. domesticus and the wild-derived inbred strains as M. m. molossinus and M. spretus. B6 and BALB were purchased from Clear Japan (Tokyo, Japan). One wild-derived inbred mouse strain, MSM/Ms as M. m. molossinus, was obtained from the National Institute of Genetics (Mishima, Japan). Another wild-derived strain, M. spretus, was purchased from the Jackson Laboratory (Bar Harbor, ME). All inbred strains were maintained by brother-sister mating. Animals were housed in air-conditioned rooms (25 ± 1°C and 60 ± 10% humidity, with a lighting schedule of 12 h of light from 0000 to 0800) and fed a commercial diet (P2, Funabashinojo, Chiba, Japan). All experimental protocols were approved by the Administrative Panel on Laboratory Animal Care of the University of Tokyo.

**Growth curve and anatomic data.** All male mice were measured to determine the change in body weight from 3 to 15 wk of age. The results were compared among the four mouse strains (B6, BALB, MSM/Ms, and M. spretus) to determine the effect of genetic background on growth rate and body size. The growth rate was calculated as (body wt of the present week – body wt of the previous week)/body wt of the previous week × 100. At 12 wk of age, the mice were euthanized after weighing, and the weights of the heart, liver, and abdominal (mesenterical, retroperitoneal, and epididymal) adipose tissue and tail length were recorded (5, 16).

**Muscle fiber typing and the cross-sectional areas of soleus.** The fiber types of soleus muscles from B6, BALB, MSM/Ms, and M. spretus were analyzed at 12 wk of age. A small block of soleus muscle was frozen in isopentane cooled in liquid nitrogen. The frozen muscles were serially cryosectioned to a thickness of 7 μm at −20°C. Individual fibers in the soleus were histochemically classified as type I, IIa, IIb, and IIc fibers by myosin ATPase activity (4, 10, 25). Samples were preincubated for a total of 30 min in sodium barbital buffer (0.03 M) at the following pH levels: 4.0, 4.2, and 4.4 (5 min each), 10.3 (15 min). After the enzyme reaction, we measured the total fiber numbers, and the composition and area of each fiber type group in the soleus muscles of all four inbred mouse strains (4, 25). The cross-sectional area of each fiber type per muscle was analyzed by using National Institutes of Health Image software.

**Physical performance test.** Maximal running time until exhaustion, with the use of an incremental protocol, was measured as an index of physical performance among the four strains at 10 wk of age (11, 24). All mice were adapted to the treadmill for a period of 10 days. During the adaptation period, the mice were made to run on the treadmill for 10 min at a speed of 20 m/min. After treadmill practice, each mouse was carried to the grid and trained to associate the grid with an electrical shock. Each mouse was then placed in a small chamber equipped with an electric shock grid that was held on the treadmill belt (Clea Japan, Tokyo, Japan). Running speed was gradually increased by 10 m/min every 1 min from
Table 1. Anatomic records in 12-wk-old male classical laboratory mice (Mus musculus domesticus: C57BL/6J and BALB/c) and wild mice [Mus musculus molossinus (MSM/Ms) and Mus spretus]

<table>
<thead>
<tr>
<th>Organ weight, g</th>
<th>M. spretus (n = 5)</th>
<th>M. m. molossinus (MSM/Ms) (n = 7)</th>
<th>C57BL/6J (n = 7)</th>
<th>BALB/cA (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>14.7 ± 0.3a</td>
<td>12.4 ± 0.6b</td>
<td>26.5 ± 0.7c</td>
<td>27.1 ± 0.4c</td>
</tr>
<tr>
<td>Heart</td>
<td>0.072 ± 0.004</td>
<td>0.053 ± 0.004</td>
<td>0.109 ± 0.006</td>
<td>0.114 ± 0.001</td>
</tr>
<tr>
<td>Liver</td>
<td>0.724 ± 0.014</td>
<td>0.618 ± 0.053</td>
<td>1.215 ± 0.070</td>
<td>1.235 ± 0.042</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.118 ± 0.019</td>
<td>0.123 ± 0.011</td>
<td>0.296 ± 0.012</td>
<td>0.383 ± 0.012</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.115 ± 0.006</td>
<td>0.089 ± 0.006</td>
<td>0.149 ± 0.007</td>
<td>0.198 ± 0.006</td>
</tr>
<tr>
<td>Abdominal adipose (mesenterical + retroperitoneal + epididymal)</td>
<td>0.648 ± 0.029</td>
<td>0.404 ± 0.053</td>
<td>1.065 ± 0.154</td>
<td>1.417 ± 0.214</td>
</tr>
<tr>
<td>Tail length, cm</td>
<td>7.3 ± 0.1a</td>
<td>5.5 ± 0.1b</td>
<td>9.0 ± 0.1c</td>
<td>9.5 ± 0.1d</td>
</tr>
</tbody>
</table>

