Anabolic steroids in part reverse glucocorticoid-induced alterations in rat diaphragm

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Van Balkom, Roland H. H., P. N. Richard Dehuijzen, Hans T. M. Folgering, Jacques H. Veerkamp, Herman T. van Moerkerk, Jack A. M. Fransen, and Cees L. A. van Herwaarden. Anabolic steroids in part reverse glucocorticoid-induced alterations in rat diaphragm. J. Appl. Physiol. 84(5): 1492–1499, 1998.—Animal and clinical studies have shown respiratory muscle dysfunction caused by treatment with glucocorticoids. The present study was designed to investigate whether anabolic steroids are able to antagonize the loss of diaphragm force induced by long-term low-dose methylprednisolone (MP) administration. Male adult rats were randomized to receive saline or MP (0.2 mg·kg−1·day−1 sc) during 9 mo, with or without nandrolone decanoate (ND; 1 mg·kg−1·wk−1 im) during the last 3 mo. The ~10% reduction in force generation of isolated diaphragm bundles induced by MP was completely abolished by addition of ND. The MP-induced decrease in number of fibers expressing type IIb myosin heavy chains was not reversed by ND. MP slightly reduced type I, IIa, and IIx fiber cross-sectional areas (CSA), but not type IIb fiber CSA. Addition of ND abolished the reduction in IIa and IIx fiber CSA. The MP-induced alterations in glycolytic activity and fatty acid oxidation capacity were not reversed by ND. In conclusion, the marked reduction in diaphragm force caused by long-term low-dose MP was completely abolished by addition of ND. ND in part also antagonized the effects of MP on diaphragm morphology but showed no beneficial effects on biochemical changes.

glycogenolytic activity; force production; muscle function; myosin heavy chains; respiratory muscles

ANIMAL STUDIES have shown evidence of respiratory muscle dysfunction induced by treatment with non-fluorinated glucocorticoids. Previously, high dosages were studied for short periods of time, resembling acute glucocorticoid myopathy (9). Subsequent studies, with use of lower dosages, showed no changes in rat diaphragm contractile properties after 0.5 mg·kg−1·day−1 of methylprednisolone (MP) for 6 wk (8) or after 1.25 mg·kg−1·day−1 of prednisolone for 4 wk (10).

In contrast, our laboratory recently showed that administration of a low dose of MP (0.2 mg·kg−1·day−1) for 6 mo caused a significant (~15%) reduction in rat diaphragm force generation (31). These functional alterations were accompanied by a reduction in the number of type IIb fibers. Besides, there was a small but significant reduction in type I and IIa fiber cross-sectional area (CSA), whereas type IIx and IIb CSA did not change. These changes resulted in a reduction in the relative contribution of type IIb fibers to total diaphragm muscle area. In addition, MP decreased glycogenolytic activity, whereas fatty acid oxidation and oxidative capacity were increased. It was speculated that the differences from the data in the above-mentioned studies were related to the prolonged period (i.e., 6 mo) of administration of MP in this study (31).

These observations in animal diaphragm after administration of low, clinically relevant dosages of MP for prolonged periods were recently confirmed in clinical studies in patients with chronic obstructive pulmonary disease (COPD) (6, 7). Indeed, a significant decrease in respiratory (and peripheral) muscle strength was observed after treatment with 4.3 mg MP for 6 mo (7). Because treatment with glucocorticoids is sometimes inevitable in these patients, interventions that attenuate or even abolish these alterations in respiratory muscles may be of importance.

In this respect, the use of anabolic steroids may be of interest. Anabolic steroids are able to raise muscle protein by increasing protein synthesis (16). This is of importance because the decrease in muscle protein caused by glucocorticoids is believed to be a major cause of glucocorticoid-induced muscle dysfunction (23). A negative nitrogen balance, indicating a catabolic condition, can be the result of glucocorticoid treatment or malnutrition, both not uncommon in patients with COPD (19). This may in part be reversed by anabolic steroids under the condition that protein intake is adequate. Indeed, Schols and co-workers (26) recently showed that anabolic steroids improved respiratory muscle function in undernourished COPD patients who were refed. However, to our knowledge, the effects of anabolic steroids in clinically relevant dosages on existing glucocorticoid-induced myopathic changes in the diaphragm have not yet been reported.

