# Steroid receptor concentration in aged rat hindlimb muscle: effect of anabolic steroid administration

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Carson, James A., Won Jun Lee, Joseph McClung, and Gregory A. Hand. Steroid receptor concentration in aged rat hindlimb muscle: effect of anabolic steroid administration. J Appl Physiol 93: 242-250, 2002; 10.1152/japplphysiol.01212.2001.-Skeletal muscle is a target of anabolic steroid action; however, anabolic steroid's affect on aged skeletal muscle is not well understood. The effect of 4 wk of nandrolone decanoate (ND) administration on hindlimb muscles of 5- and 25-mo-old Fischer 344/Brown Norway rats was examined. ND (6 mg/kg body wt) was injected every 7th day for 4 wk. Controls received an oil injection. ND significantly reduced 25-mo-old rat perirenal fat pad mass by 30%. Soleus (Sol) and plantaris (Plan) muscle-to-body weight ratios were reduced in 25-mo-old rats. ND did not affect Sol or Plan muscle-to-body weight ratios at either age. Sol DNA concentration was reduced by 25% in 25-mo-old rats, and ND increased it to 12% greater than 5-mo-old rats. ND did not affect Plan DNA content. Sol androgen receptor (AR) protein in 25-mo-old rats was reduced to 35% of 5-mo-old values. ND increased AR protein by 900% in 25-mo-old rat Sol. Plan AR concentration was not affected by aging but was induced by ND in both age groups. Aging or ND treatment did not affect glucocorticoid receptor levels in either muscle. These data demonstrate that fast- and slow-twitch rat hindlimb muscles differ in their response to aging and ND therapy.

hypertrophy; nandrolone decanoate; sarcopenia; muscle wasting

SKELETAL MUSCLE'S ROLES as metabolic sink (i.e., glucose), secreted protein manufacturer (i.e., lipoprotein lipase), and facilitator of body locomotion make it vital for human health. Muscle mass loss is linked to frailty, morbidity, and mortality in humans (25). However, skeletal muscle is composed of highly oxidative, postmitotic fibers, which make it a target for aging sensitivity (38). Skeletal muscle mass decreases with advancing age, and this sarcopenia is associated with decreased muscle protein synthesis, existing fiber atrophy, and muscle fiber loss (38, 39, 42, 46). Muscle fiber loss is associated with spinal motoneuron death, which also contributes to motor unit size expansion and mosaic fiber pattern loss in aged muscle (42). Another cause of muscle mass loss with aging is that due to decreased muscle fiber cross-sectional area or

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atrophy. A portion of muscle fiber atrophy with advancing age may be related to systemic or intrinsic biological phenomena related to the aging process and independent of physical activity levels.

It is clear that aging induces changes to skeletal muscle that are independent of an age-induced loss of muscle mass. These age-related changes manifest themselves long before any alteration in muscle mass due to age are reported. Skeletal muscle is a dynamic tissue, and aging decreases its plasticity to many stimuli, such as those requiring regeneration or remodeling (6, 9, 12, 40). Deficits in skeletal muscle plasticity can occur at relatively young ages in the rat. Recovery from toxin-induced injury in skeletal muscle is decreased in both 18- and 32-mo-old rats compared with young rats (40). There is strong evidence that age-induced decreases in muscle regenerative capacity are not intrinsic to the muscle itself but rather are dependent on the aging organism as a whole (11, 12, 18, 44). These facts suggest that signaling stimuli targeting muscle may be deficient in the aged organism, and, therefore, aged muscle's plasticity and regenerative capacity could be restored or improved if provided the appropriate stimulus. Although this theory fits well with skeletal muscle plasticity and/or adaptability to a regenerative stimuli, it is less certain how this impacts the ageinduced loss of muscle mass.

Testosterone and its pharmaceutical derivatives are potent regulators of skeletal muscle mass. Anabolicandrogenic steroids are structural derivatives of testosterone manufactured to maximize anabolic and minimize and rogenic effects. Anabolic steroid administration has a growth effect on female (21), normal male (4, 22, 30), and hypogonadal male muscle (5). Anabolic steroid therapy for patients with chronic wasting diseases can maintain or increase muscle mass (34, 51). Skeletal muscle protein synthesis increases in elderly men administered testosterone (56). The rat has proven to be a useful model for studying anabolic steroid's action on skeletal muscle (47). The effect of testosterone administration on basal young rat muscle mass is mixed (2, 8, 55, 58) and likely due to drug type, dosage, and administration schedules. Exogenous tes-

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tosterone administration and skeletal muscle loading act synergistically to increase skeletal muscle mass in healthy and diseased humans (4, 57). A synergistic relationship between testosterone and muscle loading is also present in functionally overloaded rat muscle subject to disuse atrophy (54). Anabolic steroid administration can reduce rat hindlimb muscle atrophy due to unloading (55) and abolish unloaded atrophy in the quadriceps muscle (58). Although testosterone is a potent skeletal muscle mass effector, little is understood about its molecular regulation and the effect of aging on this regulation.

