Hypertrophy of rat plantaris muscle fibers after voluntary running with increasing loads

AKIHIKO ISHIHARA,1 ROLAND R. ROY,2 YOSHINOBU OHIRA,4 YASUHIKO IBATA,5 AND V. REGGIE EDGERTON.2,3

1Laboratory of Neurochemistry, Faculty of Integrated Human Studies, Kyoto University, Kyoto 606-01, Japan; 2Brain Research Institute, University of California School of Medicine, Los Angeles 90095-1761; 3Department of Physiological Science, University of California, Los Angeles, California 90095-1527; 4Department of Physiology and Biomechanics, National Institute of Fitness and Sports, Kanoya 891-23, Japan; and 5Department of Anatomy and Neurobiology, Kyoto Prefectural School of Medicine, Kyoto 602, Japan

Ishihara, Akihiko, Roland R. Roy, Yoshinobu Ohira, Yasuhiro Ibata, and V. Reggie Edgerton. Hypertrophy of rat plantaris muscle fibers after voluntary running with increasing loads. J. Appl. Physiol. 84(6): 2183–2189, 1998.— There have been no systematic comparisons of skeletal muscle adaptations in response to voluntary wheel running under controlled loading conditions. To accomplish this, a voluntary running wheel for rats and mice was developed in which a known load can be controlled and monitored electronically. Five-week-old male Sprague-Dawley rats (10 rats/group) were assigned randomly to either a 1) sedentary control group (Control); 2) voluntary exercised with no load (Run-No-Load) group; or 3) voluntary exercised with additional load (Run-Load) group for 8 wk. The load for the Run-Load group was progressively increased to reach ~60% of body weight during the last week of training. The proportions of fast glycolytic (FG), fast oxidative glycolytic (FOG), or slow oxidative (SO) fibers in the plantaris were similar in all groups. The absolute and relative plantaris weights were greater in the Run-Load group compared with the Control and Run-No-Load groups. The mean fiber cross-sectional areas of FG, FOG, and SO fibers were 20, 25, and 15% greater in the Run-Load than in Control rats. In addition, these fiber types were 16, 21, and 12% larger in Run-Load than in Run-No-Load rats. The muscle weights and mean cross-sectional areas of each fiber type were highly correlated with the average running distances and total work performed in the Run-Load, but not the Run-No-Load, group. The slope of the relationship between fiber size and running distance and total work performed was significant for each fiber type but was higher for FG and FOG fibers compared with SO fibers. These data show that the load on a rat running voluntarily can determine the magnitude of a hypertrophic response and the population of motor units that are recruited to perform at a given loading condition.

fiber cross-sectional area; fiber type distribution; running wheel; skeletal muscle loading

EXERCISE TRAINING IN RATS results in a large number of adaptations in the contractile, morphological, and metabolic properties of skeletal muscle that are specific to the type and amount of exercise. In general, skeletal muscles of rats that are trained by using a relatively low-intensity and long-duration type of work show endurance-related adaptations, including increased percentages of oxidative fibers, higher oxidative capacities, higher myoglobin levels, higher mitochondrial levels, and increased capillarization (1, 6). In contrast, training in which a strength or power type activity is used (relatively high intensity and short duration) results in fewer apparent metabolic adaptations but elicits muscle fiber hypertrophy (1, 25). However, many of the procedures used to train rats, such as running on a motorized treadmill, forced swimming with added weights, and weight lifting, provide little quantitative information on overload parameters that can be interpreted with respect to the “size principle” of recruitment of muscles during normal movements. Furthermore, most of the training paradigms involve physical and psychological stressors, i.e., electrical shock, loud sounds, air blasts, and food deprivation and reward to keep the rats exercising at a constant level. In addition, the exercise training is often performed during daytime hours when rats are not normally active. Because of these inherent problems with forced-exercise protocols, it is difficult to distinguish between the contribution of the exercise training alone vs. the training plus the associated physical and psychological stresses to the muscle adaptations.

Rats are a highly active species and will run spontaneously in running wheels at their own pace if given the opportunity (12, 13, 17, 22, 24). These studies indicate that rats spontaneously run distances greater than normally performed during typical treadmill exercise-training regimens. Chronic voluntary wheel running results primarily in “aerobic” adaptations in the skeletal muscles (10, 17, 23), but some hypertrophy (12–20%) has been reported in the predominantly slow soleus muscle of young male (15) and female (13) rats. The plantaris muscle, a predominantly fast agonist of the soleus, appears to hypertrophy in female (13), but not in male (15–17), rats in response to voluntary running. In these studies, the total distance run is usually recorded, but the running rates and loads are
unknown, and, therefore, these adaptations cannot be interpreted with respect to use or recruitment patterns.

