Exercise interrupts ongoing glucocorticoid-induced muscle atrophy and glutamine synthetase induction

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Falduto, M. T., A. P. Young, and R. C. Hickson. Exercise interrupts ongoing glucocorticoid-induced muscle atrophy and glutamine synthetase induction. Am. J. Physiol. 263 (Endocrinol. Metab. 26):E1157–E1163. 1992.—This study was undertaken to determine whether regular endurance exercise is a deterrent to a developing state of muscle atrophy from glucocorticoids and to evaluate whether the contractile activity antagonizes the hormonal actions on glutamine synthetase, alanine aminotransferase, and cystolic aspartate aminotransferase (cAspAT). Adult female rats were administered cortisol acetate (CA, 100 mg/kg body wt) or an equal volume of the vehicle solution for up to 15 days. Exercise (treadmill running at 31 m/min, 10% grade, 90 min/day) was introduced after 4 days of CA treatment, at which time plantar们 and quadriceps muscle mass had been reduced to 90% of control levels. Running for 11 consecutive days prevented 40 mg of the 90-mg loss and 227 mg of the 808-mg loss that were subsequently observed in plantar们 and quadriceps muscles, respectively, in the sedentary animals. Glutamine synthetase mRNA and enzyme activity were elevated threefold by glucocorticoid treatment in the deep quadriceps (fast-twitch red) muscles after 4 days. Initiating exercise completely interfered with the further hormonal induction (to ~5-fold) of this enzyme and, after 11 consecutive days of the exercise regimen, glutamine synthetase mRNA and enzyme activity were 58 and 68% of values from CA-treated sedentary animals. In vehicle-treated groups, basal levels of glutamine synthetase expression were also diminished by exercise to ~40% of the values in sedentary controls. Hormone treatment did not alter either aminotransferase enzyme activity but reduced cAspAT mRNA in fast-twitch red muscles by 50%. Exercise abolished the glucocorticoid effect on cAspAT mRNA. These results demonstrate that exercise is capable of inhibiting an existing and progressive condition of muscle atrophy. The data also support the hypothesis that the contractile activity effects are mediated through antagonism of a subset of hormone-responsive upregulated and downregulated genes that contribute to the atrophy.

alnine aminotransferase; aspartate aminotransferase; fast-twitch red muscle; gene expression; female rats

INCREASED CONTRACTILE ACTIVITY has the potential to alter the course of muscle wasting associated with excess glucocorticoids. In laboratory studies, significant atrophy prevention has been demonstrated when endurance exercise regimens have been introduced either before or simultaneously with chronic administration of glucocorticoid hormones (6–10, 16, 18, 19, 32). It is not known whether this condition can be attenuated or stopped if exercise is initiated during the period of progressive muscle catabolism and atrophy. This question appears to have obvious importance from a clinical perspective in the treatment of disorders associated with elevated steroid levels. Consequently, one purpose of this study was to determine whether starting an exercise program of endurance running after the onset of significant and progressive muscle wasting would be an effective deter

rent to additional muscle atrophy development.

Glutamine synthetase (GS), the enzyme that catalyzes the ATP-dependent condensation of glutamic acid and ammonia to form glutamine, is glucocorticoid inducible in skeletal muscle (9, 10, 12, 22–24). Along these lines, the biochemical events that trigger muscle atrophy and atrophy prevention are not known. However, the hormonal induction of GS may be a key participant to the extent of muscle wasting. Specifically, skeletal muscle is a major site of glutamine synthesis. Glutamine levels are higher than the total remaining amino acid pool combined, and this quantity cannot be accounted for by the 5–7% proportion of glutamine in major muscle proteins (21). Glutamine is released by muscle at high rates during catabolic conditions (25, 29, 30). A number of studies have now shown that elevated expression of GS mRNA and enzyme activity in skeletal muscle closely parallels the atrophy due to both glucocorticoid treatment and denervation (9, 10, 23). Furthermore, we have demonstrated that endurance exercise antagonizes the glucocorticoid-induced levels of GS mRNA and enzyme activity primarily in mixed muscle or muscle regions that are extensively recruited during the exercise (9, 10). Thus these data suggest that the downregulation of GS by exercise represents a major regulatory site in the prevention of muscle atrophy. Based on this information, a second aim was to observe whether the ability of exercise to retard a developing state of atrophy would also be effective at inhibiting or reversing the elevated GS enzyme activity and mRNA or whether either of these effects occur independently of each other.