Androgen receptors (AR) and glucocorticoid receptors (GR) are potential modulators of skeletal muscle mass. Glucocorticoid- and testosterone-induced cellular regulation involves binding with its specific cytosolic steroid receptor, translocating to the nucleus, and then altering gene transcription by binding to its corresponding DNA response element (29, 41). Signaling cascades, including RhoA-mediated signaling, can alter AR and GR transcriptional activity (52). AR interactions with coactivators and other DNA-binding proteins, including serum response factor, at the level of DNA binding to alter its transcriptional activity (37, 41). Circulating testosterone levels have been well characterized in the Fischer 344/Brown Norway rat, decreasing dramatically between 4 and 28 mo of age (17). AR expression appears sensitive to circulating testosterone concentration (2). However, the influence of circulating testosterone levels on AR expression appears to be muscle specific (2).

Like other aging phenomena, sarcopenia will likely be related to both environmental and genetic/biological factors (38). There has been considerable focus on the ability of aged skeletal muscle to adapt to exercise training without a clear understanding of the limitations biological aging places on this response. Anabolic steroids target skeletal muscle, maintain muscle mass in many wasting disease states (34, 51), and can act synergistically with resistance exercise in humans (4, 57); however, their effect on age-induced muscle mass loss or sarcopenia is less certain. Additionally, the therapeutic use of anabolic steroids is limited by their broad spectrum of biological targets (3). At this time, the anabolic steroid-induced signaling mechanisms influencing skeletal muscle mass regulation are not well understood. A better understanding of anabolic steroid action on skeletal muscle will improve both drug design and the use of combined environmental (i.e., diet, exercise) and pharmaceutical interventions to offset sarcopenia in the aged individual. The purpose of the present study was to examine whether 4 wk of nandrolone decanoate (ND) administration had an anabolic effect on primarily fast- and slow-twitch aged rat hindlimb muscles. It was hypothesized that anabolic steroid treatment would reduce age-related losses in muscle mass of the soleus and plantaris muscles from 25-mo-old rats. Additionally, it was hypothesized that AR concentration would be reduced in the 25-mo-old rats, and anabolic steroid administration would return AR levels to young adult levels.

#### **METHODS**

Animals and housing. Thirty-six male Fischer 344XF1/ Brown Norway rats were acquired from the National Institute on Aging aged rodent colony. Eighteen rats were  $\sim 4$  mo old at the start of the study, and 18 rats were  $\sim$ 24 mo old at the start of the study. Animals were housed individually, kept on a 12:12-h light-dark cycle, and given ad libitum access to normal rodent chow and water for the duration of the study at fully accredited animal care facilities at the University of South Carolina, Columbia. All rats in this study had a small (1 cm) incision on the lateral aspect of the hindlimb at the start of the fourth week of treatment. These animals also served as sham controls for a separate study. Briefly, animals were anesthetized for  $\sim 2$  h by a ketamine/ xylazine-acepromazine injection. Under sterile conditions, a 1-cm incision through the skin was made on the lateral aspect of each hindlimb and then sutured. No muscles analyzed in this current study were impacted by the procedure. and animals resumed normal locomotion 1-2 h postsurgery. The University of South Carolina Animal Care and Use Committee approved all procedures.

Anabolic steroid administration. The anabolic steroid ND (Deca-Durabolin, Oranon) was used in the present study because of its long biological half life and previous studies demonstrating its anabolic effect in rat skeletal muscle (54, 55, 58). The selected dose of ND administration has been previously demonstrated to prevent hindlimb suspension-induced rat skeletal muscle atrophy (58). ND was injected (6 mg/kg body wt) intramuscularly into the hip region every seventh day, including day 1, and the right and left hip region alternated each week. Control animals received a similar-volume intramuscular injection of sesame seed oil. Each animal received four injections of either ND or oil and was killed at the end of 4 wk (28 days).

Total DNA. Total muscle DNA was quantified as previously described (40). Briefly, a frozen soleus or plantaris muscle ( $\sim$ 25–50 mg) was cut and weighed. This same piece of muscle was then used to quantify total DNA, total RNA, and total protein. Muscle was homogenized in 0.2 N HClO<sub>4</sub> and centrifuged (4°C, 12,000 g for 10 min). After several washes, the pellet was resuspended in 0.3 N KOH. At this stage, an aliquot was removed for total protein (see Total protein below). A 0.75 volume of 1.2 N HClO<sub>4</sub> was than added to the supernatant. After centrifugation (4°C, 12,000 g for 10 min), the supernatant was transferred to a new tube. The pellet was washed two more times with 1.2 N HClO<sub>4</sub>, and the supernatants from all washes were combined for total RNA concentration. The pellet was then resuspended in 1 M NaOH and incubated for 30 min at 50°C. DNA content was determined by a standard fluorometric assay using bis-benzimide and salmon sperm DNA standards. DNA is expressed as the concentration per milligram of muscle and total DNA as that per whole muscle.

