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Elevated endogenous testosterone concentrations potentiate muscle androgen receptor responses to resistance exercise

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ABSTRACT

The purpose of this study was to determine the influence of endogenous circulating testosterone (T) on muscle androgen receptor (AR) responses to acute resistance exercise (RE). Six healthy men (26 ± 4 years; 176 ± 5 cm; 75.8 ± 11.4 kg) performed a knee extension exercise protocol on two occasions separated by 1–3 weeks. Rest preceded one trial (i.e., control [CON] trial) and a high-volume upper-body RE protocol designed to increase circulating T preceded the other trial (i.e., high T [HT] trial). Serial blood samples were obtained throughout each trial to determine circulating T concentrations. Biopsies of the *vastus lateralis* were obtained pre-RE (REST), 10-min post-RE (+10), and 180-min post-RE (+180) to determine muscle AR content. Circulating T concentrations remained stable during CON. Alternately, HT significantly ($p \leq 0.05$) increased T concentrations above resting values (+16%). Testosterone area-under-the-time curve during HT exceeded CON by 14%. AR content remained stable from REST to +10 in both trials. Compared to the corresponding +10 value, muscle AR content at +180 tended to decrease during CON (−33%; $p = 0.10$) but remained stable during HT (+40%; $p = 0.17$). Muscle AR content at +180 during the HT trial exceeded the corresponding CON value. In conclusion, acute elevations in circulating T potentiated muscle AR content following RE.

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1. Introduction

Resistance exercise (RE) potently stimulates skeletal muscle growth [1]. This favorable adaptation results from protein accretion within existing muscle fibers, thus promoting fiber hypertrophy. Although numerous physiological mechanisms contribute to the regulation of muscle size (e.g., endocrine, immune/inflammatory, and intrinsic muscle factors), several experimental approaches substantiate the importance of androgen signaling for mediating RE training-induced muscle growth: (1) exogenous testosterone (T) administration during RE training potentiates gains in muscle strength and muscle mass [2]; (2) gonadotropin-releasing hormone analogs, which inhibit endogenous T release, prevent gains in muscle strength and attenuate gains in muscle mass during RE training [3]; and (3) androgen receptor (AR) antagonists, which inhibit endogenous T from binding to the AR, impair muscle growth during synergist overload [4]. Therefore, androgen signaling represents an important target for research aimed at promoting muscle growth and/or attenuating the muscle atrophy characteristic of aging, unloading, and various diseases.

Resistance exercise affects androgen signaling via several mechanisms. First, RE transiently elevates endogenous T concentrations [5], which increases the probability of T–AR interactions. Transient RE-induced elevations in circulating T potentiate gains in muscle strength following long-term training [6]; this underscores the physiological importance of endogenous T for mediating RE adaptations. Second, muscle contraction/overload up-regulates muscle AR content via enhanced transcription of AR mRNA [7–9]. Increased AR content sensitizes the overloaded muscles to circulating T and, in turn, contributes to the selective hypertrophy of the overloaded muscle fibers [4]. Third, preliminary evidence indicates that transient elevations in endogenous T following RE could feasibly increase muscle AR content independently of the influence of muscle contraction. Exogenous androgen administration (without RE) increases muscle AR content in rats [10] and in humans [11]. Unlike muscle overload, however, androgen administration

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up-regulates AR content by increasing AR mRNA translation [12,13] and by increasing AR half-life [13]. Therefore, acute RE-induced elevations in circulating T represent an additional mechanism by which RE might regulate muscle AR content. This supposition is supported by animal research demonstrating that chronic exogenous androgen administration enhances muscle AR adaptations to muscle overload [14].

The purpose of this study was to determine the influence of transiently elevated endogenous T concentrations on muscle AR responses to acute RE. Because muscle contraction and T influence muscle AR content via distinct mechanisms (via transcription and translation/AR half-life, respectively), we hypothesized that combining muscle contraction with elevated endogenous T concentrations would potentiate AR responses to acute RE.

