Nandrolone decanoate does not enhance training effects but increases IGF-I mRNA in rat diaphragm

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MATERIALS AND METHODS

Animals, Training, and Treatment

Forty 15-wk-old male (360 ± 34 g, n = 20) and female (229 ± 57 g, n = 20) Wistar rats were randomly assigned to an

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RESPIRATORY MUSCLE WEAKNESS plays an important role in the pathophysiology of ventilatory failure in patients with chronic obstructive pulmonary disease or chronic myopathy. In an attempt to improve respiratory muscle function in these patients, specific inspiratory muscle training (IMT) was used to ameliorate endurance and/or force of the inspiratory muscles, as well as exercise performance, and to decrease dyspnea (6, 21, 39). However, motivation of the patients is an important factor influencing the results of such tests, such that data are difficult to compare among studies. Moreover, direct assessment of training effects on respiratory muscle function is difficult in patients. Therefore, to better understand the relationship between the training stimulus and its resulting effects on inspiratory muscles, our laboratory previously developed an animal model to simulate IMT as applied in patients (9). After 8 wk of intermittent inspiratory resistive loading (5 times/wk, 30 min/day), type II fiber hypertrophy was obtained in the diaphragm of trained rats (9).

Another way to induce muscle hypertrophy and to expect improvement in muscle function is to treat patients with anabolic agents. The role of such medication meant to improve respiratory muscle contractility has never been investigated in chronic obstructive pulmonary disease patients or in patients with corticosteroid-induced myopathy. The effects of anabolic agents, in combination with training, have, to the best of our knowledge, never been investigated in the diaphragm, whereas they are well documented in peripheral muscles, both in humans and in animals (2, 7, 17, 18, 24, 32, 34, 35).

Finally, hypertrophy as a response to training and/or anabolic treatment is expected to result from changes in muscle proteins, thereby suggesting a potential role of the growth factors. Of the various growth factors, insulin-like growth factor I (IGF-I) is of particular interest because of its ability to stimulate growth and differentiation of skeletal muscle cells (19). IGF-I is not only secreted in liver where its production is under control of growth hormone but is also produced in a variety of extrahepatic tissues, including skeletal muscles (13, 38). This latter production of IGF-I has been shown to be involved in hypertrophic adaptation of muscle after mechanical loading, and this is independent of growth hormone modulation (1). Enhanced local expression of the IGF-I gene has also been demonstrated after work-induced compensatory hypertrophy in rat skeletal muscle (13).

The present study was thus designed to examine whether concomitant treatment with an anabolic steroid (nandrolone decanate) might enhance the effects produced by intermittent inspiratory muscle loading in rat diaphragm and whether IGF-I mRNA may be involved in this process. The study was performed in adult male and female rats trained separately by sex.

Animals, Training, and Treatment

Forty 15-wk-old male (360 ± 34 g, n = 20) and female (229 ± 57 g, n = 20) Wistar rats were randomly assigned to an

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IMT (n = 30, 15 each sex) or to a control (C) group (freely moving, n = 10, 5 each sex). Animals subjected to IMT breathed through a Hans-Rudolph valve attached to a rigid mask while they were trained in a whole body cylinder, as previously described (9). During the training period, these animals had to overcome a load applied to the inspiratory limb of the Hans-Rudolph device, which consisted of a needle of decreasing diameter, while the expiratory limb was left free. After 11 days of conditioning, animals in the IMT group were able to breathe through the training device for 30 min uninterrupted. Thereafter, each animal in the IMT group was held in the training device for 30 min/day, at a frequency of 5 times/wk, for 8 wk (training period). Initially, the internal diameter of the needle placed at the inspiratory port of the valve was 0.8 mm (equivalent to resistance of 0.7 cmH2O·ml⁻¹·s⁻¹ at a constant flow of 5 ml/s), and it was progressively decreased, reaching 0.15 mm (equivalent to 28 cmH2O·ml⁻¹·s⁻¹) at the end of the training period. Animals were trained by sex, males apart from females.