Total RNA. Total muscle RNA was quantified as previously described (14, 23). Briefly, after initial muscle homogenization and centrifugation (see *Total DNA*), the pellet was washed two more times with 1.2 N HClO<sub>4</sub>, the supernatants from all washes were combined, and the RNA was quantified by ultraviolet absorbance at 260 nm. Total RNA is expressed as the concentration per milligram of muscle and total RNA as that per whole muscle.

*Total protein*. Total muscle protein was quantified as previously described (14, 23). Briefly, an aliquot of muscle homogenate (see *Total DNA*) was analyzed for protein content by the standard Bradford assay (Bio-Rad). Total protein is expressed as the concentration per milligram of muscle and total protein as that per whole muscle.

*Crude protein extracts.* Crude protein extracts were made as previously described (24). Frozen soleus and plantaris muscles were homogenized in Mueller buffer (50 mM HEPES, pH 7.4, 0.1% Triton X-100, 4 mM EGTA, 10 mM EDTA, 15 mM Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>, 100 mM β-glycerolphosphate, 25 mM NaF, 1 mM Na<sub>3</sub>VO<sub>4</sub>, 0.5 µg/ml leupeptin, 0.5 µg/ml pepstatin, and 0.3 µg/ml aprotinin), 2 ml per 1 g of tissue. Tissue was homogenized on ice with a Polytron homogenizer (Kinematica Switzerland) using three 15-s pulses at a low setting. Homogenates were fractionated into soluble and insoluble fractions by centrifugation, and the protein concentration was determined by DC Lowery assay (Bio-Rad) and aliquoted at -80°C until use for Western blotting.

Western blot analysis. Western blot analysis was performed as previously reported (14). Forty micrograms of crude homogenate protein were incubated (15 min. 65°C) with an equal volume of protein sample buffer, fractionated on an 8% SDS-polyacrylamide gel (150 V, 25°C, 1 h), and electrophoretically transferred to a nitrocellulose membrane (300 mA, 4°C, 14 h). Transfer was verified by Ponceau S staining. Dose-response analysis of the AR and GR demonstrated that 40 µg of crude protein extract gave the signal in a linear range for quantification (data not shown). The membrane was then probed with either AR (N-20) or GR (M-20) polyclonal rabbit antibodies (Santa Cruz Biotechnology, Santa Cruz, CA), as previously described (14). The donkey anti-rabbit IgG horseradish peroxidase-linked secondary antibody was visualized by enhanced chemiluminescence (Amersham Life Sciences) per manufacturer instructions and quantified by densitometry scanning.

*Data analysis.* Results are reported as means  $\pm$  SE. All variables were analyzed by two-way ANOVA (steroid treatment  $\times$  age) for each variable to determine significant main effects and interactions ( $P \leq 0.05$ ). Post hoc analysis of significant interactions was done with a Bonferroni test ( $P \leq$ 



Fig. 1. Nandrolone decanoate (ND) treatment significantly reduces rat body weight. The graph represents the change in body weight over a 14-day period. Rat body weight was taken at the beginning of week 2 and the beginning of week 4 of the study. Data are representative of 9–10 animals per treatment group. Data are mean changes  $\pm$  SE. %BW change, percent change from body weight recorded at week 2; 5-m, 5-mo-old rat receiving weekly sham injections; 5-m+ND, 5-mo-old old rat receiving weekly ND (6 mg/kg) injections. 25-m+ND, 25-mo-old old rat receiving weekly ND (6 mg/kg) injections. <sup>a</sup>Significant main effect of age. (P < 0.05). <sup>n</sup>Significant main effect of ND treatment (P < 0.05).



Fig. 2. ND treatment significantly reduces fat pad-to-body weight ratio in 25-mo-old rats. The graph represents the change in fat pad mass corrected for body weight mass. Fat pad and body weights were taken at the time of death after 4 wk of treatment. Data are representative of 5–6 animals per treatment group. "Significant main effect of age (P < 0.05). "Significant main effect of ND treatment (P < 0.05).

0.05). A priori planned comparisons analyzed 5- and 25-mo baseline values of the AR and GR protein with one-way ANOVA (Age) to determine the effect of age on these variables.

## RESULTS

Rat body weight. Aged rats (24 mo) were significantly heavier than the adult rats (4 mo) at the beginning of the study (556  $\pm$  8 g, n = 20 vs.  $300 \pm 6$ , n = 18, respectively). Body weights were not different among control 5-mo-old ( $300 \pm 10$  g and  $301 \pm 9$  g) or 25-moold ( $560 \pm 12$  and  $557 \pm 12$ ) rats randomly assigned to 5 wk of ND treatment. There were significant main effects of both age and ND treatment on the percent change in body weight between the second and fourth weeks of the study (Fig. 1). Twenty-five-month-old control rats maintained a stable body weight over this period, whereas adult rats grew rapidly. ND administration significantly reduced body weight changes over the entire study for both age groups (week 1 vs. week 5).

