Effect of resistance exercise on muscle steroidogenesis

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Submitted 15 September 2008; accepted in final form 1 October 2008

Vingren JL, Kraemer WJ, Hatfield DL, Anderson JM, Volek JS, Ratamess NA, Thomas GA, Ho JY, Fragala MS, Maresh CM. Effect of resistance exercise on muscle steroidogenesis. J Appl Physiol 105: 1754–1760, 2008. First published October 2, 2008; doi:10.1152/japplphysiol.91235.2008.—Circulating testosterone is elevated acutely following resistance exercise (RE) and is an important anabolic hormone for muscle adaptations to resistance training. The purpose of this study was to examine the acute effect of heavy RE on intracrine muscle testosterone production in young resistance-trained men and women. Fifteen young, highly resistance-trained men (n = 8; 21 ± 1 yr, 175.3 ± 6.7 cm, 90.8 ± 11.6 kg) and women (n = 7; 24 ± 5 yr, 164.6 ± 6.7 cm, 76.4 ± 15.6 kg) completed 6 sets of 10 repetitions of Smith machine squats with 80% of their 1-repetition maximum. Before RE and 10 and 70 min after RE, muscle biopsies were obtained from the vastus lateralis. Before RE, after 3 and 6 sets of squats, and 5, 15, 30, and 70 min into recovery from RE, blood samples were obtained using venipuncture from an antecubital vein. Muscle samples were analyzed for testosterone, 17β-hydroxysteroid dehydrogenase (HSD) type 3, and 3β-HSD type 1 and 2 content. Blood samples were analyzed for glucose and lactate concentrations. No changes were found for muscle testosterone, 3β-HSD type 1 and 2, and 17β-HSD type 3 concentrations. However, a change in protein migration in the Bis-Tris gel was observed for 17β-HSD type 3 postexercise; this change in migration indicated an ∼2.8 kDa increase in molecular mass. These findings indicate that species differences in muscle testosterone production may exist between rats and humans. In humans, muscle testosterone concentrations do not appear to be affected by RE. This study expands on the current knowledge obtained from animal studies by examining resting and postexercise concentrations of muscle testosterone and steroidogenic enzymes in humans.

intracrine; testosterone; hydroxysteroid dehydrogenase

TESTOSTERONE (17β-hydroxy-4-androstene-3-one) is a potent anabolic hormone that stimulates muscle protein synthesis (15) and intramuscular amino acid uptake (5). The main sites of testosterone production are the testis in men and the ovaries and adrenal cortex in women. Leydig cells, which have the largest capacity for testosterone production, are found only in the testis and thus largely explain the up to 10-fold greater circulating testosterone concentration in men compared with women. Testosterone is produced from cholesterol via a series of conversions catalyzed by specific enzymes (9). Several of the intermediates in this process are hormones that can exert their own physiological actions, including progesterone, dihydroepiandrosterone (DHEA), and androstenedione. Some of the intermediates in testosterone production are also precursors for other steroid hormones such as cortisol and aldosterone; testosterone is itself a precursor to estradiol (9). This helps explain how the adrenal gland and the ovaries can produce testosterone despite the absence of Leydig cells in these tissues.

Testosterone is also produced in small quantities from peripheral conversion of the weak androgens androstenedione, androstenediol, and DHEA, which are mainly produced in the gonads and the adrenal cortex (27–29). The key enzymes involved in this conversion are 3β-hydroxysteroid dehydrogenase (HSD) and 17β-HSD (30). These enzymes are found in many different tissues, including brain, liver, kidney, and skeletal muscle of rats (1, 2, 36, 50), rhesus monkeys (35), and humans (36, 55). There are several different variants of these enzymes, each with specific functions in steroidogenesis. In humans, the variants that catalyze the reactions leading to testosterone formation are the 3β-HSD type 1 and type 2 (30), which catalyze the conversion of DHEA to androstenedione, and 17β-HSD type 3 and type 5, which catalyze the reduction of androstenedione to testosterone (30, 40).