On the basis of the metabolic effects of anabolic steroids described above, we hypothesized that anabolic steroids are able to reverse the loss in diaphragm force production observed after prolonged administration of MP in clinically relevant dosages (31). To test this hypothesis, we examined in vitro contractile properties of the diaphragm in rats treated with nandrolone decanoate (ND) during the last 3 mo of a 9-mo treatment period with a low dose of MP. Morphological and biochemical parameters were measured to evaluate cellular adaptations to the drugs tested.
METHODS

Study Design, Animals, and Treatment

Adult male outbred Wistar rats (n = 30), age 18–20 wk and weighing 380 ± 25 (SE) g, were randomized into three treatment groups: Saline (0.9% NaCl, 0.2 ml/day sc for 9 mo); MP (0.2 mg·kg−1·day−1 sc for 9 mo); and MP+ND (0.2 mg·kg−1·day−1 sc MP for 9 mo, combined during the last 3 mo with 1 mg/kg im ND every week).

The dose of MP (Sigma Chemical, Bornem, Belgium) used in the present study was based on the observation of similar anti-inflammatory potency and metabolism of MP in rats and humans. If an absorption of 100% is assumed, 0.2 mg kg−1·day−1 of MP would be equivalent to a dose of ~14 mg/day in a 70-kg human. However, the actual biologically available dose may be less because an absorption of only 60% was found after intramuscular injections (22). In addition, the subcutaneous route requires higher doses to produce effects similar to those for intramuscular administration. ND (Organon, Oss, The Netherlands) was selected as an anabolic agent because this drug is a long-acting steroid ester that is slowly hydrolyzed and therefore provides a constant tissue level. The dose used in the present study falls within the range recommended by the manufacturer for humans (50 mg im once every 3 wk and 200 mg im every week) and is one that has been proven to be effective in clinical studies (26).

With each subcutaneous injection (saline or MP), all animals received a similar volume (~0.20 ml). During 9 mo the animals were subcutaneously injected in the neck daily between 8:30 and 10:00 AM. ND was administered, alternating in left and right upper hindlimb. The rats were fed ad libitum, held on a 12:12-h light-dark regime, and were weighed once weekly. Although daily food intake was not accurately quantified (animals were not held in metabolic cages), food intake appeared to be similar in all groups. At the end of the treatment period, contractile properties, and histological, morphometrical, and biochemical characteristics of the diaphragm were examined. The soleus (containing predominantly type I fibers) and extensor digitorum longus (EDL; containing predominantly type IIx/b fibers) were extracted and weighted. All animals were investigated between 23 and 30 h after the last subcutaneous injection. Animals treated with ND were studied 3–5 days after the last ND injection. The study was approved by the Animal Experiments Committee of the University of Nijmegen.

