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Functional and Muscle Size Response to 5 Days of Treadmill Training in Young Rats

Alon Eliakim, Mark Y. Moromisato, David Y. Moromisato, and Dan M. Cooper

In this study, the hypothesis that improvements in functional and structural measures could be detected in the young, female rat with only 5 days of moderate treadmill training was tested. Eight-week-old female Sprague-Dawley rats were divided randomly into control ($n = 10$) and training groups ($n = 11$). Over the 5-day period, running duration and treadmill speed increased progressively. Maximal running time and gas exchange were measured on Day 6. In trained compared with control rats, maximal running time was 54% greater ($p < .005$), right hindlimb muscle was 16% heavier ($p < .01$), and end-exercise respiratory exchange ratio (RER) was 17% lower ($p < .05$). Substantial metabolic and structural adaptations occurred in young female rats after only 5 days of treadmill training. This protocol may be useful in discovering the initiating mechanisms of the training response in the young organism.

Exercise and physical activity modulate tissue anabolism (e.g., muscle hypertrophy) and catabolism (e.g., disuse atrophy), but the underlying processes involved in these responses, particularly in the young developing organism, are not fully understood. The growth hormone–insulin-like growth factor-I (GH-IGF-I) axis seems to play a role since there is increasing evidence that the “fit state” is marked by higher circulating levels of GH and IGF-I (7, 9, 11). However, a sudden imposition of a training intervention might, in the short term, lead to a reduction in the same circulating growth factors, even in the presence of local tissue growth (e.g., muscle hypertrophy) (5). More recent investigations have focused on molecular mechanisms including the role of growth factors like IGF-I at the local tissue level (4, 12) and the factors that trigger increases in oxidative capacity (1). Such studies usually require direct tissue sampling and, therefore, the use of animal models such as the rat.

The majority of protocols in the rat consist of training periods lasting at least 4 weeks. Protocols of this duration are problematic for developmental scientists. The primary reason is that 4 weeks in the life of a young rat spans a much longer

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developmental stage than does 4 weeks in the human (e.g., puberty in the rat is completed by about postnatal Day 45). Moreover, it would be critically important to determine the *triggering* factors or earliest signals and messengers through which mechanical work alters cellular growth in the intact organism, and how these factors are affected by maturational state. Even in those recent studies that are focused on the earliest training responses (e.g., Town and Essig [10]) there is virtually no information about whether cellular adaptations were associated with functional or cardiorespiratory responses, such as maximal treadmill running time or maximal oxygen uptake ($\dot{V}O_2\text{max}$).

In the present report, we tested the hypothesis that functional indices of training could be detected in the young, female rat within 5 days of moderate treadmill training. The aim of the study was to test a short-duration training protocol. This protocol could be used to investigate the initial cellular and molecular events that lead to the training effect in the young, still developing organism.

Methods

The study was approved by the institutional review board at Harbor-UCLA Medical Center, and all national principles of laboratory animal care were adhered to. In designing this study, we took into consideration our previous experience that the cooperation of rats in treadmill training and in the performance of maximal tests is quite varied. Endurance training is performed in an open system, and approved interventions (such as noise or other mild electrical or tactile stimuli) can be used if the animal stops running before the session is completed. In contrast, $\dot{V}O_2\text{max}$ is measured in a closed system, and little can be done when a particular rat simply stops running. Interestingly, the unwillingness of a given rat to complete a maximal test is, in our experience, more common after the animal is accustomed to the treadmill (i.e., untrained rats appear to give maximal efforts more readily than do some trained rats). Finally, those rats *unwilling* to complete maximal tests are easy to distinguish: They stop running very early on at relatively low speeds, often lower than those that had been successfully completed by the same rat during the endurance training phase of the studies.

Sample Population

Twenty-six 8-week-old female Sprague-Dawley rats participated in the study. All rats were housed in steel cages and kept on a 12:12 hour light–dark schedule. Food and water was provided to all rats ad libitum. Rats were brought to the laboratory 7 days before the protocol began so that they could become familiar with their surroundings (e.g., treadmill) and handlers. The rats were weighed at the beginning and at the end of the experimental period.

Rats were divided randomly into control ($n = 10$) and training groups ($n = 16$). We chose an unequal number because, as noted above, we anticipated that 4–5 of the rats in the training group would not be willing to complete the training, perform a maximal test, or both. The training protocol consisted of the following schedule:

- Day 1: 21 m/min for 30 min
- Day 2: 21 m/min for 45 min
- Day 3: 24 m/min for 45 min

- Day 4: 24 m/min for 60 min
- Day 5: 27 m/min for 60 min

No inclination was used throughout the training protocol.