2. Materials and methods

2.1. Experimental approach

Subjects performed a knee extension exercise protocol on two occasions separated by 1–3 weeks. One trial was preceded by rest and the other was preceded by a bout of high-volume upper-body RE using short rest periods (Fig. 1). Given the physiological demands, one trial (knee extension exercise only) did not change circulating T concentrations (i.e., CON trial) and the other trial (knee extension exercise preceded by high-volume upper-body RE using short rest periods) significantly elevated circulating T concentrations (i.e., HT trial). Because the knee extension exercise protocol during the two experimental trials was identical, any difference between trials in *vastus lateralis* AR content could be attributed to differences in systemic (e.g., hormonal) factors.

2.2. Subjects

Six healthy men participated in this study (mean ± S.D.; age: 26 ± 4 years; height: 176 ± 5 cm; body mass: 75.8 ± 11.4 kg; body fat: 16 ± 6%). Exclusion criteria included previous involvement in a RE training protocol (within 6 months); any orthopedic or other medical issues that would impact performance; and previous use of anabolic hormones or prohormones. Analysis of dietary records indicated that none of the subjects were taking nutritional supplements (e.g., creatine). Subjects received a verbal explanation of the study procedures and associated risks. Subsequently, subjects provided written informed consent. The University of Connecticut Institutional Review Board for use of human subjects approved all procedures.

2.3. Procedures

2.3.1. Dietary controls

Subjects recorded all dietary intakes during the 2 days preceding each experimental trial. During this time, subjects refrained from exercise and the ingestion of alcohol and stimulants (including caffeine). Diet records from the first trial were photocopied and returned to subjects prior to the second trial with instructions to match previous intake as closely as possible. Analysis of dietary records indicated that subjects were consuming a typical American diet of ∼55% carbohydrate, ∼30% fat, and ∼15% protein.

All experimental trials were performed in the morning after a 12-h over-night fast (except for water). Time of day was standardized (±1 h) to avoid confounding influences of diurnal hormonal variations. Additionally, subjects were instructed to drink ∼0.5 l of water the night before and ∼0.5 l of water the morning of the experimental trials to ensure adequate hydration. Adequate hydration (urine specific gravity ≤1.020 [15]) was confirmed before the experimental protocol via urine refractometry. This study control was based on previous research from our laboratory showing that hydration status can influence testosterone responses to RE [16].

2.3.2. Anthropometrics, familiarization, and one-repetition maximum (1-RM) testing

Height, body mass, and percent body fat were measured during the initial visit via stadiometer, calibrated scale, and skin-fold thicknesses, respectively. Subjects were then familiarized with each of the resistance exercises. The knee extension exercise began with the subject in a seated position, with the fulcrum of the machine aligned with the knee joint, the leg pad positioned ∼5 cm above the ankle, and their knees at ∼90° of flexion. On command, the subject fully extended their knees and then lowered the weight back to the starting position. The bench press exercise began with the subject lying supine on a bench, holding a barbell at full elbow extension. On command, the subject lowered the bar to mid-chest and then lifted the bar back to full elbow extension. Subjects began the bench row exercise by lying prone on a bench, holding a barbell at full elbow extension. On command, the subject raised the bar to the bottom of the bench and then lowered the bar back to full elbow extension. The shoulder press began with the subject in a seated position, holding a barbell at full elbow extension. On command, the subject lowered the bar past his chin and then raised the bar back to full elbow extension.

Subjects were then tested for one repetition maximum (1-RM) strength in the knee extension, bench press, bench row, and overhead press exercises. Briefly, subjects performed a warm-up on a cycle ergometer (∼5 min) followed by self-directed stretching. Then, subjects performed 8–10 repetitions at ∼50% of estimated 1-RM followed by another set of 3–5 repetitions at ∼85% of 1-RM. Three to four maximal trials separated by 2–3 min of rest were used to determine individual 1-RM for each exercise (i.e., knee extension, bench press, bench row, and overhead press). Mean (±S.D.) 1-RM strength for the knee extension, bench press, bench row, and overhead press was 112 ± 21 kg, 64 ± 12 kg, 65 ± 11 kg, and 50 ± 11 kg, respectively.