Fat pad weight. A target of androgen administration in the rat is the perirenal fat pad found in the rat abdominal cavity (50). Perirenal fat pad mass from the abdominal region was weighed at the time of death. Age had a significant effect on fat pad mass. Twentyfive-month-old control rats (12.7  $\pm$  0.4 g) averaged 400% greater fat mass than the 5-mo-old  $(3.1 \pm 0.3 \text{ g})$ group. ND treatment had a significant main effect (P =0.024) on fat pad mass across both ages, and there was a significant interaction (P = 0.023) between age and ND treatment. Fat pad mass was significantly reduced by 30% in 25-mo-old rats receiving ND (8.9  $\pm$  1.0 g) compared with 25-mo-old controls (12.7  $\pm$  0.4 g). ND treatment did not affect 5-mo-old rat  $(3.1 \pm 0.3 \text{ g vs.})$  $3.1 \pm 0.4$  g) fat pad weight. In the 25-mo-old animals, ND treatment induced a greater decrease in fat pad mass (-30%) than decrease in body weight (-2%).

Because of the ND treatment having a significant effect on body weight, fat pad weight was also corrected for body weight (Fig. 2). There was a significant main

Table 1. Organ weights

	5-mo Control Tissue Wt, mg	5 mo + ND Tissue Wt, mg	25-mo Control Tissue Wt, mg	25 mo + ND Tissue Wt, mg
Heart Testes Prostate	$\begin{array}{r} 883 \pm 27 \\ 3{,}490 \pm 98 \\ 857 \pm 92 \end{array}$	$\begin{array}{c} 948 \pm 42 \\ 3,236 \pm 64 \dagger \\ 844 \pm 75 \dagger \end{array}$	$\begin{array}{c} 1,375\pm23^{*}\\ 4,416\pm313^{*}\\ 896\pm37^{*} \end{array}$	$\begin{array}{c} 1,404\pm37^{*}\\ 3,642\pm177^{*}\\ 1,230\pm40^{*}\\ \dagger\\ \vdots \end{array}$

Values are mean changes  $\pm$  SE. 5-mo, 5-mo-old rat receiving weekly sham injections; 5 mo + ND, 5-mo-old rat receiving weekly nandrolone decanoate (6mg/kg) injections; 25-mo, 25-mo-old rat receiving weekly sham injections; 25 mo + ND, 25-mo-old rat receiving weekly nandrolone decanoate (6mg/kg) injections. \*Significant main effect of age (P < 0.05). †Significant main effect of ND treatment (P < 0.05). ‡Significantly different from young 5-mo control group (P < 0.05).

effect of age (P = .0001) on the fat pad-to-body weight ratio, with the 25-mo-old group being significantly greater. ND treatment also had a significant effect on the fat pad-to-body weight ratio across both age groups (P = 0.05). This effect appeared to be mainly due to the ND-induced 22% reduction in the fat pad to body mass ratio in 25-mo-old rats.

Heart, prostate, and testes weight. There was a significant effect of age (P = 0.0001) on heart weight (Table 1), with 25-mo control hearts being 55% larger than the 5-mo group. There was no effect of ND treatment on total heart weight. However, when heart weight was corrected for body weight, there was a significant (P = 0.0085) 9% increase in ND treatment hearts across both groups. Prostate weight was significantly affected by age (P = 0.0037) and ND treatment (P = 0.021) (Table 1). Regardless of ND treatment, prostates from 25-mo-old rats were 25% larger on average than those from the 5-mo-old animals. The main effect of ND treatment was due to the response of the 25-mo-old rats. There was a significant interaction of age and ND treatment (P = 0.0138), and 25-mo-old rats receiving ND treatment had significantly larger prostates than the other groups. ND treatment induced a 37% increase in prostate weight of the 25-moold rats, whereas it had no effect on 5-mo-old rats.

Testes weight was significantly affected by age (P = 0.0048; Table 1). Regardless of ND treatment, testes

from 25-mo-old rats were 20% larger on average than those from the 5-mo-old animals. There was also a significant main effect of ND treatment (P = 0.031; Table 1), with testes weight being significantly reduced 13% across both age groups.

Skeletal muscle weight. There was a significant effect of age on soleus and plantaris muscle weights. Muscles from 25-mo-old rats were significantly larger (Table 2), which was likely related to the significantly higher (54%) body weights in these rats. However, the differences in soleus (22%) and plantaris (10%) muscle weights were not as great as the difference in body weight. Because of differences in body weight between groups, muscle mass was also expressed relative to body weight (Table 2). Age had a significant effect on the muscle-to-body weight ratio, which was reduced in both the soleus (-21%) and plantaris (-31%) muscle from 25-mo-old animals. There was no effect of ND treatment on soleus or plantaris muscle weight or muscle-to-body weight ratio at either age. ND treatment appeared not to be sufficient for restoring the age-induced decrement in the muscle-to-body weight ratio of the rat hindlimb muscles. Muscle weights were also examined for the gastrocnemius, tibialis anterior, and extensor digitorum longus (EDL). These muscles showed a similar pattern of results as the soleus and plantaris muscles (Table 2). There was no significant effect of age on the gastrocnemius or EDL muscle weights, although the 25-mo-old rats had significantly higher body weights. ND treatment had no effect on total muscle weight for the gastrocnemius, EDL, or tibialis anterior muscles in either age group.

