Overload-induced androgen receptor expression in the aged rat hindlimb receiving nandrolone decanoate

WON JUN LEE, JOSEPH McCLUNG, G. A. HAND, AND JAMES A. CARSON

Integrative Muscle Biology Laboratory, Exercise Science Department,
University of South Carolina, Columbia, South Carolina 29208

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Lee, Won Jun, Joseph McClung, G. A. Hand, and James A. Carson. Overload-induced androgen receptor expression in the aged rat hindlimb receiving nandrolone decanoate. J Appl Physiol 94: 1153–1161, 2003; 10.1152/japplphysiol.00822.2002.—This study’s purpose was to examine whether functional overload with nandrolone decanoate (ND) administration increased muscle mass and steroid receptor concentration in aged rat soleus (Sol) and plantaris (Plan) muscle. ND (6 mg/kg body wt) was administered once a week for 4 wk, whereas control rats received sesame seed oil injections. Functional overload of the hindlimb Sol and Plan was induced by synergistic gastrocnemius muscle ablation at the beginning of the fourth week. Adult (5 mo of age) and aged rats (25 mo of age) were randomly assigned to four groups: control, overload, control-ND, and overload-ND. Seven days of functional overload increased adult Sol muscle mass 27%, whereas the aged Sol muscle mass did not change. The aged overloaded Sol muscle receiving ND significantly increased muscle weight by 35% and total muscle protein by 24%. Aged Plan muscle did not increase muscle weight with overload or ND treatment. Androgen receptor protein was induced by ND treatment and functional Ov, and combining the two treatments induced Sol androgen receptor protein concentration above either alone. Sol glucocorticoid receptor protein concentration increased in overload groups of both ages. ND administration can increase aged Sol muscle mass and protein content after 7 days of functional overload, and the cooperative induction of androgen receptor may be important for this response.

steroid receptor; hypertrophy; nandrolone decanoate; sarcopenia; muscle wasting

AGING RESULTS IN A PROGRESSIVE decrease in muscle strength, which can in part be attributed to muscle mass loss (31). Skeletal muscle is primarily composed of highly oxidative, postmitotic fibers, which undergo constant remodeling, and aging can induce an imbalance within this remodeling process (29). Age-induced muscle mass loss is associated with decreased muscle protein synthesis, muscle fiber loss, α-motoneuron loss, and a decline in fiber cross-sectional area (35). Although skeletal muscle is a dynamic tissue, aging decreases its plasticity to many stimuli, and this is especially true of stimuli requiring muscle regeneration or remodeling (5, 8, 14, 30). 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 (9, 22). Systemic-related changes with aging include alteration in cardiovascular, endocrine, and immune systems (31). Signaling stimuli targeting muscle may be deficient in the aged organism; therefore, aged muscle’s regenerative capacity could possibly be restored if provided the appropriate stimuli.

Functional work overload of rat hindlimb muscle induces a rapid remodeling response, which includes structural damage, fiber growth, satellite cell activation, and macrophage infiltration (10, 16, 28, 41). Each of these responses has been hypothesized to be an important component for the large increases in muscle mass and protein content induced by functional overload. Aging alters rat hindlimb muscle growth induced by functional overload. Plantaris (Plan) muscle from 36-mo-old rats does not adapt to 8 wk of functional overload in the same manner as muscles from young rats. However, the response of aged rat hindlimb muscle during the onset of functional overload has not been determined.

Anabolic steroids are structural derivatives of testosterone, which can increase skeletal muscle mass and protein synthesis in adult and aged individuals (3, 38, 47). Exogenous testosterone administration with muscle loading can synergistically increase skeletal muscle mass in healthy and diseased humans (4). A synergistic relationship between testosterone and muscle loading is present in functionally overloaded rat muscle subject to disuse atrophy (45). Anabolic steroid administration can also abolish unloaded atrophy in the rat quadriceps muscle (48). Although anabolic steroids are a potent skeletal muscle mass effector, the intracellular signaling mechanisms induced by the interaction of overload and anabolic steroids have not been demonstrated.