Contractile Properties

The rats were anesthetized with pentobarbital sodium (70 mg/kg ip), and a polyethylene cannula was inserted through a tracheotomy. The animals were mechanically ventilated with an O2-enriched gas mixture (flow 0.5 ml·g body wt−1·min−1, respiration frequency 70 breaths/min, duty cycle 50%). The diaphragm was quickly removed through a combined laparotomy and thoracotomy and was immediately immersed in a cooled, oxygenated Krebs solution at a pH of 7.4. This solution consisted of the following (mM/l): 137 NaCl, 4 KCl, 2 MgCl2, 1 KH2PO4, 24 NaHCO3, 2 CaCl2, and 7 glucose. d-Tubocurarine chloride (25 µM; Sigma Chemical) was added to prevent spontaneous neuromuscular activity. Two small rectangular bundles, parallel to the long axis of the muscle fibers, were dissected from the middle part of the lateral costal region of each hemidiaphragm. Silk sutures were firmly tied to both ends of the bundle to serve as anchoring points. Each bundle was placed in a tissue bath between two large platinum stimulating electrodes. The tissue baths were filled with Krebs solution at 37°C and were oxygenated with 95% O2-5% CO2. The central tendon insertion of the bundles was tied to a fixed point and the costal margin origin to an isometric force transducer (model 31/1437, Sensotec, Columbus, OH). Data acquisition and storage were performed by using a Dash-16 interface and Twist-Trigger software (Instrumental Dept.-electronics, University of Nijmegen). The stimulator (Instrumental Dept.-electronics) was activated by a personal computer. To ensure supramaximal stimulation, subsequent stimulations were performed 20% above the voltage at which maximal forces were obtained. The pulse duration was set at 0.2 ms. Twitch stimuli were used to determine the optimal length, followed by a 15-min thermodilution period. The following measurements were made.

Twitch characteristics. Two twitches were recorded at optimal length to obtain maximal twitch force (P0), contraction time (CT), and one-half relaxation time (RT1/2). The averages were used for statistical analysis.

Maximal tetanic contraction. Two maximal tetanic stimuli (with a frequency of 160 Hz and a train duration of 250 ms) were generated to obtain maximal tetanic force (P0).

Force-frequency protocol. Muscle bundles were stimulated every 2 min with the following frequencies: 25, 50, 80, 120, and 160 Hz (train duration 250 ms). Data were expressed as absolute values (N/cm²) and as percentage of initial P0.

The generated forces were expressed per CSA (N/cm²). CSA was measured by dividing diaphragm bundle weight by muscle density (1.056 mg/mm³) and bundle length.

Histological and Immunohistochemical Procedures

Muscle strips obtained from the costal part of the right hemidiaphragm were embedded in Tissue-Tek in a plastic holder. The muscle fibers were oriented parallel to the long side of the holder. Subsequently, these specimens were quickly frozen in isopentane cooled in liquid N2, followed by further freezing in liquid N2. During this procedure, the diaphragm muscle bundles were not fixed at optimal length. Serial cross sections were cut at 7 µm with a cryostat kept at −30°C. Diaphragm sections of five animals in each group were taken for routine hematoxylin-eosin staining.

Anti-myosin heavy chain (MHC) antibodies (Regeneron Pharmaceuticals) were used for morphometric examination of serial diaphragm sections. The following antibodies were used: BA-DS reactive with type I MHCs; SC-71 reactive with type IIa MHCs; BF-35 reactive with type I, IIa, and IIb but not with type IIx MHCs; and BF-F3 reactive with type IIb MHCs (25). Incubation with anti-MHC antibodies was performed at room temperature for 1 h. Antibodies were subsequently labeled with ultra-small immunogold reagent followed by silver enhancement (Aurion, Wageningen, The Netherlands). A minimum of 300 fibers was analyzed from each diaphragm by using a Sprynt-based, PC-image digital analysis system (Bos, Waddinxveen, The Netherlands). Fiber-type distribution and CSA were analyzed for type I, IIa, IIx, and IIb diaphragm muscle fibers. The relative contribution to total diaphragm muscle area per fiber type was calculated as the product of the mean CSA and fiber distribution in the diaphragm.

