Muscle adaptations to hindlimb suspension in mature and old Fischer 344 rats

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HINDLIMB SUSPENSION OF RATS is a model of simulated microgravity that has been used to investigate a variety of skeletal muscle adaptations during non-weight-bearing conditions (31). Particularly notable during suspension is the atrophy of antigravity hindlimb muscles, especially the soleus (Sol), which has a high percentage of slow oxidative fibers (17, 29–31). Alternatively, mass is affected less by hindlimb suspension in muscles that are not normally involved in weight bearing or are composed of predominantly fast-glycolytic fibers, such as the extensor digitorum longus (EDL). Previous animal studies using simulated (4, 11, 12, 16, 22, 29, 30) or actual (3, 32) microgravity have also suggested that the capacity of hindlimb muscles to take up, phosphorylate, and oxidize glucose is increased per unit mass. Evidence for this comes from studies utilizing hindlimb perfusion (29, 30) and isolated muscle incubation techniques (11–13, 32), as well as from the examination of muscle enzyme activities (4, 11, 12, 22) and GLUT-4 protein concentrations (11, 12, 16).

GLUT-4 is the predominant glucose transporter protein expressed in skeletal muscle, and its concentration is associated with glucose transport capacity (10, 19). GLUT-4 concentration has been shown to increase in Sol muscles from young rats after hindlimb suspension periods of 7 days or less (11, 12, 16). In addition, the ability to phosphorylate glucose in the cytosol as measured by hexokinase activity is increased in rat Sol muscles after hindlimb suspension (3, 11, 27) and spaceflight (3). Previous enzyme data also suggest an increase in the glycolytic capacity of individual fibers from Sol muscles (3, 4) after various periods of hindlimb suspension (4) and after spaceflight (3). Finally, Sol (4, 11, 12) and gastrocnemius (4) muscles appear to maintain or increase citrate synthase activity after non-weight-bearing conditions.

Conversely, aging is associated with significant decreases in glycolytic capacity and in mitochondrial enzyme activities of several rat hindlimb muscles (2). Hexokinase activity has been shown to decrease with age in some studies (20, 26) but remained unchanged in others (2, 15) for a variety of muscles. Furthermore, GLUT-4 protein concentration decreases in skeletal muscles of rats over their life span. However, most of this decrease occurs during development from juvenile (1–3.5 mo) to young adult (10–13 mo) ages (2, 5).

Recent studies have suggested that the regulation of GLUT-4 protein levels may be coregulated with hexokinase (14, 21) and citrate synthase (9, 25) under conditions of increased neuromuscular activity. However, some dissociation in expression of these elements has been observed in Sol muscles from young rats with renewed weight bearing after hindlimb suspension (12). In addition, nearly every previous hindlimb suspension study examined rats <8 mo of age that had not attained peak muscle mass. Therefore, the purpose of this study was to compared mature (8 mo) with aged senescent (23 mo) male Fischer 344 rats for responses of hindlimb muscle GLUT-4 glucose transporter protein concentration and of hexokinase and citrate synthase enzyme activities to 14 days of hindlimb suspension. We hypothesized that GLUT-4 protein concentration and hexokinase and citrate synthase enzyme activities would increase in rat antigravity Sol and plantaris (PI) muscles after hindlimb suspension. Furthermore, changes in GLUT-4 concentration and hexokinase and citrate synthase enzyme activities would be similar in magnitude for mature and old rats. We selected the...
antiglucocorticoid and PI (53% fast glycolytic and 40% fast oxidative glycolytic) muscles, and the non-weight-bearing tibialis anterior (TA; 79% fast glycolytic) and EDL (79% fast glycolytic) muscles to compare differences based on fiber type composition (1) as well as on participation in weight-bearing function.

METHODS

Animal care. Mature (8 mo) and old (23 mo) Fischer 344 rats were obtained from the National Institute on Aging and housed in the animal care facility at the University of Arizona. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Arizona. Animal quarters were maintained with 12:12-h light-dark daily and with temperatures between 22 and 24°C. Rats were provided with rat chow pellets (Wayne, Rodent Blox, Bartonsville, IL), mash prepared from the rat chow pellets (1.85 kcal/g), apple slices, and water ad libitum. Mature and old rats were randomly assigned to either hindlimb suspension or cage control groups. Groups were designated mature control (MC; n = 10), mature suspended (MS; n = 10), old control (OC; n = 9), and old suspended (OS; n = 9). All animals were maintained in suspension or control conditions for 14 days.