Steroid receptors, a subgroup of the nuclear receptor superfamily, are transcription factors showing high homology in their DNA-binding domain (44). The androgen receptor and glucocorticoid receptor are both potential modulators of skeletal muscle mass. These receptors can alter cellular gene expression by binding

Address for reprint requests and other correspondence: J. A. Carson, Univ. of South Carolina, Dept. of Exercise Science, 1300 Wheat St., Columbia, SC 29208 (E-mail: carson@sc.edu).

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ligand in the cytosol, translocating to the nucleus, and binding to their corresponding DNA response element to alter gene transcription (33, 44). Steroid receptors have also been shown to have ligand-independent effects on cellular gene expression, which can be modulated by several signaling pathways (25). Targets of androgen and glucocorticoid receptor-induced transcriptional activity related to skeletal muscle mass regulation have not been well characterized. The expression of the skeletal muscle androgen receptor is sensitive to circulating hormone levels, age of the animal, and muscle loading conditions. In humans, the skeletal muscle androgen receptor is downregulated after puberty (36), and androgen receptor levels are also decreased in 25-mo-old Fischer 344 × Brown Norway rat soleus (Sol) muscles compared with 5-mo-old animals (12). This change in androgen receptor concentration coincides with a dramatic reduction in circulating testosterone levels in Fischer 344 × Brown Norway rats between 4 and 28 mo of age (15). Skeletal muscle androgen receptor protein expression increases in aged men administered a physiological dose of testosterone for 1 mo (18). Rat Sol and Plan androgen receptor concentrations are induced by anabolic steroid administration in both young and old rats (12). Androgen receptor ligand binding capacity in rat skeletal muscle increases in functionally overloaded muscle (6, 23). However, the androgen receptor expression induction during overload induced muscle growth in aged rats is not known.

To our knowledge, the interaction between anabolic steroid administration and functional overload in aged rat hindlimb muscle has not been examined previously. The purpose of this study was to examine whether functional overload with nandrolone decanoate pretreatment increases muscle mass and steroid receptor concentration in aged rat Sol and Plan muscles. It was hypothesized that aging would attenuate muscle mass increases and androgen receptor protein induction after 7 days of functional overload. We also hypothesized that nandrolone decanoate pretreatment would interact with functional overload to increase muscle mass and steroid receptor concentration in aged skeletal muscle. The effects of synergist ablation-induced functional overload and nandrolone decanoate treatments on muscle mass, total DNA, total RNA, protein concentration, and steroid receptor protein concentration were examined in 5- and 25-mo-old Fischer 344 × Brown Norway rats.

METHODS

Animals and housing. Forty-three male Fisher 344 × F1 Brown Norway rats were acquired from the National Institutes on Aging aged rodent colony. Twenty rats were ~4 mo at the start of the study, and twenty-three rats were ~24 mo 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 the fully accredited animal care facilities at the University of South Carolina, Columbia. Rats were randomly assigned to four treatment groups as follow: 1) control-oil (Con), 2) overload-oil (Ov), 3) control-steroid (Con-S), 4) overload-steroid (Ov-S). All procedures were approved by the University of South Carolina Animal Care and use Committee. A subset of the animals in the study was used in a study examining differences due to age. Data regarding a subset of control-oil and control-steroid treatment groups have been published elsewhere (12). As stated in the present study’s purpose statement, the emphasis of this study is on interactions between functional overload and nandrolone decanoate pretreatment.

Anabolic steroid administration. The anabolic steroid nandrolone decanoate (Deca-Durabolin, Oralon) was used in these studies because of its long biological half-life and because previous studies have demonstrated an anabolic effect in rat skeletal muscle (12). The selected dose of nandrolone decanoate administration has been previously demonstrated to prevent hindlimb suspension-induced rat skeletal muscle atrophy (48). Nandrolone decanoate was injected (6 mg/kg body wt) intramuscularly into the hip region every 7 days, 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 injections of either nandrolone decanoate in sesame seed oil or sesame seed oil alone. Sham or synergist ablation surgeries were performed after the third week of nandrolone decanoate treatment, and the fourth injection was given at the time of surgery.

