Effects of nandrolone decanoate on respiratory and peripheral muscles in male and female rats

ANJA BISSCHOP,1 GHISLAINE GAYAN-RAMIREZ,2 HÉLÈNE ROLLIER,2 P. N. RICHARD DEKHUIJZEN,3 RENÉ DOM,3 VERA DE BOCK,2 AND MARC DECRAMER2
1Faculty of Physical Education and Physiotherapy, 2Respiratory Muscle Research Unit, Laboratory of Pneumology, and Respiratory Division, 3Department of Neuropathology, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

Nandrolone decanoate (Deca-Durabolin, 25 mg/ml; Organon) was administered in an oil vehicle. Nandrolone decanoate study. To 18 female rats (6 of each group) and all the male rats (10 of each group), food and water were given ad libitum. Animals were housed three per cage and were weighed weekly. Diaphragm contractile properties were examined in vitro, and samples of the diaphragm and gastrocnemius were removed for histological and histochemical analyses after treatment.

Pair-fed study. The remaining 24 female Wistar rats, 3 control and 18 low-dose-treated animals, were housed in individual cages in a temperature-controlled room. Individual food intake was measured every day at the same time, as well as body weight. Control animals and eight low-dose-treated animals were given food and fluid ad libitum. The other eight low-dose-treated animals [pair fed (PF)] each received the same amount food as did controls. In addition, in vitro diaphragm contractile properties were examined, and samples of the diaphragm and gastrocnemius were removed for histological and histochemical analyses after treatment.

Experimental Procedures
One week after the last injection, rats were anesthetized with pentobarbital sodium (Nembutal; 55 mg/kg ip), tracheotomized, and mechanically ventilated (Harvard pump respirator, South Natick, MA) with an O2-enriched gas mixture.

Contractile properties. The diaphragm was quickly removed through a laparotomy and was immediately immersed in a cooled, oxygenated Krebs solution [containing (in mM) 137 NaCl, 4 KCl, 2 CaCl2, 1 MgCl2, 1 KH2PO4, 12 NaHCO3, 6.5 glucose, and 0.03 d-tubocurarine chloride]. A small rectangular bundle was carefully dissected from the middle part of the lateral costal region of each hemidiaphragm, parallel to the long axis of the fibers. Silk sutures were firmly tied to both ends of the bundle to serve as anchoring points. Each bundle

ANABOLIC STEROIDS might have more extensive effects on the diaphragm compared with other skeletal muscles because of its higher cytosol androgen-receptor density (9) and its continuous cyclic contraction during respiration. Previous studies using sedentary young rats showed that anabolic steroids induced changes in mass (22), histochemistry (2, 8), biochemistry, and capillary density (9) of the diaphragm, whereas changes in histochemistry have been disputed (22). Recently, the effects of anabolic steroids on contractile and fatigue properties of the diaphragm have also been examined (22). Conversely, anabolic-androgen compounds may have only limited effects in mature animals, as previous studies suggested (3, 14). Further study of this issue in adult mammals is of particular interest to patients with respiratory muscle dysfunction. Indeed, because respiratory muscle weakness may be an important cause of respiratory failure (27), anabolic steroid therapy may improve diaphragmatic intrinsic strength and endurance in patients with respiratory failure.

The present study was designed with the following objectives: 1) to assess the effects of anabolic steroids on body weight, mass, and histochemistry of some respiratory and peripheral muscles and on in vitro contractile and fatigue characteristics of the diaphragm in adult postpubescent male and female rats; 2) to investigate dose-specific effects and their interaction with gender; and 3) to isolate the effects of anabolic steroids from nutritional changes by studying pair-fed animals.

MATERIALS AND METHODS

Animals and Treatment
Forty-two female (14 wk old, weighing 200–230 g) and 30 male (15 wk old, weighing 370–440 g) sedentary Wistar rats were divided by gender and randomized into three groups to receive weekly, during 5 wk, an intramuscular injection into the left hindlimb of either 0.05 ml saline [control (C); 10 males, 14 females], 1.5 mg/kg nandrolone decanoate [low dose (LD); 10 males, 22 females], or 7.5 mg/kg nandrolone decanoate [high dose (HD); 10 males, 6 females].