In addition, 3 wk after training was started, rats (IMT and C) were randomized into three groups to receive weekly 5, 15, or 25 wk intramuscular injection into the left hindlimb of the following: 1) 0.1 and 0.08 ml saline (IMT and C groups), for male and female rats, respectively; 2) 1.5 mg/kg nandrolone decanoate [low dose (LD)], or 3) 7.5 mg/kg nandrolone decanoate [high dose (HD)].

Nandrolone decanoate was selected as the anabolic agent because it is a long-acting steroid ester that is slowly hydrolyzed to give a constant tissue level of steroid. This steroid form is reported to have a lower incidence of liver toxicity than orally absorbed anabolic steroids. As the manufacturer-recommended (Organon) dose of nandrolone decanoate in humans falls within the 50- to 100-mg range every 3–4 wk, a dose of 1.5 or 7.5 mg/kg body wt given every week, as used in the present study, corresponds to a high therapeutic and supertherapeutic dose, respectively. Male and female animals were chosen because of different basal levels of circulating endogenous androgens. Finally, because anabolic effects are believed to be more pronounced in subjects previously trained (2, 16), drug administration started after 3 wk of training.

**Variables Measured During Training**

During each training session, snout pressure (P_snout) was measured continuously via a pressure transducer (range ±250 cmH2O, Validyne MP45) and recorded on computer by using Labdat (Labdat/Anadat, RHT-InforDat, Montreal, PQ). Signals were analyzed by applying Anadat software. For each animal and over the 30-min training session, the following measurements were made with anadat on the middle and the last day of training, days at which the same load was applied: 1) maximal pressure swing (P_max), 2) the sum of positive and negative pressures (P_neg), and 3) breathing rate. Finally, randomized samples were taken to determine inspiratory time (T_i) and total duration of respiratory cycle (T_t). These data were used to determine the tension-time index of the diaphragm, which is T_t/T_max × P_max/P_t, where P_max is the maximal inspiratory pressure, which is the predicted maximal pressure generated by the respiratory muscles of the rat, and was assumed to be ~80 cmH2O (unpublished data).

**Removal and Dissection of Muscle Bundles**

One week after the last injection, rats were anesthetized with pentobarbital sodium (Nembutal, 60 mg/kg ip), tracheotomized, and mechanically ventilated (Harvard pump respirator, Harvard Apparatus, South Natick, MA) with an O2-enriched gas mixture. The diaphragm was quickly removed through a laparotomy and immediately immersed in a coded, curarized, oxygenated Krebs solution containing (in mM) 137 NaCl, 4 KCl, 2 CaCl2, 1 MgCl2, 1 KH2PO4, 12 NaHCO3, and 6.5 glucose. Two small rectangular bundles (width <2 mm) from the middle part of the lateral costal region of each hemidiaphragm were obtained by careful dissection parallel to the long axis of the fibers. Both ends of each bundle were tied with silk sutures to serve as anchoring points. The bundles were suspended in a tissue bath containing Krebs solution and were continuously aerated with 95% O2–5% CO2. Temperature was maintained at 37°C by using a thermostatically controlled water pump. The bundles were placed in two large platinum stimulating electrodes and were anchored at the bottom to a rigid support and fastened at the top to an isometric force transducer (Maywood, Hampshire, UK) connected to a micrometer. Signals were amplified and recorded on computer via analog-to-digital conversion (DT-2801A) by using Labdat (Labdat/Anadat). Stimulations were delivered through a Harvard 50–5016 stimulator (Edenbridge, Kent, UK) connected to a power amplifier made from power one model HS24–4.8, which was developed by the computer technology resources center, University of Virginia (R. J. Evans, 1983). Optimal muscle length (L_o) for peak twitch force was established for each bundle.

The following measurements were performed at L_o after a thermoequilibration period of 15 min: twitch characteristics, maximal tetanic tension, force-frequency curve, and fatigue run.

**Twitch characteristics.** Maximal twitch tension (P_t) was obtained from two successive twitch stimulations (1 Hz). The twitch force was established for each bundle.

**Force-frequency curve.** The force-frequency relationship was measured by using the following order of frequencies with a 2-min interval between stimulations: 25, 160, 50, 160, 80, 160, 120, and 160 Hz.

**Fatigue properties.** First, the tension developed at 160 Hz after each stimulus during the force-frequency protocol was measured, and, second, fatigue was induced by a 5-min stimulation run consisting of repeated 25-Hz stimulations of 330 ms applied every 3 s.