Surgical ablation of synergists. The hindlimb Sol and Plan muscles were functionally overloaded for 7 days by surgical ablation of the distal third of the lateral and medial gastrocnemius muscle as previously reported (13). Rats were anesthetized with an intramuscular injection of a cocktail containing ketamine hydrochloride (75 mg/kg body wt), xylocaine (3 mg/kg body wt), and acepromazine (5 mg/kg body wt). After centripetal dissection, the dorsal surface of the hindlimb was shaved and cleaned, and the gastrocnemius muscles were then exposed by a posterior longitudinal incision through the skin and biceps femoris muscle of each lower hindlimb, and the distal two-thirds of heads of each gastrocnemius muscle were excised. The nerve and vasculature supplies to the remaining musculature were undisturbed. Control animals underwent sham surgeries, which consisted of the same procedure, except for gastrocnemius excision. Incisions were closed by using wound clips, with no postoperative complications being observed over the course of the study. After the rats recovered from the anesthetic, they were returned to their cages. Rats were weighed weekly and observed for signs of failure to thrive, such as precipitous weight loss, disinterest in the environment, or unexpected gait alterations. Sol and Plan muscles were removed at 7 days after the initial surgery, frozen in liquid nitrogen, and stored at −80°C until further analysis.

Total DNA. Total muscle DNA was quantified as previously described (12). Briefly, a frozen Sol or Plan muscle (~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 HClO4 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) and 0.75 vol of 1.2 N HClO4 was then added to the supernatant. After centrifugation (4°C, 12,000 g for 10 min), the supernatant was transferred to a new tube, and the pellet was washed two more times with 1.2 N HClO4. The supernatants from all washes combined for total RNA concentration. The pellet was resuspended in 1 M NaOH and incubated for 30 min at 50°C. DNA content was determined by a standard
fluorimetric assay using bis-benzimide and salmon sperm DNA standards. DNA is expressed as the concentration per milligrams of muscle and as total DNA per whole muscle.

Total RNA. Total muscle RNA was quantified as previously described (12). Briefly, after initial homogenization centrifugation (see DNA assay), the pellet was washed two times with 1.2 N HClO₄, the supernatants from all washes were combined, and the RNA was quantified by UV absorbance at 260 nm. Total RNA is expressed as the concentration per milligrams of muscle and as total RNA per whole muscle.

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

Crude protein extracts. Crude protein extracts were made as previously described (12, 19, 20). Frozen Sol and Plan 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 Na₃PO₄, 100 mM β-glycerolphosphate, 25 mM NaF, 1 mM Na₂VO₄, 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) by 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 Lowrey assay (Bio-Rad) and aliquoted at ~80°C until use for Western blotting.

Western blot analysis. Western blot analysis was performed as previously reported (11). Forty micrograms of crude homogenate protein (40 µg) were incubated (15 min, 65°C) with an equal volume of protein sample buffer and fractionated on a 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 androgen receptor and glucocorticoid receptor demonstrated that 40 µg of crude protein extract gave a signal in a linear range for quantification (data not shown). The membrane was then probed with either androgen receptor (N-20, Santa Cruz Biotechnology, Santa Cruz, CA) or glucocorticoid receptor (M-20, Santa Cruz Biotechnology) and polyclonal rabbit antibodies as previously described (16). The donkey anti-rabbit IgG horseradish peroxidase-linked secondary antibody was visualized by enhanced chemiluminescence (Amersham Life Sciences) as per manufacturer’s instructions and quantified by densitometry scanning.

Data analysis. Results are reported as means ± SE. For each age group, all variables were analyzed by a two-way ANOVA analysis (steroid treatment × functional overload) for each variable to determine significant effects and interactions (P ≤ 0.05). When a significant main effect of overload was found without any significant effect of steroid treatment or any steroid × overload interaction, data were presented as the pooled sham controls (oil and steroid) vs. the pooled 7-day overload (oil and steroid). This was also done if the main effect was steroid treatment. Post hoc analysis on significant interactions was done with a Bonferroni test (P ≤ 0.05).