A model that has been proposed as a possible mechanism for the biochemical basis of muscle atrophy prevention by exercise is that glucocorticoids elevate a number of genes in skeletal muscle to produce atrophy, while exercise diminishes the expression of a subset of those genes (9). To our knowledge, other glucocorticoid-inducible proteins with potential relevance to muscle atrophy have not been examined. Therefore, a third aim was to investigate the glucocorticoid and exercise responsiveness of alanine aminotransferase and the cytosolic form of aspartate aminotransferase in skeletal muscle, since these enzymes exhibit glucocorticoid inducibility in other tissues (27, 33).

METHODS

Animal care and experimental treatments. Female Sprague-Dawley rats, 250–300 g body wt, were obtained from Harlan Sprague-Dawley (Indianapolis, IN). They were housed individually in rodent cages (Wahmann, Baltimore, MD) on a 12:12-h light-dark cycle and were provided Purina rat Chow and water ad libitum. Rats were arbitrarily assigned to six sedentary groups (n = 6–18) that received daily subcutaneous injections of

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cortisol acetate (CA) at a dose of 100 mg/kg body wt for 1, 3, 4, 7, 11, or 15 days. Two other sedentary groups (n = 12) received daily subcutaneous injections of the dosing vehicle, 1% aqueous carboxymethyl cellulose in 0.9% NaCl for 4 or 15 days. The remaining two groups (n = 12–18) received the steroid or vehicle injections for 4 days, during which they remained sedentary. Starting on the 5th day, these two groups were subjected to an exercise protocol consisting of treadmill running at 31 m/min, 10% grade for 90 min daily while receiving simultaneous treatment with CA (CA + exercise) or vehicle (vehicle + exercise). Every 15 min during the training session the speed was increased to 50 m/min for 2 min. Exercise and CA or vehicle treatments continued for 11 additional days in these groups. Therefore, the total treatment duration (4 days sedentary + 11 days exercise) was 15 days. Entry into different treatment groups was sequential so that one animal from every group (10/day) could be killed on the same day, thereby eliminating age and minimizing body mass differences (all groups were the same at the start of the studies). Before beginning the experimental treatments, all animals received treadmill acclimation treatments, all animals received treadmill acclimation by running up to 31 m/min, 10 min/day, 3 days/wk for at least 3 wk. At death, which was ~18–20 h after the last treatment, whole plantaris and quadriceps muscles were removed and weighed. The deep (fast-twitch red) region was further dissected from the quadriceps muscles, quick-frozen in liquid nitrogen, and stored at -80°C until assayed. The fiber population typical of this muscle region was studied because it has previously been shown to be both hormone and exercise responsive (10).

**GS and aminotransferase assays.** GS was measured by determining the formation of [14C]glutamine from [14C]glutamate with modifications of the method of Pishak and Phillips (28), as recently described (10). Alanine aminotransferase (AAT) activity was measured on muscle homogenates using the procedure of Bergmeyer and Bernit (3). For the measurement of the cytosolic form of aspartate aminotransferase (cAspAT), muscle homogenates were heated for 15 min at 70°C to inactivate the mitochondrial isoenzyme (28). Aspartate aminotransferase activity was then measured on the heated homogenates by using [3H]aspartate as substrate and by monitoring the formation of [3H]H2O and [3H]oxaloacetate as described by Garrison et al. (13). All enzyme activities were expressed relative to protein content (4).

**RNA isolation, Northern analysis, and quantitation of specific mRNAs.** Total cellular RNA was isolated from the fast-twitch red region of the quadriceps muscle by the guanidine thiocyanate phenol-chloroform method of Chomczynski and Sacchi (5). Northern blot analysis was used to provide an assessment of the integrity of each RNA preparation. Both Northern and dot-blot analyses were performed exactly as described previously (9, 10). Blots were initially hybridized with 32P-labeled pBB3.2, a plasmid containing a 3.2-kb BamH I fragment that encodes the 3′-terminus of the murine GS gene (22). After removal of the 32P-labeled pBB3.2, blots were probed again with 4B21, a plasmid containing the cDNA for the cytosolic isoenzyme of cAspAT (27) kindly provided by Dr. Robert Barouki. Finally, blots were rehybridized with 32P-labeled oligo(dT)16, as previously described (9, 15), to quantitate the proportion of specific mRNAs in the population of poly(A)+ RNA in addition to the population of total RNA. The signal obtained with this probe reflects the amount of polyadenylated RNA present in the total RNA sample (15). Moreover, the data can be expressed as the ratio of the signal obtained with the specific mRNA probes to that obtained with the oligo(dT)16 probe. Because this ratio is independent of the amount of total RNA assayed, potential errors in sample loading will not contribute to the observed differences among treatment groups. To quantitate the relative level of GS mRNA, cAspAT mRNA, and poly(A)+ RNA, autoradiographs were scanned using a computer scanner (Apple Scanner) followed by analysis with densitometry software (Scan Analysis, Biosoft). For each sample, at least three loadings yielded a signal that was above background and linearly proportional to the RNA loaded.

