Detrimental Effects of Short-term Glucocorticoid Use on the Rat Diaphragm
Jane M Eason, Stephen L Dodd, Scott K Powers and A Daniel Martin
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Background and Purpose. The purpose of this study was to determine the effect of short-term, high doses of glucocorticoids on both body and diaphragm weights as well as contractile characteristics of the rat diaphragm. Subjects. Adult, female Sprague-Dawley rats were divided into 2 groups: a control group (n=16) and a prednisolone group (n=16). Methods. The prednisolone group received prednisolone at a dosage of 5 mg/kg, and the control group received sham saline injections for 5 days. Animals were weighed prior to and after completion of the drug injection period. At the completion of the drug injection period, the animals were sacrificed, and the diaphragm, soleus, and extensor digitorum longus muscles were removed and weighed. A small strip of the costal diaphragm was connected to a force transducer, and the following contractile characteristics were measured: maximal specific isometric tetanic tension, peak isometric twitch specific tension, one-half relaxation time, and time to peak tension. Results. Both body and diaphragm weights decreased by 15% in the prednisolone group as compared with the control group. Maximal specific isometric tetanic tension was reduced 13% in the prednisolone group as compared with the control group. There was no difference in any twitch contractile characteristics between the 2 groups. Conclusion and Discussion. These data support the hypothesis that glucocorticoid treatment over a 5-day period results in a decrease in specific tension as well as diaphragm and body weight. These results may have implications for the treatment of patients receiving high doses of glucocorticoids for acute medical conditions. [Eason JM, Dodd SL, Powers SK, Martin AD. Detrimental effects of short-term glucocorticoid use on the rat diaphragm. Phys Ther. 2000;80:160–167.]

Key Words: Contractile properties, Glucocorticoid, Muscle atrophy, Rat, Skeletal muscle.

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Glucocorticoids are commonly used for the treatment of patients with a variety of disorders, including pulmonary diseases. Because of their potent anti-inflammatory effects, glucocorticoids are useful in the treatment of inflammation. Glucocorticoids act by binding to a receptor in the cytoplasm of a cell, and this drug-receptor complex is transported into the nucleus, where it binds to specific elements on the DNA. This binding results in enhanced transcription of the DNA into RNA and ultimately leads to increases in protein synthesis. A potentially serious side effect of glucocorticoids, however, is the development of muscle atrophy. Although glucocorticoids stimulate protein synthesis in some tissues, glucocorticoids have catabolic effects on muscle tissue. Glucocorticoids stimulate the breakdown of muscle into amino acids, which results in muscle atrophy.

Glucocorticoids are used to treat a variety of pulmonary disorders; therefore, it is possible that the diaphragm may develop muscle atrophy. Because the diaphragm is considered the primary inspiratory muscle, any decrease in force production as a result of muscle atrophy may have deleterious effects in people with lung disease. The decreased force production in the diaphragm may have clinical implications. Impairment in force production of the diaphragm may place these patients at risk for prolonged mechanical ventilation and length of stay in an intensive care unit. Physical therapy intervention may need to include treatments designed to increase the force generated by the diaphragm.

It is generally well accepted that prolonged treatment (8–14 days) with glucocorticoids at either moderate or high doses results in a reduction of both diaphragm muscle mass and force production in the rat diaphragm. Moore et al and Dodd et al found 28% and 15% reductions in diaphragm mass in rats treated with glucocorticoids for 10 days. Dodd et al and Sasson et al found 26% and 12% declines in diaphragm force production in rats treated with glucocorticoids. Furthermore, administration of glucocorticoids results in atro-
High doses of glucocorticoids for short periods of time (5 days or less) are commonly used in the treatment of both lung transplant rejection and status asthmaticus. Very little is known regarding the potential effects that short-term, high doses of glucocorticoids may have on respiratory muscles. Nava et al reported that very high doses of glucocorticoids over a 5-day period resulted in a decrease in diaphragm mass but did not affect maximal specific isometric tetanic tension (Pmax). Those researchers, however, used glucocorticoid dosages that were 5 times greater than the dosage commonly used in clinical practice to treat patients with status asthmaticus. Problems may occur, however, even when glucocorticoids are administered at dosages used in clinical practice. These problems are related to possible differences in how the drug is metabolized in individuals once it has been transported into the cell. Furthermore, it is often difficult to separate the iatrogenic effect of these drugs from the effects of the disease.