RESULTS

Body weight, muscle mass, and protein concentration. Body weight was not affected by nandrolone decanoate or functional overload in adult animal. There was a significant 6% decrease (P = 0.041) in body weight in aged animal (566 ± 11 vs. 530 ± 11 mg/kg). In adult animals, there was no effect of nandrolone decanoate treatment on Sol and Plan muscle weight. However, across both ND and oil treatment, there was a significant 27% (P = 0.0025) and 14% (P = 0.01) increase in Sol and Plan muscle weight in the overloaded group (155 ± 6 vs. 196 ± 9 and 371 ± 12 vs. 423 ± 17 mg, respectively; Fig. 1). Aged Sol muscle wet weight was not increased after 7 days of functional overload, although adult Sol muscle mass increased. However, aged Sol muscle from rats receiving nandrolone decanoate and functional overload treatments increased muscle weight by 35% (P = 0.0024; Fig. 1A). Aged Plan muscle mass was not affected by functional overload or nandrolone decanoate treatments (Fig. 1B). Muscle-to-body weight ratio (mg/kg) data showed a similar trend as muscle weight. Aged Sol muscle from rats receiving nandrolone decanoate and functional overload treatments increased muscle-to-body weight.

Fig. 1. Soleus (A) and plantaris (B) muscle weights. Values are means ± SE. 5-m, 5-mo-old rats; 25-m, 25-mo-old rats; 5-m Con, 5-m receiving oil only and nandrolone decanoate (ND) only treatments; 5-m Ov, 5-m receiving oil + overload (Ov) and ND + Ov treatments; 25-m Con, 25-m receiving oil only; 25-m Ov, 25-m receiving Ov + oil; 25-m Con-S, 25-m receiving ND only; 25-m Ov-S, 25-m receiving Ov + ND. Where there was no main effect of steroid treatment or interaction of overload and steroid treatments, the significant main effect of overload across the pooled oil and ND is shown.* Significant main effect of functional overload across pooled oil and steroid treatment groups, P < 0.05. #Significantly different from other same-age treatment groups, P < 0.05.
Table 1. Soleus muscle-to-body weight ratio and protein concentration

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<td>Muscle weight/body weight ratio, mg/kg</td>
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<td>5 mo</td>
<td>0.46 ± 0.03</td>
<td>0.58 ± 0.02</td>
<td>0.44 ± 0.01</td>
<td>0.63 ± 0.04</td>
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<td>25 mo</td>
<td>0.33 ± 0.02</td>
<td>0.35 ± 0.01</td>
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<td>Protein concentration, µg/mg</td>
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<tr>
<td>5 mo</td>
<td>154 ± 10.9</td>
<td>140 ± 9.1</td>
<td>173 ± 11.9</td>
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<tr>
<td>25 mo</td>
<td>151 ± 8.4</td>
<td>146 ± 3.4</td>
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Values are means ± SE. Con, oil injections only; Ov, functional overload only; Con-S, nandrolone decanoate only; Ov-S, functional overload with nandrolone decanoate; 5 mo, 5-mo-old rats; 25 mo, 25-mo-old rats.

The RNA-to-DNA ratio is related to the quantity of RNA per nuclei in the muscle, because nuclei have a finite amount of DNA. This ratio also can be used as an indicator of transcriptional activity per nuclei. The RNA-to-DNA ratio in rat Plan and Sol muscle differed in response to functional overload and nandrolone decanoate treatment (Fig. 3). The Sol muscle the RNA-to-DNA ratio was not affected by either functional overload or nandrolone decanoate treatment in adult animals, whereas nandrolone decanoate treatment induced a significant decrease in RNA-to-DNA ratio in aged animals as averaged across control and overload treatment groups (Fig. 3A). The Plan muscle significantly increased the RNA-to-DNA ratio in both age groups after functional overload as averaged across

Fig. 2. Change in RNA concentration (Conc) in soleus (A) and plan- taris (B) muscle. Values are means ± SE. Con, pooled oil only and ND only treatments; Ov, oil + Ov and ND + Ov treatments. Graphs represent the significant main effect of overload across the pooled oil and ND treatments. There was no effect of steroid treatment or interaction of overload and ND treatments on RNA concentration in either the soleus or planataris muscles. *Significant effect of functional overload, P < 0.05.

