Adaptations to high-intensity intermittent exercise in rodents

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Bexfield NA, Parcell AC, Nelson WB, Foote KM, Mack GW. Adaptations to high-intensity intermittent exercise in rodents. J Appl Physiol 107: 749–754, 2009. First published July 16, 2009; doi:10.1152/japplphysiol.91446.2008.—In humans, exercise-induced plasma volume (PV) expansion is typically associated with an increase in plasma albumin content, due in part to an increase in hepatic albumin synthesis. We tested the ability of a 12-day high-intensity intermittent exercise protocol to induce an increase in PV in rodents. Since albumin synthesis is transcriptionally regulated, we tested the hypothesis that exercise training would induce an increase in hepatic albumin gene expression. Fifty adult male Sprague-Dawley rats weighing between 245 and 350 g were randomly assigned to one of five groups: cage control (CC), sham exercise (sham), continuous moderate-intensity exercise training (MI), high-intensity intermittent exercise training (HI), or a single day of HI training (1-HI). Twenty-four hours after the last training session, rats were anesthetized. PV was determined, and the liver was removed, flash frozen, and stored for later analysis. Citrate synthase (CS) activity of the red quadriceps muscle, a marker of aerobic adaptation, increased with training (MI and HI) and in response to 1-HI (P < 0.05). We did not see a significant exercise-induced PV expansion as PV averaged 23.6 ± 2.7 ml/kg body wt in the CC group and 26.6 ± 1.3 ml/kg body wt in the HI group (P > 0.05). However, hepatic albumin mRNA expression, as determined by real-time PCR, increased 2.9 ± 0.4- and 4.1 ± 0.4-fold after MI and HI, respectively, compared with CC. A single bout of HI (1-HI) did not alter hepatic albumin mRNA expression. These data demonstrate an increase in both CS activity and hepatic albumin gene expression with 12 days of aerobic exercise training in the rodent with a rapid (within 24 h) adaptation in the skeletal muscle to high-intensity intermittent exercise.

Although exercise-induced PV expansion has been reported in the rabbit (5), dog (17), horse (18), and rat (34), there is no animal model that appears to replicate the human response to aerobic training. For example, Tipton et al. (34) demonstrated exercise-induced PV expansion in hypertensive rats but not in their normotensive cohort. In humans, specific exercise stimuli (i.e., exercise intensity and duration) are known to produce PV expansion, but data on rodents (or other animals) are less clear. The purpose of this study was to induce PV expansion in rodents using training stimuli that replicate human studies. Thus, we used a 12-day moderate-intensity aerobic training protocol (MI) (3, 4) and a single bout of high-intensity [85% maximal O2 consumption (V̇O₂max)] intermittent (8 bouts of 4-min exercise/5-min recovery) exercise (1-HI) (8, 23) to stimulate PV expansion in the rodent. Furthermore, to examine the impact of exercise intensity on PV expansion in the rodent, we also included a 12-day high-intensity aerobic training protocol (HI). To verify aerobic adaptations to each training protocol, we monitored citrate synthase (CS) activity of the red quadriceps (RQ) muscle of the rats. Since albumin synthesis is transcriptionally regulated (28), any increase in albumin synthesis during exercise training should be reflected by an increase in albumin gene expression (19, 22, 23, 28, 36). As such, we also tested the hypothesis that aerobic exercise training would stimulate liver albumin mRNA expression in the rodent.

MATERIALS AND METHODS

Fifty adult male Sprague-Dawley rats from the breeding facility of Charles Rivers Laboratory (Portage, MI) weighing between 245 and 350 g were randomly assigned to one of five groups: cage control (CC; n = 10), sham exercise (sham; n = 10), MI (n = 10), HI (n = 10), and 1-HI (n = 10). All rats acclimatized to the new housing facilities for 14 days before any treatment. CC animals were handled minimally and only in association with daily animal care procedures. The animal studies were reviewed and approved by the Brigham Young University Institutional Animal Care and Use Committee. Rats in all the exercise groups (sham, MI, HI, and 1-HI) then participated in a 14-day exercise acclimation period. The sham group walked on a motorized treadmill for 10 min/day at 9.8 m/min (≈46 ± 2% V̇O₂max) for the entire 14-day acclimation period. Rats in the MI, HI, or 1-HI groups participated in a pretraining program that increased in speed (from 9.9 to 20.1 m/min) and duration (10–60 min) over the 14-day acclimation period.