Nandrolone decanoate (Deca-Durabolin, 25 mg/ml; Organon) was administered in an oil vehicle.

Pair-fed study. The remaining 24 female Wistar rats, 3 control and 18 low-dose-treated animals, were housed in individual cages in a temperature-controlled room. Individual food intake was measured every day at the same time, as well as body weight. Control animals and eight low-dose-treated animals were given food and fluid ad libitum. The other eight low-dose-treated animals [pair fed (PF)] each received the same amount food as did controls. In addition, in vitro diaphragm contractile properties were examined, and samples of the diaphragm and gastrocnemius were removed for histological and histochemical analyses after treatment.

Experimental Procedures
One week after the last injection, rats were anesthetized with pentobarbital sodium (Nembutal; 55 mg/kg ip), tracheotomized, and mechanically ventilated (Harvard pump respirator, South Natick, MA) with an O2-enriched gas mixture.

Contractile properties. The diaphragm was quickly removed through a laparotomy and was immediately immersed in a cooled, oxygenated Krebs solution [containing (in mM) 137 NaCl, 4 KCl, 2 CaCl2, 1 MgCl2, 1 KH2PO4, 12 NaHCO3, 6.5 glucose, and 0.03 d-tubocurarine chloride]. A small rectangular bundle was carefully dissected from the middle part of the lateral costal region of each hemidiaphragm, parallel to the long axis of the fibers. Silk sutures were firmly tied to both ends of the bundle to serve as anchoring points. Each bundle

ANABOLIC STEROIDS might have more extensive effects on the diaphragm compared with other skeletal muscles because of its higher cytosol androgen-receptor density (9) and its continuous cyclic contraction during respiration. Previous studies using sedentary young rats showed that anabolic steroids induced changes in mass (22), histochemistry (2, 8), biochemistry, and capillary density (9) of the diaphragm, whereas changes in histochemistry have been disputed (22). Recently, the effects of anabolic steroids on contractile and fatigue properties of the diaphragm have also been examined (22). Conversely, anabolic-androgen compounds may have only limited effects in mature animals, as previous studies suggested (3, 14). Further study of this issue in adult mammals is of particular interest to patients with respiratory muscle dysfunction. Indeed, because respiratory muscle weakness may be an important cause of respiratory failure (27), anabolic steroid therapy may improve diaphragmatic intrinsic strength and endurance in patients with respiratory failure.

The present study was designed with the following objectives: 1) to assess the effects of anabolic steroids on body weight, mass, and histochemistry of some respiratory and peripheral muscles and on in vitro contractile and fatigue characteristics of the diaphragm in adult postpubescent male and female rats; 2) to investigate dose-specific effects and their interaction with gender; and 3) to isolate the effects of anabolic steroids from nutritional changes by studying pair-fed animals.
was suspended in a water-jacketed tissue bath filled with Krebs solution, maintained at 37°C, and continuously aerated with 95% O₂-5% CO₂. The central tendon side of the bundle was anchored to a rigid support, whereas the rib margin side was fastened to an isometric force transducer (Maywood, Hampshire, UK) mounted to a vertical micrometer.

Field stimulation was delivered via two large platinum-stimulating electrodes placed along both sides of each bundle.

The bundles were placed at their optimal length (L₀), defined as the length at which peak twitch force was obtained. Stimulation were delivered through a Harvard 50-5016 stimulator (Edenbridge, Kent, UK), connected in series to a power amplifier from power one model HS24-4.8. Pulse duration of 0.2 ms at supramaximal voltage was used for all stimulations. The force transducer output was amplified and recorded on computer via analog-to-digital conversion (DT 2801-A) by using Labdat (Labdat/Anadat, RHT-InfoDat, Montreal, PQ). Data analysis was performed by means of Anadat.