**Statistical analysis.** Results are expressed as means ± SE for the number of animals indicated. Data were analyzed by multivariate analysis of variance with multiple linear contrasts of significant F ratios using the Systat program of Wilkinson (36). Where appropriate, the Wilcoxon signed ranks test was used to test for significance between relative mRNA means compared with control values arbitrarily set to a value of 1.0. Statistical significance was set at the 95% level of confidence.

**RESULTS**

**Effectiveness of exercise as therapy against developing muscle atrophy.** In glucocorticoid-treated sedentary animals, plantaris and quadriceps muscle weights were reduced to ~90% of vehicle-treated values after 4 days and 60% after 15 days (Figs. 1 and 2). The loss of muscle mass in the 4-day time interval is consistent with previous data that employed the same steroid dosage (17). In animals who began the exercise program after the initial 4 days of steroid treatment, there was 36% less atrophy in plantaris muscles as compared with these same muscles of sedentary hormone-treated animals. This level of protection from muscle atrophy is consistent with that observed in previous studies when exercise was initiated simultaneously with glucocorticoid treatment or when an endurance training regimen of 12-16 wk was used before 11 days of hormone treatment (6, 8–10). Of equal importance, exercise for 11 days after 4 days of glucocorticoid treatment prevented 46% of the further decline in plantaris muscle mass, which occurred from 4 to 15 days in animals that remained sedentary (Fig. 1).

In the quadriceps muscles of glucocorticoid-treated rats, exercise prevented 23% of the total loss in muscle mass over the 15-day period. Compared with the decline in muscle weight from the 4-day time point in sedentary animals, the daily exercise prevented 28% of the atrophy due to glucocorticoids in this muscle (Fig. 2). Based on the fiber-type composition of quadriceps muscles (1), it is estimated that fast twitch red fibers comprise ~27% of the total fiber population. Consequently, the relative atrophy prevention observed is similar to the proportion of

![Fig. 1. Plantaris muscle mass responses to 4 days of cortisol acetate (CA) followed by 11 additional days of CA treatment and exercise (Ex).](http://ajpendo.physiology.org/ by 10.1152/ajpendo.10332.2017)
fast-twitch red fibers present and is consistent with previous data that shows the atrophy sparing occurs to a greater extent in muscle types that are highly recruited by this running program (8).

GS activity. After 4 days of hormone treatment, GS enzyme activity in fast-twitch red muscle was threefold higher in glucocorticoid-treated than in vehicle-treated animals. Enzyme activity peaked by 7 days, at which time GS levels were elevated by approximately fourfold over vehicle-treated groups (Fig. 3). The 11-day exercise program, which began after 4 days of CA treatment, interfered with further induction of GS. GS activity was diminished by 42% in the exercised hormone-treated animals as compared with that observed in sedentary CA-treated animals over the entire 15-day period. These values were even somewhat lower than those in animals hormone treated for 4 days. Similarly, GS activity was reduced to 44% in muscles from vehicle-treated exercised animals compared with those of animals that remained sedentary (Fig. 3).

The fast-twitch white region of quadriceps muscles was not currently investigated. In preliminary studies, we found GS activity in this muscle region was not responsive to an 11-day exercise regimen similar to that currently used.

In plantaris muscles, the results of two separate experiments showed that GS responses in exercised animals were reduced to 80% of that in sedentary hormonally treated animals. GS was reduced to 46% in vehicle-treated exercised animals when compared with their sedentary counterparts (data not shown). These results are in close agreement with that observed in plantaris muscles after an endurance training program (9) as well as with other unpublished results from this laboratory employing the same type of exercise regimen for 11 consecutive days.

GS mRNA. The expression of GS mRNA followed the same pattern as that for GS enzyme activity (Fig. 4, Table 1). After 4 days of CA treatment, GS mRNA in fast-twitch red muscle was threefold higher than in vehicle-treated animals and continued to increase to values fivefold higher by 15 days. Exercise interfered with the hormone-mediated increase in GS mRNA such that the final level was 69% in CA-treated animals (3.6/5.3). Likewise, the basal level of GS mRNA was reduced to 40% in vehicle-treated animals (0.4/1.0) compared with the respective sedentary controls (Table 1).