At the end of the in vitro experiment, each muscle bundle was removed from the bath, and its length, width, and thickness were measured at L_o. They were blotted dry and weighed. All tensions were normalized for cross-sectional area (CSA).

The remaining diaphragm tissue, scalenus medius, para-sternal intercostals (including sternum and chondral parts of the ribs), muscular gastrocnemius, muscle soleus, musculus plantaris, musculus extensor digitorum longus of the right hindlimb, and the heart were dissected, trimmed, blotted, and weighed.

**Muscle Area and Fiber Type Analysis**

The right costal region of the diaphragm, the middle part of the scalenus and gastrocnemius, was folded, cut transversely, and placed at excised length on “tissue glue” (Tissue-Tek, Elkhart, IN) on a cork holder, with the fibers oriented perpendicularly to the surface of the cork. The preparations were...
frozen in isopentane cooled with liquid N₂. Afterwards, serial sections parallel to the cork were cut in 10-µm thicknesses with a cryostat kept at −20°C. Two sections of each muscle were stained for routine hematoxylin and eosin staining, whereas the other serial sections were stained for ATPase after acid preincubation at pH 4.5 and 4.3. On the basis of their histochemical reactions, fibers were identified as slow-twitch type I, fast-twitch type IIa, or fast-twitch type IIx/b fibers. CSAs were determined from the number of pixels within the outlined borders by using a Leitz Laborlux S microscope (Wetzlar, Germany) at ×20 magnification, connected to a computerized image system (Quantimet 500, Leica, Cambridge, UK). Between 100 and 250 fibers of each muscle were used to calculate CSA of all fiber types and proportions. In addition, for all muscles studied, CSAs were corrected for the shortening occurring from L₀ according to a formula obtained in our laboratory from a study with 32 rats.

RNA Extraction

Samples of diaphragm obtained from each group were frozen in liquid N₂. Total RNA was isolated by using a modified guanidinium isothiocyanate-CsCl method (12). Quality and quantity of the RNA preparations were determined by measurement of absorbance at 260 and 280 nm and by Northern blot analysis.

Northern Blotting

Samples of 20 µg of RNA were separated on a 1% agarose gel containing formaldehyde as described (30). After electrophoresis, the RNA was transferred to Biotrans (Bethesda, MD) was excised from the pGEM3 vector with restriction endonucleases (Bethesda, MD) was excised from the pGEM3 vector with restriction endonucleases and subsequently analyzed. Comparisons among the different groups in male and female animals were performed by using one-way analysis of variance. Differences among means were assessed by using Gabriel/Dunnett’s multiple range test. Statistical significance was set at P < 0.05. Data are expressed as means ± SD.

RESULTS

Respiratory Variables During Training

Data from the middle day of training were similar to those of the last day. Table 1 illustrates the breathing characteristics on the last day of training (day 40) in male and female rats of all groups together. Neither dose nor treatment had an effect on the breathing pattern.

Body and Muscle Weight

Starting body weight was similar among the groups, both in male (C: 344 ± 14, IMT: 376 ± 20, LD: 377 ± 9, HD: 371 ± 43 g) and female rats (C: 231 ± 3, IMT: 228 ± 9, LD: 230 ± 2, HD: 229 ± 6 g). Similarly, no differences in body weight were observed at the beginning of training in male (C: 356 ± 11, IMT: 368 ± 18, LD: 367 ± 7, HD: 370 ± 46 g) and female animals (C: 234 ± 8, IMT: 227 ± 7, LD: 228 ± 7, HD: 229 ± 7 g).