Table 2. Plantaris muscle-to-body weight ratio and protein concentration

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<tr>
<td>Muscle weight/body weight ratio, mg/kg</td>
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<tr>
<td>5 mo</td>
<td>1.14 ± 0.09</td>
<td>1.30 ± 0.06</td>
<td>1.04 ± 0.04</td>
<td>1.36 ± 0.15</td>
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<td>25 mo</td>
<td>0.70 ± 0.03</td>
<td>0.73 ± 0.03</td>
<td>0.69 ± 0.03</td>
<td>0.79 ± 0.03</td>
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<tr>
<td>Protein concentration, µg/mg</td>
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<tr>
<td>5 mo</td>
<td>156 ± 6.4</td>
<td>123 ± 21.7</td>
<td>158 ± 19.4</td>
<td>97 ± 14.6</td>
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<tr>
<td>25 mo</td>
<td>160 ± 6.3</td>
<td>136 ± 9.4</td>
<td>155 ± 4.3</td>
<td>129 ± 8.1</td>
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Values are means ± SE.
Steroid receptor concentration. The expression of androgen receptor and glucocorticoid receptor protein in response to functional overload and nandrolone decanoate treatment differed between the Plan and Sol muscle. Across both nandrolone decanoate and oil treatments, adult animals subjected to functional overload increased Sol androgen receptor protein concentration by 113% ($P < 0.001$; Fig. 4B). Nandrolone decanoate treatment had no effect on adult Sol androgen receptor protein concentration (Fig. 4B). In aged animals, functional overload and nandrolone treatments had significant effects ($P < 0.001$) on Sol androgen receptor protein concentration as averaged across treatment group (Fig. 4B). Aged Sol muscle functionally overloaded and receiving nandrolone decanoate treatment increased androgen receptor protein concentration by 27-fold above the aged controls. When nandrolone decanoate and functional overload treatments were combined, adult Plan androgen receptor concentration increased threefold greater than the increase observed with nandrolone decanoate treatment alone (Fig. 4C). Adult Plan muscle, functionally overloaded and receiving nandrolone decanoate treatment,

Fig. 3. RNA concentration-to-DNA concentration ([RNA]/[DNA]) ratio in soleus (A) and plantaris (B) muscle. Values are means ± SE. A: graph represents the significant main effect of ND treatment across the pooled sham and overload treatments. Oil, oil injections only and oil injections + Ov; Steroid, ND injections only and ND injections + Ov. There was no significant effect of overload treatment or interaction of overload and ND treatments. *Significant main effect of ND treatment, $P < 0.05$. B: graph represents the significant main effect of overload across the pooled oil and ND. Con, oil only and ND only treatments; Ov, oil + Ov and ND + Ov treatments. There was no significant effect of ND treatment or interaction of overload and ND treatments. *Significant main effect of either Ov or ND treatment, $P < 0.05$.

Fig. 4. Western blot analysis of androgen receptor (AR) protein in rat soleus and plantaris muscles. Forty micrograms of crude muscle protein homogenate were fractionated by 8% SDS-PAGE. AR protein was quantified by immunoblotting and antibody detection. A: representative blot showing aged soleus AR protein levels (arrow). Ponceau-stained membrane (bottom) demonstrates even loading and transfer. Con, receiving oil only; Ov, receiving oil + Ov; Con-S, receiving ND only; Ov-S, receiving ND + Ov; B: soleus muscle AR protein concentration. 5-m Con, 5-m receiving oil only and ND only treatments; 5-m Ov, 5-m receiving oil + Ov and ND + Ov treatments; 25-m Con, 25-m receiving oil only; 25-m Ov, 25-m receiving Ov + oil; 25-m Con-S, 25-m receiving ND only; 25-m Ov-S, 25-m receiving Ov + ND. C: plantaris muscle AR concentration. 5-m Con, 5-m receiving oil only; 5-m Ov, 5-m receiving Ov + oil; 5-m Con-S, 5-m receiving ND only; 5-m Ov-S, 5-m receiving Ov + ND; 25-m Con, 25-m receiving oil only and ND only treatments; 25-m Ov, 25-m receiving oil + Ov and ND + Ov treatments. Values are means ± SE. Where there was no main effect of steroid treatment or interaction of overload and steroid treatments, the significant main effect of overload across the pooled oil and ND is shown. *Significant main effect of functional overload, $P < 0.05$. #Significantly different from other same-age treatment groups, $P < 0.05$. 