Biochemistry

Parameters of the bioenergetic capacity of the diaphragm included the activities of the glycolytic enzymes phosphofructokinase, lactate dehydrogenase, the mitochondrial enzymes 2,4-diketoglutarate dehydrogenase (HADH), a marker for the fatty acid oxidation capacity, and citrate synthase (CS), as an index of citric acid cycle activity.
The procedures used to determine biochemical activities were recently described in detail (32). Briefly, remaining tissues and right hemidiaphragms were frozen in liquid N₂ and stored at −80°C. Segments of fresh-frozen diaphragm were thawed in ice-cooled buffer containing (in mM) 250 sucrose, 2 EDTA, and 10 Tris·HCl (pH 7.4). In this buffer muscle homogenates (5% wt/vol) were prepared by using a Potter-Elvehjem glass-Teflon homogenizer. Total phosphorylase (a+b) activity was assayed at 37°C and expressed as micromoles of NADPH oxidized per minute per gram tissue. CS activity, determined at 25°C, was expressed as micromoles of CoA formed per minute per gram tissue. HADH activity, assessed at 50 µM acetoacyl-CoA at 37°C, was expressed in nanomoles of HADH oxidized per minute per gram tissue. CS activity, determined at 25°C, was expressed as micromoles of CoA formed per minute per gram tissue. The assays for metabolic enzymes were performed spectrophotometrically in duplicate. The coefficient of variation for the assays applied was ~5%.

Data Analysis

Data of contractile properties of the two bundles obtained from one rat were averaged and compared among groups by using one-way analysis of variance followed by Duncan’s multiple-range test. Repeated-measures analysis of variance was used for growth curve analysis. Morphometric analysis was performed by using an average per fiber type per animal that was utilized as a single value in the statistical analysis. All tests were performed by using the SPSS/PC package, version 5.0.1 (Chicago, IL). Results were considered significant at P < 0.05. All data are expressed as means ± SE.

RESULTS

Body and Muscle Weight

At the start of the study, body weight did not differ among the groups. Repeated-measures analysis of variance showed a small but significant effect of treatment on rat body weight during the 9-mo study period (Fig. 1). Body weight gains in Saline, MP, and MP + ND groups were 54 (from 384 ± 7 to 592 ± 9 mg), 44 (from 390 ± 4 to 564 ± 7 mg), and 41% (from 387 ± 5 to 546 ± 9 mg), respectively. Rat body length, measured as nose-anus as well as nose-tail length, was significantly reduced in both the MP and MP + ND groups compared with in the Saline group (Table 1). Total diaphragm muscle weight was not measured because of the speed of handling and the multiple purposes of the tissue.

Diaphragm Bundle Dimensions and Contractile Properties

Diaphragm bundle dimensions were similar in all groups (data for the Saline, MP and MP + ND groups: length 22.7 ± 0.2, 22.6 ± 0.3, and 22.9 ± 0.2 mm; thickness 0.67 ± 0.01, 0.67 ± 0.01, and 0.68 ± 0.01 mm, respectively). After MP treatment, P₁ and P₀, significantly decreased by 10 and 13%, respectively, in comparison with the Saline group. This reduction in diaphragm force generation in the MP group was completely abolished by the addition of ND (Table 2). No changes were found in CT or RT₁/₂. The P₁-to-P₀ ratio was significantly lowered in the MP + ND group compared with in the MP group, but both values did not differ from those in the Saline group.

The force-frequency curves, expressed in N/cm², showed a significant decrease in force generation in the MP group compared with in the Saline group. This downshift was completely reversed by addition of ND to MP (Fig. 2). When force-frequency curves were expressed as a percentage of initial P₀, no differences were observed among the three groups (data not shown).

Histology and Immunohistochemistry

Histological examination of hematoxylin-eosin-stained slides showed a normal muscular pattern in all three groups. No signs of myogenic alterations such as an increase in the number of nuclei, excessive variations in fiber dimensions, or excess of connective tissue were observed.

Although the proportions of the type IIb fibers in the control animals were small, morphometric analysis of the immunohistochemically stained slides showed a significant reduction in the number of type IIb fibers

Table 1. Rat body length

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Body Length</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nose-anus, cm</td>
</tr>
<tr>
<td>Saline</td>
<td>26.3 ± 0.5</td>
</tr>
<tr>
<td>MP</td>
<td>25.8 ± 0.5*</td>
</tr>
<tr>
<td>MP + ND</td>
<td>25.9 ± 0.5*</td>
</tr>
</tbody>
</table>

Values are means ± SE. MP, methylprednisolone; ND, nandrolone decanoate. *P < 0.05 compared with Saline group.