The following measurements were performed at L₀, after a thermoequilibration period of 15 min.

**Twitch Characteristics.** The highest value of two successive 1-Hz stimulations was used to determine maximal twitch tension (Pₜ) and its corresponding time to peak tension (TPT) and half relaxation time (RTₜₚ).

**Maximal Tetanic Tension (Pₜₒ).** Stimulation frequency was set at 160 Hz to reach maximal force generation in rats at 37°C (24). The highest value of two successive stimulations (train = 250 ms) was used for further analysis. In between the two tetanic stimulations, a time interval of 2 min elapsed.

**Force-Frequency Curve.** Each bundle was successively stimulated (train = 250 ms) every 2 min at 25, 50, 100, 150, 160, 120, and 160 Hz (modified after Reid and Miller (24)).

**Fatigue Properties.** First, high-frequency fatigue was assessed by measuring the decline in force output at 160 Hz during the force-frequency curve. Second, low-frequency fatigue was induced by a 5-min stimulation run consisting of repeated 25-Hz stimulations of 330 ms, applied every second (modified after Burke et al. (4)).

At the end of the in vitro experiment, length, thickness, and width of each muscle bundle were measured at L₀, by means of a caliper, and bundles were weighed after being blotted dry. All tensions were normalized for cross-sectional area (CSA) (5). Finally, the ratio of twitch to tetanic tension (Pₜ/Pₜₒ) was calculated for each muscle bundle.

**Muscle mass.** The remaining diaphragm tissue, the para-episternal intercostals (including sternum and chondral parts of the ribs), the right medial scalene, and also the soleus and gastrocnemius of the right hindlimb and the heart were dissected, trimmed, blotted, and weighed. For the pair-fed study, masses of the right extensor digitorum longus and plantaris were also measured.

**Histological and histochemical procedures.** Material for histochemical analysis was obtained from the same animals. Adjacent parts of the diaphragm were used for determination of contractile properties and for histological and histochemical analyses.

The right costal region of the diaphragm was placed at excised length on tissue glue (Tissue-Tek, Elkhart, IN) over a cork holder, with the fibers oriented perpendicular to the surface of the cork. The preparations were frozen in isopentane cooled with liquid N₂. The same was done for a midbelly section of gastrocnemius. Afterward, serial sections (10 μm), parallel to the cork, were cut with a cryostat (−20°C). Two sections of each muscle were taken for routine hematoxylin and eosin staining, whereas the other serial sections were stained for adenosinetriphosphatase (ATPase) after acid (pH 4.3 and 4.5) preincubation. A preliminary study showed that slides preincubated at this pH offered a clear separation of the three fiber types. On the basis of their histochemical reactions, fibers were identified as slow-twitch type I (low-myosin ATPase, high oxidative capacity), fast-twitch type IIa (high-myosin ATPase, high oxidative capacity), or fast-twitch type IIx/b (high-myosin ATPase, low oxidative capacity) (20).

It should be noted that this standard histochemical analysis is not adapted to detect type IIx fibers, recently described in the rat diaphragm (30). Therefore, we will refer to the type IIb fibers as type IIx/b fibers.

Morphometric examination was carried out with a Leitz microscope (Wetzlar, Germany) at ×25 magnification, connected to a camera and digitizing board (model 2207, Numerics, Montgomeryville, PA). For each muscle, at least 250 CSAs (determined from the number of pixels within the boundaries of a delineated muscle fiber) and diameters (determined by measuring the maximum diameter perpendicular to the long axis of the cross-sectional fiber) of individual muscle fibers were calculated. However, because diaphragm was fixed at excised length, diameters and CSAs were corrected for the shortening occurring from L₀, according to the formula of Prakash et al. (21). Peripheral muscles were not corrected because the L₀ has generally been found to be between 100 and 120% of their resting length (5).