The exercise training for the MI and HI group consisted of 6 days of training and 1 day of rest followed by 6 more days of training. Rats in the 1-HI group participated in a single bout of high-intensity intermittent exercise after the acclimation period. Rats ran on a standard multilane animal treadmill (Quinton, Bothell, WA). Exercise intensities for each exercise group were estimated based on the linear relationship between treadmill speed and O2 consumption generated from data in the literature on male Sprague-Dawley rats running on a treadmill at a 10% grade [V̇O₂max (in ml·min⁻¹·kg body wt⁻¹) = 1.61 × speed (in m/min) + 29.8] (1, 21, 27, 37). To estimate the relative metabolic cost of each exercise protocol, we assumed the average V̇O₂max for a male Sprague-Dawley rat to be ~100 ml O2·min⁻¹·kg body wt⁻¹ (1, 21, 27, 37).

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EXERCISE-INDUCED plasma volume (PV) expansion is a hallmark adaptation to aerobic exercise training in humans (30). In addition, PV expansion can occur within 24 h after a single day of high-intensity intermittent exercise training without a significant change in red blood cell volume (7, 8). This exercise-induced PV expansion is associated with an increase in plasma albumin content that acts to retain water in the vascular compartment via its colloidal osmotic properties. The increase in plasma albumin content is associated with several physiological responses including a redistribution of albumin from the interstitial fluid to the plasma compartment (8, 9, 13, 16, 22, 23, 25, 38), a reduction in the rate of albumin escape from the vascular compartment (13), and an increase in hepatic albumin synthesis (22, 38). The cellular and molecular signaling pathways involved in stimulating an increase in the hepatic albumin synthetic rate in response to exercise are unknown and are unlikely to be elucidated without an appropriate animal model of exercise-induced PV expansion.
The MI group ran continuously for 60 min at 30.4 m/min and 10% grade (≈78 ± 3% \( \text{VO}_{2\text{max}} \)). The HI group performed a high-intensity intermittent exercise protocol that consisted of running at 42.1 m/min and 10% grade (≈98 ± 4% \( \text{VO}_{2\text{max}} \)) for 4 min followed by a 5-min recovery period of running at 20.1 m/min (≈62 ± 2% \( \text{VO}_{2\text{max}} \)). The HI group repeated this exercise pattern 8 times/session (total time: 72 min). The 1-HI group performed a single day of HI training. CC animals remained in the cages for 14 days and were killed after that period.

Twenty-four hour after the last training session, rats were anesthetized with a ketamine-xylazine cocktail. The right jugular vein was cannulated, and a 0.45-ml control blood sample was collected. A Texas red albumin solution (0.25 ml) was injected and flushed with 0.20 ml saline. After a 5-min mixing period, a second blood sample of 0.5 ml was taken to determine blood volume using the dye dilution method described by Gillen et al. (10). The liver was exposed through a midline incision, and the animal was euthanized with the method described by McCray et al. (25). The liver was harvested to analyze maximal CS enzyme activity using a colorimetric assay (kit no. CS0720, Sigma-Aldrich). Maximal CS activity was monitored as an indicator of improved aerobic metabolism in the active skeletal muscle. Finally, the heart was excised, rinsed, and weighed.

Muscle tissue was crushed into a fine powder on liquid nitrogen, and 50 g of powdered muscle tissue were homogenized in Tris buffer. The frozen liver was also crushed into a fine powder on liquid nitrogen, and RNA was isolated from 100–150 g of liver powder using a guanidine thiocyanate method (Trizol reagent, Sigma). cDNA was synthesized from 2 μg of total RNA with oligo(dT) and Stratascript RT (Stratagene, La Jolla, CA) using an MJ Research PTC-200 Peltier Thermal Cycler (Global Medical Instruments, Ramsey, MN). cDNA was quantified using a picogreen reagent (Invitrogen-Molecular Probes, Carlsbad, CA) based on a standard curve derived from a serial dilution of a known concentration of double-stranded DNA. Real-time PCR was performed using 100 ng cDNA derived from a serial dilution of a known concentration of double-stranded DNA. Real-time PCR was performed using 100 ng cDNA derived from a serial dilution of a known concentration of double-stranded DNA. Real-time PCR was performed using 100 ng cDNA derived from a serial dilution of a known concentration of double-stranded DNA. Real-time PCR was performed using 100 ng cDNA derived from a serial dilution of a known concentration of double-stranded DNA. Real-time PCR was performed using 100 ng cDNA derived from a serial dilution of a known concentration of double-stranded DNA. Real-time PCR was performed using 100 ng cDNA derived from a serial dilution of a known concentration of double-stranded DNA. Real-time PCR was performed using 100 ng cDNA derived from a serial dilution of a known concentration of double-stranded DNA. Real-time PCR was performed using 100 ng cDNA derived from a serial dilution of a known concentration of double-stranded DNA. Real-time PCR was performed using 100 ng cDNA derived from a serial dilution of a known concentration of double-stranded DNA. The amplification product was detected using the PicoGreen dye (Invitrogen-Molecular Probes, Carlsbad, CA) based on a standard curve produced using a serial dilution of cDNA derived from pooled liver samples for the target gene albumin and the endogenous reference gene (GAPDH). The efficiencies of the PCR for albumin and GAPDH were determined from duplicate standard curves and averaged 89% and 92%, respectively.