Table 2. Diaphragm contractile properties

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>P₁, N/cm²</th>
<th>CT, ms</th>
<th>RT₁/₂, ms</th>
<th>P₀, N/cm²</th>
<th>P₁/P₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>7.9 ± 0.1</td>
<td>26.4 ± 0.4</td>
<td>23.5 ± 0.4</td>
<td>27.1 ± 0.3</td>
<td>0.29 ± 0.02</td>
</tr>
<tr>
<td>MP</td>
<td>7.1 ± 0.1*</td>
<td>26.8 ± 0.4</td>
<td>24.3 ± 0.5</td>
<td>23.2 ± 0.5*</td>
<td>0.31 ± 0.03</td>
</tr>
<tr>
<td>MP + ND</td>
<td>7.8 ± 0.2</td>
<td>26.3 ± 0.4</td>
<td>23.6 ± 0.3</td>
<td>27.6 ± 0.3</td>
<td>0.28 ± 0.02†</td>
</tr>
</tbody>
</table>

Values are means ± SE. P₁, maximal twitch force; CT, contraction time; RT₁/₂, one-half relaxation time; P₀, maximal tetanic force; P₁/P₀, P₁-to-P₀ ratio. *P < 0.01 compared with Saline and MP + ND. †P < 0.05 compared with MP group.

![Fig. 1. Growth curve. O, Saline group; ■, methylprednisolone (MP) group; □, MP + nandrolone decanoate (ND) group. *P < 0.05, MP and MP + ND groups compared with Saline group.](image-url)
(Table 3) induced by MP. This reduction was not reversed by ND. Type I, IIa, and IIx fiber CSA significantly decreased after MP treatment. In the MP+ND group, the reduction in type IIa and IIx fiber CSA was completely abolished, whereas ND had no effect on type I CSA (Table 3). Type IIb fiber CSA was not significantly changed by MP treatment. Addition of ND to MP resulted in an increase in type IIb fiber size compared with the Saline group. The distribution of fiber CSA per fiber type is shown in Fig. 3. The histogram for type IIb fibers illustrates that the MP-induced decrease in number of IIb fibers occurred without preference for fiber size (Fig. 3D).

As a result of the changes in number and CSA of the different fiber types, there was an increase in the relative contribution of type IIx fibers to total diaphragm muscle area after MP. This increase was not reversed by administration of ND. The reduced contribution of type IIb fibers in the MP group was in part reversed by addition of ND (Table 3).

Table 3. Fiber-type distribution, CSA, and relative fiber-type contribution to total diaphragm muscle area.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Type I</th>
<th>Type IIa</th>
<th>Type IIx</th>
<th>Type IIb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber-type distribution, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>39.6±1.1</td>
<td>31.2±1.0</td>
<td>24.9±0.9</td>
<td>4.3±0.8</td>
</tr>
<tr>
<td>MP</td>
<td>41.6±1.5</td>
<td>30.4±1.6</td>
<td>27.1±1.2</td>
<td>0.9±0.9*</td>
</tr>
<tr>
<td>MP+ND</td>
<td>41.4±0.9</td>
<td>31.0±1.0</td>
<td>24.6±0.6</td>
<td>2.1±0.9*</td>
</tr>
<tr>
<td>Fiber CSA, μm²</td>
<td>1,308±422</td>
<td>1,519±160</td>
<td>4,471±549</td>
<td>7,667±935</td>
</tr>
<tr>
<td>Saline</td>
<td>1,197±335*</td>
<td>1,451±125†</td>
<td>4,177±481†</td>
<td>8,650±894</td>
</tr>
<tr>
<td>MP</td>
<td>1,195±386*</td>
<td>1,513±151</td>
<td>4,716±580</td>
<td>9,514±804*</td>
</tr>
<tr>
<td>MP+ND</td>
<td>215±1.2</td>
<td>19.7±1</td>
<td>45.5±1</td>
<td>13.3±2.1</td>
</tr>
<tr>
<td>Fiber-type contribution to total diaphragm area, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>23.2±0.7</td>
<td>20.7±0.9</td>
<td>52.7±0.9*</td>
<td>3.4±0.9*</td>
</tr>
<tr>
<td>MP</td>
<td>21.2±0.6</td>
<td>20.9±0.7</td>
<td>49.5±0.6*</td>
<td>8.4±1</td>
</tr>
<tr>
<td>MP+ND</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Values are means ± SE. CSA: cross-sectional area. *P < 0.05 compared with Saline group. †P < 0.05 compared with Saline and MP+ND groups.