Hindlimb suspension. Suspension of rat hindlimbs was accomplished by using a modification of the method first described by Morey (23). Briefly, the rat's tail was cleaned with alcohol, allowed to dry, and sprayed with benzoin. An adhesive strip with a triangular metal clip was affixed to the caudal half of the tail. The metal clip was attached to the suspension apparatus above the cage so that the rat's hindlimbs were elevated off the floor into a non-weight-bearing position. The rats were able to use their forelimbs for support, movement, feeding, and grooming. In addition, the apparatus allowed the rat 360° free rotation and access to the entire cage. Control animals occupied similar cages, but all four legs were weight bearing. The rats were examined daily by the investigators and by veterinarians affiliated with the University of Arizona.

Preparation and analysis of muscle samples. After the experimental period, the rats were injected with pentobarbital sodium (5 mg/100 g body mass ip), and the Sol, Pl, EDL, and TA muscles were removed from the hindlimbs, frozen in ice-cold saline solution, blotted and weighed, frozen in liquid nitrogen, and stored at −70°C. In addition, a portion of the left ventricle was excised and prepared as above.

The frozen muscles were prepared for analysis by homogenizing each in 40 vol of ice-cold 20 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid buffer, 1 mM EDTA, and 250 mM sucrose (pH 7.4). A portion of the homogenate was used to determine total protein concentration by using the bicinchoninic acid assay method (Sigma Chemical, St. Louis, MO). GLUT-4 protein was determined essentially as described by Rodnick et al. (25). Briefly, homogenate samples containing 25 μg of protein were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis by using a 12% gel. The gels were then transferred to nitrocellulose filter paper. The nitrocellulose papers were blocked with 5% nonfat dry milk (Carnation, Los Angeles, CA) in phosphate-buffered saline (PBS; pH 7.4) containing 0.2% sodium azide and stored overnight at 4°C. The nitrocellulose papers were then incubated at 37°C for 1 h in PBS containing 1% powdered milk and a 1:250 dilution of an antisera (East-Acres Biologicals, Southbridge, MA) specific for the COOH-terminal peptide sequence of the GLUT-4 protein (residues 498–509). Thereafter, the blots were washed in PBS containing 1% Triton X-100 and incubated with 0.30 μCi/ml goat anti-rabbit 125I-labeled immunoglobulin G (ICN Radiochemicals, Irvine, CA) in PBS for 1 h at 37°C. After a final wash, the papers were exposed to Kodak XAR-5 film at −70°C for 48 h. Autoradiographs were analyzed, and GLUT-4 was quantified relative to controls by scanning densitometry (Hoefer model GS300 with GS370 version 2.3 software; Hoefer, San Francisco, CA).

Total hexokinase and citrate synthase activities were assayed spectrophotometrically (12) from the homogenates described above.

Data analysis. All values are expressed as means ± SE. The differences among treatment and age groups were evaluated using analysis of variance procedures and Dunnett's multiple-range post hoc tests. Statistical significance was set at the 0.05 probability level.

RESULTS

Body mass and hindlimb muscle mass. Body mass for the MC rats was 374 ± 9 g after 14 days of control conditions. This was essentially unchanged from the mean body mass for this group before the experimental period but was 11% lower than the OC rats after 14 days, 420 ± 9 g (P ≤ 0.05). Hindlimb suspension for 14 days resulted in 20% decrease in body mass for the MS rats to 295 ± 5 g and an 18% decrease for the OS rats to 352 ± 5 g compared with presuspension values for these groups (P ≤ 0.05).

Absolute and relative hindlimb muscle masses are shown in Table 1. Absolute mass (mg) was significantly lower in the hindlimb-suspended mature rats for the Sol (42%), PI (29%), TA (25%), and EDL (15%) muscles compared with the controls (P ≤ 0.05). Similar percent differences in Sol (38%), PI (31%), TA (22%), and EDL (12%) mass were noted when comparing the OS with the OC rats. When muscle mass was expressed relative to body mass (mg/100 g), the Sol muscle continued to exhibit significantly lower values after suspension com-

<table>
<thead>
<tr>
<th>Group</th>
<th>Sol</th>
<th>PI</th>
<th>TA</th>
<th>EDL</th>
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<tr>
<td>MC</td>
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<td>632</td>
<td>149</td>
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<tr>
<td>MS</td>
<td>79</td>
<td>251</td>
<td>477</td>
<td>126</td>
</tr>
<tr>
<td>OC</td>
<td>134</td>
<td>347</td>
<td>608</td>
<td>146</td>
</tr>
<tr>
<td>OS</td>
<td>84</td>
<td>238</td>
<td>476</td>
<td>128</td>
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Table 1. Effect of old age and hindlimb suspension on muscle mass and protein concentration