Due to technical problems, we were unable to determine PV (and thereby plasma albumin) in four animals. For these variables, the number of rodents in each group was 9 rodents in the CC group, 8 rodents in the sham group, 10 rodents in the MI group, 9 rodents in the HI group, and 10 rodents in the 1-HI group. We used ANOVA to compare the effect of treatment group on albumin mRNA expression, PV, blood volume, and other variables. When a significant \( F \) value was obtained, we used a least-significant-difference post hoc test to compare differences between each group. Significance levels were set at \( P < 0.05 \). Least-squares regression was used to identify significant relationships between variables.

**RESULTS**

At the time of death, animals in the CC (294 ± 9 g) and sham (298 ± 7 g) group were significantly (\( P < 0.05 \)) heavier than those in the MI (269 ± 4 g), HI (274 ± 5 g), or 1-HI (257 ± 11 g) groups. However, heart weight-to-body weight ratios were similar for all treatment groups.

**Training stimulus.** The exercise training stimulus was characterized by three parameters: exercise intensity (treadmill speed, in m/min), exercise volume (treadmill speed \times time = m/workout), and total exercise volume (m/workout \times number of training days = m). Exercise intensity averaged 9.8, 30.4, 42.1, and 42.1 m/min for animals in the sham, MI, HI, and 1-HI groups, respectively. Exercise volume per workout averaged 98, 1,824, 2,151, and 2,151 m/workout for animals in the sham, MI, HI, and 1-HI groups, respectively. Total exercise volume averaged 1,176, 21,888, 25,812, and 2,151 m for animals in the sham, MI, HI, and 1-HI groups, respectively.

**CS activity.** CS activity in the RQ skeletal muscle increased in proportion to the exercise training intensity (\( P < 0.05 \); Fig. 1 and Table 1). The 12-day training stimulus produced a 40% increase in CS activity in the RQ muscle of the HI group compared with the CC group (\( P < 0.05 \); Fig. 1). In addition, we saw a similar increase in CS activity in the RQ muscle after a single day of HI training (1-HI group, \( P < 0.05 \)).

**Hematological responses.** Hematocrit increased from 46.8 ± 0.5% in the CC group to 48.9 ± 0.3%, 47.4 ± 0.7%, and 50.4 ± 0.4% in the sham, MI, and HI groups, respectively (\( P < 0.05 \); Table 1). Hematocrit in the sham group was higher than in the CC group (\( P < 0.05 \)) but lower than in the HI group (\( P < 0.05 \)). PV and blood volume averaged 7.3 ± 0.6 and 13.9 ± 1.3 ml in the CC group, respectively. PVs in the MI, HI, and 1-HI groups were similar to the CC group despite a change of +15%, +12%, and +10%, respectively (\( P > 0.05 \); Table 1). Blood volumes in the MI, HI, and 1-HI groups were similar to the CC group, although there were some small changes (+16%, +20%, and +9%, respectively, \( P > 0.05 \)). Plasma
albumin content was similar for all treatment groups (Table 1).
There was, however, a significant association between PV and plasma albumin content ($P \leq 0.05$, $r = 0.94$; Fig. 2).

Liver responses. Albumin mRNA expression increased above CC levels for the MI ($P \leq 0.05$) and HI ($P \leq 0.05$) groups but was unaffected in the 1-HI group (Fig. 3).

DISCUSSION
A significant new finding of this study is that hepatic albumin mRNA expression was increased after 12 days of moderate- or high-intensity exercise training. The aerobic nature of the training stimulus was verified by an increase in CS activity in RQ skeletal muscle after exercise training. Another significant finding of this study was that 1-HI was sufficient to induce an increase in CS activity in RQ skeletal muscle. In contrast, hepatic albumin mRNA expression was not changed after 1-HI. Finally, despite the apparent aerobic training stimulus, we were unable to identify a significant increase in PV in any of our training protocols.