Training and nandrolone decanoate affected body weight differently in male and female rats (Fig. 1). In

Table 1. Breathing characteristics in male and female animals on the last day of training (day 40)

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pmax, cmH₂O</td>
<td>−14.6 ± 4.1</td>
<td>−13.6 ± 5.6</td>
</tr>
<tr>
<td>Ti/Tt</td>
<td>0.61 ± 0.04</td>
<td>0.61 ± 0.07</td>
</tr>
<tr>
<td>TTI</td>
<td>0.03 ± 0.01</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>BR, breaths/min</td>
<td>177 ± 24</td>
<td>156 ± 29</td>
</tr>
</tbody>
</table>

Values are means ± SD of pooled data of trained and treated rats. Pmax, maximum pressure swing; Ti/Tt, ratio of inspiratory time to total duration of breathing cycle; TTI, tension-time index; BR, breathing rate.
male rats, body weight increased more in controls than in the other groups (Fig. 1A). In the IMT and LD groups, body weight remained stable over time, whereas it slightly increased in the HD group (Fig. 1A). Thus, at the end of the study, compared with respective initial value, body weight increased by 17 ± 8% in controls (406 ± 19 g, P < 0.001, control vs. other groups) and only by 4 ± 7% in the HD group (386 ± 47 g), whereas it was unchanged in the IMT (368 ± 12 g) and LD groups (375 ± 7 g).

In female rats, growth was enhanced from the third injection with nandrolone, and the body weight curve was similar in C and IMT groups (Fig. 1B). At the end of the study, compared with respective initial value, body weight increased by 12 ± 5 and 9 ± 4% in C (260 ± 8 g) and IMT (251 ± 2 g) groups and by 16 ± 6 and 18 ± 1% in LD (265 ± 14 g) and HD (265 ± 10 g) groups, respectively.

The weight response of the respiratory and peripheral muscles was similar in male and female rats compared with their respective controls (Table 2). In fact, nandrolone treatment failed to induce any changes in muscle weights, except for the heart, which increased dose dependently after treatment in females only (Table 2). This effect was still present in HD-treated female rats when heart mass was expressed as a percentage of respective body weight (C: 0.253 ± 0.014, IMT: 0.252 ± 0.014, LD: 0.249 ± 0.011, HD: 0.273 ± 0.013% body wt; P < 0.05, HD vs. other groups).

Geometry and Weight of Diaphragm Bundles

In males as well as in females, individual diaphragmatic bundle dimensions and bundle weight were similar in the four groups.

Diaphragmatic Contractile Properties

For either sex, diaphragmatic twitch kinetics did not change with treatment, neither did P1, P0, or P1/P0 (Table 3).

Compared with C, nandrolone decanoate did not modify the diaphragm response to increasing stimulus frequencies when data were expressed either in grams per centimeters squared or as a percentage of instantan-

### Table 2. Muscle weight in male and female animals

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>IMT</th>
<th>LD</th>
<th>HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diaphragm</td>
<td>0.583 ± 0.062</td>
<td>0.595 ± 0.057</td>
<td>0.599 ± 0.064</td>
<td>0.612 ± 0.051</td>
</tr>
<tr>
<td>Scalenus medius</td>
<td>0.557 ± 0.032</td>
<td>0.520 ± 0.043</td>
<td>0.510 ± 0.055</td>
<td>0.503 ± 0.061</td>
</tr>
<tr>
<td>Parasternals</td>
<td>2.531 ± 0.324</td>
<td>2.686 ± 0.059</td>
<td>2.751 ± 0.186</td>
<td>2.751 ± 0.324</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>1.891 ± 0.146</td>
<td>1.918 ± 0.228</td>
<td>1.954 ± 0.046</td>
<td>1.955 ± 0.167</td>
</tr>
<tr>
<td>Plantaris</td>
<td>0.375 ± 0.033</td>
<td>0.415 ± 0.072</td>
<td>0.393 ± 0.014</td>
<td>0.403 ± 0.052</td>
</tr>
<tr>
<td>Extensor digitorum longus</td>
<td>0.174 ± 0.018</td>
<td>0.183 ± 0.016</td>
<td>0.184 ± 0.008</td>
<td>0.189 ± 0.025</td>
</tr>
<tr>
<td>Soleus</td>
<td>0.157 ± 0.021</td>
<td>0.156 ± 0.015</td>
<td>0.154 ± 0.009</td>
<td>0.167 ± 0.019</td>
</tr>
<tr>
<td>Heart</td>
<td>0.839 ± 0.102</td>
<td>0.828 ± 0.036</td>
<td>0.867 ± 0.063</td>
<td>0.908 ± 0.076</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diaphragm</td>
<td>0.440 ± 0.034</td>
<td>0.463 ± 0.011</td>
<td>0.465 ± 0.047</td>
<td>0.481 ± 0.043</td>
</tr>
<tr>
<td>Scalenus medius</td>
<td>0.390 ± 0.061</td>
<td>0.375 ± 0.046</td>
<td>0.396 ± 0.035</td>
<td>0.372 ± 0.037</td>
</tr>
<tr>
<td>Parasternals</td>
<td>1.742 ± 0.104</td>
<td>1.762 ± 0.096</td>
<td>1.923 ± 0.116*</td>
<td>1.929 ± 0.145*</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>1.296 ± 0.032</td>
<td>1.341 ± 0.061</td>
<td>1.384 ± 0.190</td>
<td>1.347 ± 0.081</td>
</tr>
<tr>
<td>Plantaris</td>
<td>0.274 ± 0.021</td>
<td>0.277 ± 0.010</td>
<td>0.274 ± 0.041</td>
<td>0.284 ± 0.023</td>
</tr>
<tr>
<td>Extensor digitorum longus</td>
<td>0.125 ± 0.009</td>
<td>0.132 ± 0.006</td>
<td>0.133 ± 0.016</td>
<td>0.124 ± 0.008</td>
</tr>
<tr>
<td>Soleus</td>
<td>0.114 ± 0.013</td>
<td>0.116 ± 0.004</td>
<td>0.103 ± 0.014</td>
<td>0.112 ± 0.010</td>
</tr>
<tr>
<td>Heart</td>
<td>0.653 ± 0.051</td>
<td>0.627 ± 0.036</td>
<td>0.666 ± 0.033</td>
<td>0.727 ± 0.039</td>
</tr>
</tbody>
</table>