In the nandrolone decanoate study, besides 48 diaphragms, consisting of 8 muscle preparations for each group of males and females, also 58 gastrocnemii (males: C, 10; LD 9; HD 12; and females: C, 10; LD, 11; HD, 6) were studied. In the pair-fed study 22 diaphragm (C, 7; PF, 7; LD, 8) and 20 gastrocnemii samples (C, 7; PF, 5; LD, 8) were examined.

**Statistical Analysis.** Differences among, and interactions between, gender (male-female) and dose (0-1.5-7.5 mg·kg⁻¹·wk⁻¹ nandrolone decanoate) were assessed by using multifactorial analysis of variance. Differences among means of combinations of levels of the primary factors as drug (saline-nandrolone decanoate) and low vs. high dose were determined by using CONTRAST statements. Multivariate analysis of variance with repeated measurements was used for assessment of differences as a function of time or stimulation frequency. Data derived from two separate bundles from the same diaphragm were averaged and subsequently analyzed as such. P < 0.05 was accepted as statistically significant. In the text, Table 1, and Figs. 1–5, values are presented as means ± SD.

**RESULTS**

**Nandrolone Decanoate Study.**

**Body and muscle weights.** Figure 1A shows that starting body weight was not different among treatment groups. In addition, treatment affected body weight change differently in males and females (gender-dose interaction; P < 0.0001). It reduced weight in males (P < 0.01), whereas it enhanced growth in females (P < 0.0001). Males were heavier than females throughout the entire experimental period (gender-specific effect; P < 0.0001). In male rats, low-dose and control animals showed a similar growth curve. In contrast, the high-dose-treated animals showed stunted growth from 2 wk of treatment on (P < 0.05), leading to a final body weight of 94% of both other groups (P < 0.01). In contrast, steroid-treated female rats responded after 1 wk of treatment by an enhanced growth (P < 0.001; Fig. 1), reaching up to 120% of control at the
end of therapy \( (P < 0.001) \). No differences appeared between low- and high-dose-treated female animals. Figure 1B presents the body weight change of all groups at the end of treatment, illustrating the stunted growth in high-dose-treated males and the enhanced growth in nandrolone-treated females.

Because final body weight was different among groups, masses of heart, respiratory, and peripheral muscles were expressed as a percentage of body weight (Fig. 2). Of all examined muscles, only the weight of the gastrocnemius was affected differently in males [i.e., no significant change (NS)] and females (i.e., an increase; \( P < 0.01 \)). The scalenus medius and gastrocnemius were heavier (\( P < 0.0001 \)) and the soleus and heart were lighter (\( P < 0.0001 \)) in males compared with females. The weights of the diaphragm and parasternal intercostals did not differ between genders. Except for the gastrocnemius, no significant drug-specific effect was observed in the muscles investigated. Finally, no differences were seen between low- and high-dose-treated animals.

**Diaphragmatic contractile properties. Twitch characteristics and \( P_0 \).** Gender and dose interactions were not significant. In males compared with females, \( P_t \) (491 ± 114 vs. 294 ± 82 g/cm²; \( P < 0.0001 \)), \( P_o \) (1,955 ± 387 vs. 1,664 ± 374 g/cm²; \( P < 0.05 \)), and \( P_t/P_o \) (0.249 ± 0.024 vs. 0.181 ± 0.019; \( P < 0.0001 \)) were significantly greater and \( R_T \) was significantly smaller (22 ± 2 vs. 30 ± 3 ms; \( P < 0.0001 \)), whereas TPT was not different between genders (20 ± 1 vs. 20 ± 3 ms; NS). Diaphragm bundles contracted faster with nandrolone treatment as shown by decreased TPT (C: 21 ± 2, LD: 19 ± 1, HD: 19 ± 2 ms; \( P < 0.0005 \)) and \( R_T \) (C: 26 ± 5, LD: 25 ± 5, HD: 23 ± 3 ms; \( P < 0.01 \)), whereas \( P_t \), \( P_o \), and \( P_t/P_o \) showed no significant drug-specific effect. None of these parameters showed significant differences between low- and high-dose-treated animals.