*Muscle DNA content*. Muscle DNA content is directly related to the number of nuclei in the muscle (26). Nuclei may be associated with myofibers, as well as other cell types within the muscle. The response of DNA content to aging and ND treatment differed between the soleus and plantaris muscles. ND treatment had a significant effect on soleus muscle DNA concentration (Fig. 3A) across both ages. Soleus muscle DNA concentration was significantly reduced by 26% by aging. However, ND administration significantly induced the 25-mo soleus DNA concentration 51% above the

Table 2. Muscle weights and muscle weight relative to body weight

	5-mo Control		5  mo + ND		25-mo Control		25  mo + ND	
	MW, mg	MW/BW, mg/g	MW, mg	MW/BW, mg/g	MW, mg	MW/BW, mg/g	MW, mg	MW/BW, mg/g
EDL	$152 \pm 22$ ( <i>n</i> = 9)	$0.44\pm0.05$	$162 \pm 8^{+}_{+}$ ( <i>n</i> = 9)	$0.47\pm0.01$	$182 \pm 18$ ( <i>n</i> = 9)	$0.32 \pm 0.03^*$	$162 \pm 14$ ( <i>n</i> = 9)	$0.31 \pm 0.03^{*}$ †
ТА	$647 \pm 30$ (n = 9)	$1.89\pm0.08$	$669 \pm 22$ ( <i>n</i> = 9)	$1.90\pm0.01$	$740 \pm 21^{*}$ (n = 9)	$1.29\pm0.03^*$	$695 \pm 10^{*}$ (n = 9)	$1.34 \pm 0.06^{*}$
Gas	$1,652 \pm 121$ (n = 9)	$4.86\pm0.41$	$1,758 \pm 99$ ( <i>n</i> = 9)	$5.01\pm0.16$	$1,823 \pm 100$ ( <i>n</i> = 9)	$3.14 \pm 0.18^{*}$	$1,910 \pm 81$ ( <i>n</i> = 9)	$3.63\pm0.12^*$
Sol	$143 \pm 15$ ( <i>n</i> = 9)	$0.43 \pm 4.94$	$149 \pm 3$ ( <i>n</i> = 9)	$0.45\pm0.01$	$185 \pm 8^{*}$ (n = 9)	$0.34 \pm 0.01^{*}$	$189 \pm 6^{*}$ ( <i>n</i> = 9)	$0.368\pm0.01$
Plan	$361 \pm 9$ $(n = 9)$	$1.08\pm0.03$	$348 \pm 8$ (n = 9)	$1.04\pm0.02$	$402 \pm 10^{*}$ (n = 10)	$0.74 \pm 0.03^{*}$	$399 \pm 16^{*}$ (n = 10)	$0.785\pm0.04$

Values are mean changes  $\pm$  SE; *n*, number of animals sampled. MW, muscle weight; MW/BW, muscle weight-to-body weight ratio; EDL, extensor digitorum longus muscle; TA, tibialis anterior muscle; Gas, gastrocnemius muscle; Sol, soleus muscle; Plan, plantaris muscle. \*Significant main effect of age (P < 0.05). †Significant main effect of ND treatment (P < 0.05).

А

25-mo control and 12% above the 5-mo control. ND administration had no affect on 5-mo soleus muscle DNA concentration. The total muscle DNA content of the soleus was not significantly different between the 5-mo-old (265  $\pm$  21 µg) and 25-mo-old (239  $\pm$  26 µg) groups, although soleus muscles from the 25-mol-old group were significantly larger (22%). ND treatment induced a 45% increase in the total muscle DNA content of the 25-mo soleus (346  $\pm$  20 µg), whereas not altering the 5-mo (264  $\pm$  28 µg) levels.

Muscle DNA concentration in 5-mo-old rats was greater in the soleus than in the plantaris muscle, whereas no difference between these muscles was found in the 25-mo-old rats. DNA concentration was reduced by 44% in 5-mo plantaris muscle (0.96  $\pm$  0.1  $\mu$ g) compared with the 5-mo soleus muscle (1.70  $\pm$  0.13  $\mu$ g). The 25-mo plantaris (1.32  $\pm$  0.21  $\mu$ g) and soleus  $(1.27 \pm 0.17 \ \mu g)$  muscle DNA concentrations were not different. ND treatment had no effect on plantaris muscle DNA concentration, regardless of age (Fig. 3A). However, total plantaris muscle DNA content was significantly greater in 25-mo-old (562  $\pm$  78 µg) rats compared with the 5-mo-old group (365  $\pm$  31 µg). ND treatment did not affect total DNA content in the 25-mo (555  $\pm$  113 µg) or in the 5-mo (403  $\pm$  104 µg) plantaris muscles.