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increased androgen receptor protein concentration ninefold compared with the adult controls. In the aged Plan muscle functional overload as averaged across nandrolone decanoate and oil treatment significantly increased androgen receptor protein concentration 145% ($P = 0.0006$; Fig. 4C).

Sol muscle glucocorticoid receptor protein concentration was increased by functional overload in both age groups as averaged across both nandrolone decanoate and oil treatments, increasing by 77% in adult animals and by 56% in aged animals ($P < 0.0001$; Fig. 5B). Nandrolone decanoate treatment had no effect on Sol glucocorticoid receptor concentration in both age groups. However, adult Plan muscle glucocorticoid receptor protein concentration was only induced by functional overload as averaged across nandrolone decanoate and oil treatment (49%), and it did not significantly change in the aged animals (Fig. 5C).

**DISCUSSION**

Anabolic steroid administration and functional overload interactions have not been previously examined in aged rat hindlimb muscle. This study’s primary finding is that aged animals subjected to 3 wk of nandrolone decanoate pretreatment before 7 days of functional overload increased Sol muscle mass and protein content, whereas aged animals receiving placebo did not. This anabolic steroid and functional overload interaction appears to be phenotype specific because Plan muscle mass did not change in aged animals after functional overload regardless of nandrolone decanoate treatment. Another important finding resulting from this study is that the combination of functional overload and nandrolone decanoate pretreatment induces androgen receptor concentration in aged rat Sol muscle above either treatment alone but that it has no effect on the Plan muscle.

In the rat hindlimb, the examination of the Sol, primarily a slow-type postural muscle, and the Plan, primarily a fast-type muscle, allows comparisons between ankle plantar flexors that are primarily different phenotypes. Understanding the response of primarily fast and slow muscle phenotypes to aging and overload are important endeavors because human muscle is a heterogeneous mix of slow and fast fibers, and fiber-type composition has been shown to be altered with advancing age (31). Both the Sol and Plan muscles respond to functional overload with a rapid and large increase in muscle mass, which consists mainly of enlarged muscle fiber cross-sectional area (28, 45). However, rat hindlimb muscle’s capacity to enlarge in response to functional overload is compromised by advancing age (5). The Plan muscle from very aged male Fischer 344 × Brown Norway rats does not undergo hypertrophy after 8 wk of functional overload (5). The present study adds to this previous observation in several ways. The age-induced deficit in skeletal muscle adaptation to functional overload can occur at a considerably younger age, 25 vs. 36 mo. The present study also demonstrates that both primarily fast and slow muscle phenotypes have an attenuated response to functional overload, and this difference can be seen after only 1 wk of functional overload. However, the first week of functional overload includes a large regenerative response in fast- and slow-type rat skeletal muscle that is characterized by edema, macrophage infiltration, connective tissue proliferation, and satellite cell proliferation (28). Because of the lack of protein accretion at 7 days of overload in all treatment groups.
except the aged Sol receiving anabolic steroid, the lack of aged Sol and Plan muscle mass induction after 7 days of overload may be related to an altered damage and/or inflammation response. Sol and Plan muscle appear to respond differently to 1 wk of functional overload. Regardless of age, functional overload increased the ratio of RNA to DNA in the Plan muscle but not in the Sol muscle. This muscle-type difference appears to be due more to alterations in the muscle DNA pool with overload, rather differential induction of total RNA. Microarray analysis of gene expression in 3-day functionally overloaded Sol muscle from young rats has identified 112 differentially expressed genes compared with the control, and this list includes many local inflammation markers (13). Age-related alterations in skeletal muscle regenerative capacity are a promising candidate for rat hindlimb muscle’s attenuated plasticity at the onset of functional overload. Advancing age can diminish skeletal muscle’s regenerative response to stimuli producing muscle damage (8, 22, 28, 30).