Aminotransferase activities. There were no major changes due to glucocorticoids in the activities of AAT and cAspAT (Table 2). Small significant increases (~30%) due to exercise were observed in AAT activity in fast-twitch red fibers from CA-treated runners. Neither enzyme activities were changed by exercise or hormone treatments in fast-twitch white muscle (data not shown).

cAspAT mRNA. Although there was no response in cAspAT enzyme activity to glucocorticoids, a consistent effect of glucocorticoids to suppress cAspAT mRNA was observed in animals treated for 4 and 15 days (Fig. 5, Table 3). There was a 30 and 50% reduction in cAspAT mRNA at these respective time points in the sedentary animals. Exercise stimulated basal mRNA content by twofold and completely negated the hormone-mediated attenuation of cAspAT mRNA expression. AAT analyses were not performed, since a cloned fragment, complementary to AAT mRNA, has not been isolated.

DISCUSSION

A substantial amount of data exists that demonstrates the importance of endurance exercise in altering the course of muscle wasting due to elevated circulating glucocorticoids (6–10, 16, 18, 19, 32). The therapeutic effects of exercise in counteracting this type of muscle atrophy may also have relevance to the muscle wasting associated with various disease states such as Cushing’s syndrome, cancer, or severe injury, in which glucocorticoids are potentially implicated in the physiological responses (2, 14, 20, 34). In a number of previous investigations, however, glucocorticoids were administered only after several weeks of endurance training, and atrophy prevention was
Table 1. Reduction of GS mRNA in fast-twitch red muscle by exercise

<table>
<thead>
<tr>
<th>Group</th>
<th>GS mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA (4 days)</td>
<td>3.3±0.9</td>
</tr>
<tr>
<td>CA (15 days)</td>
<td>5.3±1.4</td>
</tr>
<tr>
<td>CA + Ex (15 days)</td>
<td>3.6±1.1*</td>
</tr>
<tr>
<td>Veh + Ex (15 days)</td>
<td>0.4±0.1†</td>
</tr>
</tbody>
</table>

Values are means ± SE for 5 observations/group. CA, cortisol acetate treated; Veh, vehicle treated; Ex, exercise; GS, glutamine synthetase. Values were arbitrarily set at 1.0 for vehicle-treated sedentary animals. *Significantly different from 15-day CA-treated sedentary group, P < 0.05. †Significantly different from vehicle-treated sedentary animals, P < 0.05.

Table 2. Alanine and aspartate aminotransferase enzyme activities in fast-twitch red muscle after glucocorticoid treatment (15 days) and exercise (11 days)

<table>
<thead>
<tr>
<th>Group</th>
<th>Aspartate Aminotransferase</th>
<th>Alanine Aminotransferase</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA sedentary</td>
<td>178±26</td>
<td>90±9</td>
</tr>
<tr>
<td>CA + Ex</td>
<td>181±15</td>
<td>123±9*</td>
</tr>
<tr>
<td>Veh sedentary</td>
<td>164±18</td>
<td>99±9</td>
</tr>
<tr>
<td>Veh + Ex</td>
<td>210±18*</td>
<td>111±11</td>
</tr>
</tbody>
</table>

Values are means ± SE for 5 observations/group and are expressed as nmol product formed·min⁻¹·mg protein⁻¹. *Significantly different from respective sedentary animals, P < 0.05.

observed in animals that were already highly trained (9, 10, 16, 18, 19, 32). Subsequent studies indicated that the muscle-sparing effects of the increased contractile activity did not require that the animals attain a training state (i.e., increased mitochondria as a prerequisite), since a similar magnitude of muscle preservation was found when the steroid and exercise treatments were administered simultaneously (6–8). The present results add to the
EXERCISE, GLUCOCORTICOIDS, AND GLUTAMINE SYNTHETASE

Table 3. Cytosolic aspartate aminotransferase mRNA in fast-twitch red muscle after glucocorticoid treatment and exercise

<table>
<thead>
<tr>
<th>Group</th>
<th>cAspAT mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA (4 days)</td>
<td>0.7±0.1*</td>
</tr>
<tr>
<td>CA (15 days)</td>
<td>0.5±0.1*</td>
</tr>
<tr>
<td>CA + Ex (15 days)</td>
<td>1.0±0.2†</td>
</tr>
<tr>
<td>Veh + Ex (15 days)</td>
<td>2.0±0.7*</td>
</tr>
</tbody>
</table>

Values are means ± SE for 5 observations/group. cAspAT, cytosolic aspartate aminotransferase. Values were arbitrarily set at 1.0 for vehicle-treated sedentary animals. *Significantly different from vehicle-treated sedentary animals, P < 0.05. †Significantly different from 15-day CA-treated sedentary group, P < 0.05.

importance of exercise as a preventive modality, since the running program halted the rate of atrophy already in progress so that nearly 50% of the further wasting was prevented in one case. At the time exercise was implemented, after 4 days of steroid injections, up to one-third of the total muscle mass loss had occurred. Thus a pre-existing state of muscle catabolism is not an impediment to the exercise effects.