We became interested in the potential effects of the short-term administration of clinically recommended high doses of glucocorticoids on the diaphragm muscle. Thus, the purpose of our study was to assess the effects of the administration of short-term, high doses of glucocorticoids for 5 days on the morphologic and contractile properties of the rat diaphragm. We hypothesized that administration of glucocorticoids at a dosage of 5 mg/kg/d for 5 days would result in a decrease in both body and diaphragm weights. Furthermore, we hypothesized that this drug regimen would result in a decrease in diaphragm Pmax.

**Method**

**Experimental Design**

Twenty-nine adult (4-month-old), female Sprague-Dawley rats were individually housed and fed rat chow and water ad libitum while being maintained on a 12-hour light/dark photoperiod for approximately 7 days prior to beginning the experiments. During this 7-day period, animals were handled daily to reduce the possibility that contact stress would occur during the study. The animals were assigned to 1 of 2 groups: animals that received daily sham saline injections for 5 days (control group, n=16) and animals that received daily prednisolone injections (5 mg/kg) for 5 days (prednisolone group, n=16). Three of the animals in the prednisolone group expired before the end of the injection series; thus, only 13 animals in the prednisolone group completed the full injection series. We do not know why the 3 animals in the prednisolone group expired, but because the 2 groups of animals were housed in separate rooms, we do not believe this event affected the results.

Prednisolone was chosen because it is prototypical of the nonfluorinated glucocorticoids used to treat human disease. The dosage of glucocorticoids used in this study is one that is commonly given to patients in clinical practice for the treatment of status asthmaticus. The rat diaphragm is an appropriate model to study characteristics of the diaphragm because the fiber type composition is similar to that of humans. Prednisolone was suspended in 0.9% saline and injected subcutaneously daily for 5 days. All injections were done at approximately the same time of the day. Animals were weighed prior to initiation of injections. Thereafter, they were weighed daily. The final weight was obtained 24 hours following the final injection. Guidelines for animal use established by the American Physiological Society were followed.

**Measurement of Contractile Characteristics**

Animals were anesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg) 24 hours following the final injection, and the entire diaphragm was removed and placed in a dissecting dish containing a Krebs-Henseleit solution equilibrated with a 95% O2/5% CO2 gas mixture. A small strip of the costal diaphragm was cut with a portion of the central tendon on one end and rib attachment on the other end. The strip was used to determine in vitro contractile measurements of peak isometric twitch specific tension (Pt), time to peak tension (TPT), one-half relaxation time (1/2 RT), and Pmax. The twitch contractile measurements of Pt, TPT, and 1/2 RT are generally indicative of calcium handling by the muscle. The measurement of TPT is the amount of time it takes the muscle to develop Pt and is generally indicative of calcium release by the sarcoplasmic reticulum. The measurement of 1/2 RT is the time it takes for Pmax force to return to one half of the force generated during the peak isometric twitch following removal of the stimulus. This measurement is generally thought to be indicative of calcium uptake by the sarcoplasmic reticulum. The measurement of Pmax is the amount of tetanic tension developed by the muscle when a stimulus of sufficient frequency and duration is delivered to the muscle.

All contractile measurements were collected with the muscle at optimal length (L0). Optimal length was defined as the muscle fiber length at which maximal
twitch tension was generated by a 2-millisecond pulse. The \( L_o \) was obtained by systematically lengthening the muscle with a micrometer while evoking single twitch contractions. The muscle was considered to be at \( L_o \) when maximal twitch tension was generated. Following contractile measurements, the mass of the muscle strip and its length (\( L_o \)) were measured in order to determine the muscle cross-sectional area (CSA). Expressing tension as the amount of force generated per muscle CSA allows for comparison of force generation between muscle strips of different sizes. Muscle CSA was determined using the following formula:

\[
\text{CSA (cm}^2\text{)} = \frac{\text{muscle mass (g)}}{\text{[muscle length (cm) \times muscle density (g/cm}^3\text{)]}}
\]

assuming muscle density = 1.056 g/cm\(^3\)

The remaining costal diaphragm was trimmed of connective tissue and fat, blotted, and weighed on an analytical balance. Total weight of the diaphragm was obtained by adding the weight of the muscle strip to the weight of the remaining costal diaphragm. Because the diaphragm contains a mixture of muscle fiber types, we were also interested in comparing the effects of short-term, high doses of glucocorticoids on muscles composed primarily of one fiber type. Thus, the soleus and extensor digitorum longus (EDL) muscles were excised, cleaned of connective tissue and fat, and weighed on an analytical balance. These muscles are composed primarily of type I and IIb muscle fibers, respectively. The animal was then sacrificed with an overdose of pentobarbital.

