Cardiac and skeletal muscle adaptations to voluntary wheel running in the mouse

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Allen, David L., Brooke C. Harrison, Alexander Maass, Matthew L. Bell, William C. Byrnes, and Leslie A. Leinwand. Cardiac and skeletal muscle adaptations to voluntary wheel running in the mouse. J Appl Physiol 90: 1900–1908, 2001.—In this paper, we describe the effects of voluntary cage wheel exercise on mouse cardiac and skeletal muscle. Inbred male C57/Bl6 mice (age 8–10 wk; n = 12) ran an average of 4.3 h/24 h, for an average distance of 6.8 km/24 h, and at an average speed of 26.4 m/min. A significant increase in the ratio of heart mass to body mass (mg/g) was evident after 2 wk of voluntary exercise, and cardiac atrial natriuretic factor and brain natriuretic peptide mRNA levels were significantly increased in the ventricles after 4 wk of voluntary exercise. A significant increase in the percentage of fibers expressing myosin heavy chain (MHC) IIa was observed in both the gastrocnemius and the tibialis anterior (TA) by 2 wk, and a significant decrease in the percentage of fibers expressing IIb MHC was evident in both muscles after 4 wk of voluntary exercise. The TA muscle showed a greater increase in the percentage of IIa MHC-expressing fibers than did the gastrocnemius muscle (40 and 20%, respectively, compared with 10% for nonexercised). Finally, the number of oxidative fibers as revealed by NADH-tetrazolium reductase histochemical staining was increased in the TA but not the gastrocnemius after 4 wk of voluntary exercise. All results are relative to age-matched mice housed without access to running wheels. Together these data demonstrate that voluntary exercise in mice results in cardiac and skeletal muscle adaptations consistent with endurance exercise.

myosin heavy chain; endurance exercise; plasticity; cardiac hypertrophy; transgenic mice

INCREASES IN MUSCULAR ACTIVITY, such as those occurring during endurance exercise, are associated with rapid and dramatic adaptations of the cardiovascular and musculoskeletal systems (29, 34). These physiological adaptations result in more efficient delivery, uptake, and utilization of energy substrates to produce sustained physical work. Endurance exercise adaptation in the rat is characterized by an increase in relative heart weight (2, 19, 26, 35, 43) as well as shifts in skeletal muscle metabolic (2–5, 9, 18, 20, 23, 27, 32, 33, 37) and contractile (8, 14, 17, 22, 36, 39) protein expression toward a slower contracting, more oxidative fiber profile that is better suited for prolonged muscle activation.

Studies on voluntary endurance exercise adaptation in the mouse have been less numerous, confirming some but not all of the results obtained from rats (21, 46). Specifically, evidence of cardiac hypertrophy (25) and increased skeletal muscle oxidative capacity (21, 44) have been observed in mice after voluntary endurance exercise training. Less well characterized, however, are the changes in fiber contractile protein expression in response to endurance exercise training in mice. Wernig and co-workers (45) demonstrated an increase in histochemical type I (slow) fibers in the soleus with endurance exercise but no change in fiber composition of the extensor digitorum longus, a fast flexor muscle. A recent study demonstrated no appreciable change in immunohistochemically typed fiber percent in the medial gastrocnemius after 8 wk of voluntary cage-wheel exercise in selectively bred house mice (46). Others have also reported no significant difference in gastrocnemius fiber-type composition between laboratory and wild house mice (15). Together these studies suggest that the contractile element of mouse skeletal muscle may be less adaptive than that of rats. This contention is supported by interspecies comparisons of the adaptive response to chronic electrical stimulation, which show that mouse tibialis anterior (TA) is much less responsive than rat TA to this supraphysiological stimulus (38). Given the relative dearth of studies on the exercise response of mice, as well as the current position of the mouse as the genetic model of choice, the purpose of this study was to systematically evaluate the time course of both cardiac and skeletal muscle adaptations to voluntary wheel-running exercise in the mouse. Our results demonstrate that mice will run distances on a cage wheel that compare favorably to those achieved by rats undergoing involuntary treadmill or voluntary wheel-running exercise. Voluntary wheel running in the mouse resulted in both cardiac hypertrophy as well as an increase in the expression of mRNAs associated with cardiac hypertrophy. Moreover, voluntary running ex-

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exercise produced a significant shift from IIb to IIA myosin heavy chain (MHC) expression in both the gastrocnemius and TA muscles. This shift in skeletal muscle MHC expression is accompanied by an increase in the percentage of NADH-positive fibers in the TA but not the gastrocnemius, as well as hypertrophy of the more oxidative type I and IIA fibers. Thus voluntary wheel running produces changes in murine cardiac and skeletal muscle consistent with endurance exercise adaptation. We believe that this adaptive response, combined with the relative ease and affordability of the voluntary wheel-running model, makes this an extremely useful model system for elucidating the molecular mechanisms underlying cardiac and skeletal muscle adaptation.