At the end of the training period, rats from each group underwent a progressive treadmill exercise test to exhaustion in order to determine $\dot{V}O_2$ max and maximal running time. Because untrained rats were unfamiliar with the treadmill, they were allowed 5-min periods of low-speed treadmill running (10 m/min, equivalent to a very slow run) on the day before the $\dot{V}O_2$ max test. Four hours after the $\dot{V}O_2$ max test, the animals were killed. The whole hindlimb musculature (from its origin to its insertion) was carefully dissected from bone and other tissues.

Measurements of $\dot{V}O_2$ max and Maximal Running Time

As previously described (3), gas exchange was measured using an open-circuit, constant room air flow-through system in which flow was measured using pneumotachographs, and O_2 , CO_2 , and N_2 concentrations were measured with a mass spectrometer. Standard Haldane correction were applied to the final calculation of $\dot{V}O_2$. The system was calibrated by administering gases of known O_2 , CO_2 , and N_2 concentrations at precisely controlled flow rates. The error of measurements was $\pm 3\%$.

Maximal tests were performed at 5% incline. A 4-min acclimation period was followed by an initial treadmill speed of 15 m/min for 4 min. Thereafter, treadmill speed was increased by 6 m/min every 4 min until the rats were unable to continue running. $\dot{V}O_2$ max and $\dot{V}CO_2$ max values were normalized to body weight. Resting (preexercise) metabolic rate is a much higher proportion of maximal metabolic rate in the rat compared with the human; therefore, resting changes in the preexercise value could mask maximal changes observed during exercise. Thus, we subtracted the preexercise $\dot{V}O_2$ from the maximal value and used this variable [$\dot{V}O_2(\text{max} - \text{rest})$] to represent the actual increase in the metabolic rate resulting from the exercise. The same method was used to calculate $\dot{V}CO_2(\text{max} - \text{rest})$. We then calculated the respiratory exchange ratio (RER) specifically using these rest-to-maximal exercise differences as: $RER(\text{max} - \text{rest}) = \dot{V}CO_2(\text{max} - \text{rest})/\dot{V}O_2(\text{max} - \text{rest})$. Maximal running time was taken as the total running time following the initial 4-min acclimation period.

Statistical Analysis

Paired *t* tests were used to compare the pre- and postprotocol changes in body weight within the control and trained groups. Unpaired *t* tests were used to compare organ weights, maximal running time, $\dot{V}O_2(\text{max} - \text{rest})$, and end-exercise RER between the two groups. Standard techniques of linear regression were used to determine the correlation between running time and $\dot{V}O_2(\text{max} - \text{rest})$. Data are presented as mean \pm SE. Statistical significance was taken at $p < .05$.

Results

As anticipated, 5 of the 16 rats assigned to the training group failed to perform a maximal treadmill test at the end of the protocol. These 5 rats stopped running at speeds equal to or less than speeds that had been successfully performed for an hour's duration on the previous day. The data from these rats were not used in the following analysis.

Somatic Growth

There were no significant differences in body weight between control and trained rats at the beginning of the experimental period. In both control and trained rats over the experimental period, there was a significant increase ($p < .0009$) in body weight (control, 208.5 ± 3.0 g to 223.9 ± 5.8 g; trained, 211.6 ± 2.9 g to 226.3 ± 4.9), but this increase did not differ between the two groups.

Hindlimb Muscle Weight

Right hindlimb muscle weight was significantly greater ($p < .02$) in trained rats following the 5 days of training (Figure 1). We also analyzed the difference in muscle hindlimb weight between control and trained rats including the 5 trained animals who had failed to perform the maximal exercise protocol. There continued to be a highly significant difference in muscle hindlimb weight between the control and trained groups (trained animals, $n = 16$, 5.5 ± 0.13 g, virtually the same value as for the trained animals, $n = 11$, shown in Figure 1).