A strong and growing body of scientific evidence suggests that age-induced decrements in muscle regenerative capacity are not intrinsic to the muscle but are dependent on the aging organism as a whole (9, 22). Systemic changes with aging that have implications for skeletal muscle regeneration include the cardiovascular, immune, and endocrine systems (31). Alterations in the endocrine system with age, including decreased testosterone levels and altered growth hormone release, continue to be targets of antiaging therapies (21, 31, 47). The numerous biological targets of anabolic steroids, synthetic derivatives of testosterone, limit their therapeutic use in aged individuals (21, 47). The regulation of skeletal muscle mass by anabolic steroid-induced cellular signaling and the potential for interactions with mechanical signaling pathways have not been well defined. Studies examining exogenous testosterone administration’s effect on young rat skeletal muscle mass have reported mixed results, and even less is understood about targets of altered gene expression (1, 7, 45, 48). Differences in results between studies are likely due to drug type, dosage, and administration schedules. Nandrolone decanoate, used in the present study, is known to have a greater proportional effect in tissues with low α5-reductase activity such as the muscle, compared with tissues with high α5-reductase activity, such as prostate (2). However, the nandrolone decanoate administration used in the present study did significantly increase rat prostate weight in the aged rats but not in the 5-mo-old animals (12). An interactive effect on muscle mass has been shown between anabolic steroid administration and mechanical overload in both resistance-trained humans and rat skeletal muscle subject to disuse atrophy (45). The present study also finds an interaction between muscle loading and anabolic steroid administration; however, this interaction was dependent on both the age of the animal and the primary muscle phenotype. No anabolic steroid and overload interactions were present on muscle mass and protein content in the young Sol muscle. The RNA-to-DNA ratio responded differently to nandrolone decanoate treatment in the Plan and Sol muscle. Only the aged Sol RNA-to-DNA ratio was significantly reduced by nandrolone decanoate treatment, which appears to be mainly due to an increase in the muscle DNA pool. Regardless of age, Plan muscle mass or protein content did not respond to anabolic steroid administration with or without functional overload, which is in agreement with the findings of others (36, 45). Several variables appear to modulate anabolic steroid action on skeletal muscle, including animal gender, animal age, primary muscle phenotype, anabolic steroid type, and anabolic steroid dosage. All of these variables are critical and must be accounted for when examining anabolic steroid administration’s effect on skeletal muscle.

The androgen receptor and associated signaling pathways that modulate its biological actions are excellent candidates for regulating gene expression that influences skeletal muscle mass. However, signaling pathways related to androgen receptor action during functional overload-induced muscle growth are not well described. Because steroid hormone actions are mediated by their cognate receptors, receptor abundance can influence the steroid-mediated response within a cell (44). Androgen receptor expression in skeletal muscle is sensitive to muscle growth-promoting stimuli. The androgen receptor ligand binding capacity increases in functionally overloaded rat skeletal muscle (6, 23). Our data demonstrate that this increase in ligand binding is in part due to an induction of androgen receptor protein level after 7 days of functional overload. Intense exercise can induce transient elevation of circulating testosterone level in humans, and corresponding increases in skeletal muscle androgen receptor binding (43). Increased androgen receptor abundance could serve to increase the muscles’ sensitivity to androgens. Androgen receptor induction may play an important role in overload-induced growth, because inhibition of androgen receptor action can partially attenuate overload-induced muscle growth in rats (26). However, the present data also support the hypothesis that androgen receptor induction is not sufficient for inducing muscle growth. Overload or nandrolone decanoate treatments induced androgen receptor protein concentration in the absence of any significant change in muscle mass. Exogenous testosterone administration has also been shown to increase androgen receptor protein concentration (18, 39). However, the present study extends these observations by demonstrating an interaction between overload and nandrolone decanoate treatment on androgen receptor protein induction. The combination of nandrolone decanoate and functional overload resulted in a 27-fold induction of androgen receptor protein concentration. This large induction of androgen receptor protein in the aged Sol by the combination of overload and steroid treatments may be important for the corresponding increase in muscle mass. The aged Plan did not increase muscle mass in response to nandrolone decanoate administration and overload, and there was also no

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corresponding increase in androgen receptor protein with both treatments as in the aged Sol.