2.3.3. Experimental trials

During the CON trial, subjects performed 5 sets of 5-RM (90–95% of 1-RM) bilateral, concentric-eccentric knee extensions with 3 min

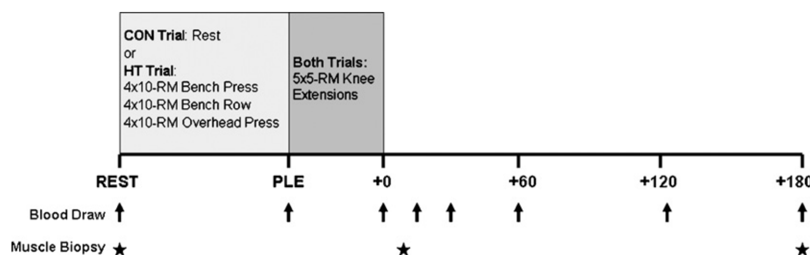

Fig. 1. Study protocol. The CON and HT trials were randomized and separated by 1–3 weeks. Acronyms: CON: control trial; HT: high testosterone trial; PLE: prior to lower-body exercise; RM: repetition maximum. Numerical values refer to number of minutes post-exercise (e.g., +60 = 60 min post-exercise).

of rest between sets. This heavy-load knee extension exercise protocol involved low total work and long rest periods, thus stimulating a minimal hormonal response [5].

During the HT trial, subjects performed an upper-body exercise protocol consisting of 4 sets of 10-RM each of the bench press, bench row, and seated overhead press exercises (in that order) immediately prior to the knee extension exercise protocol. For each upper-body exercise, the initial load was 80% of individual 1-RM. If muscle failure occurred during a set, then spotters provided assistance until the subject completed the remaining repetitions and resistance was reduced for subsequent sets. In all cases, 2 min of rest separated sets and exercises. This high-volume, short rest period, upper-body protocol maximized the hormonal response to resistance exercise [5]. Immediately (within 2 min) following the upper-body exercise protocol, subjects began the knee extension exercise protocol identically as performed during the CON trial.

2.3.4. *Blood draws*

Prior to commencing experimental trials, a trained phlebotomist inserted an indwelling Teflon cannula into a superficial forearm vein of the subject. The cannula was kept patent with a 10% heparin-saline solution. During the CON and HT trials, venous blood samples were obtained at rest (REST), immediately after the upper-body exercise protocol/immediately prior to the lower-body exercise protocol (PLE), immediately post-RE (+0), and at 15-min (+15), 30-min (+30), 60-min (+60), 120-min (+120), and 180-min (+180) post-RE. Prior to each blood draw, 3 ml of blood was drawn and discarded to avoid inadvertent dilution of the blood sample. For each blood draw, ~10 ml of blood was collected and transferred into appropriate tubes for obtaining serum and then centrifuged at $1500 \times g$ for 15 min at 4 °C. Resulting serum was aliquoted and stored at −80 °C until subsequent analyses.

2.3.5. *Muscle biopsies*

During each experimental trial, small (30–50 mg) muscle samples were obtained from the *vastus lateralis* using the percutaneous needle biopsy technique with suction [17]. Briefly, the skin over the muscle was gently shaved with a razor, disinfected using betadine, and anesthesized using 2% lidocaine. A small (~1 cm) incision was made through the skin and muscle fascia with a scalpel and then a sterile biopsy needle was introduced into the muscle to obtain the muscle sample. Muscle samples were cleared of excess blood and connective tissue, flash frozen in liquid nitrogen, and stored at −80 °C until subsequent analyses. During a given experimental trial, each biopsy was obtained from the same leg, but through a separate incision. Each incision was separated by ~3 cm to avoid potential confounding influences of immune/inflammatory responses to the biopsy procedure on muscle AR content. The contra-lateral leg was biopsied during the subsequent experimental trial, again using 3 separate incisions. During each trial, biopsies were obtained at REST, at exactly 10 min post-RE (+10), and at 180 min post-RE (+180).