**METHODS**

**Voluntary cage-wheel exercise.** Voluntary running was performed by inbred male C57BL/6J mice, age 8–10 wk at the start of exercise. Founder mice were originally obtained from Jackson Laboratories; all mice used in the present study were offspring of our breeding colony. Hamster-sized metal cage wheels with a diameter of 11.5 cm (model no. 6208; Petsmart, Phoenix, AZ) were fitted with digital magnetic counters (model BC 600, Sigma Sport, Olney, IL) and placed into 47 × 26 × 14.5-cm cages. The digital counters measure maximum running speed, total distance run, and total time run. The sampling frequency for maximum speed was based on the amount of time required for one revolution of the wheel, which was ~1–3 s. Each morning these data were recorded from the computer and logged for each animal, and the counter was reset. For a given litter, mice were randomly assigned to either a particular exercise duration (1, 2, or 4 wk) or nonexercise littermate control. Control mice were housed in cages without a cage wheel. All animals were supplied with food and water ad libitum.

**Body, heart, and muscle mass data.** At different times after the initiation of voluntary exercise (1, 2, and 4 wk), exercised and nonexercised mice were euthanized by cervical dislocation under inhaled anesthesia. All nonexercised mice were euthanized on the same day as exercised mice. Body, heart, and muscle mass for selected muscles were all recorded. Hearts and muscles were frozen either in liquid nitrogen (for biochemical analysis) or in isopentane cooled in liquid nitrogen (for histochemical analysis).

**Gel electrophoresis.** Muscle samples were prepared for high-resolution gel electrophoresis by mild homogenization by hand in 5 vol of myosin extraction buffer (0.3 M NaCl, 0.1 M NaH₂PO₄, 0.05 M Na₂HPO₄, 0.01 M sodium pyrophosphate, 1 mM MgCl₂, 10 mM EDTA, and 1 mM dithiothreitol, pH 6.5) and extraction on ice for 1 h as described by Butler-Browne and Whalen (6). Samples were microfuged for 5 min to remove cellular debris, mixed 1:1 in extraction buffer containing 50% glycerol, and stored at −20°C. Protein concentration was determined using the Bradford method, and protein concentration for all samples was adjusted to 2.5 mg/ml.

Gel electrophoresis was carried out as described by Talmedge and Roy (41). Briefly, muscle samples were electrophoretically separated on gels containing 30% glycerol and 8% acrylamide for 40 h at 80 V at 4°C. Gels were stained in Express Coomassie (R&D Systems, Mount Prospect, IL) to visualize the bands. Using this system, consistent separation of the IIA and IId/x protein bands could not be achieved; the combined signal for both bands was therefore used as a single value to gauge the shift from IIb to IId/x and IIA MHC isoforms. Gels were dried between cellophane sheets, digitally scanned using a computer scanner, and densitometrically analyzed using NIH Image software. For each sample, the IIB and IId/x+IIA bands were outlined and quantified; background was eliminated by outlining a portion of the lane not containing protein bands and subtracting this from each band value.