Biochemistry

Glycogenolytic activity, measured by phosphorylase, was decreased in the diaphragm of the MP-treated animals (P < 0.05) (Table 4). Addition of ND to MP further reduced phosphorylase. HADH, a marker for β-oxidation capacity, increased after MP treatment. In the MP+ND group, HADH significantly decreased compared with MP. MP alone did not change rat diaphragm oxidative capacity, indicated by CS activity. After addition of ND to MP, however, CS activity was reduced (P < 0.05).

DISCUSSION

The present study was designed to investigate whether the anabolic steroid ND was able to reduce changes in rat diaphragm observed after 6 mo of MP therapy, both in low, clinically relevant dosages. The results show that, despite continuation of MP administration for a total period of 9 mo, the reduction in force generation was completely abolished by ND. ND reversed the MP-induced atrophy of type IIa and IIx fibers but had no effect on type I fiber atrophy. Both MP and MP+ND reduced the number of type IIb fibers in the diaphragm. Biochemically, addition of ND to MP decreased oxidative capacity in the diaphragm muscle.

Interaction Between ND and Glucocorticoids: Mechanism of Action

The blunting capacity of ND on MP-induced changes, as observed in the present study, may be due either to a direct anabolic effect of ND on muscle fibers or to an antagonistic action at the receptor level of ND against glucocorticoids, or to a combination of these two actions.

With regard to the first possibility, it is known that anabolic steroids have an effect on normal skeletal muscles, i.e., independent of glucocorticoid treatment. Anabolic steroids promote amino acid incorporation into muscle proteins, decrease amino acid catabolism, and cause nitrogen retention and tissue growth (16). This results in an increase in muscle protein synthesis and an increase in myosin and myofibrillar protein fraction. This may be important in the protection against glucocorticoid-induced fiber atrophy because glucocorticoids are known to reduce protein synthesis (23). The direct effect of anabolic steroids seems to be more pronounced in fast muscle fibers (11).

Second, several interactions between anabolic steroids and glucocorticoids have been described. Anabolic steroids are believed to act via muscle glucocorticoid receptors rather than via muscle androgen receptors in antagonizing the catabolic effects of glucocorticoids (5). Mayer and Rosen (21) proposed a binding competition between androgens and glucocorticoids for the same side of the receptor responsible for mediating the catabolic action of glucocorticoids. Inhibition of glucocorticoid action at the gene level (17) or downregulation of the glucocorticoid receptor content (27) has also been reported as an anabolic effect counteracting glucocorticoid-induced muscular changes.
Besides these specific effects of anabolic steroids on muscle fibers, it has been shown that anabolic steroids increase capillary supply in the diaphragm, resulting in an increase in endurance (12). In androgen-sensitive muscles like the levator ani, anabolic steroids may affect neuromuscular structure and function. The density of acetylcholine receptors at the endplates is increased in the levator ani (3), probably as an adaptive adjustment to the androgen-induced increase in muscle fiber size. This adaptation may be required to maintain a normal synaptic function. Whether such mechanism also occurs in diaphragm neuromuscular junctions is unknown.