2.3.6. *Blood biochemical analyses*

Serum samples were analyzed in duplicate for total T via a commercially available enzyme-linked immunosorbent assay (Diagnostic Systems Laboratory, Webster, TX). Serum samples were thawed only once before analysis. All samples were analyzed in one run to avoid inter-assay variation. Coefficient of variation for this assay was 3.6%.

2.3.7. *Muscle biochemical analyses*

Muscle samples were homogenized on ice in Tissue Extraction Reagent I (BioSource, Carlsbad, California) (containing 50 mM Tris, pH 7.4, 250 mM NaCl, 5 mM EDTA, 1 mM NaF, 2 mM Na_3VO_4, 20 mM $Na_4P_2O_7$, 0.02% NaN_3, and a proprietary detergent) and a protease inhibitor cocktail (MiniComplete, Roche, Indianapolis, IN) using a glass tissue grinder (Duall® 21, Kontes, Vineland, NJ). Samples were then spun at $10{,}000 \times g$ for 15 min at 4 °C to obtain supernatant. Samples were analyzed for total protein concentration using a detergent-compatible, reducing reagent-compatible protein assay (BioRad, Hercules, CA). Forty micrograms of homogenate was incubated (85 °C, 5 min) with an equal volume of protein sample buffer and then fractionated on a 4–20% tris–glycine gel (125 V, room temperature, 90 min). Protein was then electrophoretically transferred to a nitrocellulose membrane (30 V, 4 °C, 16–20 h). Transfer was verified by Ponceau S stain of the membrane and Coomassie Blue stain of the gel. The membrane was then blocked in 5% fat-free dry milk (Bio-Rad, Hercules, CA) in Tris-buffered saline (TBS) for 2 h at room temperature, washed 2× 10 min in TBS with 0.35% Tween^{20} (TBS–T), and incubated with a rabbit IgG primary antibody for AR (sc-816, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) in 1.7% fat-free dry milk in TBS-T for 2 h at room temperature. The membrane was then washed 3× 10 min in TBS-T and subsequently incubated with an anti-rabbit IgG horseradish peroxidase-linked secondary antibody (sc-2004, Santa Cruz Biotechnology) in 1.7% fat-free dry milk in TBS–T for 1 h at room temperature. AR protein was then visualized using enhanced chemiluminescence (Pierce, Rockford, IL) and film (Biomax light, Kodak, Rochester, NY). The developed film were scanned and the intensity of the blots were measured by absorptometry using ImageJ software (National Institute of Health, Washington, DC); the resulting AR pixel density values were normalized such that REST AR protein concentrations were given an arbitrary value of 1.0.

2.4. *Statistical analyses*

Measures of central tendency and variation were calculated for all variables. Area-under-the-time-curve (AUC) was calculated for circulating total T using standard trapezoidal methods. Data were tested to ensure that they met the appropriate statistical assumptions (e.g., normalcy, sphericity) and then analyzed using a trial × time repeated-measures ANOVA. In the event of a significant F score, the Fisher LSD post-hoc test was used to determine pairwise differences. Cohen's d effect size was calculated for selected comparisons using the pooled SD. The criterion for statistical significance was set *a priori* at $p \leq 0.05$.

3. Results

Circulating T concentrations remained stable during CON. Alternately, the HT protocol significantly elevated circulating T concentrations above REST values (+16% at +0; $p = 0.02$; effect size = 0.61) (Fig. 2). Moreover, T values during HT were significantly greater than CON at PLE, +0, and +60 ($p = 0.01$–0.04; effect size = 0.63–1.03). The T AUC was 14% greater during the HT trial than the CON trial ($p < 0.01$; effect size = 0.60) (Fig. 2 insert).