Organ indexes*:
- Heart: 0.49 ± 0.02a, 0.42 ± 0.02b, 0.41 ± 0.02b, 0.42 ± 0.01b
- Liver: 4.94 ± 0.19, 4.93 ± 0.22, 4.61 ± 0.29, 4.62 ± 0.11
- Pancreas: 0.79 ± 0.12a, 0.99 ± 0.06a–b, 1.12 ± 0.03b, 1.41 ± 0.05b
- Kidney: 0.78 ± 0.03a, 0.71 ± 0.02b, 0.56 ± 0.03c, 0.73 ± 0.02b
- Abdominal adipose (mesenterical + retroperitoneal + epididymal): 4.40 ± 0.17a–b, 3.28 ± 0.16a, 4.11 ± 0.67a,b, 5.23 ± 0.78b

Values are means ± SE. *Organ indexes are indicated as organ weight/body weight × 100. Values with different letters are significantly different among them by one-way ANOVA following Fischer’s protected least-significant difference test, P < 0.05.

Table 2. Total fiber numbers, fiber type percentage, and the cross-sectional areas in soleus muscle differentiated by myosin ATPase activity

<table>
<thead>
<tr>
<th>Mus musculus</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. m. domesticus</td>
</tr>
<tr>
<td>C57BL/6J (n = 5)</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Total fiber no.</td>
</tr>
<tr>
<td>Fiber type percentage, %</td>
</tr>
<tr>
<td>Type I</td>
</tr>
<tr>
<td>Type IIa</td>
</tr>
<tr>
<td>Type IIb</td>
</tr>
<tr>
<td>Type Iic</td>
</tr>
<tr>
<td>Cross-sectional areas of muscle fiber, μm²</td>
</tr>
<tr>
<td>Type I</td>
</tr>
<tr>
<td>Type IIa</td>
</tr>
<tr>
<td>Type IIb</td>
</tr>
<tr>
<td>Type Iic</td>
</tr>
</tbody>
</table>

Values are means ± SE. Myosin ATPase activity consisted of a 15-min preincubation at pH 10.3 and a 45-min preincubation at pH 4.0, 4.2, and 4.4 in 12-wk-old male classical laboratory mice and wild-derived inbred mice. Values with different letters are significantly different among them by one-way ANOVA followed Fischer’s protected least-significant difference test, P < 0.05.
the laboratory mouse strains from 3 through 15 wk of age. The mean body growth rates of MSM/Ms and *M. spretus* at 15 wk of age were about half those of B6 and BALB. The whole body growth rates between the laboratory mouse and wild-derived inbred mouse also differed markedly from 3 to 4 wk of age. The differences between B6 (50.0%) and BALB (53.0%) were marginal, as were the differences between MSM/Ms (32.7%) and *M. spretus* (34.7%). After an analysis of the rates of weight gain in all mouse strains, a significant change in growth rate was no longer evident after the time of sexual maturation (~7 wk of age).

**Tissue weights and tail length at 12 wk of age.** Mean tissue weights of all strains at 12 wk of age are shown in Table 1. Organ indexes (organ weight/body weight × 100) of heart, pancreas, kidney, and abdominal adipose tissues, also shown in Table 1, differed significantly (*P* < 0.05) among the four strains. In particular, the organ index of heart of *M. spretus* was the heaviest, whereas the pancreas index of *M. spretus* was the lightest among all mouse strains. The tail length, which is one of skeletal size (5, 16), at 12 wk of age also differed significantly (*P* < 0.05) among the four inbred strains.

**Total fiber number, type, and the cross-sectional areas of soleus muscle.** There are significant differences in the fiber number of soleus muscle between the two wild-inbred mouse strains, *M. m. molossinus* and *M. spretus* (*P* < 0.05), with that of *M. spretus* being approximately one-half that of *M. m. molossinus* and one-third that of *M. m. domesticus* (Table 2). The soleus muscle fiber types differed significantly (*P* < 0.05) at 12 wk of age among *M. m. domesticus*, *M. m. molossinus*, and *M. spretus* (Table 2 and Fig. 2). The main fiber types in the soleus muscle were type I for *M. spretus*, whereas type IIa dominated for the classical laboratory strains. No difference was observed within species. The soleus muscle of *M. m. molossinus* consisted of almost equivalent percentages of type I and type IIa. Type IIb and IIc could not be recognized in the soleus muscle of *M. spretus*. The cross-sectional area of type I of *M. spretus* was significantly larger than the area of the other three mouse strains (Table 2 and Fig. 2). There is not a significant difference in all mouse strains at the each subtype in type II.