**Immunohistochemistry.** Cryosections (10-μm thickness) for immunohistochemical analysis were cut from the midbelly of frozen muscle samples and placed on gelatin-coated microscope slides. Slides were stored at −20°C until use. Slides were air dried for 20 min and then incubated in a blocking solution (0.12% BSA, 0.12% nonfat dry milk, and 0.01% Triton X-100 in PBS) containing unlabelled Fab' fragments (10 mg/ml) for 1 h to block nonspecific binding. After being rinsed with PBS, sections were placed in primary antibody solution overnight at 4°C or for 1 h at room temperature. The antibodies used were the following: SC-71, which labels IIA MHC-containing fibers (16); MHCs (Novocastra, which labels type I MHC-containing fibers; and BF-F3, which labels type IIB MHC-containing fibers (16). After primary incubation, sections were rinsed three times in PBS followed by three 5-min incubations in PBS. The secondary antibody was goat anti-mouse IgG-peroxidase conjugate (Vector Laboratories, Burlingame, CA) used at a 1:200 dilution in blocking solution. Sections were incubated in secondary antibody for 1 h at room temperature followed by numerous washes with PBS. Immunostaining was visualized using a peroxidase 3,3'-diaminobenzidine reaction kit (Vector Laboratories) for 5 min followed by several rinses in distilled H₂O and mounting in Permount. Fiber percents were determined from counts of 500–1,000 fibers in 4–6 randomly chosen fields. Because of the lack of an antibody specific to type IId/x MHC, the percentage of type IId/x expressing fibers was determined by subtracting the percentage of I, IIA, and IIB fibers from 100%. In our hands, the antibodies used yielded strong signals, which make interpretation quite easy. However, to check this point, two independent observers scored the same slides from the same animal, and we found very little difference between the two interpretations (data not shown).

Fiber cross-sectional area (CSA) was measured for type I and type IIA fibers using a video camera (Videoscope International) mounted on a Zeiss bright-field microscope attached to a Macintosh compatible PowerBase 200 computer (Power Computing, Austin, TX). Fiber CSA was determined by using a computer mouse to trace the outline of positively immunostained muscle fibers using NIH Image software. The analysis software was calibrated by outlining defined areas on a slide micrometer. A total of 50 fibers of each type per muscle were analyzed from 5–10 randomly chosen areas in each muscle. Histochemical staining for NADH-tetrazolium reductase (NADH-TR) was carried out by incubation for 30 min at 37°C in NADH-TR reaction solution (0.2 M Tris, 1.5 mM NADH, and 1.5 mM nitrotetrazolium blue) and then dehydrogenation through serial dilutions of acetone and mounting in Aquamount (Lerner Laboratories, Pittsburgh, PA). The number of NADH-TR-positive fibers was counted for approximately six fields per section (500–1,000 fibers per muscle), and a mean was calculated for each mouse and each time point. With NADH-TR staining, three types of fibers were observed (see Fig. 4): unstained, moderately stained, and darkly stained. Fibers that were moderately or darkly stained were classified as NADH-TR positive.

**Northern blotting.** Northern blotting was carried out using standard methods. RNA was isolated from individual mouse
ventricles by using the RNA STAT-60 reagent (Tel-Test, Friendswood, TX) according to the manufacturer’s recommendations, dissolved in sterile H₂O, and stored at −70°C until use. Ten micrograms of total RNA were run on 1.2% agarose-formaldehyde gels and then transferred by capillary action to a nylon membrane where they remained overnight. Membranes were cross-linked by ultraviolet radiation and then rinsed in Perfect-Hyb prehybridization solution (Sigma Chemical) for 3–4 h. Blots were hybridized overnight to radiolabeled cDNA fragments specific to either atrial natriuretic factor (ANF; 650 bp) or brain natriuretic peptide (BNP; 380 bp) at 60°C and then washed two times with 2 × sodium chloride-sodium citrate (SSC)-0.1% SDS and two times with 0.2 × SSC-0.1% SDS at 60°C. Membranes were visualized by exposure to a phosphoimager, and signal intensity was analyzed using Image Quant software (Molecular Dynamics). Membranes were then stripped and reprobed with a radiolabeled 250-bp cDNA fragment to glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and the ANF and BNP signals were normalized to GAPDH signals. Results are reported as means ± SE.

Statistical analysis. All data are reported as means ± SE. One-way ANOVA with Fisher’s post hoc test for least squares differences was used to evaluate the differences between exercised and nonexercised animals at different time points. To minimize variability, littersmates were used for each time point as either exercised or nonexercised. However, because we observed no significant differences for any variable among nonexercised animals at the different time points, these animals were pooled for statistical comparison. Statistical significance was taken as P < 0.05.