After MI and HI exercise training, we noted a large three- to four-fold increase in hepatic albumin mRNA. The mRNA expression of our housekeeping gene (GAPDH) remained constant for all treatment groups, and the efficiencies of the PCR for GAPDH and albumin were quite similar. Hepatic albumin mRNA expression in chronically instrumented rodents can be downregulated by 50% after an acute increase in plasma albumin content (28). Larger effects on albumin mRNA expression (2.3- to 2.5-fold increase) have been seen in cultured hepatocytes exposed to hydrocortisone or cAMP derivatives (2). As such, the three- to four-fold increase in albumin mRNA expression we saw in response to 12 days of exercise seems reasonable. The increase in albumin mRNA expression with MI and HI implies some adaptation to the exercise training stimulus. The sham group, which walked on the treadmill for 10 min/day at 9.8 m/min, did not show an increase in albumin mRNA expression, indicating that some

Table 1. Hematological and muscle changes after 12 days of exercise training

<table>
<thead>
<tr>
<th>Variable</th>
<th>CC</th>
<th>Sham</th>
<th>MI</th>
<th>HI</th>
<th>1-HI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit, %</td>
<td>46.8±0.6</td>
<td>48.9±0.3*</td>
<td>47.4±0.7*</td>
<td>50.4±0.4‡</td>
<td>46.3±0.6§</td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Plasma volume, ml/kg</td>
<td>23.6±2.7</td>
<td>23.4±2.8</td>
<td>27.1±3.1</td>
<td>26.4±1.2</td>
<td>26.0±0.7</td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>8</td>
<td>10</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Blood volume, ml/kg</td>
<td>44.2±5.4</td>
<td>46.2±5.5</td>
<td>51.6±5.8</td>
<td>53.1±2.5</td>
<td>48.2±4.9</td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>8</td>
<td>10</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Albumin content, g/kg</td>
<td>0.70±0.09</td>
<td>0.67±0.13</td>
<td>0.83±0.11</td>
<td>0.84±0.07</td>
<td>0.78±0.10</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>8</td>
<td>10</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Citrate synthase activity of the red quadriceps muscle, μmol·min⁻¹·μg⁻¹</td>
<td>74.0±4.9</td>
<td>85.3±6.3</td>
<td>90.6±7.5</td>
<td>105.8±10.6*</td>
<td>104.2±7.5*</td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
<td>10</td>
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</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals/group. Treatment groups were as follows: cage control (CC), sham exercise (sham), moderate-intensity continuous exercise (MI), high-intensity intermittent exercise training (HI), and 1 day of high-intensity intermittent exercise training (1-HI). *$P < 0.05$, significantly different from the CC group; †$P < 0.05$, significantly different from the sham group; ‡$P < 0.05$, significantly different from the MI group; §$P < 0.05$, significantly different from the HI group.
threshold level of training must be provided to induce this adaptation. We included an acclimation phase before the start of each training program, so the increase in albumin mRNA expression may be due to the combined effect of the acclimation protocol and training stimulus. However, the 1-HI group showed no increase in albumin mRNA expression despite participating in the 14-day acclimation period. Thus, we conclude that it was the training protocol that induced the significant increase in albumin mRNA expression.

CS activity in the RQ muscle was measured as a marker of skeletal muscle adaptation to aerobic exercise training. In our study, the 1-HI and HI groups produced a significant increase in CS activity in RQ skeletal muscle. The fact that CS activity was enhanced 24 h after 1-HI points to a rapid adaptive response of skeletal muscle to the high-intensity exercise. Others have identified rapid skeletal muscle protein responses to exercise. For example, Ren et al. (29) reported rapid increase in glucose transporter 4 protein content after 1 day of exhaustive swim exercise and an increase in CS activity in the epitrochlearis muscles after 2 days of swim training. However, in previous treadmill training studies involving rodents, it took much longer than 12 days (6, 20, 33) or a greater volume of exercise (15) to produce an increase in CS activity in the RQ muscle. Specifically, Holloszy et al. (15) produced a significant increase in skeletal muscle CS levels in 11 days, but they performed two 2-h workouts/day during the training period. The CS levels in the MI group were similar to those in the CC or sham groups. This observation is consistent with the literature, which indicates that significant skeletal muscle adaptations to moderate intensity continuous treadmill exercise in the rodent requires >8 wk to establish (14). The HI group performed slightly more work (2,152 m/workout) than the MI group (1,822 m/workout). The acclimation procedure, which is necessary for success in the training protocol, may contribute to the CS response in the RQ muscle. We noted significant increases in skeletal muscle CS activity levels after HI and 1-HI protocols. However, CS levels in RQ muscle were similar in the CC and MI groups. This observation indicates that the acclimation period used before MI did not significantly impact CS levels before training. On the other hand, CS levels in the HI and 1-HI groups were greater than in the CC group but not different from the MI group. As such, we cannot attribute the increase in CS levels after training solely to any specific training protocol but to the net impact of the combined effects of acclimation and training. We did note a moderate degree of association between RQ skeletal muscle CS activity and the intensity of the training stimulus.