**Relationship between treadmill exercise performance and body weight or characteristics of soleus muscle in wild-derived and laboratory mouse strains.** Maximal running time was compared across strains at 10 wk of age (Fig. 3). The exercise performance time was significantly different (*P* < 0.05) between subspecies and/or interspecies, i.e., subspecies of the laboratory mouse showed higher performance levels than those of the other three inbred mouse strains. On the other hand, running time until exhaustion of *M. spretus*, a closely related species to the laboratory mouse, was shorter than the running time of the other three inbred mouse strains. The maximal running speed at exhaustion was 60–70 m/min for B6 and BALB/c, 70–90 m/min for MSM/Ms, and 40–60 m/min for *M. spretus*.
not significantly correlated with body weight within any mouse strains.

**DISCUSSION**

The classical laboratory mouse strains have an extensive history of experimental use and are excellent tools for genetics and other areas of science by virtue of the huge accumulation of genetic information from these strains (1). However, the wild-derived inbred mouse strains (21) have greatly expanded research possibilities because of their peculiar genetic background. In the present study, we used MSM/Ms and *Mus spretus* as representative wild-derived inbred mouse strains. MSM/Ms, which was trapped in Mishima City, Japan, and inbred by Kazuo Moriwaki, is an Asian subspecies (*M. m. molossinus*) that is a natural hybrid of *M. m. musculus* and *M. m. castaneus* (36). *M. spretus* is a species closely related to *M. musculus*, the source of the classical laboratory mouse. Although a great deal is known about the genetics of the wild-derived inbred mouse strains, little information regarding the physical abilities and other physiological characteristics had been obtained. A few reports have shown great differences in behavioral (15) and/or reproductive (30) characteristics of *M. spretus* relative to *M. m. domesticus*.

**Growth rate and anatomic characteristics.** The growth pattern of the classical laboratory mouse is generally divided into four growth phases (29): phase 1 is postembryonic development, phase 2 is the highest growth period preceding sexual maturation, phase 3 comprises skeletal maturation, and phase 4 constitutes the completion of maturation. A difference in body weight between the classical laboratory and wild-derived inbred mouse strains was clearly observed after weaning and continued until 15 wk of age (Fig. 1). In particular, the growth rate of *M. spretus* appeared to differ from the other inbred strains at phase 2. In addition to growth differences, we also observed differences in some anatomic characteristics. For example, several significant differences in organ indexes were found. The organ index of the pancreas was significantly smaller in *M. spretus* than in the other three inbred mouse strains, which suggests differential glucose metabolism (*P* < 0.05; Table 1). The organ index of the heart also differed significantly from that of the classical laboratory mouse strains (*P* < 0.05; Table 1), suggesting a difference in circulatory function between laboratory and wild-derived inbred mouse strains, especially *M. spretus* (*P* < 0.05; Table 1). From these results, we predict a large difference in physiological function between wild-derived and laboratory mice.
Fig. 4. Relationship between running time until exhaustion on maximal exercise test and body weight or characteristics of soleus muscle in laboratory strains [M. m. domesticus: B6 (●) and BALB/c (○)] and wild-derived inbred strains [M. m. molossinus (MSM/Ms; □) and M. spretus (■)]. Correlation between running time until exhaustion and body weight (A), total fiber number (B), proportion of type I (C), proportion of type IIa (D), cross-sectional areas of type I (E), and cross-sectional areas of IIa (F).
Characteristics of the soleus muscles of wild-derived mice. We examined muscle characteristics of the soleus muscle, which is an important hindlimb muscle because of its participation in the maintenance and regulation of postural activity (6). Mammalian skeletal muscles consist of fast- and slow-twitch muscle fibers. The soleus of the wild-derived inbred mouse strains, especially M. spretus, at 12 wk of age showed interesting fiber types and cross-sectional areas (Table 2 and Fig. 2). Unlike the other three inbred mouse strains, the fibers of the M. spretus soleus muscle were dominated by type I. Wigston et al. (32) reported that the relative proportions of the fibers of mouse soleus staining positively with fast and slow myosin antibodies were similar at all ages studied, with ~60–70% being fast and 30–40% being slow. Then, the type I of M. spretus had the cross-sectional area of the close double for those of the other three mouse strains (Table 2). Therefore, most of the cross section of soleus muscle was closed by the slow-twitch muscle fibers. M. spretus did not have type IIc fibers that have characteristics intermediate to the slow- and fast-twitch muscle fiber type (12). The muscle fiber type of MSM/Ms was also surprising; type I and IIa fibers were found in almost equal proportions in the soleus muscle of MSM/Ms (Table 2). The fast- and slow-twitch muscle fibers seem to equally exist, when cross-sectional areas of type I and IIa are considered (Table 2). Thus soleus muscle fiber type of these wild-derived mouse strains was clearly different from those of the laboratory mouse strains. Not only did fiber composition and the area differ, but fiber number differed among groups as well (Table 2). M. spretus soleus muscle contained the smallest number of fibers of all mouse strains. Thus the soleus muscles of M. m. domesticus (B6 and BALB), M. m. molossinus (MSM/Ms), and M. spretus indicated a peculiarity of species. Although determining the complete relationship between skeletal muscle characteristics and physical performance from a single skeletal muscle is not possible, our results suggest the potential utility of wild-derived mouse strains in examining the genetics of skeletal muscle characteristics.