**FORCE-FREQUENCY CURVE.** The effect of nandrolone on the force-frequency curve was not different in males and females. But the diaphragm of males responded differently to increasing stimulus frequencies com-
pared with females (Fig. 3; \( P < 0.0001 \), repeated measurements). Diaphragmatic force generation, corrected for CSA, was higher in males at low stimulation frequencies (1 Hz: \( P < 0.0001 \), 25 Hz: \( P < 0.001 \), and 50 Hz: \( P < 0.05 \); Fig. 3A). When expressed as a percentage of the interposed 160-Hz stimulations (Fig. 3B), the force response in males was higher than in females at all stimulation frequencies (\( P < 0.0001 \), except \( P < 0.01 \) for 120 Hz). Finally, nandrolone treatment did not modify the diaphragm response to increasing stimulus frequencies.

**Fatigue properties.** During the force-frequency protocol, no gender and dose interaction was found for 160-Hz fatigue. But diaphragm strips of males fatigued 2.9 times more than did those of females (\( P < 0.0001 \)). Finally, nandrolone did not affect diaphragmatic 160-Hz fatigability.

During the low-frequency fatigue run, no gender and dose interaction was found. Diaphragm strips of both genders fatigued to a similar degree, reaching, after 5 min of 25-Hz stimulations, 17.1 ± 4.2 and 20.7 ± 3.8% of initial force in males and females, respectively. Finally, nandrolone did not change diaphragmatic fatigue induced by repeated 25-Hz stimulations.

**Histology and histochemistry.** Within one gender, histological examination of routine hematoxylin and eosin-stained slides of the diaphragm and gastrocnemius showed no obvious differences between control and treatment groups.

No significant gender-dose interaction was found for proportions, CSAs, and diameters of types I, IIA, and IIX/b fibers of the diaphragm. As a consequence, statistical analysis for drug-specific effects was performed gender independently. Fiber proportion was greater for type I (40 ± 4 vs. 37 ± 3%; \( P < 0.05 \)) and was not significantly different for type IIA (34 ± 3 vs. 36 ± 4%; NS) and IIX/b fibers (27 ± 4 vs. 28 ± 5%; NS) in males compared with females. Fiber proportion was not influenced by drug treatment. The CSAs of the three fiber types were greater in males compared with females, but this was only significant for type IIA (742 ± 71 vs. 623 ± 78 \( \mu m^2 \); \( P < 0.0001 \)) and IIX/b fibers (1,968 ± 191 vs. 1,534 ± 261 \( \mu m^2 \); \( P < 0.0001 \)). Nandrolone treatment resulted in an increase of the CSA of type IIX/b fibers (C: 1,654 ± 370 vs. treated: 1,799 ± 280 \( \mu m^2 \); \( P < 0.05 \)), whereas that of type I (pooled values: 606 ± 84 \( \mu m^2 \)) and type IIA fibers (pooled values: 682 ± 96 \( \mu m^2 \)) did not change significantly. The same gender- and drug-specific effects were seen for the diameters of the fiber types. Finally, the relative contribution of type IIX/b fibers to the total CSA of the diaphragm was greater in males compared with females (51.1 ± 4.8 vs. 48.5 ± 4.2%; \( P < 0.05 \)) but did not change with treatment. That of the other fiber types was not affected by gender or dose.