It is well established that skeletal muscle can produce peptide hormones such as insulin-like growth factor I in animals (19, 54) and humans (18). Recently, Aizawa et al. (1) showed that rat skeletal muscle cell cultures incubated with DHEA produced testosterone in a DHEA dose-dependent manner. Muscle joins a growing list of tissues found to be capable of steroidogenesis. The concentration of testosterone in rat muscle tissue is similar to that found in the brain, kidney, and liver of rats but is much lower compared with rat ovarian and testicular testosterone concentrations (1). It was suggested by Labrie et al. (27) that muscle-derived testosterone functions in an intracrine manner and not in an autocrine and/or paracrine manner. Interestingly, female rats expressed higher levels of muscle 3β-HSD, 17β-HSD, P-450 aromatase mRNA, and 17β-HSD and P-450 aromatase protein than male rats (2). Despite these higher levels of steroidogenic enzymes in female rats, the muscle concentration of testosterone was threefold higher in male rats (2). This difference in testosterone concentration did not appear to be due to a greater conversion of testosterone to estradiol by P-450 aromatase, since no sex difference existed for muscle estradiol concentration. The larger muscle concentration of testosterone might be due to cellular infiltration of testosterone from the circulation or a greater activity of steroidogenic enzymes.

Testosterone is important for the development and maintenance of muscle mass in males. When circulating testosterone
is suppressed, as with long-term pharmacological androgen deprivation therapy, men experience a loss of muscle mass (7, 49) and muscle strength (4). The importance of normal circulating testosterone concentrations for the development and maintenance of muscle mass in women has not received much attention in the literature; however, in androgen-deficient women with hypopituitarism, testosterone administration leads to an increase in muscle mass (38). Similarly, supraphysiological injections of anabolic steroids lead to increases in muscle mass in women (21) and female rats (53); this potent anabolic effect of testosterone in women is exemplified by the large muscle accretion in women body builders who use anabolic steroids. Thus testosterone appears to have a role in the maintenance of muscle mass in women, although the importance of this role has not yet been fully established.

The importance of testosterone for muscle adaptations to resistance exercise training was recently highlighted by the finding that suppression of circulating testosterone concentrations prevented resistance training-induced hypertrophy in young, healthy men (26). In addition, it has been shown that impediment of testosterone-androgen receptor interaction by administration of an androgen receptor antagonist attenuates hypertrophy from 2 wk of electrical stimulation in rats (20). Circulating testosterone concentrations are generally elevated following a bout of resistance exercise in men (24, 31, 46, 52), whereas findings for the effect of resistance exercise on circulating testosterone in women are equivocal, with increases (10, 42) and no changes observed (22, 31). No study has investigated the effect of a bout of resistance exercise on muscle testosterone concentration; however, it has been found that swimming (51) and treadmill running (2) can significantly increase muscle testosterone concentrations in male and female rats. This upregulation of muscle testosterone in rats appears, at least in part, to be due to an increase in 3β-HSD and 17β-HSD type 1 expression (2). No study has examined the effect of exercise on muscle testosterone or steroidogenic enzymes in humans. The purpose of this study was to examine the effect of a single bout of heavy resistance exercise on muscle testosterone production and signaling in young resistance-trained men and women. This study expands on the current knowledge by examining resting and postexercise muscle steroidogenesis in humans.