Body Weight and Muscle Masses

The effects of anabolic steroids on body weight are gender related. Anabolic steroids increased body weight gain in female animals (2, 5, 11), whereas in males a reduction (2, 24) or similar (5) body weight gain was found compared with control. In the present study design, ND was not able to abolish the small reduction in weight gain caused by MP. The small increase in relative EDL muscle weight in the MP+ND group compared with in the Saline and MP groups and the lack of changes in soleus muscle weight can be explained by a more-pronounced effect of anabolic steroids on fast muscle fibers (11).

It has been suggested that malnutrition is, in part, responsible for the glucocorticoid-induced diaphragm impairment. Indeed, in most animal studies, including the present one, glucocorticoid treatment attenuated in body weight gain. It must be noted, however, that in the present study the differences in body weight gain between the Saline and MP groups were small (54 and

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Table 4. Diaphragm enzyme activities

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Phosphorylase, U/g</th>
<th>CS, U/g</th>
<th>HADH, U/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>44.3 ± 1.3</td>
<td>29.4 ± 1.3</td>
<td>7.97 ± 0.4</td>
</tr>
<tr>
<td>MP</td>
<td>39.9 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.0 ± 0.7</td>
<td>8.83 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MP + ND</td>
<td>33.9 ± 0.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24.8 ± 0.7&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.49 ± 0.16&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± SE. CS, citrate synthase; HADH, 3-hydroxyacyl-CoA dehydrogenase. <sup>a</sup>P < 0.05 compared with Saline group; <sup>b</sup>P < 0.01 compared with Saline group; <sup>c</sup>P < 0.01 compared with Saline and MP groups; <sup>d</sup>P < 0.05 compared with MP group; <sup>e</sup>P < 0.01 compared with MP group.
44% of initial weight in Saline and MP animals, respectively). Addition of ND to the MP-treated animals further decreased body weight, whereas muscle contractile properties were improved. This might, in part, be explained by the increase in fat-free body mass observed as a result of anabolic steroid administration (1, 26). However, rat diaphragm muscle function was reduced after prednisolone administration, whereas contractile properties were not affected in a pair weight control group (33). This suggests that the beneficial effect of ND is related to its property to reverse corticosteroid-induced changes.

Contractile Properties

Previous studies have shown that glucocorticoids can reduce diaphragm force generation, depending on the dose and duration of the administration (10, 32). In the present studies (31) we used a very low glucocorticoid dosage that was 2.5 times lower than the lowest dosage previously reported (8), but the period of administration was prolonged fourfold. The data confirm that the duration of administration is an important factor contributing to the onset of glucocorticoid-induced changes in contractile properties.

The effects of anabolic steroids on skeletal muscle force generation are inconsistent (16). Administration of durabolin increased twitch force and improved fatigue resistance in the EDL muscle of female rats (11). The MP-induced reduction in specific force in the present study was completely reversed by addition of ND to MP. The direct anabolic action of ND on muscle fibers, as well as the antagonizing action of ND on MP, as described above, may be responsible for these drug-induced changes in diaphragm muscle function. This direct effect of ND on muscle fibers results in an increase in muscle protein synthesis and an increase in myosin and myofibrillar protein fraction. This may be an important mechanism in regaining force generation because glucocorticoids are known to reduce these proteins responsible for muscle contraction (23).

Morphometry

In the present study, ND did not reverse the MP-induced decrease in the number of fibers expressing type IIb MHCs. Such an effect would not be expected because ND did not alter the expression of the different MHC genes (30). Moreover, anabolic steroids are not believed to stimulate satellite cells in muscles (4). Thus it is unlikely that new fibers are generated by ND.

The type IIa and IIx fiber atrophy caused by MP was completely abolished by ND. In contrast, ND had no effect on MP-induced type I fiber atrophy. The observation that the effects of anabolic steroids on fast fibers are more pronounced is in accordance with the findings by Egginton (11). This author found hypertrophy of fast fibers in the diaphragm muscle after nandrolone phenylpropionate treatment (1 mg/kg every other day for 5–6 wk), whereas the CSA of slow fibers did not change.