**Experimental Protocol**

The experimental protocol for collection of contractile data was similar to that described by previous investigators. These methods are the standard methods of measuring these muscle characteristics and can be judged to yield valid and reliable measurements based on their extensive use in the literature. Briefly, the dissected muscle strip was suspended vertically between 2 Plexiglas clamps in a jacketed tissue bath containing Krebs-Henseleit solution and connected to a force transducer (model 300B). In order to produce complete neuromuscular blockade, 12-\(\mu\)M d-tubocurarine was added to the tissue bath. The jacketed tissue bath was aerated with gas (95\% \(\text{O}_2/5\% \text{CO}_2\)), pH was maintained at 7.4, and the osmolality of the bath was approximately 290 mOsm. The temperature in the organ bath was maintained at 25°C. The muscle strip was stimulated along its entire length with platinum wire electrodes using a modified Grass Instruments S48 stimulator. After a 15-minute equilibration period, the muscle strip was adjusted to \( L_o \), and maximal twitch tension was obtained by applying a supramaximal voltage (140 V) to stimulate the strip.

The force transducer output was amplified and differentiated by operational amplifiers and underwent analog-to-digital conversion for analysis using a computer-based data acquisition system (GW Instruments Series II). All contractile measurements were measured in triplicate and averaged, and the mean was used for statistical analysis. The twitch contractile measurements of \( P_t \), TPT, and \( \frac{1}{2} \text{RT} \) were obtained by applying twitches of 2 milliseconds duration to the strip at supramaximal voltage. The tetanic contractile measurement of \( P_o \) was produced by using a supramaximal stimulus train of 80 Hz and 330 milliseconds duration. Maximum tension generated during both twitch and tetanic contractions was normalized to muscle CSA.

**Data Analysis**

Comparisons between the control and prednisolone groups were made by unpaired \( t \) tests. Variables measured were diaphragm and body weights, \( P_o \), \( P_t \), TPT, and \( \frac{1}{2} \text{RT} \). A repeated-measures analysis of variance was used to test changes in weight across groups over time. Data were analyzed by the Statview 4.1 statistical package on a Macintosh computer. Significance level was set at \( P<.05 \).

**Results**

**Morphological Characteristics**

Physical characteristics (\( \bar{X} \pm \text{SEM} \)) of the animals are summarized in Table 1. Mean body weights were similar in both the control and prednisolone groups prior to administration of glucocorticoids (280.3±2.4 g versus 280.2±4.4 g). The Figure illustrates the decrease in body weight over the 5-day treatment period. By the third day of drug treatment, the body weights of the rats in the prednisolone group were 7\% less than those of the rats in the control group. The body weights of the rats in the prednisolone group decreased by 2\% to 3\% daily throughout the remainder of the drug treatment protocol. Thus, by the end of the 5-day drug treatment, the body weights of the rats in the prednisolone group were 15\% less than those of the rats in the control group.

Similarly, diaphragm weights in the prednisolone group were smaller than diaphragm weights in the control

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1 Grass Instruments, Div of Astro-Med Inc, 600 E Greenwich Ave, West Warwick, RI 02883.
2 Rohm & Haas Co, Independence Mall W, Philadelphia, PA 19105.
4 Abacus Concepts Inc, 1918 Bonita Ave, Berkeley, CA 94704.
5 Apple Computer Inc, 1 Infinite Loop, Cupertino, CA 95014.
The EDL muscle contains a predominance of type IIb fibers, but these results indicate that it is likely that muscle atrophy was not assessed in individual muscle samples. Maximal specific isometric tetanic tension was lower in the prednisolone group as compared with the control group (18.6 ± 0.7 N cm⁻² versus 21.4 ± 0.7 N cm⁻²). There were no differences in twitch properties (P₀, TPT, ½ RT) between the 2 groups.