METHODS

Study Overview

Fifteen resistance-trained men and women were recruited for this study. Fifteen apparently healthy men (n = 8; 21 ± 1 yr, 175.3 ± 6.7 cm, 90.8 ± 11.6 kg) and women (n = 7; 24 ± 5 years, 164.6 ± 6.7 cm, 76.4 ± 15.6 kg). Only highly resistance-trained individuals who were involved with a structured resistance training program were recruited for this study. Twelve of the fifteen subjects were current or recent National Collegiate Athletic Association collegiate or nationally competitive athletes, including two national champions. The three remaining subjects were involved in resistance training programs that were similar to those of the competitive athletes. In addition, the high training status of these three subjects was substantiated by their Smith machine squat performance, which was comparable to that of the competitive athletes and to that found in the literature for highly resistance-trained individuals (46). Each subject completed a medical history questionnaire and a physical activity questionnaire to ensure that they met the inclusion/exclusion criteria of the study. Upon review of the medical history forms by a physician, subjects were excluded if a preexisting medical condition put them at risk while performing the exercise protocol or might influence the outcomes of this study. The medical exclusion criteria included preexisting heart conditions or anomalies, respiratory conditions, blood pressure problems, musculoskeletal problems, or previous orthopedic injuries that would limit the range of motion about the shoulder, elbow, hip, knee, or ankle joint. Special attention was given to exclude potential volunteers with lower back problems, including herniated intervertebral disks. In addition, women had to be eumenorrheic. Potential volunteers were excluded from participation in the study if they had recently taken, or were currently taking, or planned to take any hormonal substances such as anabolic steroids or growth hormone. Individuals using hormonal birth control or other hormonal medication were excluded from participation due to the potential interference with the outcome of the study. Subjects were screened to exclude subjects who were taking any nutritional supplements or adhering to atypical diets that might have confounded the results of this study. This study was approved by the University of Connecticut Institutional Review Board, and all volunteers signed an institutional approved informed consent document to participate.

Procedures

Anthropometric measurements, familiarization, and 1-RM test. Approximately 1 wk before the AHRET, subjects reported to the laboratory for anthropometric measurements, familiarization, and 1-RM determination. After measurement of height and weight, subjects were familiarized with the exercise procedures, and subsequently, their squat 1-RM was determined. Height was measured to the nearest 0.5 cm using a stadiometer (Seca, Hamburg, Germany), and total body mass was measured to the nearest 0.1 kg on a digital scale (Ohaus, Florham Park, NJ). Participants wore only light athletic clothes and no shoes for measurements of height and body mass. Subjects performed a standardized warm-up consisting of 5 min of cycling on a stationary bike followed by light dynamic stretches (heel kicks, lunges, high knees, high kicks, and unweighted squats). Subjects were then familiarized with the proper technique for performing the squat exercise using the Plyometric Power System. The Plyometric Power System utilizes a modified Life Fitness (Franklin Park, IL) Smith machine that allows only vertical translation of the bar. Linear bearings attached to either side of the bar allow it to slide up and down two steel shafts with minimal friction. Once subjects demonstrated proper technique in the squat exercise, their 1-RM strength was measured using the methods described by Kraemer and Fleck (23). Briefly, subjects performed squats for 8–10 repetitions at ~50% of their estimated 1-RM followed by another set of 2–5 repetitions at ~85% of estimated 1-RM. Subsequently, four to five one-repetition trials were used to determine the 1-RM. All exercise protocols were supervised by a National Strength and Conditioning Association Certified Strength and Conditioning Specialist. The 1-RM
in the Smith machine squat was 150 ± 15 kg for the men and 102 ± 15 kg for the women.

**AHRET visit.** Subjects reported to the laboratory after a 12-h overnight fast. Subjects then provided a urine sample for measurement of hydration status. Subjects were then placed on the biopsy bed, and an indwelling Teflon catheter was inserted into an antecubital forearm vein. Subsequently, the Pre muscle biopsy and blood sample were collected. Subjects then performed the same standardized warm-up as for the familiarization visit, followed by one warm-up set of squat at 50% of their 1-RM. Subjects performed 6 sets of 10 repetitions of the squat with 2 min of rest between sets at an initial load of 80% of 1-RM. Immediately after the sixth set of squats, subjects were transported back to the biopsy bed for the first postexercise muscle biopsy (10 min postexercise) and remained in the bed or in a comfortable chair (resting or reading) until after the final biopsy and blood draw at 70 min postexercise. In addition to the Pre blood sample, blood samples were obtained after the third set (Mid), after the sixth set (IP), and 5, 15, 30, and 70 min into recovery from exercise. Heart rate (HR) and rating of perceived exertion (RPE) were assessed at Pre and immediately after each set of squats. HR was measured using a standard heart rate monitor (Polar, Lake Success, NY) with telemetry. The category-ratio (CR-10) scale of perceived exertion (43) was used.