In the gastrocnemius, no gender-dose interaction was found for the proportions, CSAs, and diameters of the three fiber types. The proportion of type IIA fibers was greater in males compared with females (27 ± 6 vs. 21 ± 6%; \( P < 0.05 \)), whereas that of type I (pooled values: 36 ± 9%) and IIX/b fibers (pooled values: 40 ± 9%) did not differ significantly. All fiber types were greater in males compared with females (I: 2,723 ± 453 vs. 2,523 ± 312 \( \mu m^2 \)) as well as in females (C: 2,174 ± 282 vs. LD and HD pooled: 2,437 ± 275 \( \mu m^2 \); \( P < 0.05 \)). As in the diaphragm, nandrolone did not change fiber proportions but increased type IIX/b dimensions (C: 2,456 ± 566 vs. treated: 2,681 ± 446 \( \mu m^2 \); \( P < 0.05 \)). In addition, type I fiber CSA increased with low-dose treatment in males (C: 2,738 ± 475 and HD: 2,523 ± 312 vs. LD: 3,004 ± 498 \( \mu m^2 \); \( P < 0.05 \)) as well as in females (C: 2,174 ± 282 vs. LD and HD pooled: 2,437 ± 275 \( \mu m^2 \); \( P < 0.05 \)).

**Pair-Fed Study**

**Food intake.** Before treatment, the food intake was similar in the three groups, averaging 113 ± 18 g/wk. During treatment, food intake in the control group was stable and averaged 122 ± 37 g/wk. The food intake of the pair-fed group followed the same course as that of controls, averaging 112 ± 12 g/wk. By contrast, already during the first week of treatment, food intake was significantly increased in the treated animals fed ad libitum (133 ± 12 g) compared with both other groups (\( P < 0.001 \)). This increase further increased to 144 ± 11
g during the second week of treatment, and remained stable afterward. Significant differences between treated animals fed ad libitum and both other groups (with no differences between the latter groups) were observed during the entire treatment period (P < 0.001 to < 0.0001).

Body and muscle weights. Starting body weight was similar among the three groups, averaging 208 ± 14 g. One week after the first injection, treated animals fed ad libitum were significantly heavier than control and pair-fed groups (LD: 230 ± 16 vs. C: 209 ± 16 and PF: 208 ± 11 g; P < 0.01). This enhanced growth further increased, leading to a final body weight of 273 ± 19 g during the second week of treatment, and remained stable afterward. Significant differences between treated animals fed ad libitum and both other groups (with no differences between the latter groups) were observed during the entire treatment period (P < 0.001 to < 0.0001).

Except for soleus and heart weights, all muscles of pair-fed group represented a greater proportion of body weight than did controls, reaching statistical significance in the plantaris (P < 0.05) and the extensor digitorum longus only (P < 0.01; Fig. 4). No differences appeared between both treated groups, whatever their nutritional intake.

Diaphragmatic contractile properties. No statistical differences in Pt and its kinetics or in TPT were found among the three groups (Table 1). However, nandrolone tended to decrease TPT and RT½, whatever the nutritional intake of the animals (PF: −11 and −14% of C; LD: −15 and −15% of C, respectively; P = 0.06 vs. C).

Similarly, no changes appeared in the force-frequency curve in the three groups (Fig. 5) or in the fatigability of the diaphragm during the low-frequency fatigue run.

Histology and histochemistry. For both muscles studied, the muscular pattern seen on hematoxylin- and eosin-stained slides was similar in pair-fed and treated animals fed ad libitum and was comparable to that of controls.

ATPase staining revealed no significant changes in the fiber type proportions in respect to treatment in the muscles studied. Pooled values of all groups together were 39 ± 5, 31 ± 4, and 30 ± 4% for types I, IIA, and IIX/b, respectively, for the diaphragm. For the gastrocnemius, these values were 41 ± 4, 18 ± 4 and 41 ± 5% for types I, IIA, and IIX/b, respectively.

On the other hand, diaphragmatic type IIX/b fiber dimensions increased in both treated groups, whatever their nutritional intake. Nevertheless, this increase was only significant in treated animals fed ad libitum (23% of control; P < 0.05) and not in pair-fed animals (14%; NS). Finally, diaphragm type I and IIA dimensions were similar in the three groups, being 520 ± 49 and 554 ± 71 μm², respectively (pooled values).