Maximal Running Time and Gas Exchange Measurements

Maximal running time was correlated with $\dot{V}O_2(\text{max} - \text{rest})$ ($r = .62$, $p < .002$). Maximal running time was significantly greater in the trained versus untrained rats

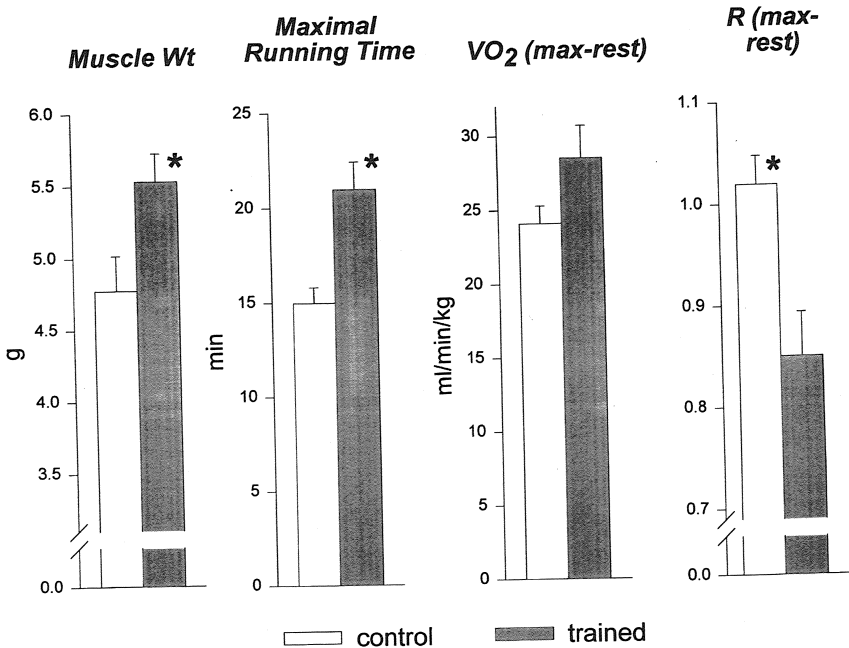


Figure 1 — Effect of treadmill training on hindlimb muscle weight, maximal running time, $\dot{V}O_2(\text{max} - \text{rest})$, and end-exercise respiratory exchange ratio [calculated as $\dot{V}CO_2(\text{max} - \text{rest})/\dot{V}O_2(\text{max} - \text{rest})$] in control (open boxes) and trained (hatched boxes) rats. Muscle weight and maximal running time were significantly greater and end-exercise R was significantly lower in the trained rats. * $p < .05$.

(Figure 1). Resting $\dot{V}O_{2\max}$ was greater in the control rats but not significantly so (22.8 ± 1.6 vs. 19.8 ± 1.7 ml \cdot min⁻¹ \cdot kg⁻¹ in control and trained rats, respectively). There were no significant differences in $\dot{V}O_{2\max}$ (47 ± 1.6 vs. 48.4 ± 3.2 ml \cdot min⁻¹ \cdot kg⁻¹ in control and trained rats, respectively) and RER_{max} (0.93 ± 0.03 vs. 0.9 ± 0.04 in control and trained rats, respectively) between the groups. $\dot{V}O_2(\max - \text{rest})$ was greater in the trained rats, but not significantly so (Figure 1). RER(max - rest) was significantly greater in the control rats, even though their running times were significantly less than in the trained animals.

Discussion

This study demonstrates that 5 days of endurance type treadmill training in female rats resulted in increased maximal treadmill running time and a greater hindlimb muscle weight. The protocol was feasible in most of the young female rats, and represented a relatively moderate training input. This is important because it is unlikely that either rats or children would be capable of successfully completing the brief but intensive training protocols that have been used in cooperative adults, such as reported by Green et al. (6), whose subjects performed cycle ergometry for 2 h per day at work rates comparable to 67% of $\dot{V}O_{2\max}$.

The control animals had a higher RER(max - rest) at the end of maximal exercise, even though the running time was significantly less and $\dot{V}O_2(\max - \text{rest})$ tended to be lower than in the trained group. RER values tended to be lower than what might have been expected for maximal efforts. Although we have no ready explanation for this, it is possible that after 5 days of training rats improve functional capability, but still do not easily exercise to their true maximal values. Indeed, as noted, it is our experience that the maximal exercise test is stressful for the rat.

We did not measure serum lactate in these rats. However, it is known that lactic acidosis during exercise is accompanied by increased CO₂ production (due to the buffering of lactic acid) and an increase in RER(max - rest). Thus, to the extent that RER(max - rest) reflects high-intensity and, possibly, anaerobic metabolism, substantial changes in muscle size, metabolic response, and cardiorespiratory function were observed with only 5 days of training.