Androgen receptor protein induction could reside in either myofibers and/or activated satellite cells within the aged Sol muscle. Satellite cells, quiescent myoblasts residing between the basal lamina and the muscle fiber sarcolemma, are the source of nuclei for growing and regenerating muscle fibers (37). Aging can alter the proliferation potential and number of satellite cells in rat muscle (21, 40). Decreased IGF-I induction in the aged rat may decrease satellite cell proliferation and subsequent fusion (22, 46). Androgens directly target satellite cells and can increase satellite cell activation and myonuclei accumulation in the rat levator ani muscle (27). Nandrolone decanoate administration alone can increase aged Sol total DNA content (12), which could represent an altered nuclei to cytoplasm ratio due to increased satellite cell proliferation. In the present study, anabolic steroid administration in combination with functional overload increased aged Sol DNA concentration, leaving the possibility that at least a portion of the androgen receptor induction may be in proliferating and/or differentiating satellite cells. However, androgen receptor protein was not quantified until day 7 of functional overload, and satellite cell proliferation would have been occurring at much earlier time points. Further work will be needed to better associate androgen receptor induction with satellite cell differentiation.

Glucocorticoid action is associated with cellular catabolism, and elevated levels of circulating glucocorticoids can result in muscle atrophy (23). However, resistance exercise and functional overload can prevent glucocorticoid-induced muscle atrophy (24). It has been hypothesized that supraphysiological doses of anabolic steroids may act on skeletal muscle by androgen receptor-independent mechanism, such as interaction with glucocorticoid receptors (17, 24). Anabolic steroid interaction with the glucocorticoid receptor could prevent glucocorticoid binding, providing a stimulus for androgen-induced muscle hypertrophy (32). However, there are several confounding variables associated with this hypothesis. Anabolic steroids have low binding affinity for the glucocorticoid receptor (24), and glucocorticoid receptor protein concentration significantly increases in the functionally overloaded Plan muscle. Glucocorticoid receptor binding capacity is not affected by either castration or androgen treatment (42), and this suggests that anabolic steroids do not participate in hypertrophy-induced glucocorticoid receptor proliferation. The present study demonstrates that Plan muscle glucocorticoid receptor concentration is induced by functional overload in adult animals. Our data suggest that glucocorticoid receptor levels do not appear to be as sensitive to circulating androgen levels as the androgen receptor protein, because anabolic steroid administration had no effect on glucocorticoid receptor expression in either the Sol or Plan muscle. However, in the present study, anabolic steroid administration increased androgen receptor abundance in both the Sol and Plan muscles, thus increasing the number of androgen-specific receptors. The relationship of glucocorticoid receptor induction by functional overload and increased muscle mass is also not certain, because aged Sol increased glucocorticoid concentration without increasing muscle mass.

In summary, aged Fischer 344 × Brown Norway rats can increase Sol muscle mass and total protein content with the combination of 7 days of functional overload and nandrolone decanoate treatment. In aged animals, neither functional overload nor ND treatment alone induced the wet muscle weight and total protein content of either the Sol or Plan muscles. Androgen receptor expression was synergistically induced by the combination of functional overload and nandrolone decanoate treatments in the aged rat Sol but not in the Plan. Nandrolone decanoate treatment had no effect of glucocorticoid receptor expression Sol or Plan muscles from aged animals. These data demonstrate that both animal age and muscle phenotype have an effect on nandrolone decanoate interaction with functional overload. The androgen receptor and associated signaling pathways remain an excellent candidate for mediating this interaction during muscle regeneration and subsequent growth.

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