AR content did not change from REST to +10 in either trial (Fig. 3). Compared to the corresponding +10 value, muscle AR content at +180 tended to decrease during CON (−33%; $p = 0.10$; effect size = 1.41) but did not significantly change during HT (+40%; $p = 0.17$; effect size = 0.68). AR content at +180 during the HT trial exceeded that of the CON trial ($p < 0.01$; effect size = 1.82).

4. Discussion

In accord with our hypothesis, elevated endogenous T concentrations potentiated AR responses to acute RE. Specifically, transient RE-induced elevations in circulating T prevented catabolism of muscle AR content following RE. These results indicate that the combination of muscle contraction and elevated T concentrations enhances AR content in the overloaded muscles, which, as postulated by Inoue et al. [18], concentrates the anabolic effects of

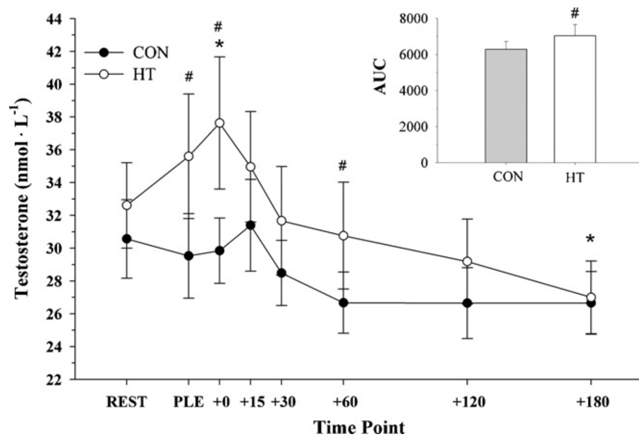

Fig. 2. Circulating testosterone responses (mean ± S.E.) to the control (CON) and high-testosterone (HT) trials. Testosterone area-under-the-time-curve (AUC) is presented in the panel insert. * = significantly ($p \leq 0.05$) different than corresponding REST value; # = significantly ($p \leq 0.05$) different than corresponding CON value.

androgens to the overloaded muscles. This implies that maximizing the T response to RE (by using multiple exercises, multiple sets, heavy loads, and short rest periods) enhances muscle AR content and, ultimately, promotes tissue anabolism and/or recovery.

Muscle overload and androgens up-regulate muscle AR content via distinct mechanisms. Overload increases AR mRNA transcription via RhoA (a member of the Rho family of small GTPases) and serum response factor signaling [8]. Alternately, T increases muscle AR via (i) enhanced AR mRNA association with polyribosomes, thus increasing AR mRNA translation [12]; and (ii) increased AR half-life (in the absence of androgens, AR half-life is 3.1 h; while, in the presence of androgens, AR half-life is 6.6 h [13]). Because muscle contraction and T influence muscle AR content via distinct mechanisms, we hypothesized, and subsequently demonstrated, that combining muscle contraction with elevated endogenous T concentrations potentiates AR responses to acute RE. Previous research [8,13,19] indicates that, in the present study, endogenous T-induced potentiation of muscle AR content was likely due to enhanced AR mRNA translation and/or increased AR half-life.

Significant between-trial differences in muscle AR content were found within 3 h following a bout of RE. Although the effects of exercise/androgens on AR content might appear surprisingly fast-acting, previous research sets a precedence for rapid changes in AR content following exercise/androgens: exercise affects AR protein content within 1–2 h post-exercise [20–22]; and, within 1 h, androgen exposure increases AR mRNA association with polyribosomes