Muscle total RNA and protein content. Total RNA concentration is an indicator of protein synthetic capacity since the majority of RNA is ribosomal (23). RNA concentration in the soleus and plantaris muscles had different responses to aging and ND treatments (Fig. 3B). Soleus muscle RNA concentration was significantly affected by age (P = 0.0136), which was decreased by 10% in 25-mo-old rats, whereas age had no effect on plantaris muscle RNA concentration. ND treatment did not affect soleus muscle RNA concentration, regardless of age. However, there was a trend (P = 0.07) for ND treatment to increase plantaris muscle RNA concentration across both age groups.

ND treatment had no affect on soleus or plantaris total muscle protein concentration, regardless of age (Fig. 3C). There was a significant effect of age on soleus muscle protein concentration (P < 0.05), which was reduced by 10% in the 25-mo soleus. There was no affect of age on plantaris muscle total protein concentration.

Soleus and plantaris muscle AR and GR concentration. AR and GR protein concentrations (see Fig. 4) in rat plantaris and soleus muscle differed in their response to aging and ND treatment. Soleus AR concentration was significantly reduced (P = 0.0001) in the 25-mo-old control rats to 35% of the levels found in the 5-mo-old controls (Fig. 5A). ND treatment had a significant main effect (P = 0.0001) on AR protein concentration in rat soleus muscle regardless of age. ND treatment increased soleus AR protein concentration compared with same-age controls by 250% in the 5-moold rats and by 940% in the 25-mo-old rats. In contrast to the soleus muscle, plantaris AR concentrations in 5and 25-mo-old control rats were not significantly dif-

2.5 n (µg DNA/mg muscle) 2 DNA Conc. 1.5 1 0.5 0 5-m+ND 25-m+ND 5-m 25-m Treatment в con) 1.5 RNA Conc. (Normalized to 5-m 1 0.5 0 5-m+ND 5-m 25-m 25-m+ND Treatment С Protein Conc. (normalized to 5-m con) 0.5 0 25-m 5-m 5-m+ND 25-m+ND Treatment

□ soleus ■ plantaris

Fig. 3. A: ND treatment increases 25-mo-old old rat soleus muscle DNA concentration. The graph represents soleus and plantaris muscle DNA concentration (µg DNA/mg muscle). Data are representative of 5-6 animals per treatment group and were measured in one soleus and plantaris muscle from each animal. B: RNA concentration in soleus and plantaris muscle. The graph represents the change in soleus and plantaris muscle total RNA concentration. Total RNA values (µg RNA/mg muscle) are shown normalized to the 5-mo-old control group for each muscle. Data are representative of 9-10 animals per treatment group and were measured in a soleus and plantaris muscle from each animal. C: protein concentration in soleus and plantaris muscle. The graph represents the change in soleus and plantaris muscle protein concentration. Protein concentration values (µg protein/mg muscle) are shown normalized to the 5-mo-old control group for each muscle. Data are representative of 9-10 animals per treatment group and were measured in a soleus and plantaris muscle from each animal. Data are expressed as means  $\pm$  SE. "Significant main effect of ND treatment (P < 0.05). \*Significantly different from other treatment groups (P < 0.05). <sup>a</sup>Significant main effect of age (P < 0.05).

ferent (Fig. 5A). However, there was a trend for a main effect of age (P = 0.067) across all treatment groups. Like the soleus, plantaris muscle AR concentration had a significant main effect of ND treatment (P = 0.0001)



Fig. 4. Western blot analysis of androgen receptor (AR) and glucocorticoid receptor (GR) protein in rat soleus muscle. Forty micrograms of crude muscle protein homogenate were fractionated by 8% SDS-PAGE. AR and GR protein quantified by immunoblotting and antibody detection. A: representative blot showing AR protein levels (see arrow) in 5- and 25-mo control soleus muscles. Ponceau-stained membrane below demonstrates even loading and transfer. B: representative blot showing AR protein levels (see arrow) in 25-mo control soleus and 25-mo soleus receiving nandrolone decanoate (ND) treatment. Ponceau-stained membrane below demonstrates even loading and transfer. C: representative blot showing GR protein levels (see arrow) in 5- and 25-mo control soleus muscles. Ponceau-stained membrane below demonstrates even loading and transfer.

across both ages. Plantaris AR protein concentrations in ND-treated rats compared with same-age controls were 430% (5 mo) and 310% (25 mo) greater.