<table>
<thead>
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<th>TA</th>
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<td>84</td>
<td>238</td>
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Muscle mass relative to body mass, mg/100 g

<table>
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Muscle protein concentration, mg/g

<table>
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<th>TA</th>
<th>EDL</th>
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<td>185.4</td>
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<td>180.1</td>
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<tr>
<td>OS</td>
<td>155.6</td>
<td>180.3</td>
<td>199.0</td>
<td>170.8</td>
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</table>

Values are means ± SE. MC, mature control (8 mo); MS, mature suspended; OC, old control (23 mo); and OS, old suspended; Sol, soleus; Pl, plantaris; TA, tibialis anterior; and EDL, extensor digitorum longus. *Significantly different from corresponding control value; †OC value significantly different from MC value, P < 0.05.
pared with control: 26% less in the mature rats and 25% less in the old rats. This was also the case for the Pl muscle, which was 9% less in the mature rats and 18% less in the old rats after suspension. TA muscle was significantly less in relative mass after suspension only in the old rats (6%), whereas the relative mass value for the EDL muscles from MS rats was higher than the mean from MC rats by 7%. Furthermore, muscle mass relative to body mass was significantly less for the old compared with the mature animals for all four hindlimb muscles examined (12–14%).

Muscle protein content and concentration. Protein content (mg) for the hindlimb muscles examined is shown in Fig. 1. The trend parallels the absolute muscle mass data discussed above. Protein was significantly lower in the MS rats compared with the MC rats for the Sol (44%), Pl (30%), TA (22%), and EDL (21%) (P ≤ 0.05). Similarly, protein content was significantly less in the OS compared with the OC rats for the Sol (43%), Pl (33%), TA (22%), and EDL (17%). Muscle protein concentration (mg/g) was not significantly different between the MC and OC rats for any of the muscles examined (Table 1). However, the OS group had a significantly lower protein concentration value for the Sol muscle compared with the OC group (9%), and the MS protein concentration value for the EDL was significantly less than the MC value (7%).

GLUT-4 concentration. Hindlimb muscle GLUT-4 protein concentration relative to total protein (arbitrary units/µg protein) was significantly less for the OC rats for the Sol (43%), Pl (33%), TA (22%), and EDL (17%) (Fig. 2). Generally, hindlimb suspension was not associated with a change in GLUT-4 concentration for the mature or old animals. The only exception was for the TA, which was 76% higher in the OS compared with the OC rats (P ≤ 0.05).

Hexokinase and citrate synthase enzyme activities. Hexokinase and citrate synthase activities expressed relative to total protein (nmol·mg⁻¹·min⁻¹) are shown in Figs. 3 and 4, respectively. Significantly lower hexokinase activities were found in the OC rats for the Pl (19%) and TA muscles (33%) compared with the MC rats (P ≤ 0.05). Similarly, citrate synthase activities were lower in the OC compared with the MC rats for the Sol (14%), Pl (17%), and TA (38%). Hindlimb suspension was associated with significantly higher hexokinase activities for all four muscles examined in both mature and old animals. Hexokinase activities were higher for the MS compared with MC rats in the Sol (43%), Pl (26%), TA (18%), and EDL (10%) and were higher in the OS compared with the OC rats for Sol (31%), Pl (19%), TA (57%), and EDL (16%). Conversely, hindlimb muscle citrate synthase activity tended to be lower after suspension in both the mature and old rats. Statistically significant differences between the MS and MC groups were detected for the Sol (20%) and EDL (18%) muscles and between the OS and OC groups for the Sol (25%), Pl (27%), and EDL (25%).