Compared with in the Saline group, MP treatment shifted the fiber contribution to total diaphragm area from type IIb to IIx fibers. Addition of ND did reverse this reduction in type IIb contribution, whereas the increase in type IIx contribution to total diaphragm area compared with the Saline group was still present. Because fiber-type distribution was similar in the MP and MP + ND groups, the changes in fiber contribution to total diaphragm muscle area are the result of the ND-induced changes in fiber CSA. Although changes in type IIb fiber CSA and proportion were statistically significant among the treatment groups, it must be noted that this is probably of minor clinical relevance because the amount of type IIb fibers in the rat diaphragm is small.

The morphometric data in the present study may have been influenced by the fact that muscle strips were not fixed at optimal length during freezing. The excised diaphragm bundle, therefore, was allowed to assume its equilibrium length, resulting in shortening of the muscle. The degree of shortening is associated with loss of passive tension present in vivo (29). In our study this passive muscle tension was similar in the Saline, MP, and MP + ND groups (0.038 ± 0.01, 0.037 ± 0.01, and 0.038 ± 0.01 N, respectively). As a consequence, the degree of muscle shortening (and thus the change in fiber CSA) is not likely to be different among the groups. This, however, does not exclude the possibility of a disproportion in degree of shortening among fiber types. The differences in CSA among type I, IIa, IIx, and IIb fibers in the Saline group, however, were in proportion to the differences in CSA when muscle strips were fixed at optimal length (28). Thus the physiological differences in size among the different fiber types did not appear to be affected by muscle shortening in the present study.

Biochemistry

Treatment with glucocorticoids alone has been shown to increase glycogen storage in rabbit diaphragm muscle (14). In the diaphragm of MP-treated rats (1 mg·kg⁻¹·day⁻¹ for 8 wk), glycolytic activity decreased, oxidative capacity increased, and β-oxidation capacity (HADH) remained unchanged in comparison with the Saline group (32). Short-term (10 days) low-dose prednisolone (0.5, 1, and 2 mg·kg⁻¹·day⁻¹ sc) administration did not change HADH enzyme capacity or CS activity in diaphragm muscle (20).

In soleus or superficial vastus muscle of male rats, nandrolone phenylpropionate (0.5 mg every 2nd day) did not change CS activity or glycogen content (34). Methandienone did not change glycolytic activity or oxidative capacity in the gastrocnemius muscle of male guinea pigs (13). Other investigators observed an increase in oxidative capacity in the EDL muscle of male rats, whereas no such change was found in the soleus muscle (18). Thus anabolic steroids appear to cause little or no change in muscle biochemistry.

In the present study, addition of ND to MP had no beneficial effects on biochemical activities. ND addition even reduced glycolytic activity, β-oxidation capacity, and oxidative capacity compared with MP alone. The mechanism of the additional negative effects on...
diaphragm biochemistry in the ND+MP group is unclear and warrants additional study.

Clinical Significance

The observed reduction in diaphragm force generation after MP administration in this study may be of clinical importance in patients with severe COPD. In these patients, respiratory muscle function may be compromised by factors like hyperinflation, malnutrition, physical inactivity, disturbances in blood gases, and cardiac failure (15).

With regard to the effects of ND, Schols et al. (26) provided evidence that anabolic steroids may be beneficial in regaining respiratory muscle strength in malnourished COPD patients. This was probably the result of an increase in muscle mass in patients receiving ND in addition to the nutritional support. A recent study by Bhasin and co-workers (1) showed a beneficial effect of a high dose of testosterone (600 mg/wk) on fat-free body mass, muscle size, and peripheral muscle strength in normal men. The clinical applicability of anabolic drugs in glucocorticoid-induced myopathy has yet to be evaluated.

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REFERENCES