We have developed a running wheel that allows the imposition of a known load during the voluntary running activity. The purpose of the present study was to determine the effects of 8 wk of voluntary wheel running, with and without an additional load, on the plantaris muscle weight and fiber type composition and size of male rats. The plantaris muscle was studied because it is a primary ankle extensor and thus is activated during the running task (8) and because it has a mixture of fiber types and thus allows for the determination of fiber type-specific effects (2). In addition, although the plantaris is responsive to some conditions of chronic increased or decreased loading (18, 19), it appears to be unresponsive to unloaded wheel running in the male rat (15–17). We hypothesized that the high-intensity activation associated with increased loading during voluntary running would heavily recruit the fast plantaris muscle and that the level of hypertrophy in a fiber type would reflect the recruitment patterns of each motor unit type and their sensitivity to the level of loading during the recruitment. The results show that muscle fiber size is much more sensitive to load and total work performed than the total amount of recruitment or activity and that the fast fibers are considerably more responsive to the added loads than are the slow fibers.

METHODS

Experimental animals. Thirty male Sprague-Dawley rats (5 wk old; initial body weight, 115 ± 6 g) were used in this 8-wk study. The rats were assigned randomly and equally into three groups: 1) a cage-confined sedentary control group (Control); 2) a voluntary running group with free access to a rotating wheel (Run-No-Load); and 3) a voluntary running group with free access to a rotating wheel plus additional loading, as described below (Run-Load). All rats were individually housed in similar cages, except that there was no rotating wheel in the Control cages (see below). Food and water were provided ad libitum. The rats were kept in a controlled environment of a fixed 12:12-h light-dark cycle (lights off from 1900 to 0700), with the room temperature maintained at 22 ± 2°C. Body weight was recorded weekly. All procedures were approved by the University Committee for the Care and Use of Animals for Research Purposes and followed the Guidelines of the National Institutes of Health for the Care and Use of Laboratory Animals.

Running-wheel apparatus and protocol. The running-wheel apparatus includes a standard rat plastic cage (30 × 40 × 20 cm) and a running wheel (diameter, 31.8 cm; width, 10 cm) attached vertically to a freely rotating shaft inserted into a metal controller box that is supported on a metal base (Fig. 1). The wheel rotates on the shaft whenever the rat walks or runs in either direction in the wheel, and the number of revolutions and load on the wheel are continuously recorded. A transducer in the controller box (Fig. 1A) connected to the wheel produces an electronic signal for each revolution of the wheel. This signal then is sent to, and subsequently

![Fig. 1. Loaded running-wheel apparatus: control box (A) and data-sequence system (B). a, Calibrated current value of load added to a wheel; b, load-adjustment knob; c, wheel-revolution counter; d, switch for no-load (free) or load condition; e, output signal to the sequencer; f, power switch; g, running wheel (diameter, 31.8 cm and width, 10.0 cm); h, plastic cage (30.0 × 40.0 × 20.0 cm); i, drinking bottle; j, food box; k, output to any computer via RS232C; l, input for signals from the control box; m, power switch. Note that the sequencer can simultaneously collect signals from 20 different cages.](image-url)
stored by a computer via a sequencer (Fig. 1B), which is equipped to continuously monitor the number of signals from up to 20 wheels simultaneously. The time interval for data collection is set by a time-mark generator (from 5 s to 24 h), and the number of wheel revolutions during this interval is recorded as a computer text file. The load attached to the wheel can be changed arbitrarily (see below), and the rats have free access to the wheel.

The load on the wheel is adjusted by varying the current load from a control panel (Fig. 1A,a). The load on the wheel is calibrated by hanging known weights on one of the bars on the wheel until the wheel is displaced. The range in loading on the wheel is from 0 to 350 g, and the relationship between the calibrated value of the load added to a wheel and the actual current load on the wheel is linear and highly correlated (r = 0.995). The regression line for this relationship is y = 0.86x – 9.5, where x is the current load on the wheel and y is the calibrated value in Fig. 1A,a. Therefore, when the current load on the wheel is set on 100 g, the calibrated value is calculated as follows: 76.5 = 0.86(100) – 9.5. The load necessary to overcome the inertia of the wheel at “no load” was 4.5 g; therefore, the force used to calculate work in the no-load condition was 4.5 g (see below).