**Blood draws.** An indwelling Teflon cannula was inserted into an antecubital forearm vein by a trained phlebotomist. The cannula was kept patent with a 10% heparin lock/saline solution. Before each blood draw, 3 ml of blood were extracted and discarded to avoid inadvertent saline dilution of the blood sample. Whole blood was centrifuged at 1,500 g at 4°C for 15 min, and resulting EDTA plasma was aliquoted and stored at −80°C until analysis.

**Muscle biopsies.** Three separate incisions 3 cm apart on the right thigh (vastus lateralis) were used to obtain the three biopsies during the AHRET visit. Three separate sites were used to avoid confounding influences of immune/inflammatory responses. Muscle samples (~100 mg) were obtained from the superficial portion of the vastus lateralis using a percutaneous needle biopsy procedure and the technique described by Bergstrom (6) while suction was applied (14). Briefly, the skin over the muscle was cleaned with betadine, and a local anesthetic (1% lidocaine) was injected under the skin and into the muscle. A small (1 cm) incision was made through the skin and muscle fascia with a scalpel. A 4- to 5-mm-diameter sterile biopsy needle was introduced into the muscle. An inner cannula was advanced to cut the tissue, and the needle was removed. The incision was covered with sterile gauze, and compression was applied to prevent bleeding. After each biopsy, the skin was closed via suture. All biopsy procedures were performed by a trained physician. Muscle samples were immediately flash frozen in liquid nitrogen and stored at −80°C until analysis.

**Experimental controls.** Subjects were instructed to refrain from resistance or exhaustive exercise, alcohol, and caffeine during the 3 days preceding the AHRET trial. To account for hormonal variations during the menstrual cycle, women were “phased” using the methods of Desouza et al. (11) so that they completed the AHRET during their early follicular phase (days 2–7 after the start of menses).

All AHRET trials were performed in the early morning (0600–0800) after a 12-h overnight fast (except for water). Time of day was standardized (±1 h) to avoid confounding influences from diurnal hormonal variations. In addition, subjects were instructed to drink ~0.5–1 l of water the night before and ~0.5–1 l the morning of the AHRET to ensure adequate hydration. Hydration state was determined before the AHRET via urine refractometry (model A300CL; Spartan, Tokyo, Japan); subjects were considered euhydrated if they had urine specific gravity < 1.020 g/ml.

**Biochemical Analyses**

**Blood.** Hemoglobin and hematocrit were measured in whole blood immediately following blood collection. Hemoglobin was measured in duplicate using an automated analyzer (Hb501; Hemocue, Lake Forest, CA), and hematocrit was measured in triplicate by centrifugation of heparinized microhematocrit capillary tubes (Fisherbrand, Pittsburgh, PA). From hemoglobin and hematocrit values, plasma volume shifts were calculated using the methods of Dill and Costill (13). The plasma volume changed −19.8 ± 5.4% from Pre to Mid and −18.0 ± 3.6% from Pre to IP for the men and −21.9 ± 4.2% from Pre to Mid and −17.0 ± 4.0% from Pre to IP for the women. After IP, the plasma volume change from Pre gradually decreased until it reached 2–5% above Pre levels at 30–70 min postexercise; circulating metabolic biomarker concentrations were not corrected for plasma volume changes due to the molar exposure at the tissue level. For the women, baseline (Pre) plasma samples were analyzed for estradiol and progesterone using enzyme immunoassay (EIA; Cayman Chemicals, Ann Arbor, MI) to confirm menstrual cycle phase. Plasma lactate and glucose were measured using an automated lactate-glucose analyzer (YSI-2300; Yellow Springs Instruments, Yellow Springs, OH) to assess the metabolic demand of the AHRET.