Values are means ± SD in g. IMT, inspiratory muscle trained rats; LD, trained and low-dose nandrolone decanoate-treated rats; HD, trained and high-dose nandrolone decanoate-treated rats. *P = 0.05 vs. control; †P = 0.01 vs. other groups.
neous $P_o$, in either male (Fig. 2A) or female animals (Fig. 2B).

The response of the diaphragm to fatigue was treatment and dose independent. The course and magnitude of the force decline of the 160 Hz during the force-frequency protocol was not different among groups, reaching a decline of 18 ± 9 and 7 ± 6% of baseline in male and female animals, respectively (pooled values of the 4 groups).

In male animals, the fatigue profile during the fatigue run was similar among groups, such that diaphragmatic tension decreased by 54 ± 4% at the end of the run. In females, the diaphragmatic force generated by the C group during the fatigue run was higher compared with that generated by the other groups (Fig. 3A). These effects disappeared when force was expressed as a percentage of the initial value, so that fatigue was similar among the four groups at the end of the run (51 ± 8%) (Fig. 3B).

Histology and Histochemistry

Within the same sex, histological examination of routine hematoxylin- and-eosin-stained slides of diaphragm, scalenus medius, and gastrocnemius showed no obvious differences among groups.

In male rats, compared with the C group, IMT was associated with an increase in diaphragm type I CSA (126%; $P < 0.005$, IMT vs. C and HD) and type IIa (122%; $P < 0.05$, IMT vs. C) and, to a lesser extent, type IIx/b CSA [19%, not significant (NS)]. Additional treatment with nandrolone decanoate did not result in further increases in CSA. In fact, HD resulted in a moderate decrease (-15%) in the CSA of type I fibers compared with IMT ($P < 0.05$; Fig. 4A). Finally, fiber proportion in male diaphragm remained unchanged.

In female animals, diaphragm fiber dimensions were not affected by training or treatment with anabolic steroids (Fig. 4B). However, independent of training, the proportion of type IIa fibers in the diaphragm significantly increased after LD (+35%; $P < 0.05$, LD