In the gastrocnemius, nandrolone also resulted in increased type IIX/b fiber dimensions, reaching statistical significance in animals fed ad libitum only (18% of control, P < 0.01; vs. PF: 9% of control, NS). In addition, type I fiber hypertrophy was seen in the low-dose group only (20% of control; P < 0.01), whereas type IIA dimension was unchanged (pooled values: 1,948 ± 170 μm²).

DISCUSSION

The present data demonstrate that in healthy sedentary adult rats, 5 wk of treatment with 1.5 and 7.5 mg·kg⁻¹·wk⁻¹ nandrolone decanoate affected body and muscle weights (especially gastrocnemius) positively in female treated rats and negatively or not at all in male treated rats. In vitro contractile and fatigue character-
The observed anabolic effects. In the gastrocnemius, type I fiber CSA and diameter were also increased with nandrolone treatment. Finally, although control of nutritional intake is likely to be needed in anabolic steroid studies, a pair-fed study showed that changes in oral food intake only partly explained the observed anabolic changes.

It should generally be emphasized that comparison of results in literature is rather difficult because anabolic effects are influenced by a series of conditions, such as the studied species (2, 3), age (3, 14), circulating synergistic hormones (7), muscles (3), androgen (19), dose (2, 15, 19, 29), mode of administration (17), duration of treatment (22, 26), activity level (12, 15), and diet (11). Indeed, these factors explain to a large extent the inconsistencies in the literature.

In the present study, in keeping with other studies performed on young animals (2, 19, 29), no linear relationship within one gender between dose and effect was found. Consequently, injection of a five times higher dose did not produce an increased effect. Except for the dose-dependent changes in body weight, no systematic differences appeared in the present study between low- and high-dose-treated animals.

In our study in adult rats, body weight increased in females, whereas it was not affected or decreased in males. This is similar to what has been reported in young rats in which body weight was increased (7, 8, 19, 22) or unchanged (14, 19) in females, whereas it was not affected (7, 14, 22, 26) or stunted (14, 16) in males, depending on the dose used. The exact mechanisms for this gender-dependent anabolic effect is not known and is beyond the aim of the present investigation. The intrinsic difference in the basal level of circulating endogenic androgens may play a role because it is higher in sexually normal male animals compared with females and seems to result in a smaller number of androgen-binding sites on the muscle cytosol (6). Furthermore, surpassing the physiological level of androgens in males has been reported to result in 1) depression of the natural production of testosterone (25), 2) downregulation of androgen-binding receptors (23), 3) decrease in appetite (16), and 4) a metabolic conversion to excess estradiol (15). Indeed, all these factors may inhibit body growth. Nevertheless, the present study does not allow us to determine the relative significance of these mechanisms.

Our pair-fed study illustrates that female rats increased their food intake with nandrolone treatment, leading to enhanced body growth. This is in keeping with studies performed in prepubescent rats in which food consumption varied with changes in body weight (13, 16). Furthermore, in the present study no significant differences in muscle weights and in diaphragmatic contractile and histochemical characteristics were found between treated animals fed ad libitum and pair-fed animals. These data illustrate that the increased ingestion of food alone was not fully responsible for the observed anabolic effects.

In prepubescent rats, muscle weights changed in proportion to body weight (8, 19, 22). In the present study, only the weight of the gastrocnemius failed to do so, and, moreover, it changed gender dependently. A graded response of different muscles to androgen administration was previously reported as being the result of differences in the level of androgen-binding receptors within the muscle cytosol (3, 6). Different anabolic effects in males compared with females were observed in young animals (7, 10, 22). Nevertheless, to the best of our knowledge, a greater effect of anabolic agents on gastrocnemius has not yet been described in rats.