PV measured in the MI, HI, and 1-HI groups averaged 14.8%, 11.9%, and 10.2% higher, respectively, than in the CC group. While this magnitude of change in PV was similar to that seen in human studies, these changes were not statistically significant. One interpretation of these data is that the adaptation in body fluid compartments with training in the rodent requires more time to develop because of postural difference between the quadruped rat and biped human. We know that in humans the supine posture limits exercise-induced PV expansion (23). However, PV expansion can occur in humans within 24 h after a single day of high-intensity intermittent training in humans (4, 8, 23, 26). An additional explanation may be a lack of statistical power related to PV measurement error or experimental design (cross-sectional vs. longitudinal sampling).

Dye dilution techniques for measuring PV can resolve relatively small changes in PV in training programs when PV is monitored pre- and posttraining on the same individual. For example, a small (5–7%) increase in PV can be detected in human studies using the Evan’s blue dye dilution techniques 24 h after 1-HI (8). The cross-sectional design of the present experiment and possibly a slightly greater error in the PV determination in the rat by dye dilution limited our ability to detect group differences in PV by ANOVA. This limitation should be overcome in the future by implementing a longitudinal study design using chronically instrumented rodents.

Since albumin synthesis is transcriptionally regulated (36), the three- to four-fold increase of albumin mRNA expression should have also been reflected by an increase in plasma albumin content. We did not see a significant change in plasma albumin content. However, we did note a significant association between PV and plasma albumin content, with a slope equal to 26 ml/g albumin. This slope value exceeds the expected ability of albumin to attract water into the vascular space (18 ml water/g albumin) as described by Scatchard et al. (31). As such, factors other than increased plasma albumin content may be acting to increase PV with training. This observation is consistent with human studies (4, 8, 9, 12, 13, 16, 23, 24).

We noted an increase in hematocrit in all the groups compared with the CC group except for the 1-HI group. In addition, the increase in hematocrit in the HI group was larger than in the sham or MI group. Increased hematocrit could be caused by either an increase in red blood cell volume or a decrease in PV. Measured PV remained the same or increased slightly; thus, the increase in hematocrit in the sham, MI, and HI groups was most likely due to an increase in red blood cell volume. Data from Gollnick et al. (11) and Tobin and Beard (35) indicated that 12 wk of aerobic exercise training did not induce an increase hematocrit or hemoglobin concentration in the rodent. Our data indicated an adaptation in hematocrit after our acclimation and 12 days of training in the rodent. However, two observations limit our interpretation of these data. First, we noted an increase in hematocrit in the sham group, which performed minimal work on the treadmill during the study period. Second, the hematocrit of the 1-HI group was similar to the CC group although this group had demonstrated an increase in CS activity in skeletal muscle.

Our measurement of PV in the anesthetized rodent was ≈20% lower than that reported for chronically instrumented, awake rodents using a similar Texas red albumin dilution technique (10, 32). The contribution of both acute trauma and anesthesia may have impacted the size of the albumin distribution space, leading to reductions in our estimates of absolute PV by dye dilution. Since all animals were treated the same, this problem should not have impacted the outcome of the analysis, unless it contributed to a larger error in the PV measurement. Gillen et al. (10) reported a SE for repeated PV measurements by Texas red-albumin conjugate of 0.9 ml/kg. In the present study, the SE for PV measurements within each group of animals ranged from 0.7 to 3.1 ml/kg and may explain, in part, our
inability to detect difference in absolute PVs between training groups. In the future, a longitudinal approach monitoring PV before and after training in chronically instrumented rodents (32) should allow sufficient resolution to detect smaller changes in PV and plasma albumin content with the MI or HI training model.

In conclusion, our high-intensity intermittent exercise protocol increased CS activity in RQ skeletal muscle within 24 h of the first exercise bout and albumin mRNA expression in the liver after 12 days of training. Although high-intensity intermittent exercise promoted an increase in liver albumin mRNA expression, we were unable to identify a significant increase in PV or plasma protein content. Our exercise model and experimental design produced promising results, and, by implementing a longitudinal design, we should improve our ability to detect the small changes in PV (≈10–14%) associated with this 12-day HI rodent training program. Our HI rodent training model provides us with the opportunity to test hypotheses related to cellular and molecular signaling mechanisms in the control of hepatic albumin gene expression during exercise-induced PV expansion.

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