Table 3. Diaphragm contractile properties in male and female rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>IMT</th>
<th>LD</th>
<th>HD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_t$, g/cm²</td>
<td>559 ± 111</td>
<td>543 ± 149</td>
<td>519 ± 37</td>
<td>510 ± 107</td>
</tr>
<tr>
<td>$P_o$, g/cm²</td>
<td>2,487 ± 432</td>
<td>2,430 ± 483</td>
<td>2,341 ± 212</td>
<td>2,389 ± 361</td>
</tr>
<tr>
<td>TPT, ms</td>
<td>20.90 ± 0.93</td>
<td>18.18 ± 2.68</td>
<td>17.53 ± 2.06</td>
<td>18.95 ± 2.75</td>
</tr>
<tr>
<td>RT&lt;sub&gt;½&lt;/sub&gt;, ms</td>
<td>23.83 ± 2.30</td>
<td>23.25 ± 4.18</td>
<td>26.78 ± 5.86</td>
<td>24.38 ± 2.02</td>
</tr>
<tr>
<td>$P_t/P_o$</td>
<td>0.224 ± 0.019</td>
<td>0.221 ± 0.027</td>
<td>0.223 ± 0.020</td>
<td>0.212 ± 0.025</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_t$, g/cm²</td>
<td>601 ± 77</td>
<td>576 ± 89</td>
<td>599 ± 118</td>
<td>537 ± 85</td>
</tr>
<tr>
<td>$P_o$, g/cm²</td>
<td>2,578 ± 195</td>
<td>2,511 ± 245</td>
<td>2,514 ± 162</td>
<td>2,441 ± 471</td>
</tr>
<tr>
<td>TPT, ms</td>
<td>24.1 ± 1.95</td>
<td>21.4 ± 2.16</td>
<td>23.1 ± 2.68</td>
<td>21.8 ± 1.92</td>
</tr>
<tr>
<td>RT&lt;sub&gt;½&lt;/sub&gt;, ms</td>
<td>27.1 ± 3.51</td>
<td>26.1 ± 2.82</td>
<td>23.1 ± 2.53</td>
<td>23.7 ± 3.56</td>
</tr>
<tr>
<td>$P_t/P_o$</td>
<td>0.231 ± 0.028</td>
<td>0.229 ± 0.02</td>
<td>0.240 ± 0.04</td>
<td>0.223 ± 0.008</td>
</tr>
</tbody>
</table>

Values are means ± SD. $P_t$, twitch tension; TPT, time to peak tension; RT<sub>½</sub>, half relaxation time; $P_o$, tetanic tension; $P_t/P_o$, ratio of $P_t$ to $P_o$. 

Fig. 2. Force-frequency curve of diaphragm in male (A) and female (B) rats. Values are expressed as a percentage of interposed 160-Hz tetanic force ($P_o$). Symbols defined as in Fig. 1 legend.

Fig. 3. Diaphragm force in female rats developed during fatigue run. Values are expressed in absolute terms (A) or as a percentage of initial values (B). Symbols defined as in Fig. 1 legend. *$P < 0.03$ vs. other groups; **$P < 0.04$ vs. IMT and HD.
vs. C and IMT). Similarly, the proportion of type IIx/b fibers decreased after LD (216%; P < 0.05, LD vs. IMT) and HD (220%; P < 0.05, HD vs. IMT).

For the scalenus medius, CSA of type IIx/b fibers significantly decreased by 17% after HD compared with the C group (P < 0.05) in male rats. By contrast, in female animals, IMT resulted in an increase in type IIx/b CSA compared with the C group (120%; P < 0.01, IMT vs. C) and an even greater increase in the LD group (137%; P < 0.01, LD vs. C), whereas HD did not further enhance fiber dimensions of the scalenus medius.

Increased type I CSA of the gastrocnemius (112%, NS, LD vs. C) was present in female rats after LD. In male animals, both type I CSA (115%, NS, other groups vs. C) and type IIx/b CSA (114%, NS, other groups vs. C) increased after nandrolone decanoate, but these increases failed to reach statistical significance.

Diaphragm IGF-I mRNA Measurement

Northern analysis of RNA extracted from the diaphragm muscle demonstrated that multiple IGF-I mRNAs were present in the tissue examined (Fig. 5). The apparent sizes of these transcripts were 7–7.8, 1.6–2.1, and 0.8–1.2 kb, corresponding to IGF-I transcripts in skeletal muscles as previously mentioned (31). After hybridization with an 18S cDNA probe, dot-blot data showed that IMT did not increase IGF-I expression for either sex (Fig. 6). However, in male animals (Fig. 6A), HD increased the levels of IGF-I mRNA, compared with IMT and C, by 73 and 77%, respectively (P < 0.05). In female animals (Fig. 6B), both LD and HD increased IGF-I mRNA compared with C (by 85 and 129%, respectively, P < 0.001) and IMT (by 58 and 96%, respectively, P < 0.001).