In designing this protocol, we considered a number of methodological factors. First, as noted above, an important, but not often discussed problem is the variability among rats in their ability or willingness to participate in training protocols and to perform maximal tests. Five of the original 16 trained rats completed the training but failed to perform a maximal test. It is noteworthy that a training effect was observed in all the training group rats by an increased hindlimb muscle weight, this despite the absence of maximal functional data for comparison with control animals.

The training protocol had to be intense enough to result in a measurable training effect, but not too intense to cause high dropout rate from the program. The fact that all of the rats completed the training program with a demonstrable training effect indicates that this goal was accomplished with the present protocol. Nor, subjectively, did we need to rely excessively on devices like tactile or electrical stimuli for the rats to complete the training protocol. This is important because a protocol that is very *stressful* could lead to physiological responses other than those associated solely with training. In our experience, and from our discussions with other investigators, it seems that female rats generally participate more "willingly" in training protocols than do male rats; and young rats are more cooperative than old. Documentation for this hypothesis is not currently available.

Finally, although under ideal circumstances we would have performed both pre- and post-maximal-exercise testing, we purposefully subjected each rat to only one maximal test (at the end of the experimental period). We did this for several reasons: First, the performance of a maximal test could, in and of itself, be a sufficient stimulus to trigger some of the cellular components of the training response. Second, since the maximal test is, in our experience, stressful for the rat, we were concerned that by beginning with a maximal test we would further reduce the number of rats willing to undergo the training itself. However, we recognize that by not performing maximal tests before and after the training protocol, we limited our ability to gauge the effectiveness of training.

Although we hypothesized that the short, but relatively intense, training protocol would increase functional indices of exercise performance, we were surprised to find significant differences in hindlimb weight after such a short period of training. Our data suggests that at least part of the early training response to running exercise is related to muscle size. In the present study we did not determine muscle water or protein content, or mitochondrial enzyme activity which can contribute to the increased muscle size in the trained rats.

Chromiac et al. (2) demonstrated elevations in mitogenic growth factors in serum and muscle-derived extracts from rats after only 7 days of climbing a vertical grid with progressively greater loads. In studies from DeVol et al. (4) and our own laboratory (12) involving longer training intervals, increases in local muscle IGF-I, a growth factor known to stimulate muscle growth were observed. Thus, to the extent that growth factors like IGF-I regulate the muscle hypertrophy that accompanies training, we hypothesize that differences in growth factor gene expression could be detected by as early as five days of training.

Town and Essig (10) examined the effect of treadmill training of different intervals on muscle mitochondrial enzymes. They showed increased aminolevulinatase synthase (ALAS) activity after only 3 days of training in young rats, and elevated cytochrome oxidase activity following 7 days of training. They also noted that ALAS activity was actually lower after 28 days of training compared with 7 days, although a statistical analysis was not presented. Thus, as reviewed recently by Booth and Thomason (1), the molecular response to training is characterized by gene expression, and by translational and post translational steps which likely vary with the duration and intensity of the training input.

Although maximal running time was correlated with $\dot{V}O_2(\max - \text{rest})$ and maximal running time was greater in the trained rats, $\dot{V}O_2(\max - \text{rest})$ was not significantly greater in the trained compared with the control group. It is, of course, possible that the variability among the rats was too great to detect the magnitude of change in $\dot{V}O_2(\max - \text{rest})$ resulting from the 5 days of training. Indeed, maximal running time was 54% greater in the trained rats compared with the control rats, while $\dot{V}O_2\max$ was only 18% greater (again, but not significantly so). It is also possible that the ability of the rats to run longer may have resulted from a learned improvement in running efficiency such that greater running times were achieved at less oxygen cost. Nonetheless, the observations of greater muscle size and lower peak RER at greater efforts in the trained rats suggests that both anabolic and metabolic adaptations had occurred during the 5 days of training.

In summary, we devised a brief treadmill training protocol for young female rats that led to longer maximal running times, lower respiratory exchange ratios at peak exercise, and increased hindlimb muscle size compared with control, untrained rats after only 5 days of training. All rats completed the training with a minimal

need for prodding; however, 31% of the rats in the original training group did not perform an acceptable maximal test at the end of the training period. The long-term consequences of exercise training during childhood has been a focus of research in this and other laboratories (e.g., 3, 8), but the duration of training intervention often encompasses much, if not all, of the period of maturation in the rat. The protocol described here may be useful in studies designed to probe the triggering cellular and molecular events that lead to the variety of cellular adaptations comprising the training effect in younger organisms.

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