Discussion

Our results support our hypothesis that a decrease in both body and diaphragm weight occurs with short-term, high doses of prednisolone. Our results also show that administration of short-term, high doses of prednisolone results in a decrease in diaphragm P₀.

The validity of the twitch properties data is questionable because statistical power calculations indicate that the sample size was too small to determine whether a meaningful difference existed between these 2 groups. We believe that a power of approximately 0.80 or greater indicates that a true difference existed. Power analyses for the twitch data produced values ranging from approximately 0.04 to approximately 0.10, thus indicating an inability to detect a difference. These data were collected to supplement the P₀ data. Thus, we believe these data are less important to the overall question of this study. A power analysis, however, revealed a power of 0.80 to detect a difference in P₀ between the 2 groups at the .05 level. Therefore, we believe that the P₀ data indicate that a true difference existed between the 2 groups and that the magnitude of this difference might have been greater with a larger sample size.

Our data show that short-term, high doses of prednisolone result in a 15% loss of body weight. Several studies have used pair-fed controls to test the notion that decreases in body and muscle weights are due to a reduction in caloric intake. The results of studies by Sasson et al⁴ and Van Balkom et al,⁶ however, suggest that glucocorticoids have a detrimental effect on body and muscle weights that cannot be accounted for by a caloric deficit. In both of these studies, glucocorticoid-treated animals lost more weight than did pair-fed controls, leading the authors to suggest that administration of glucocorticoids has a catabolic effect on muscle independent of atrophy due to decreased caloric intake.⁴,⁶ Furthermore, the results of studies by Moore et al⁵ and Gardiner et al⁶ showed that pair-fed control rats actually gained weight during the course of the studies, whereas the glucocorticoid-treated animals lost weight. Because we did not measure caloric intake in our study, we acknowledge that a caloric deficit may have played a role in the weight loss we observed in the glucocorticoid-treated animals. Based on the evidence of previous investigators,²⁴,⁶,²⁰ however, we believe that the decrease in body weight observed in our study was primarily the result of the effects of glucocorticoids.

The study of Nava et al¹⁴ is most similar to our study in terms of treatment duration. Nava and colleagues, however, used a drug dosage (ie, 80 mg/kg/d) that was much greater than our dosage (ie, 5 mg/kg/d). They reported a 20% decrement in body weight after 5 days of glucocorticoid treatment. Comparison with other studies is difficult due to differences in type of glucocorticoid administered (fluorinated versus nonfluorinated) and duration and dosage of treatment.

Both the diaphragm and the EDL muscle weights of the prednisolone group were less than those of the control group. There was no difference, however, in soleus muscle weights between the 2 groups. The degree of muscle atrophy was not assessed in individual muscle fibers, but these results indicate that it is likely that atrophy of both type IIX and type IIB fibers occurred. The EDL muscle contains a predominance of type IIB fibers.

Table 1. Morphological Characteristics of Adult Female Sprague-Dawley Rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control Group (n=16)</th>
<th>Prednisolone Group (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>SEM</td>
</tr>
<tr>
<td>Initial body weight [g]</td>
<td>280.3</td>
<td>2.4</td>
</tr>
<tr>
<td>Final body weight [g]</td>
<td>285.6</td>
<td>2.9</td>
</tr>
<tr>
<td>Costal diaphragm muscle weight [mg]</td>
<td>598.7</td>
<td>11.5</td>
</tr>
<tr>
<td>Extensor digitorum longus muscle weight [mg]</td>
<td>121.6</td>
<td>3.2</td>
</tr>
<tr>
<td>Soleus muscle weight [mg]</td>
<td>115.5</td>
<td>2.8</td>
</tr>
</tbody>
</table>

* Significantly different from control group (P<.05, unpaired t test).
fibers, whereas a large proportion of the fibers of the diaphragm are type IIx fibers. Studies have shown that glucocorticoids cause a preferential atrophy of both type IIx and type IIb fibers. In regard to the soleus muscle, our results agree with data from previous studies that showed the soleus muscle is not affected by glucocorticoids. These results are consistent with the notion that type I muscle fibers are generally resistant to the atrophic effects of glucocorticoids.