RESULTS

Wheel running. Mice ran an average of 4.3 h/24 h and a distance of 6.8 km/24 h (Table 1). The range of responses was 2.4–7.1 h/24 h and 3.1–16.2 km/24 h. The average running speed was 26.4 m/min, and the average maximum running speed was 44.8 m/min (Table 1). Across all groups, both distance ran and average speed increased across the time examined; distance was significantly higher during the second and third weeks of exercise compared with the first week, and average speed was significantly higher at 2, 3, and 4 wk of exercise compared with 1 wk (Table 1). Neither total time run per night nor maximum speed differed across the time period analyzed in this study (Table 1).

For comparison, we calculated the average distance run per week for representative published studies employing involuntary treadmill running for rats (8, 14, 17, 31), voluntary wheel running for rats (22, 31, 33, 37), and voluntary wheel running in mice [observed in the present study and in a recently published study (21)]. The results demonstrate that C57/Bl6 mice run significantly farther per week than rats undergoing either involuntary or voluntary exercise (rat treadmill, 8.22 km/wk; rat wheel running, 24.9 km/wk; mouse wheel running, 47.66 km/wk). Moreover, if these distances are normalized to the stride length and body size of the animals, the differences are even more dramatic (data not shown). Our distances are comparable to those reported by Houle-Leroy et al. (21) for Hsd:ICR house mice (31.34 km/wk).

Body and muscle mass data. Voluntary running exercise resulted in no significant difference in body mass through 4 wk of voluntary running (Table 2), consistent with the results of Swallow et al. (40). Relative skeletal muscle weights were not significantly different for the TA, gastrocnemius, or plantaris muscles at any time (Table 3). However, a significant increase in absolute heart weight was observed in 4 wk exercised mice compared with nonexercised mice (Table 2). Heart weight relative to body mass was significantly increased after 2 wk of voluntary exercise and persisted through 4 wk of exercise (Table 2). This demonstrates that mice were able to run sufficiently in a voluntary model of exercise to induce physiological cardiac hypertrophy.

Changes in cardiac mRNA expression. To determine whether voluntary running exercise provided a sufficient stimulus to induce the expression of hypertrophic markers such as ANF and BNP, Northern blotting analysis was carried out. A significant increase in both ANF and BNP mRNA levels was seen in mice run for 4 wk. This increase was first evident after 2 wk of voluntary running but only reached significance after 4 wk (Fig. 1).

Table 1. Mean nightly wheel-running activity in inbred C57Bl6 mice

<table>
<thead>
<tr>
<th>Week</th>
<th>Time, h</th>
<th>Distance, km</th>
<th>Average Speed, m/min</th>
<th>Maximum Speed, m/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.0 ± 1.3</td>
<td>6.2 ± 2.7</td>
<td>25.7 ± 7.5</td>
<td>42.7 ± 5.6</td>
</tr>
<tr>
<td>2</td>
<td>4.4 ± 0.9</td>
<td>8.5 ± 3.0 a</td>
<td>31.6 ± 8.6 a</td>
<td>43.9 ± 4.3</td>
</tr>
<tr>
<td>3</td>
<td>4.3 ± 0.7</td>
<td>8.4 ± 2.2 a</td>
<td>32.8 ± 8.3 a</td>
<td>46.2 ± 4.9</td>
</tr>
<tr>
<td>4</td>
<td>3.8 ± 1.1</td>
<td>7.1 ± 2.4</td>
<td>32.5 ± 9.0 a</td>
<td>45.9 ± 4.3</td>
</tr>
<tr>
<td>Overall</td>
<td>4.3 ± 0.9</td>
<td>6.8 ± 2.6</td>
<td>26.4 ± 8.6</td>
<td>44.8 ± 5.5</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 12 mice for week 1, n = 8 mice for week 2, and n = 4 mice for weeks 3 and 4. *P < 0.0001 compared with appropriate week 1 value.

Table 2. Body and heart mass with and without voluntary free-wheel running

<table>
<thead>
<tr>
<th></th>
<th>Body Mass, g</th>
<th>Heart Mass, mg</th>
<th>Heart/Body Mass, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>25.9 ± 4.0</td>
<td>128.2 ± 13.2</td>
<td>5.0 ± 0.5</td>
</tr>
<tr>
<td>1-wk</td>
<td>24.7 ± 1.7</td>
<td>131.8 ± 16.4</td>
<td>5.3 ± 0.3</td>
</tr>
<tr>
<td>2-wk</td>
<td>22.9 ± 3.7</td>
<td>136.7 ± 20.4</td>
<td>6.0 ± 1.0†</td>
</tr>
<tr>
<td>4-wk</td>
<td>24.8 ± 1.7</td>
<td>146.9 ± 10.6†</td>
<td>5.9 ± 0.1†</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 12 mice for nonexercised and n = 4 for 1-, 2-, and 4-wk exercised. †P < 0.05 vs. nonexercised control. †P < 0.01 vs. nonexercise control.