Treadmill physical performance of wild-derived inbred mouse strains. Physical performance has been evaluated as running time and/or speed, sustaining power, and spontaneous activity to examine the characteristics of wild-derived inbred mice. We compared the running time until exhaustion of classical laboratory strains at 10 wk of age by using the maximal exercise test (11, 24) on a treadmill (Fig. 3). Our results suggest that this performance test could be an indicator of aerobic exercise capacity (18) in the mice. Although we did not measure maximal oxygen consumption (V̇O₂) directly, the differences in performance may correlate with differences in maximal V̇O₂, because V̇O₂ linearly increases as treadmill-running speed increases up to a maximal rate (27). However, the heart organ index indicates that the cardiovascular capacity of M. spretus was the largest of the mouse strains in the present study.

Relationship between physical performance and body weight and characteristics of soleus muscle in wild-derived and laboratory mouse strains. It is well known that the prevalence of type I fibers, which have a high oxidative capacity, is related to high aerobic capacity. However, in the present study, running time until exhaustion was not related to the type I fiber ratio of the soleus muscle (Fig. 4). The running time of M. spretus, which has soleus muscles mainly composed of type I (slow-twitch type), was the lowest, whereas that of MSM/Ms, which has soleus muscles with an almost equal ratio of type I and IIa fibers (fast-twitch type), was the highest. The maximal exercise test is designed to require the display of largest power with the endurance ability in each mouse. Therefore, in the present physical performance test, maximal power would be required for the mouse. Power might be affected not only by differential V̇O₂ capacity or skeletal muscle characteristics of each mouse strain but also by communicative competence of skeletal muscle and the nervous system to adapt running speed. Furthermore, clear differences in behavioral responses to novelty in an open field have been reported, with M. spretus being much less active than B6 (15). From these results, we propose that the complexity of factors affecting physical performance necessitates the use of wild-derived strains to study such polygenic traits. Moreover, the strain dependence of long-time endurance exercise ability raises an interesting possibility. As endurance runners have a significantly higher percentage of fiber types I and IIc than nonrunners (31), M. spretus should be examined for its endurance running ability.

It is without doubt that the widely used classical laboratory mice represent an important tool for identifying specific genes of interest and their functions. However, introduction of large phenotypic variation into the background of laboratory mice is also useful in precisely analyzing gene functions. For this reason, we hope our study will encourage the use of wild-derived mice for studying the genetic basis of physical performance. In other words, the characteristics of the wild-derived mouse will have better utility for the analysis of complicated genetic traits, such as physical performance and behavior. In conclusion, we have demonstrated a practical system for evaluating physical performance that includes the wild-derived mouse. Moreover, the genetic and environmental factors underlying interesting differences in physical capacity between the wild-derived mouse and the laboratory mouse should be clarified by future work.

DISCLOSURES

This study was supported in part by two Grants-in-Aid for Scientific Research from The Ministry of Education, Culture, Sports, Science, and Technology, and by Japan Science and Technology/Research Institute of Science and Technology for Society.

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