DISCUSSION

The present data demonstrate that administration of nandrolone decanoate to adult rats subjected to an IMT did not enhance the previously observed training effects observed in the diaphragm. Indeed, diaphragmatic weight, its in vitro contractility, and fatigue resistance were unaltered by concomitant administration of anabolic steroids, although the IGF-I mRNA expression increased and diaphragm type I atrophy was present in male rats.

First, it should be mentioned that anabolic effects are influenced by a series of variables, as detailed previously (see references in Ref. 8). All of these variables probably play a role in the lack of consistent results found in the literature when anabolic agents are used.

Second, it is worthwhile to mention that no systematic differences appeared in the present study between LD- and HD-treated animals. This is in keeping with the study of Tsika et al. (37) in which no additional effect was shown in young female rats treated with either 15 or 45 mg·kg⁻¹·wk⁻¹ nandrolone decanoate. Also, in studies performed in sedentary animals (5), no linear relationship within a sex between dose and effect was found.

In the present study, the training protocol and the load applied on the respiratory muscles were similar to those obtained in a previous study (9). In accordance with this study (9), the training in the present study was characterized by a high number (>150 breaths/...
min) of repetitive low-load (18% of estimated PImax) contractions, as in endurance training, and resulted in structural adaptations of the diaphragm. Because this specific training model was extensively compared with other models of respiratory muscle training, available both in humans and animals, in our previous study and because the hypertrophic response of the diaphragm to training was previously described (9), only the effects of nandrolone decanoate combined with training are discussed here.

In our study, nandrolone decanoate treatment did not affect respiratory variables during training, no matter what the dose and sex. Thus, because no differences in breathing characteristics were found among treatment groups (Table 1), observed differences between saline and nandrolone-treated animals cannot be attributed to differences in training load. Interaction between training and anabolic agents has only been investigated in peripheral muscles in both humans and animals (2, 7, 17, 18, 24, 32, 34, 35). It remains difficult, however, to compare training programs such as weight bearing, treadmill exercise, or swimming to IMT, because different groups of muscles are used with regard to exercise types. Thus, during strength training, an increase in the number of androgen binding sites has been reported, thereby making the trained muscles more susceptible to anabolic compounds (23). It is also not obvious whether anabolic steroids improve aerobic capacity more than would be expected by aerobic training alone (25). The capillary supply during strength exercises, as opposed to endurance, seems to be of minor importance in providing the muscle with adequate oxygen and blood substrates (36). Because nandrolone decanoate may have some deleterious effects on the muscle capillaries and mitochondria (32), this anabolic agent might be more effective in strength training.

In our study, body weight response to nandrolone is in keeping with other studies in which trained animals were given anabolics, showing enhanced growth in female rats (17, 37) and no additional changes in male rats (18, 22, 29). The exact mechanism for this sex-dependent anabolic effect is not known, but the basal level of circulating endogenic androgens may probably play a role, as previously described (8).

Similar to previous studies in which the effects of strength or endurance training combined with anabolic treatment were examined in skeletal muscles (4, 17, 22, 24, 26, 35), none of the respiratory or peripheral skeletal muscle weights investigated in our study was affected by nandrolone treatment. Also, in sedentary rats, skeletal muscle weight changed proportionally to body weight changes (14, 15, 27, 28). Surprisingly, heart weight in our study increased in females treated with a high dose of nandrolone decanoate, whereas, up to now, only nonsignificant changes were reported in the literature (4, 11, 18, 22, 35). Differences in training type, steroid dose, and steroid type may, in part, explain this difference in heart weight.