Table 3. Normalized muscle mass with and without voluntary free-wheel running

<table>
<thead>
<tr>
<th></th>
<th>Tibialis Anterior</th>
<th>Plantaris</th>
<th>Gastrocnemius</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.49 ± 0.16</td>
<td>0.56 ± 0.07</td>
<td>4.59 ± 0.11</td>
</tr>
<tr>
<td>1-wk</td>
<td>1.61 ± 0.03</td>
<td>0.64 ± 0.05</td>
<td>5.16 ± 0.28</td>
</tr>
<tr>
<td>2-wk</td>
<td>1.57 ± 0.13</td>
<td>0.61 ± 0.04</td>
<td>4.63 ± 0.37</td>
</tr>
<tr>
<td>4-wk</td>
<td>1.57 ± 0.18</td>
<td>0.61 ± 0.13</td>
<td>4.72 ± 0.25</td>
</tr>
</tbody>
</table>

Values are means ± SD given in mg/g body wt; n = 4 mice per condition.
Skeletal muscle MHC adaptations. Voluntary running exercise produced a shift toward greater expression of type IIa + IIId/x MHC as revealed by gel electrophoresis (Fig. 2). This shift was first evident after 1 wk of running exercise and reached a peak after 2 wk of exercise and did not change thereafter in the TA muscle; in the gastrocnemius muscle, these changes were only significant at the 2-wk time point (Fig. 2). In addition, immunohistochemistry followed by fiber percent analysis revealed that both the gastrocnemius and the TA muscles experienced a shift in MHC expression (Figs. 3 and 4). Specifically, an increase in the percentage of fibers expressing IIa MHC and a decrease in the percentage of fibers expressing type IIb MHC were observed in both muscles, and these changes were first evident after 1 wk of exercise but increased throughout the time points examined. After 4 wk of exercise, fibers expressing IIa MHC comprised ~20 and 40% of the total fibers in gastrocnemius and TA, respectively, compared with ~10% in nonexercised muscles (Fig. 3). No change in type I fiber percent was observed in either muscle at any time point with exercise (Fig. 3). Interestingly, we saw no change in the percentage of fibers expressing IIId/x MHC in the gastrocnemius muscle at any time point and only a small decrease in IIId/x fibers after 4 wk of voluntary exercise in the TA (Fig. 3B), but given the methodological limitations in analyzing IIId/x MHC expression, specifically the lack of an antibody specific to IIId/x, it is difficult to assess the relevance of this observation. Finally, we did not analyze coexpression of MHC isoforms, and it is therefore possible that some of the fibers expressing IIa were coexpressing IIId/x and even IIb MHC.

We examined fiber size for type I and IIa fibers in both the gastrocnemius and TA muscles to determine whether voluntary running exercise induced fiber hypertrophy in the most oxidative muscle fibers. In the gastrocnemius muscle, both type I and type IIa fibers were significantly larger after 4 wk of voluntary exercise (Fig. 5). In addition, type IIa fibers were significantly larger after 1 and 2 wk of voluntary exercise compared with those of nonexercised controls (Fig. 5). In the TA, in contrast, there was no significant difference in mean fiber size for IIa fibers at any time point; moreover, too few type I fibers could be found in either the nonexercised or exercised TA to allow meaningful statistical comparison.

Changes in oxidative fiber percents. Finally, we stained transverse sections from unexercised and exercised mice to determine whether there was any
change in the percentage of NADH-TR-positive fibers. In gastrocnemius muscle, there was no significant increase in the number of NADH-TR-positive fibers at any time point (data not shown). In the TA, however, there was a steady increase in the percentage of NADH-TR-positive fibers, which was significant after 4 wk of voluntary exercise (Figs. 4 and 6).