Neither age nor ND treatment had a significant effect on GR protein levels in the rat soleus muscle. However, in the plantais muscle, there was a main effect of age (P = 0.0267) on GR protein levels across both treatment groups (Fig. 5B). This appeared to be mainly due to the response of the 25-mo plantaris receiving ND treatment. There was no main effect of ND treatment, but there was a significant interaction (P = 0.0257) of age and ND treatment, with the 25-mo-old ND-treated rats having a 63% greater concentration of GR protein than the 5-mo ND-treated group.

### DISCUSSION

Skeletal muscle is a biological target of testosterone action (29). Anabolic-androgenic steroids are manufactured, structural derivatives of testosterone and can differ due to the growth (anabolic)- and androgenic (secondary sex characteristics)-inducing properties, as well as their biological half life. Anabolic steroid's effect on aged rat hindlimb skeletal muscle undergoing sarcopenia has not been previously documented. To our knowledge, this is the first study to report an ageinduced decrease in rat skeletal muscle AR concentration and that ND administration can reverse this age-



Fig. 5. A: AR protein concentration is reduced by aging in soleus muscle and induced by ND treatment in both soleus and plantaris muscles. The graph represents the change in soleus and plantaris AR protein concentrations. AR values (integrated optical denisty/40 µg protein) are the integrated optical density from densitometry scanning of enhanced chemiluminescence-detected protein. B: soleus muscle GR protein concentration is not sensitive to aging. The graph represents the change in soleus and plantaris GR protein concentrations. GR values (integrated optical denisty/40 µg protein) are the integrated optical density from densitometry scanning of enhanced chemiluminescence-detected protein. Data are representative of 6-8 animals per treatment group and were measured in a soleus and plantaris muscle from each animal. Data are means  $\pm$  SE. <sup>a</sup>Significant main effect of age (P < 0.05). "Significant main effect of ND treatment (P < 0.05). \*Significantly different from 5-mo control group (P < 0.05). #Significantly different from 5-mo ND group (P <0.05).

related decrement in AR protein concentration while not altering skeletal muscle mass. Although the skeletal muscle mass appeared to be undergoing a sarcopenia-like atrophy in the aged animals, the anabolic steroid treatment was not sufficient to restore skeletal muscle mass-to-body weight ratios to young adult levels in any rat hindlimb muscle examined.

AR and associated signaling mechanisms are excellent candidates for regulating gene expression that influences skeletal muscle mass. Testosterone-induced cellular regulation involves binding with its cytosolic AR, translocating to the nucleus, and then altering gene transcription by binding to its androgen response element (29, 41). AR expression is thought to be sensitive to circulating testosterone levels (2). Both the circulating levels of testosterone and the regulation of its release have been well characterized in the aged Fisher 344/Brown Norway rat, with advancing age circulating testosterone levels decreasing from 2.4 ng/ml at 4 mo of age to 0.3 ng/ml at 28 mo of age (17). Aged Fischer 344/Brown Norway rats also have a decreased testosterone response to gonadotropin-releasing hormone compared with young rats. The release pattern of testosterone is also altered in aged rats. which does not demonstrate a bimodal diurnal variation that is seen in young rats (31). The reduction in circulating testosterone and variations in its release could be a mechanism for decreasing aged soleus AR protein concentration; however, the regulation of AR protein abundance by circulating testosterone levels appears to be muscle phenotype specific. Aging did not reduce the fast-type plantaris muscle AR protein concentration as it did in the slow-type soleus muscle. The lack of change of plantaris AR protein concentration with a decrease in circulating testosterone supports previous research demonstrating that plantaris AR protein concentration in very young rats is not sensitive to castration (2).

AR protein induction by anabolic steroid administration has not been previously reported in the rat soleus and plantaris muscles. The plantaris muscle has previously been shown to be an unresponsive anabolic steroid administrator (2). Different abilities to induce plantaris AR concentration with anabolic steroid administration may be related to the type of anabolic steroid administered. For example, ND and Stanozolol anabolic steroids have different abilities to induce heat shock protein 72 in rat skeletal muscle (28), and this difference in induction parameters may be related to the fact that ND is an estrene derivative, whereas stanozolol is an androstane derivative of testosterone. ND was used in the present study because it has been widely examined in both rodent and human studies on anabolic steroid action and because it has documented anabolic effects on rat hindlimb muscle (53-55, 58). Additionally, ND is also a long-lasting anabolic steroid, with one injection elevating circulating levels for up to 4 wk (53). These data demonstrate the complexity of skeletal muscle regulation induced by anabolic steroid administration and the importance of controlling for

anabolic steroid type when investigating hormone action.