Cardiac muscle from the left ventricle. Total protein (mg/g) and GLUT-4 protein (arbitrary units/µg protein) concentrations were not statistically different (P > 0.05) between age or treatment groups for the left ventricle (Table 2). Citrate synthase enzyme activity in cardiac muscle was 12% lower in the OC compared with the MC rats. Conversely, hexokinase activity was significantly higher in the OC ventricles compared with MC by 14%. Hindlimb suspension had no significant effect on citrate synthase or hexokinase enzyme activities in the hearts of either the mature or old animals.
Comparing 10- to 17-mo-old rats with 24- to 29-mo-old but not for the Sol or Pl (Fig. 2). Previous studies in GLUT-4 concentration for the TA and EDL muscles longer (17, 29–31). These factors represent indexes for the transport, synthase enzymes in a variety of rat hindlimb muscles. In this study, old age was associated with a decrease in GLUT-4 concentration for the TA and EDL muscles but not for the Sol or Pl (Fig. 2). Previous studies comparing 10- to 17-mo-old rats with 24- to 29-mo-old rats did not show a significant difference in GLUT-4 concentration for any of the muscles examined (2, 5, 6, 18). The reasons for the discrepancy between the results of those studies and the findings of the present study are not clear. However, the TA was not examined previously, and the only study that examined the EDL showed a trend toward a decrease in GLUT-4 concentration when comparing 6- to 8-mo-old with 27- to 29-mo-old rats (18). The GLUT-4 results for the Sol and Pl muscles in the present study are consistent with Gulve et al. (5).

Surprisingly, non-weight-bearing-induced atrophy of the Sol and Pl (Table 1, Fig. 1) did not result in significant changes in GLUT-4 concentration for either the 8-mo-old rats or the 23-mo-old rats (Fig. 2). Previous studies using young rats have consistently shown increased Sol muscle GLUT-4 concentrations (33–107%) after 3–7 days of hindlimb suspension (10, 11, 16), the greatest increase being observed after 7 days (11). Furthermore, insulin-stimulated glucose transport activity in Sol muscle increases to a similar extent after comparable non-weight-bearing periods (11, 13).

This is consistent with other studies showing a strong correlation between GLUT-4 concentration and glucose transport capacity (10, 19). The reason GLUT-4 concentration was not increased in the Sol and Pl with hindlimb suspension in this study is obscure. It may be that the increases in GLUT-4 after non-weight-bearing-induced atrophy are transient, reaching a peak at 7 days but normalizing by 14 days. However, Stump et al. (30) demonstrated that glucose uptake remains elevated at maximally stimulating insulin concentrations after 14 days of unilateral hindlimb suspension in both the Sol and Pl muscles from 3-mo-old rats. This result may imply a dissociation between muscle GLUT-4 concentration and glucose transport or may be due to a lesser response to hindlimb suspension in mature and aged rats. Two studies reported that muscle GLUT-4 protein concentration was not increased in old rats (25–29 mo) with exercise training but was increased in mature rats (6–17 mo) (6, 18). This finding may indicate that muscles from senescent animals are generally less plastic in terms of upregulating GLUT-4 protein content. Conversely, a recent study reported a 55% increase in rat hindlimb muscle GLUT-4 concentration in 24-mo-old Fischer 344 rats after 8 wk of exercise training (33). Furthermore, a decrease in senescent muscle plasticity would not explain the lack of an increase in GLUT-4 concentration for the 8-mo-old rats after suspension in the present study.

GLUT-4 protein concentration increased in the TA muscles of the old rats after hindlimb suspension compared with controls, reaching levels not significantly different from those in the mature rats’ TA muscles (Fig. 2). Interestingly, suspension of the mature animals did not have a significant effect on TA GLUT-4 concentration. GLUT-4 protein content of the TA has not been measured previously after hindlimb suspension. However, glucose transport activity of the EDL, another anterior leg compartment muscle of similar fiber type composition, has been examined.

## DISCUSSION

To our knowledge, this is the first study to examine skeletal muscle adaptations to hindlimb suspension comparing aged senescent rats (23 mo) with mature rats (8 mo). These ages were selected to examine the effects of old age separate from the developmental period. Previously, the vast majority of studies using hindlimb suspension have examined animals <6 mo of age. However, one study has compared developing young (3 mo) with old (23 mo) rats for various skeletal muscle enzymatic adaptations (27). The present study measured GLUT-4 glucose transporter protein concentration and the activities of hexokinase and citrate synthase enzymes in a variety of rat hindlimb muscles. These ages were hindlimb suspended for 14 days so that significant changes have been examined (33). Furthermore, the animals were hindlimb suspended for 14 days so that significant changes in the PI, EDL, and TA might be observed. Previous studies have shown that significant changes in Sol mass occur within the first 3 days of suspension (11, 17, 31), but changes in other hindlimb muscles may not become apparent until after 1 wk or longer (17, 29–31).

In this study, old age was associated with a decrease in GLUT-4 concentration for the TA and EDL muscles but not for the Sol or Pl (Fig. 2). Previous studies comparing 10- to 17-mo-old rats with 24- to 29-mo-old animals did not have a significant effect on TA GLUT-4 concentration. GLUT-4 protein content of the TA has not been measured previously after hindlimb suspension. However, glucose transport activity of the EDL, another anterior leg compartment muscle of similar fiber type composition, has been examined.