DISCUSSION

The adaptational response of the cardiovascular and musculoskeletal systems to the sustained muscle activation associated with endurance exercise has been extensively documented. Studies on both involuntary treadmill exercise training and voluntary wheel running in animals have consistently demonstrated that endurance exercise is associated with cardiac hypertrophy (2, 19, 26, 43) and changes in skeletal muscle protein expression, resulting in a slower contracting, more oxidative muscle (2–5, 9, 18, 20, 22, 27, 32, 33, 37). However, to date, there have been few studies on exercise adaptation in mice. The present results demonstrate that voluntary wheel running in mice produces specific adaptations associated with endurance training in both the cardiac and skeletal muscle systems.

One initial question was whether mice would run sufficiently to produce an adaptational response. Most of the studies on voluntary running in rats have suggested that the changes are qualitatively similar but may often be quantitatively less robust than those achieved using involuntary swimming or treadmill exercise. One reason this may be true is because animals that exercise voluntarily do so at a percentage of exercise capacity insufficient to elicit the same magnitude of changes that are seen with treadmill exercise, which is typically done at a higher speed and/or inclination. Indeed, most studies examining voluntary exercise in
the rat have examined very long time courses (upward of 1–2 mo) to provide a greater overall volume of work for allowing adaptations to occur. In the present study, we demonstrate that mice undergoing voluntary running exercise will run substantially farther per night than has been reported in representative studies on rats run on either a treadmill or voluntary wheel. This is obviously an underestimate of the true difference, given the dramatic difference in body size and therefore stride length between a mouse and a rat. In addition, the distances run per week reported in the present study are quite similar to those recently reported for Hsd:ICR outbred mice (21). Thus, although the intensity of voluntary exercise may be below that of typical treadmill-based exercise, the substantial duration of the exercise stimulus appears to be sufficient to produce fairly robust adaptational responses. Significant cardiac hypertrophy was first evident after just 1 wk of voluntary running, and it reached a peak after 2 wk of exercise that was maintained through 4 wk of exercise (Table 2, Fig. 1). This hyper-

Fig. 5. Fiber size in exercised vs. nonexercised mice. Fiber cross-sectional area (CSA) was measured using NIH Image software on muscle cross sections immunohistochemically stained with antibodies specific to Ila (SC-71) and type I (MHCs) MHC. The area of 50 individual fibers was determined per muscle. Values are means ± SE; n = 3–4 animals per time point. The CSA of fibers expressing Ila MHC was significantly increased at 1, 2, and 4 wk of voluntary exercise in gastrocnemius but not TA muscle; the CSA of fibers expressing type I MHC was significantly increased at 4 wk of voluntary exercise in the gastrocnemius. *P < 0.05. **P < 0.01.

Fig. 6. The percentage of NADH-tetrazolium reductase-positive fibers (NADH +) in the TA muscle in exercised and nonexercised mice. Values are means ± SE; n = 3–4 animals per time point. NADH −, NADH-tetrazolium reductase-negative fibers. Voluntary running exercise resulted in a significant increase in the number of NADH-tetrazolium reductase-positive fibers after 4 wk of voluntary running exercise. *P < 0.05.
trophy was specific to cardiac muscle because there was no change in either body mass or in mass of the hindlimb muscles analyzed (Tables 2 and 3). In addition, we observed an increase in ventricular ANF and BNP mRNA levels after 2 wk of voluntary running that increased further after 4 wk of voluntary running (Fig. 1). Increased ANF expression is a hallmark of a number of cardiac hypertrophic states (1, 11, 24), although the relationship among exercise, cardiac hypertrophy, and ANF expression is more equivocal. An increase in ANF mRNA was observed in the atria but not ventricles of endurance-trained rats (1), whereas a modest increase in ANF mRNA but no cardiac hypertrophy was observed in response to exercise in another study (28). The present data support the contention that voluntary running exercise provides a sufficient stimulus to induce both cardiac hypertrophy as well as alterations in cardiac gene expression in mice.

Similarly, running exercise is associated with distinct metabolic adaptations to skeletal muscle (2–5, 9, 18, 20, 23, 27, 32, 33, 37). We observed a significant increase in the percentage of NADH-positive muscle fibers, although this was evident only in the TA and not the gastrocnemius muscle (Figs. 4 and 6). The increase in oxidative fibers in the TA is consistent with the increase in the activity of a number of enzymes of oxidative metabolism observed in mouse hindlimb muscle in response to voluntary running (21). It is not known why an increase in NADH-positive fibers was observed in the TA but not the gastrocnemius muscle, although the fact that the TA muscle also underwent a greater shift in MHC expression (see below) suggests that this muscle may have a greater adaptive capability in the mouse.