**Muscle homogenization.** Muscle samples (~40–60 mg) were homogenized on ice in a buffer (Tissue Extraction Reagent I; Invitrogen, Carlsbad, CA; 15 µl buffer/mg muscle) containing 50 mM Tris, pH 7.4, 250 mM NaCl, 5 mM EDTA, 2 mM Na3VO4, 1 mM NaF, 20 mM Na3P04, 0.02% NaN3, a proprietary detergent, and a protease inhibitor cocktail (MiniComplete, Roche, Indianapolis, IN) using a glass tissue grinder (Daul 21; Kontes, Vineland, NJ). The homogenate was gently mixed for 15 min at 4°C and subsequently centrifuged at 10,000 g for 15 min at 4°C. The resultant supernatant was divided into several aliquots, flash frozen in liquid nitrogen, and stored at 80°C until Western blot or EIA analysis.

**Total protein.** Total protein concentration in the supernatant was determined immediately following muscle homogenization using a detergent-compatible Lowry-based protein assay (DC Protein Assay, Bio-Rad, Hercules, CA) according to the manufacturer’s instructions. Samples were diluted (1:10) in homogenization buffer before protein analysis.

**Muscle testosterone.** Muscle homogenate supernatant testosterone concentration was measured in duplicate using a commercially available EIA (Cayman Chemicals). Samples were diluted (1:300) with EIA buffer before analysis. The resultant testosterone concentration of the muscle homogenate was corrected for total protein concentration. The sensitivity of this assay was 0.04 nM, and the coefficient of variance was 6.1%.

**Western blots.** Muscle homogenate supernatant was mixed with NuPAGE LDS-sample buffer and NuPAGE sample reducing agent (Invitrogen) and incubated at 70°C for 10 min. Protein (40 µg) was then separated on a 4–12% gradient Bis-Tris gel (NuPAGE Novex; Invitrogen) using a MOPS SDS running buffer (NuPAGE) for ~45
min at 200 V. Each gel contained duplicate samples for all three time points for one male and one female subject to minimize the effect of potential interassay variance; furthermore, the order of each sample within a gel was counterbalanced across all gels to prevent bias from potential intra-assay variance. A molecular mass standard (Kaleidoscope; Bio-Rad, Hercules, CA) was included on each gel. The separated protein was then transferred to a 0.2-μm polyvinylidene difluoride membrane (Millipore, Billerica, MA) overnight at 30 V and 4°C in a transfer buffer (NuPAGE Novex; Invitrogen) containing 10% methanol and 0.1% antioxidant (NuPAGE Novex; Invitrogen). Transfer was verified by Ponceau S stain of the membrane and Coomassie blue stain of the gel.

Each membrane was blocked in 5% fat-free dry milk (Bio-Rad) in Tris-buffered saline with 0.35% Tween 20 (TBS-T) overnight at 4°C, washed twice for 10 min in TBS-T, and incubated with primary antibody in 1.7% fat-free dry milk in TBS-T for 2 h at room temperature. The membrane was then washed three times for 10 min in TBS-T and subsequently incubated with secondary antibody in 1.7% fat-free dry milk in TBS-T for 45 min at room temperature. After 3-HSD analysis, the membrane was stripped of antibodies and reprobed for 17β-HSD.