In the present study, the rats were allowed to run voluntarily in the wheel for 12 h each day during the dark cycle. The rats in the Run-Load group were exercised with no load for the first week, and then the load was progressively increased: weeks 2 and 3 at 120 g; weeks 4 and 5 at 140 g; week 6 at 160 g; week 7 at 190 g; and week 8 at 220 g. Work was calculated and expressed relative to body weight as follows:

\[
\text{Work} = \text{force (N)} \times \text{distance (ms)} / \text{body wt (kg)}
\]

where force is the load on the wheel, and distance is the number of revolutions times the circumference of the wheel.

Tissue preparation. At the end of the 8-wk training period, the rats were anesthetized with pentobarbital sodium (50 mg/kg ip). The plantaris muscles on the right side were removed, cleaned of excess fat and connective tissue, and wet weighed. The muscles were placed on cork, stretched to approximate their in vivo length, and immediately frozen in isopentane cooled in liquid nitrogen. Serial transverse sections, 10 µm thick, from the midbelly region of each muscle were cut in a cryostat at 20°C. The sections were air-dried and stained for myosin adenosine triphosphatase following alkaline preincubation, succinate dehydrogenase, and α-glycerophosphate dehydrogenase. The muscle fibers were classified as fast glycolytic (FG), fast oxidative glycolytic (FOG), or slow oxidative (SO), as described previously (14).

Between 400 and 450 fibers were sampled consistently from a midregion of the muscle cross section. The cross-sectional area of each fiber on the muscle transverse section was measured by using a computer image-processing system. Statistical procedures. All statistical analyses were performed by using Statview. One-way ANOVA was used to determine overall differences, and a post hoc Bonferroni adjustment was used to determine individual group differences. Pearson product correlations were used to determine the relationship between running distance and muscle weight or fiber cross-sectional area by fiber type. The 0.05 probability level was established for statistical significance.

RESULTS

Body and muscle weights. At the end of the study, the mean body weight was 9 and 5% lower in the Run-Load group than in the Control and Run-No-Load groups (Table 1). Mean absolute plantaris muscle weight was 19 and 17% greater in the Run-Load group compared with the Control and Run-No-Load groups. When differences in body weights were considered, these differences were 31 and 24%, respectively.

Fiber type distribution and cross-sectional areas. There were no differences in the fiber type distribution of the plantaris muscle among the three groups (Table 2). Compared with the Control and Run-No-Load groups, the mean cross-sectional areas of the FG, FOG, and SO fibers were 20 and 16%, 25 and 21%, and 15 and 12% greater in the Run-Load group, respectively (Table 3). There were no differences in the mean fiber cross-sectional areas of any fiber type between the Control and Run-No-Load groups.

Relationships between running distance and muscle weight or fiber cross-sectional area. The average daily voluntary running distance in the Run-No-Load group increased almost threefold during the 8-wk exercise period (Fig. 2). The Run-Load rats showed three response patterns for running distance: two rats showed a response similar to that observed in the Run-No-Load group (high-distance subgroup); six rats ran a similar distance as the Run-No-Load rats during the first week and then maintained a relatively constant distance each week throughout the study (moderate-distance subgroup); and two rats ran ~50% less than the Run-No-Load group during the first week and maintained this relatively low level of running distance throughout the study (low-distance subgroup).

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<tr>
<th>Table 1. Body and plantaris muscle weights</th>
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<td>Group</td>
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<tr>
<td>Control</td>
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<tr>
<td>Run-No-Load</td>
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Values are means ± SE from 10 rats/group. See text for further group description. *, †Significantly different from Control or Run-No-Load groups, respectively, at P < 0.05.

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<th>Table 2. Fiber type distribution of the plantaris muscle</th>
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<td>Fiber Type, %</td>
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<tr>
<td>Control</td>
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<td>Run-No-Load</td>
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Values are means ± SE from 10 rats/group. FG, fast glycolytic; FOG, fast oxidative glycolytic; SO, slow oxidative.

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<th>Table 3. Fiber cross-sectional areas of the plantaris muscle</th>
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<td>Group</td>
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<td>Control</td>
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<td>Run-No-Load</td>
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Values are means ± SE from 10 rats/group. *Significantly different from Control at P < 0.05.
Plantaris muscle weights were positively correlated \((r = 0.88)\) with running distance in the Run-Load group, but not \((r = 0.16)\) in the Run-No-Load group (Fig. 3). Furthermore, the cross-sectional area of each fiber type was positively and significantly correlated with running distance in the Run-Load group, but not in the Run-No-Load group (Fig. 4). The regression equations and correlations for each fiber type in the Run-Load group were as follows: FG: \(y = 0.312x + 3,707, r = 0.88\); FOG: \(y = 0.267x + 3,567, r = 0.88\); SO: \(y = 0.116x + 3,275, r = 0.57\). All slopes for the relationship between fiber size and running distance were significantly different from zero for the Run-Load, but not the Run-No-Load, groups. In addition, the slope for this relationship in the Run-Load group was 169% \((P < 0.05)\) and 130% \((P = 0.07)\) higher in FG and FOG compared with SO fibers.