### Table 2. Effect of old age and hindlimb suspension on cardiac muscle from left ventricle

<table>
<thead>
<tr>
<th>Group</th>
<th>Protein Concentration, mg/g</th>
<th>GLUT-4 Concentration, units/μg protein</th>
<th>Hexokinase Activity, nmol·mg⁻¹·min⁻¹</th>
<th>Citrate Synthase Activity, nmol·mg⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC</td>
<td>171.1 ± 5.1</td>
<td>100 ± 3</td>
<td>30.0 ± 0.4</td>
<td>824 ± 27</td>
</tr>
<tr>
<td>MS</td>
<td>180.5 ± 3.1</td>
<td>93 ± 3</td>
<td>28.8 ± 1.6</td>
<td>853 ± 15</td>
</tr>
<tr>
<td>OC</td>
<td>174.3 ± 2.7</td>
<td>94 ± 3</td>
<td>34.1 ± 0.9*</td>
<td>723 ± 17*</td>
</tr>
<tr>
<td>OS</td>
<td>175.7 ± 3.1</td>
<td>92 ± 2</td>
<td>34.3 ± 1.0</td>
<td>775 ± 19</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Statistically significant difference between MC and OC groups, P < 0.05.

Fig. 4. Citrate synthase enzyme activity relative to total protein for select hindlimb muscles. *Significantly different from corresponding control value; †OC value significantly different from MC value, P ≤ 0.05.
Whereas no change in insulin-stimulated glucose transport was observed in the EDL of juvenile rats (1 mo) with 6 days of hindlimb suspension (13), evidence for increased glucose uptake has been observed in 3-mo-old rats after 14 days of hindlimb suspension (29, 30). The results of the latter studies have been postulated to be caused by either the increased stretch on the anterior leg compartment as the rats’ hindlimbs assume exaggerated plantar flexion after 4 days of suspension (24, 29) or by systemic factors (30). The increase in GLUT-4 in the TA muscles from the OS rats in the present study may be the result of similar mechanisms. Alternatively, the TA muscle from the OS rats was significantly less in mass relative to body mass compared with the OC rats (Table 1). This trend was not observed for the mature rats’ TA muscles or for the EDL muscles from either the mature or old rats, indicating that 14 days of hindlimb suspension may have resulted in selective atrophy of the TA muscle in the old rats. Henriksen et al. (11) have shown that GLUT-4 concentrations are increased in Sol muscles only after significant atrophy has occurred. However, as noted above, the fact that, in the present study, GLUT-4 concentrations did not change for the mature or old rats after atrophy of the Sol and Pl muscles indicates that this is not the only factor to be considered.

Hexokinase phosphorylates glucose as it enters the muscle cell and, therefore, represents the first step in glucose metabolism. Surprisingly, the effects of old age on hexokinase activity have been inconsistent in previous studies. Some studies have reported no change in hexokinase activity with aging for a variety of muscles (2, 15). In addition, Klitgaard et al. (20) did not observe a difference in hexokinase activity in rat Sol and Pl muscles when comparing 9-mo-old with 24-mo-old rats. However, a significant decrease was noted at 29 mo of age. In addition, when comparing 6-mo-old with 24-mo-old rats, Sanchez et al. (26) demonstrated a decrease in hexokinase activity in Sol and EDL muscles by 20 and 8%, respectively. Interestingly, the TA and Pl muscles were 33 and 19% lower in hexokinase activity after 23 mo compared with 8 mo in the present study, whereas the Sol and EDL muscles only exhibited a nonsignificant trend toward a decrease (Fig. 3). Conversely, hindlimb suspension resulted in significant increases in hexokinase in all four muscles examined for both the mature (10–43%) and old (16–31%) rats (Fig. 3). Similar increases in hexokinase activity have been observed in Sol (3, 27), gastrocnemius (27), and TA (3, 27) muscles from young (3–5 mo) rats after various periods of hindlimb suspension (3, 27) and spaceflight (3). In addition, hexokinase activity has been shown to increase in Sol muscles from 22-mo-old rats after 3 wk of hindlimb suspension, but increases in the gastrocnemius muscles were not statistically significant (27). Collectively, these results indicate that old age is associated with no change or a decrease in hexokinase activity, depending on the muscles examined and the ages selected. Conditions of simulated or actual microgravity consistently cause increases in hexokinase activity in most hindlimb muscles.