An important finding of this study was that the $P_o$ of the diaphragm in the prednisolone group was 13% less than that of the control group. These results are in agreement with those of other studies that have shown a decrease in $P_o$ of the diaphragm following administration of glucocorticoids. Other studies, however, have shown no decrease in diaphragm $P_o$. These conflicting results, in our opinion, are due largely to differences in drug dosage and duration and type of glucocorticoid administered. The study that is most similar to ours is that of Nava et al. Despite using doses of glucocorticoids much greater than ours for the same treatment duration, they found no decrease in diaphragm $P_o$. The mechanisms to explain this difference are unclear at this time.

A reduction in $P_o$ indicates that intrinsic changes occurred in the muscle fibers. The first possibility is that a reduction in the myofibrillar protein density in the diaphragm may have occurred due to glucocorticoid administration. Kayali et al. showed that corticosterone at a dosage of 10 mg/kg for 10 days resulted in a selective loss of myofibrillar proteins from the plantaris muscle. Such an alteration could result in a decrease in maximal isometric tetanic tension by reducing the number of crossbridges available to generate force. Further studies are necessary to confirm this possibility.

An alteration in calcium handling is another factor that may affect maximal isometric tetanic tension. A change in calcium handling would result in a modification in crossbridge cycling kinetics and could possibly affect tension development. The few studies examining the effects of glucocorticoids on calcium handling in skeletal muscle, however, demonstrated conflicting results. The results of our study appear to indicate that this glucocorticoid regimen does not affect the calcium-handling capabilities of treated muscle and support the notion that glucocorticoids directly affect the muscle fibers, as evidenced by the decrease in $P_o$.

In our study, there were no differences in $P_t$, TPT, or $1/2$ RT between the 2 groups, and this finding could have been due to our small sample. Mixed results regarding twitch characteristics have been reported. Several investigators reported no change in $P_t$ in glucocorticoid-treated animals, a decrease in $P_t$ in glucocorticoid-treated animals, or an increase in $P_t$. Similar differences exist when examining TPT and $1/2$ RT measurements. The study by Nava and colleagues is most similar to our study and, with a large sample size, their results indicate a prolongation of both TPT and $1/2$ RT. Again, comparison of studies is difficult considering the type and dosage of glucocorticoid used as well as the time over which the drug was administered.

We believe that the results observed in our study may be clinically relevant. A decrease in diaphragm muscle force may contribute to difficulty in weaning patients with cardiopulmonary dysfunction from mechanical ventilation. Acute diaphragm myopathy has been reported in people with asthma hospitalized with severe exacerbations of their disease and requiring high doses of intravenous glucocorticoids ($\geq 1,000$ mg/d) for short periods of time. These patients had difficulty weaning themselves from the ventilator and, therefore, required an increased length of mechanical ventilation and intensive physical therapy.

**Figure.** Decline in body weight over the 5-day treatment period (repeated-measures analysis of variance, $F=59.01; df=5,135; P=.001$).
Table 2.
In Vitro Costal Diaphragm Strip Characteristics in Female Sprague-Dawley Rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control Group (n=13)</th>
<th>Prednisolone Group (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>SEM</td>
</tr>
<tr>
<td>$P_e$ (N·cm$^{-2}$)</td>
<td>21.4</td>
<td>0.7</td>
</tr>
<tr>
<td>$P_i$ (N·cm$^{-2}$)</td>
<td>5.7</td>
<td>0.2</td>
</tr>
<tr>
<td>$\frac{1}{2}$ RT (ms)</td>
<td>50.0</td>
<td>2.5</td>
</tr>
<tr>
<td>TPT (ms)</td>
<td>53.0</td>
<td>1.8</td>
</tr>
</tbody>
</table>

* $P_e$=maximal specific isometric tetanic tension, $P_i$=peak isometric twitch specific tension, $\frac{1}{2}$ RT=one-half relaxation time, TPT=time to peak tension.


Conclusion
The results of our study show that short-term, high doses of glucocorticoids result in a decrease in both body and diaphragm mass as well as a decrease in diaphragm $P_e$. These results may have clinical implications for physical therapists who provide physical therapy services to patients in intensive care units. Patients may experience difficulty weaning from the ventilator or inability to clear lung secretions. Further research in this area should focus on potential mechanisms underlying glucocorticoid-induced muscle atrophy, recovery from myopathic and contractile changes due to glucocorticoid use, potential changes in endurance characteristics of steroid-treated muscle, and therapeutic interventions to combat these side effects.


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