Relationships between work and muscle weight or fiber cross-sectional area. During the 8-wk period, the amount of work for the Run-No-Load group increased only from \(-250\) to \(-400\) N-m·kg body wt\(^{-1}\)·day\(^{-1}\) (Fig. 5). In contrast, the amount of work in the low-, moderate-, and high- distance subgroups for the Run-Load group increased from \(-250\) (no load added during week 1) to \(-2,500, 7,500,\) and \(12,000\) N-m·kg body wt\(^{-1}\)·day\(^{-1}\) during weeks 6–8. Therefore, the work for these Run-Load subgroups was \(-6,\) \(-20,\) and \(-30\)-fold greater than for the Run-No-Load group during the final 3 wk of the study.

Plantaris muscle weights were positively correlated \((r = 0.85)\) with the amount of work performed in the Run-Load group, but not in the Run-No-Load group \((r = 0.17)\) (Fig. 6). However, it should be noted that the data points for the Run-No-Load group were distributed evenly around the extended regression line for the Run-Load data (see dashed line in Fig. 6). The cross-sectional area of each fiber type was positively corre-
lated with the amount of work performed in the Run-Load group (FG, $r = 0.85$; FOG, $r = 0.86$; SO, $r = 0.54$), but not in the Run-No-Load group (FG, $r = -0.03$; FOG, $r = -0.41$; SO, $r = -0.25$) (Fig. 7). The regression equations for each fiber type in the Run-Load group were as follows: FG: $y = 0.074x + 3,694$; FOG: $y = 0.063x + 3,557$; SO: $y = 0.027x + 3,272$. All slopes for the relationship between fiber size and work were significantly different from zero for the Run-Load, but not for the Run-No-Load, group. In addition, the slope for this relationship in the Run-Load group was 174% ($P < 0.05$) and 133% ($P = 0.09$) higher in FG and FOG compared with SO fibers. It also should be noted that the points for the FG fibers of the Run-No-Load group were distributed around the extended regression line for the Run-Load data, whereas the points for the FOG and SO fibers all fell below the line. Thus it appears that the FG, but not the FOG or SO, fibers showed a linear response across the no-load and load conditions.

**DISCUSSION**

The primary finding of the present study was that the plantaris muscle of male rats hypertrophied in response to loaded, but not to unloaded, voluntary wheel running. The lack of a hypertrophic response of the plantaris muscle in the Run-No-Load rats is consistent with previous results (15–17). Our interpretation of the present results is that the increment in load placed on the Run-Load rats was sufficient to incrementally recruit more motor units in the plantaris muscle at a higher frequency to accomplish the task. This interpretation is supported by the significant increase in the cross-sectional area in each fiber type in the Run-Load rats. Furthermore, the largest hypertrophy

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**Fig. 5.** Average daily work for each week is shown for rats in the Run-No-Load (●) and for rats in the Run-Load group that had a relatively high (▲, $n = 2$), moderate (▲, $n = 6$), or low (●, $n = 2$) amount of work. Bars indicate SE.

**Fig. 6.** Relationship between average daily work over 8-wk period and plantaris muscle weight of rats in Run-No-Load (●) and Run-Load (▲) groups. Each point represents an individual rat, $n = 10$ rats/group. There was a significant relationship for Run-Load (●, $r = 0.85$), but not for Run-No-Load ($r = 0.17$), group. Regression equation for Run-Load group was $y = 0.03x + 318$. Dashed line is an extension of regression line for Run-Load group and demonstrates that the points for Run-No-Load group are dispersed evenly around this line.

**Fig. 7.** Relationship between average daily work over 8-wk period and cross-sectional area of each fiber type in Run-No-Load (●) and Run-Load (▲) groups is shown. These relationships were significantly correlated in Run-Load group (FG, $r = 0.85$; FOG, $r = 0.86$; SO, $r = 0.54$) but not in Run-No-Load group (FG, $r = -0.03$; FOG, $r = -0.41$; SO, $r = -0.25$). Each point represents an individual rat, $n = 10$ rats/group. Dashed lines are extensions of regression lines for Run-Load group for each fiber type and indicate location of points for Run-No-Load group relative to this line. Note that points for the FG fibers are distributed around this line, whereas the points for the FOG and SO all fall below the line.
occurred in the rats that ran at the highest loads and was greatest in the fibers that would be recruited the least theoretically, i.e., the FG fibers. These data indicate that the responsiveness to loading differed among the fiber types, i.e., FG > FOG > SO.