For 3β-HSD protein quantification, a goat IgG primary antibody that detects 3β-HSD type 1 and type 2 (sc-30821; Santa Cruz Biotechnology, Santa Cruz, CA) and a donkey anti-goat IgG horse radish peroxidase-linked secondary antibody (sc-2020; Santa Cruz Biotechnology) were used. For 17β-HSD protein quantification, a goat IgG primary antibody for 17β-HSD type 3 (17β-HSD-3) and a donkey anti-goat IgG horseradish peroxidase-linked secondary antibody (sc-2020; Santa Cruz Biotechnology) were used. The protein of interest was then visualized using enhanced chemiluminescence (Pierce, Rockford, IL) and film (Biomax light; Kodak, Rochester, NY) according to the manufacturers’ instructions. The developed films were scanned, and the intensity of the blots was measured by absorbometry using ImageJ software (National Institutes of Health, Bethesda, MD).

**Statistical Analyses**

Data were analyzed using two-way ANOVA (sex × time) with repeated measures on time. In the event of a significant F score, Fisher’s least significant difference post hoc test was used to determine pairwise differences. The level of significance for this study was set at \( P \leq 0.05 \). Data are means ± SD. It was determined that a sample size \( n \) of 7 in each group (each sex) was adequate to defend the 0.05 alpha level of significance with a Cohen proportion level of at least 0.80 for each dependent variable (nQuery Advisor software; Statistical Solutions, Saugus, MA).

**RESULTS**

As expected, HR was significantly increased \((P \leq 0.05)\) compared with Pre following each set of squats; there were no sex differences for the HR response to exercise. Similarly, RPE was significantly increased compared with Pre following each set of squats, and there was a gradual increase after each set of squats. No sex differences were found for RPE. The load used during each set of squats was significantly reduced for sets 3–6 compared with set 1 for both sexes. Results for HR, RPE, and the load used are presented in Table 1. Circulating lactate concentrations increased significantly during exercise and stayed elevated compared with Pre throughout the 70 min of recovery; there were no sex differences in the circulating lactate response to exercise (Table 2). Blood glucose concentrations were significantly elevated compared with Pre for Mid through 30 min postexercise; there were no sex differences in the blood glucose response to exercise (Table 2). Combined, these results show that the AHRET was a very metabolically and physically demanding protocol for both sexes and that the participants were at a high level of exertion during the AHRET.

There were no effects of time or sex on muscle testosterone concentrations (Fig. 2), 3β-HSD content (Fig. 3A), or 17β-HSD-3 content (Fig. 3B). For 17β-HSD-3, a difference in protein migration during SDS-PAGE, the molecular mass of 17β-HSD-3 appeared to have increased \( \sim 2.8 \) kDa at 10 min postexercise compared with Pre for 14 of the 15 participants. At 70 min postexercise, this apparent increase in molecular mass of 17β-HSD-3 was found for all subjects.

**DISCUSSION**

The findings of this study provide unique physiological insight regarding the intracellular androgen response to resistance exercise in men and women. No study has previously examined the effect of resistance exercise on muscle steroidogenesis in human muscle. The primary finding in this study was that muscle steroidogenesis (i.e., testosterone production) in highly resistance-trained humans was not affected by an acute bout of heavy resistance exercise. This finding is in contrast to previous findings in rats suggesting that a species difference in the muscle steroidogenic response to exercise may exist. A secondary finding was that the apparent molecular mass of 17β-HSD type 3 was increased following a single bout of heavy resistance exercise.

No differences were found for muscle testosterone or steroidogenic enzyme (17β-HSD type 3 and 3β-HSD types 1 and 2) concentrations between sexes or in response to resistance exercise. These findings are in contrast to those of some studies.