In the present study, of all the contractile properties measured, nandrolone treatment solely altered diaphragmatic twitch kinetics toward a fast-twitch profile, being consistent with the observed type Ilx/b fiber hypertrophy. In young female rats, but not in young males, Prezant et al. (22) also found a decreased RT1/2 after testosterone treatment (62.5 mg·kg⁻¹·wk⁻¹) but with no concomitant changes in diaphragmatic morphometry. This suggests additional mechanisms besides changes in histochemistry induced by anabolic agents, such as modification of neural and neuromuscular function (including neurotransmitter levels) (1) and/or upregulation of the Ca²⁺ -ATPase pump (26). Moreover, Prezant et al. also reported enhanced diaphragmatic force in young female rats during short-term (2.5-wk) testosterone treatment but not if treatment was extended to 10 wk. Downregulation of muscle cytosol androgen receptors probably explains the observed discrepancies with different treatment durations (22). Other studies with young or adult rats confirm in peripheral muscles our results in the diaphragm (8, 28).

Only few studies investigated morphological adaptations of diaphragm to anabolic agents. Prezant et al. (22) found no histochemical changes in the diaphragm compared with pair-fed control male and female animals. In contrast, Egginton (8) reported increased type IIa fiber proportion and dimensions compared with female controls. In our study, a selective type Ilx/b fiber hypertrophy was present compared with controls but not compared with pair-fed animals. Thus it seems that differences in morphological adaptations were observed only when comparison was made against controls and not against pair-fed animals.

To the best of our knowledge, histochemical alterations of the gastrocnemius to anabolic steroids have previously not been reported.

Gender-related differences in fiber composition and morphometry, as well as in some enzymological characteristics, may explain differences in contractile properties and fatigue resistance of the diaphragm between male and female rats. Indeed, the dimensions of all diaphragmatic fiber types, the proportion of type I fibers, as well as the relative contribution of type Ilx/b fibers to total CSA of the diaphragm were greater in males compared with females. The reduced P0 and P0/P0 in diaphragms of female rats may additionally be related to a greater amount of connective tissue and, therefore, a greater series elastic component.
Although the present study was performed in sedentary rats, our results suggest that treatment with high therapeutic (1.5 mg·kg⁻¹·wk⁻¹) or supratherapeutic doses (7.5 mg·kg⁻¹·wk⁻¹) of nandrolone may be of benefit in patients (especially females) being in a chronic state of catabolism either combined with exercise reconditioning or not. Indeed, anabolic androgens may not only act directly on the androgen receptors but also indirectly through inhibition of the catabolic and antianabolic action of endogenous glucocorticoids (7, 26). Anabolic agents also may further enhance the myotrophic effect of exercise (12), reduce fatigability, and lead to a faster recovery as experienced by athletes, being likely to relate to anabolic effects on the central nervous system (18). This is supported by our findings because nandrolone decanoate treatment did not change fatigue resistance of the diaphragm. Whether such effects are present in patients needs to be examined. Finally, the administration of intermittent short bursts rather than continuous doses of anabolic agents may be more beneficial for muscles in terms of enhanced muscle contractility, thereby avoiding possible down-regulation of muscle cytosol androgen receptors.

In conclusion, this study shows that nandrolone decanoate when given during 5 wk at high therapeutic or supratherapeutic doses affects males and females in a similar way except for body weight, which is enhanced in females and stunted in males. Diaphragmatic contractility and fatigability were unaltered, except for twitch kinetics. The latter changed toward a fast-twitch profile, consistent with a type IIx/b hypertrophy. Gastrocnemius weight changed disproportionally fast-twitch profile, consistent with a type IIx/b hypertrophy. Finally, the anabolic steroid-induced changes in food ingestion were only partly responsible for the observed anabolic effects.

The authors thank N. Plaisance-Buts for expert technical assistance and Organon Belgium for supplying us with nandrolone decanoate.

This work was supported by a grant from the Fonds voor Geneeskundig Wetenschappelijk Onderzoek.

Address for reprint requests: M. Decramer, Professor of Medicine Respiratory Div., Univ. Hospital, Herestraat 49, B-3000 Leuven, Belgium.

Received 7 June 1994; accepted in final form 12 November 1996.

REFERENCES