Advantages of voluntary vs. forced exercise. Although forced treadmill running, swim training, or weight training are useful models for studying skeletal muscle plasticity in rats, these regimens undoubtedly include stress to the animals, and thus it is not obvious whether the observed physiological changes are due to stress, exercise, or a combination of the two. A less stressful model for rats (28) and mice (26) is a training regimen involving voluntary running exercise as used in the present study. The rats run voluntarily during their normal active hours, i.e., at night, and no stressor is used to force them to run. Running occurs in the same cage in which the rats are housed; therefore, the environment is not changed for running and nonrunning conditions. In addition, trainers are not necessary, and the rats can voluntarily exercise 7 days/wk. All of these conditions provide a more controlled and physiological environment and, thereby, facilitate the interpretation of the observed adaptations relative to the adaptation-inducing event, i.e., voluntary running.

Muscle mass and fiber size adaptations. The effects of voluntary running on extensor muscle mass and fiber size in male rats appear to be highly variable. For example, Ishihara et al. (7) showed no change in the absolute weights of the soleus or plantaris muscles of 4-wk-old male Wistar rats exercised for 45 days, although the weights relative to body weight were increased for both muscles. In addition, only the FOG fibers in the superficial (away from the bone) region of the plantaris hypertrophied. In contrast, Rodnick et al. (15–17) showed an increase in soleus, but not in plantaris, weight in young male Sprague-Dawley rats after 6 wk of voluntary running. The soleus hypertrophy ranged from 13 to 20% and was not correlated with running distance. These data are consistent with the recruitment of the soleus but not the plantaris during unloaded voluntary running. The major advantages of the load-controlled voluntary running wheel are that it provides a means of modulating the workload according to the appropriate experimental conditions and also provides a means of quantifying the work performed. The present data indicate that, through the use of this wheel, hypertrophy can be induced in the plantaris muscle by voluntary running in male rats if the rats are provided access to wheels with added resistance to rotation.

Comparison with other high-resistance exercise models. The present means of inducing muscle hypertrophy has several advantages over other methods of producing hypertrophy (11, 25). In the present study, the presence of hypertrophy in all fiber types in Run-Load rats suggests that all types of motor units in this region of the plantaris were recruited during loaded running. In addition, the mean cross-sectional area of each fiber type was positively related with running distance and total work performed in the Run-Load group. Also, the voluntary loaded running described in the present study allows the rat to exercise freely during its entire active (awake) period without being monitored closely by a trainer. Gonyea and Ericson (4, 5) operantly trained cats to lift weights with their forelimbs against progressively increasing resistances for a food reward. The flexor carpi radialis muscles of the trained limb were larger than those of the nontrained limb (16%) and those of body weight-matched controls (44%) (4). They also found that all fiber types were ~10% larger in the trained compared with nontrained muscles, indicating that the muscle was heavily recruited during the task. Kliitgaard (9) operantly trained rats for a food reward to lift (via plantar flexion) a weight bar resting on their shoulders. Training resulted in marked increases in the weights and maximum tension capability of both the soleus and plantaris muscles. Although these training procedures were successful in producing a modest amount of muscle hypertrophy without the use of behaviorally aversive techniques, both involved some form of food deprivation. Roy et al. (21) trained rats to respond to a light stimulus (to avoid a mild shock through a grid floor) by standing in such a way as to touch a bar lowered from the top of a weight-lifting chamber. The rats were made to lift loads of up to 300% body weight by having weights attached to a belt around the abdomen. The relative weights of the soleus and adductor longus, a predominantly slow adductor of the thigh, were 11 and 14% larger in the weight-lifting compared with control rats. All of the above procedures used in rats required considerable technical assistance. Other procedures that have been used to produce work-induced muscle hypertrophy in rats have included electrical stimulation of specific regions of the brain (3) or skeletal muscles (27) or a synergist removal (20), but each of these included invasive procedures that may have resulted in conditions that differ significantly from the voluntarily performed work in vivo in the awake animal.

Summary. An exercise model for small rodents in which the resistance (workload) of a voluntary running wheel can be controlled and monitored has been presented. This training regime avoids a variety of stressful stimuli associated with imposing an exercise regimen on rats, particularly when performing high-resistance training. Because low-volume voluntary running under loaded conditions results in muscle hypertrophy, this training modality appears to be a valuable tool to study the mechanisms associated with the prevention of muscle atrophy and how these and other adaptations relate to the population of motor units that must be recruited to accomplish a motor task.
REFERENCES