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**Table 1. HR, RPE, and load for the AHRET**

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Set 1</th>
<th>Set 2</th>
<th>Set 3</th>
<th>Set 4</th>
<th>Set 5</th>
<th>Set 6</th>
</tr>
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<tr>
<td>HR, beats/min</td>
<td>70±7</td>
<td>162±14*</td>
<td>171±11*</td>
<td>171±10*</td>
<td>164±13*</td>
<td>169±11*</td>
<td>172±11*</td>
</tr>
<tr>
<td>Men</td>
<td>67±9</td>
<td>162±17*</td>
<td>174±14*</td>
<td>176±13*</td>
<td>175±14*</td>
<td>176±13*</td>
<td>178±12*</td>
</tr>
<tr>
<td>Women</td>
<td>0.6±0.6</td>
<td>7.3±2.5*</td>
<td>8.3±1.4*</td>
<td>9.1±1.1*</td>
<td>9.4±1.8*</td>
<td>10.1±1.8*</td>
<td>10.8±2.2*</td>
</tr>
<tr>
<td>RPE</td>
<td>5.0±0.8</td>
<td>6.9±2.1*</td>
<td>8.3±1.5*</td>
<td>9.3±1.5*</td>
<td>9.1±1.9*</td>
<td>9.9±1.7*</td>
<td>10.0±1.2*</td>
</tr>
<tr>
<td>Load, kg</td>
<td>116±15</td>
<td>115±11</td>
<td>108±12†</td>
<td>99±15†</td>
<td>95±16†</td>
<td>90±16†</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>82±12</td>
<td>78±13</td>
<td>74±12†</td>
<td>71±10†</td>
<td>69±9†</td>
<td>67±7†</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
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</table>

Values are means ± SD of heart rate (HR), the category-ratio (CR-10) rating of perceived exertion (RPE), and load lifted for preexercise (Pre) and for each set of squats in the acute heavy resistance exercise test (AHRET) protocol. *P ≤ 0.05, significantly different from Pre (main effect of time). †P ≤ 0.05, significantly different from corresponding set 1.
in rats. Previous studies have shown that treadmill running (2) and intense swimming (51) can increase muscle testosterone content acutely after exercise and that treadmill running can increase the expression of the steroidogenic enzymes 17β-HSD type 1 and 3β-HSD (2). One study, however, has found an acute decrease in rat quadriceps testosterone concentrations after swimming to exhaustion (32). It is possible that a difference in muscle steroidogenesis in response to exercise exists between rats and humans. 17β-HSDs are a family of enzymes that modulate biological potency of estrogens and androgens via conversions at position 17. At least 12 different variants of 17β-HSD (types 1–12) have been identified; each type has a preferential substrate and thus serves specific functions within steroidogenesis (40). Each type of 17β-HSD catalyzes a unidirectional conversion and thus is involved only in either a reductive or an oxidative reaction. In humans, only types 3 and 5 are involved in testosterone production; whereas types 1, 3, and 5 are involved in testosterone production in rodents (3, 17, 33, 34, 39, 40, 45). In the present study, muscle 17β-HSD type 1 was not examined because it is not a major contributor to testosterone production in humans. It is possible that the difference in the muscle testosterone response to exercise between this study in humans and the previous findings in rats is due to the different function that 17β-HSD type 1 serves in the two species investigated. Even if exercise upregulates 17β-HSD type 1 in humans, as is the case in rats (2), this effect of exercise would not result in an increase in muscle testosterone concentrations. Instead, this would likely lead to an increase in the conversion of estrone to estradiol, since that is the primary reaction catalyzed by 17β-HSD type 1 in humans (34, 40).

The differences in muscle steroidogenesis between this study and the two previous studies that have found an increase in muscle testosterone following exercise (2, 51) also could be due to differences in the exercise modality used. The present study involved a heavy resistance exercise protocol lasting 20 min, whereas the previous studies by Aizawa et al. (2) and