In the present study, diaphragmatic contractile properties remained unchanged after nandrolone treatment. The effects of anabolic agents in combination with training have never been investigated in the diaphragm. Several studies examined effects on contractility of peripheral muscles and revealed no additional effects of anabolic agents to those obtained with training alone (14, 17, 18, 26, 34), as in the present study in the diaphragm. On the contrary, an increase in isometric force characteristics was described in the gastrocnemius of exercised rats concomitantly treated with di-anabol (26). Discrepant results may be due either to muscle specificity (26), to differences in age of the rats (immature vs. mature), thus leading to differences in contractile properties with age (18), or to a graded response of different muscles to androgen administration because of differences in the level of androgen receptors within the muscle cytosol (10), although there does not appear to be any considerable fiber type difference in androgen binding levels (23).
In our study, nandrolone treatment in addition to training induced a type I fiber atrophy of the loaded diaphragm. This is in agreement with the results of Soares and Duarte (32), which showed a decrease in type I fiber dimensions in trained soleus from mice treated for 6 wk with 15 mg·kg⁻¹·wk⁻¹ nandrolone. No other studies have examined morphological adaptations of the diaphragm after training combined with anabolic agent treatment. The repercussions of this histochemical adaptation in diaphragm in our study is, however, discrete because diaphragmatic contractile properties were unaffected. Finally, independent of training, nandrolone decanoate increased fiber dimensions of the gastrocnemius, as previously observed (8).

To the best of our knowledge, the effects of IMT, nandrolone decanoate treatment, or both combined on IGF-I mRNA levels in muscle have never been investigated. In our study, IMT alone did not alter IGF-I mRNA levels, although diaphragm fiber hypertrophy was noticed (Fig. 6, A and B). By contrast, anabolic steroid independent of IMT resulted in increased levels of IGF-I mRNA in the diaphragm, whereas no further hypertrophy was observed. In fact, nandrolone decanoate treatment was even associated with a decreased type I CSA in the diaphragm of male treated animals, whereas in female treated rats, such treatment induced a decrease in the type IIx/b proportion, which was compensated by an increase in the type IIa proportion. Obviously, the higher IGF-I levels are, therefore, likely to be due to anabolic steroid per se rather than to training. The reasons why this increase in diaphragm IGF-I mRNA content was not associated with diaphragm hypertrophy are not clear. However, our data suggest that alterations in IGF-I mRNA levels in the diaphragm are likely not to play a causal role in the muscle adaptations observed in our study. Indeed, whereas IMT alone resulted in fiber hypertrophy in the diaphragm, IGF-I mRNA levels in this muscle were unchanged compared with controls. Along the same lines, whereas anabolic steroid treatment did not induce diaphragm fiber hypertrophy, IGF-I mRNA content was enhanced. Therefore, it seems that the diaphragmatic changes in IGF-I mRNA and in fiber dimensions observed in our study are two dissociated events. Such phenomenon has been previously reported in other models such that increased (33) or unchanged (20) IGF-I mRNA levels were observed in the diaphragm while diaphragm atrophy was present. Thus decreased diaphragm fiber dimensions or proportion after nandrolone decanoate, as observed in the present study, in association with increased IGF-I mRNA is not as surprising as it could be thought at first sight.

Finally, it remains to be established whether increased IGF-I mRNA, as observed in the diaphragm in our study after nandrolone decanoate, is effectively associated with an increase in IGF-I at the protein level. In fact, because diaphragm hypertrophy was not observed after treatment, this may indicate that increased IGF-I protein production was absent. Alternatively, if IGF-I production in diaphragm was enhanced after nandrolone decanoate treatment, IGF-I action may have been impaired, e.g., by concordant changes in the production of IGF binding proteins or by changes in IGF-I receptor levels or sensitivity. Further research is, however, needed to unravel these issues.

Although the present study was performed in rats, our results suggest that administration of nandrolone decanoate to healthy individuals submitted to an IMT program would not result in additional benefit to the respiratory muscles. This does not mean that patients in a chronic state of catabolism might not benefit from anabolic steroids in addition to training, keeping in mind that the present study was performed in normal rats not under the catabolic state. Indeed, hypertrophy has been noted in anabolic-steroid-treated patients in whom tissues were in some form of a depleted state (3).

In summary, our results show that the administration of nandrolone decanoate in combination with IMT did not further enhance the effects previously observed in diaphragm with IMT alone, although anabolic steroid treatment was associated with increased IGF-I mRNA levels in the diaphragm. The latter phenomenon was thought not to play a causal role in the diaphragm changes observed in the present study.

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