Citrate synthase activity was measured as an index of hindlimb muscle oxidative capacity for the mature and old rats (Fig. 4). Activities were lower (14–38%) in the 23-mo-old compared with the 8-mo-old rats for the Sol, Pl, and TA muscles after control conditions. This decrease is consistent with previous animal studies examining a variety of mitochondrial enzymes for several muscles and is probably secondary to a decrease in the total number of mitochondria (2). Hindlimb suspension in the present study had similar effects on citrate synthase activity for the mature and old animals (Fig. 4). Generally, there was a decrease in activity for the Sol and EDL muscles (18–27%). In addition, the Pl muscle was significantly lower in citrate synthase activity in the OS compared with the OC rats. Previous studies have found that, when muscle homogenates are examined, citrate synthase activity is decreased in Sol muscles after hindlimb suspension (31). In contrast, citrate synthase activity in individual Sol and gastrocnemius fibers was increased and not changed, respectively, after a 2- to 4-wk non-weight-bearing period (4). This discrepancy is apparently due to the increase in interstitial space relative to total mass that occurs in non-weight-bearing Sol muscles (30). However, this result has not been demonstrated for other hindlimb muscles (30). Our citrate synthase activity data were obtained from muscle homogenates and are expressed relative to total muscle protein. Furthermore, we believe that the decrease in cellular oxidative capacity after 14 days of hindlimb suspension in these animals has biological significance. Although increases in muscle interstitial space may have contributed to the decrease in activity observed in the Sol, it is unlikely that this effect would have masked a significant increase in cellular enzyme activity. By using similar muscle homogenate procedures, two studies have demonstrated in juvenile rats a significant increase in citrate synthase activity after 3-day (12) and 7-day (11) non-weight-bearing periods. Therefore, the decrease in citrate synthase activity after hindlimb suspension that was observed in the present study may be related to the age of the animals.

In the present study, the difference in myocardial GLUT-4 concentration with age was not statistically significant (Table 2). Cartee (2) reported a 12% decrease in GLUT-4 between 13 and 25 mo of age in Fischer 344/Brown Norway F1 hybrid rats, and Hall et al. (7) found a 27% decrease in myocardial GLUT-4 concentration when comparing 25-mo-old with 7-mo-old Fischer 344 rats. The old rats exhibited a 14% increase in hexokinase activity and a 12% decrease in citrate synthase activity in the present study (Table 2). Hansford and Castro (8) reported a generalized decrease in oxidative capacity in hearts from 24-mo-old rats compared with 6-mo-old rats, including decreases in citrate synthase activity and fatty acid oxidation. In addition, an increase in left ventricular hexokinase activity has been reported previously for old Fischer 344 rats (28). Collectively, these results suggest an increased cardiac muscle reliance on glycolytic energy sources and less on oxidative steps, especially fatty acid
oxidation, with old age. Fourteen days of hindlimb suspension did not affect GLUT-4 concentration or hexokinase or citrate synthase activities (Table 2).

In summary, the results of this study indicate that hindlimb muscle GLUT-4 protein concentration, hexokinase activity, and citrate synthase activity tended toward lower values for the 23-mo-old compared with the 8-mo-old rats. However, the TA was the only muscle that was significantly lower for all three variables. This study also demonstrated that hindlimb muscles from mature and old rats responded similarly to suspension-induced non-weight-bearing. Decreases in muscle mass (Table 1) and total protein (Fig. 1) were nearly identical for the two age groups. In addition, hexokinase activities were increased after suspension for all four muscles examined, citrate synthase activities tended to decrease, and GLUT-4 protein levels were generally not different between the suspended and control animals for either age group. The exceptions, in which the old muscle responded to hindlimb suspension to a greater extent than the mature muscle, included a significant increase in GLUT-4 concentration, a greater increase in hexokinase activity, and a decrease in muscle mass relative to body mass for the TA. The enzyme results from this study are consistent with previous enzyme data (3, 4), suggesting that rat hindlimbs are more reliant on glycolytic metabolism after hindlimb suspension. The GLUT-4 concentration data are less convincing, although we do not have muscle glucose transport data for these animals nor do we know the transporter distribution or intrinsic activity. Finally, the results of this study did not indicate that GLUT-4 protein levels and hexokinase or citrate synthase activities were regulated together during 14 days of hindlimb suspension, as had been suggested previously with increased neuromuscular activity (9, 14, 21, 25).

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