Table 2. Plasma lactate and glucose concentrations

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Mid</th>
<th>IP</th>
<th>+5 min</th>
<th>+15 min</th>
<th>+30 min</th>
<th>+70 min</th>
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<tr>
<td><strong>Lactate, mM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>1.0±0.2</td>
<td>13.2±3.4*</td>
<td>15.3±4.1*</td>
<td>14.9±4.5*</td>
<td>11.7±4.9*</td>
<td>7.7±4.1*</td>
<td>3.5±1.8*</td>
</tr>
<tr>
<td>Women</td>
<td>1.2±0.3</td>
<td>10.0±2.1*</td>
<td>11.9±1.7*</td>
<td>10.7±1.7*</td>
<td>8.3±1.8*</td>
<td>5.4±1.2*</td>
<td>2.5±0.5*</td>
</tr>
<tr>
<td><strong>Glucose, mM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>5.4±0.7</td>
<td>6.7±0.8*</td>
<td>8.2±1.2*</td>
<td>7.7±1.2*</td>
<td>7.2±1.0*</td>
<td>6.4±1.0*</td>
<td>5.2±0.6</td>
</tr>
<tr>
<td>Women</td>
<td>4.9±0.2</td>
<td>6.0±0.5*</td>
<td>7.2±1.0*</td>
<td>7.0±1.1*</td>
<td>6.6±1.1*</td>
<td>5.8±1.0*</td>
<td>4.7±0.4</td>
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</tbody>
</table>

Values are means ± SD of lactate and glucose concentrations for Pre, after 3 sets (Mid), immediately postexercise (IP), and 5, 15, 30, and 70 min into recovery from exercise. *P < 0.05, significantly different from Pre (main effect of time).

Fig. 2. Muscle testosterone concentration per microgram of supernatant protein Pre and 10 and 70 min into recovery from exercise.

Fig. 3. Muscle 3β-hydroxysteroid dehydrogenase (3β-HSD; A) and 17β-HSD (B) content Pre and 10 and 70 min into recovery from exercise.
Tchaikovsky et al. (51) utilized 30 and 60 min of endurance exercise, respectively. Considering the anabolic potency of testosterone, it appears unlikely that endurance exercise favors stimulations of muscle testosterone production. Although endurance exercise training significantly increases muscle protein synthesis, this increase does not lead to muscle hypertrophy because net protein balance is not improved (44). In addition, endurance training attenuates muscle mass accretion from resistance training (41). This attenuation is believed to be caused, at least in part, by a reduction in contractile protein synthesis via inhibition of the Akt-mTor (mammalian target of rapamycin) pathway (8). One of the mechanisms by which testosterone induces muscle fiber hypertrophy is via an increase in myonuclei addition to the cells (48). The addition of myonuclei amplifies the muscles capacity for protein synthesis including contractile proteins. Whereas several studies have shown that resistance exercise induces an increase in myonuclei per fiber, no study appears to have investigated the independent effect of endurance exercise on myonuclear number.

A close relationship between myonuclear number and muscle fiber cytoplasmic volume has been reported, and this suggests a tight regulation of the quantity of genetic machinery based on the protein requirements of a muscle fiber (47). Since endurance training is not associated with muscle hypertrophy, it is unlikely to result in an increase in myonuclei. One could speculate that the increase in muscle testosterone found with endurance exercise in some studies is due to an accumulation because of reduced utilization and not due to increased production; however, endurance exercise has not been shown to affect androgen receptor content or binding affinity in the fast-twitch muscles examined in these studies (12, 37).

Alternatively, it is possible that the differences among findings are due to the different training levels of the participants of the present study and the animals used in the previous investigations. In the present study, all participants were highly trained with a long resistance training history. These subjects had already experienced marked muscle adaptations from resistance training that have brought them closer to their maximal genetic potential. They might therefore not experience the signals resulting in increased intracellular testosterone production; in contrast, the animals in which an increase in testosterone, 3α-HSD type 1 and type 2, and 17β-HSD type 3 in highly resistance-trained young men and women. These findings suggest that species differences in muscle testosterone production exist between rats and humans. This study expands on the current knowledge obtained from animal studies by examining resting and postexercise concentrations of muscle testosterone and steroidogenic enzymes in humans.

ACKNOWLEDGMENTS

We thank the dedicated group of highly trained men and women who participated in this study.

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