Less well established is the plasticity of the sarcomeric components of mouse skeletal muscle in response to endurance exercise. A study by Wernig et al. (45) demonstrated histochemical changes in muscle fiber ATPase in the mouse soleus but not extensor digitorum longus muscle in response to long-term voluntary wheel running, whereas a recent study on the medial gastrocnemius muscle of wild house mice revealed only negligible changes in immunohistochemically typed fiber percentages with voluntary wheel running (46). We observed a decrease in type IIb MHC and a corresponding increase in type IIa MHC that was first evident after 1 wk of exercise and continued through 4 wk of exercise (Figs. 3 and 4). This fiber-type shift was observed in both an ankle extensor (gastrocnemius) and an ankle flexor (TA), although the magnitude of change was greater for the TA than for the gastrocnemius. Moreover, we observed no increase in the percentage of type I fibers in either muscle at any time point up to 28 days of voluntary running, suggesting that the stimulus from voluntary running was insufficient to result in a complete shift from fast to slow MHC. This is consistent with the lack of “complete” fiber transformation to type I MHC expression in rats and mice even with supraphysiological models such as chronic electrical stimulation (38). The present data support the idea that contractile protein expres-

sion systems in mice are sensitive to endurance exercise training as has been previously characterized in rats (14, 17, 34, 36, 39).

Few studies to date have examined the time course of the adaptations in response to voluntary running exercise (2). Indeed, most studies have used voluntary running regimens lasting >1 mo to achieve a steady state of adaptation (4, 5, 18, 26, 45, 46). We sought to determine the time course of adaptations in response to voluntary wheel running and examined 1, 2, and 4 wk of voluntary running exercise. Our results demonstrate that cardiac and skeletal muscle adaptations first become evident as early as 1 wk after initiation of voluntary exercise, although most of these adaptations did not reach statistical significance until 2 wk or in some cases 4 wk of exercise. This suggests that the adaptational response is initiated fairly rapidly on initiation of exercise.

Much of the knowledge regarding the adaptation of physiological systems has come from involuntary treadmill exercise paradigms. This approach has several obvious advantages, such as the ability to standardize the exercise stimulus for all animals and the ability to quantify the amount of work done and increase it in a specific manner. In addition, a treadmill exercise paradigm can be used to diagnose abnormalities in cardiovascular or respiratory function (13). However, treadmill training also results in increased stress to animals (12, 30, 42). Voluntary wheel-running paradigms appear to produce relatively less stress to animals (31), presumably by allowing animals to exercise when they want, and to the extent that they want, with no handling or shocks. The voluntary wheel-running paradigm has been fairly well characterized and has been demonstrated to lead to qualitatively similar adaptations as the treadmill paradigms, i.e., a shift toward slower, more oxidative fiber types (22, 32, 33, 39). To date, most studies on voluntary wheel running in the mouse have not focused on the extent of muscle adaptation. Thus the present work is one of the first to systematically characterize both cardiac and skeletal muscle adaptations occurring with voluntary exercise in the mouse.

One of the most powerful tools for analyzing the molecular mechanisms underlying physiological function is the generation of genetically altered lines of animals, and to date the overwhelming majority of genetically altered animals have been mice, because the techniques for undertaking genetic manipulation of other animals remain less well established (7). Mice containing alterations to cardiovascular or musculoskeletal genes could be used to analyze the importance of a specific gene in the exercise adaptation process using the voluntary exercise system described here (10, 13). Indeed, we have recently observed that a number of different null and transgenic mouse lines will engage in voluntary exercise to an extent comparable to wild-type, nontransgenic mice (unpublished observations), making studies of this type feasible. This “candidate” gene approach is an excellent complement to genetic
selection and screening studies, which have already shown tremendous potential (15, 21, 46).

In summary, we have demonstrated that a voluntary wheel-running model of endurance exercise for mice produces distinct adaptational responses in both cardiac and skeletal muscle. This approach complements the existing model of involuntary treadmill running and provides another system for examining the molecular and cellular mechanisms governing the adaptation of physiological systems to increased activation states.

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