Considerable evidence has accumulated that GS expression corresponds very closely with a muscle's response to atrophy (9, 10, 23). For example, muscles that are susceptible to glucocorticoid-mediated atrophy contain high levels of GS enzyme activity and mRNA, whereas muscles that remain glucocorticoid resistant display very low GS expression (10). Several studies (9, 10) have now shown that endurance training, either separately or in combination with glucocorticoid treatment, can antagonize GS enzyme activity and mRNA content to 50-60% of the levels observed in both basal and hormone-stimulated fast-twitch red and plantaris muscles of sedentary controls. As a result of these findings, the second question associated with this work was whether the
effectiveness of exercise as therapy against muscle wasting would be related to a concomitant reduction of GS activity or whether a differential response would be found. These data clearly show that the atrophy antagonism induced by the exercise also interfered with further induction of both GS enzyme activity and GS mRNA. The magnitude of diminished GS expression was in the same range as observed when the animals had 12-16 wk of endurance training followed by glucocorticoid treatment with continued training for 11 days (9, 10). The findings add continued support to the hypothesis that GS is a contributing component to atrophy and atrophy prevention. Although general agreement existed regarding atrophy prevention and attenuation of GS induction, there was still additional loss of muscle mass after the exercise sessions were introduced. Moreover, the kinetics of induction of GS enzyme activity and GS mRNA by glucocorticoids were completely interrupted such that the levels of both indexes of GS expression were maintained or dropped below the level attained at the time that exercise was initiated. This pattern of responses suggests that GS may be just one of a number of key elements involved in the regulation of muscle atrophy. At the same time, a principal role for GS cannot be dismissed. While further induction of GS, above that seen at 4 days, was stopped by the addition of the contractile activity, GS activities remained elevated at levels that had produced atrophy over the first four treatment days. The subsequent rate of muscle mass loss may be attributed to the failure of the exercise program to abolish the entire induction of the enzyme in the fast-twitch red fiber types as well as in the fast-twitch white fibers, which have the highest GS expression and are not influenced by this type of contractile activity (10).

A third purpose of this study was to investigate the participation of known glucocorticoid responsive genes in other tissues to the steroid regulation in muscle. AAT and the cytosolic isoenzyme of aspartate aminotransferase were chosen as two other amino acid synthesizing enzymes. On the basis of previous studies in some rat tissues, the activity of both enzymes and the mRNA for cytosolic aspartate aminotransferase were induced after the administration of glucocorticoids (27, 33). In addition, besides glutamine, alanine is the second most predominant amino acid in muscle and plasma (11). It is an important gluconeogenic precursor in liver and, like glutamine, is released during catabolic conditions from muscle at a high rate (25). In human muscle, endurance training elevates aspartate aminotransferase activity by ~50% (31). Presently, the absence of any consistent steroid-mediated effects on either AAT or aspartate aminotransferase enzyme activity suggests these genes are not of primary importance to the steroid regulation involved in muscle atrophy or atrophy attenuation. The small increases (30%) of both enzymes in muscles of selected exercise groups indicates that these enzymes are capable of adapting to regular exercise. The changes observed probably do not represent final steady-state increases, since there were only 11 training days. In spite of the fact that there was no induction of aspartate aminotransferase activity by gluocorticoids in fast-twitch red muscle, cAspAT mRNA was significantly decreased at both measured time points. Exercise completely counterbalanced the downregulation by glucocorticoids. It fails to be seen how the changes in mRNA alone could contribute mechanistically to muscle atrophy. It is not unreasonable, however, to speculate that enzyme activity measured in vitro does not reflect the in vivo state of the enzyme. In any case, the regulation of cAspAT mRNA by exercise may serve as an important model toward understanding how muscle contractile activity counteracts hormone-induced downregulated gene expression.

In conclusion, the present results have demonstrated 1) that the relationship of GS, muscle atrophy, and contractile activity still exists under a new experimental system, where regular exercise antagonizes both the ongoing muscle atrophy and GS induction from glucocorticoids; 2) that these exercise effects occur primarily in fast-twitch fiber types recruited by the exercise; 3) the alanine and cAspAT results suggest that the GS responses are unique among amino acid metabolizing enzymes; and 4) the results also show clinical relevance as an existing state of muscle atrophy can be deterred.

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EXERCISE, GLUCOCORTICOIDs, AND GLUTAMINE SYNTHETASE