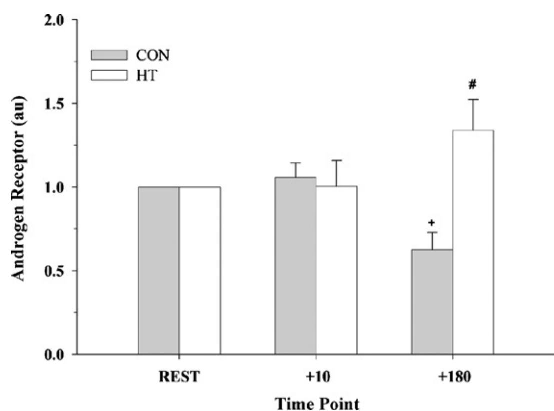

Fig. 3. Muscle androgen receptor (AR) responses (mean ± S.E.) to the control (CON) and high-testosterone (HT) trials. # = significantly ($p \leq 0.05$) different than corresponding CON value; + = trend ($p = 0.10$) for difference compared to corresponding +10 value.

[12] and AR protein content [19]. Androgen exposure also doubles AR half-life (from 3.1 h to 6.6 h [13]). These data support the rapid sensitivity of AR to exercise and circulating androgens.

A large body of evidence demonstrates that chronic muscle overload increases muscle AR content [8,9,14,18,23–28]. However, few studies describe muscle AR responses to an acute bout of exercise. In the present investigation (during the CON trial), muscle AR content tended to decrease following an acute bout of RE. Other research shows that AR has a phasic response to muscle exercise: AR content transiently increases at 2 h, returns to baseline by 4 h, then gradually peaks by 72 h post-exercise [22]. The phasic response of AR to exercise, along with the influence of circulating T concentrations, might explain why our laboratory has previously reported increases [20] and decreases [21] in muscle AR content following acute RE. Based on the current and previous research [20–22], it appears that AR content typically decreases following acute RE and then subsequently increases following chronic RE. Early AR catabolism prior to subsequent upregulation is supported by Lee et al. [8], who found that, in responses to synergist overload, AR content decreased following 1 d, returned to baseline values by 3 d, increased at 7 d, and remained elevated through 21 d.

Resistance exercise potently stimulates skeletal muscle growth and attenuates muscle atrophy during unloading. Although numerous physiological mechanisms regulate muscle size, androgen signaling represents an important component of this process [2–4]. The present research contributes to our understanding of androgen signaling by identifying another mechanism by which RE affects AR content. This research implies that (1) maximizing T responses to RE promotes muscle anabolism via T–AR interactions; and (2) impaired T responses to RE might attenuate AR responses and, subsequently, muscle anabolism. The latter might be an important contributor to aging-induced sarcopenia, as aging is associated with a gradual decline in resting T concentrations, blunted T responses to RE, reduced muscle AR content, and, ultimately, decreased muscle size [1,10]. Practical implications of the present study are that large muscle group, multi-joint exercises (e.g., bench, squats, deadlifts) are recommended to counteract aging-induced declines in T and in AR.

Small sample size ($n = 6$) indicates that this study was underpowered to detect statistical difference in some instances. Obviously, this represents the primary limitation of the present study. To compensate for the lack of statistical power, we have reported the corresponding effect sizes to describe the "meaningfulness" of various pair-wise comparisons. A secondary limitation of this study was relying on only total T measurements to reflect circulating androgen concentrations. Testosterone circulates in many forms (e.g., free, SHBG-bound, albumin-bound). Although total T serves as an indicator of androgen status, it is generally believed that only unbound, free T exerts biological activity. Therefore, future work should employ larger sample sizes and broader assays of circulating T concentrations to assess the role that androgens have on muscle AR content.

In summary, subjects performed two identical bouts of knee extension exercise during which circulating endogenous T concentrations were experimentally manipulated via upper-body exercise. We found that elevated circulating T concentrations prevented catabolism of *vastus lateralis* AR content following RE. This response was likely due enhanced AR mRNA translation and/or increased AR half-life. Ultimately, increased AR content likely improves muscle anabolism/recovery after RE by promoting T–AR interactions.

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