Aging did not affect AR protein induction in the soleus muscle treated with ND. AR ligand binding capacity increases in rat skeletal muscle induced to hypertrophy by functional overload (7, 32). AR mRNA levels also increase in resistance-exercised human muscle (56). Administration of an AR agonist can suppress exercise-induced rat gastrocnemius hypertrophy, although significant growth can still occur (33). Although there is a relationship of AR induction with hypertrophying/overloaded muscle, there was no induction of plantaris or soleus muscle mass in the present study. The induction of AR protein could be in muscle fibers or alternatively in activated satellite cells. Muscle fibers are multinucleated and are thought to have an optimal nuclei-to-cytoplasm ratio (1). Additional nuclei supplied from replicating satellite cells are needed to support postnatal muscle growth (44, 49). Satellite cell activation also appears to be required for overload-induced rat muscle hypertrophy (48). An increase in AR protein in the aged soleus, related to satellite cell proliferation, is a possibility because DNA content also increased in the aged soleus muscle. Satellite cells are direct targets of androgen action upregulating AR concentration and decreasing differentiation of porcine satellite cells in culture (20, 35). Testosterone also enhances satellite cell activity and myonuclei incorporation into growing muscle fibers of the triceps brachii from postnatal pigs (43). The rat levator ani muscle also has increased satellite cell activation and myonuclei accumulation with testosterone treatment (35). However, plantaris and adult soleus muscle AR concentrations were induced by ND even though there was no corresponding increase in muscle DNA content. Further work is needed to demonstrate an effect of anabolic steroid administration on satellite cell activation in the aged rat soleus.

Advancing age did not reduce GR protein concentration in the soleus or plantaris muscles. GR binds its steroid ligand in the cytosol and translocates to the nucleus, where it exerts specific control over gene transcription (29). Glucocorticoid action is associated with cellular catabolism, and elevated levels of circulating glucocorticoids can result in muscle atrophy (27, 36). Glucocorticoid-induced skeletal muscle atrophy can be offset by an infusion of anabolic stimuli such as insulin-like growth factor I (36). The ratio of circulating testosterone to glucocorticoids has been hypothesized to be a critical indicator of an organism's anabolic state. The skeletal GR levels do not appear to be as sensitive to circulating androgen levels as the AR protein; however, our data suggest in fast-type muscle the sensitivity of GR abundance to ND treatment is altered by aging.

Slow-type muscle fibers have a higher DNA concentration, DNA-to-cytoplasm ratio, and overall satellite cell percentage compared with fast muscle (1, 26, 49). Differences in DNA content between muscle types have been hypothesized to be due to the high degree of oxidative metabolism and the postural role of slow-type muscle, such as the soleus. These same characteristics also make the soleus a candidate for biological aginginduced decrements in cellular function since highly oxidative, postmitotic cells have been identified as aging targets (38). The aged soleus muscle's decreased DNA content has several potential implications involving muscle wasting and/or muscle regenerative capacity due to potential changes in the myonuclear domain within muscle fibers. All cell nuclei contain a finite quantity of DNA, and skeletal muscle consists of many different cell types, including muscle fibers, satellite cells, fibroblast cells, and endothelial cells, among others (26). Myofiber-associated nuclei have been reported to represent  $\sim 37-45\%$  of the total rat soleus nuclei and  $\sim$ 50% of the rat EDL muscle nuclei (26). ND administration reversed the age-induced decrements in soleus muscle DNA concentration. The reversal of age-related declines in DNA content in the aged soleus muscle may set the stage to allow for the prevention of further age-induced wasting and improved regenerative capacity for the aged soleus muscle.

The present study clearly demonstrates an effect of anabolic steroid action. Several testosterone-sensitive tissues had their mass altered. Other than a main effect of anabolic steroid treatment decreasing testicular weights across both ages, the prostates of the aged animals were dramatically increased. However, like the decreased mass of the perirenal fat pad, most steroid-induced changes were limited to the aged animals. Anabolic steroid administration in adult humans has demonstrated an anabolic growth effect on skeletal muscle (4, 5, 21, 22, 30). However, it is critical to understand the different mechanisms of anabolic steroid action that would maintain muscle mass in a catabolic state (prevent further wasting) vs. the induction of anabolic growth. Many patients with chronic wasting diseases currently receive anabolic steroid therapy for maintaining their muscle mass (34, 51). Testosterone administration has also been shown to benefit aged skeletal muscle by increasing skeletal muscle protein synthesis in elderly men (56). Anabolic steroid administration can reduce rat hindlimb muscle atrophy due to unloading (54, 55) and abolish unloaded atrophy in the quadriceps muscle (58). The molecular mechanisms behind the prevention of muscle wasting while the organism is in a catabolic state or how biological aging would affect this regulation is not known.

In summary, this study reports that the physiological effects of 4 wk of ND administration in the rat are dependent on both the age of the animal and the muscle phenotype examined. Aging reduced muscle-tobody weight ratios for all rat hindlimb muscles examined, and there was no effect of ND treatment regardless of age. Advancing age reduced soleus muscle AR concentration, and ND was able to induce AR protein concentration above adult levels. Further work is needed to understand the functional significance and complex signaling mechanisms involved in aged skeletal muscle subjected to anabolic steroid administration. The authors would like to acknowledge the technical assistance of Tyrone Washington, Ray Thompson, and Tonia Zehr.

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