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## Sex-based comparisons of myofibrillar protein synthesis after resistance exercise in the fed state

Daniel W. D. West,<sup>1</sup> Nicholas A. Burd,<sup>1</sup> Tyler A. Churchward-Venne,<sup>1</sup> Donny M. Camera,<sup>2</sup> Cameron J. Mitchell,<sup>1</sup> Steven K. Baker,<sup>3</sup> John A. Hawley,<sup>2</sup> Vernon G. Coffey,<sup>2</sup> and Stuart M. Phillips<sup>1</sup>

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**West DW, Burd NA, Churchward-Venne TA, Camera DM, Mitchell CJ, Baker SK, Hawley JA, Coffey VG, Phillips SM.** Sex-based comparisons of myofibrillar protein synthesis after resistance exercise in the fed state. *J Appl Physiol* 112: 1805–1813, 2012. First published March 1, 2012; doi:10.1152/jappphysiol.00170.2012.—We made sex-based comparisons of rates of myofibrillar protein synthesis (MPS) and anabolic signaling after a single bout of high-intensity resistance exercise. Eight men (20 ± 10 yr, BMI = 24.3 ± 2.4) and eight women (22 ± 1.8 yr, BMI = 23.0 ± 1.9) underwent primed constant infusions of L-[ring-<sup>13</sup>C<sub>6</sub>]phenylalanine on consecutive days with serial muscle biopsies. Biopsies were taken from the vastus lateralis at rest and 1, 3, 5, 24, 26, and 28 h after exercise. Twenty-five grams of whey protein was ingested immediately and 26 h after exercise. We also measured exercise-induced serum testosterone because it is purported to contribute to increases in myofibrillar protein synthesis (MPS) postexercise and its absence has been hypothesized to attenuate adaptive responses to resistance exercise in women. The exercise-induced area under the testosterone curve was 45-fold greater in men than women in the early (1 h) recovery period following exercise ( $P < 0.001$ ). MPS was elevated similarly in men and women (2.3- and 2.7-fold, respectively) 1–5 h postexercise and after protein ingestion following 24 h recovery. Phosphorylation of mTOR<sup>Ser2448</sup> was elevated to a greater extent in men than women acutely after exercise ( $P = 0.003$ ), whereas increased phosphorylation of p70S6K1<sup>Thr389</sup> was not different between sexes. Androgen receptor content was greater in men (main effect for sex,  $P = 0.049$ ). Atrogin-1 mRNA abundance was decreased after 5 h recovery in both men and women ( $P < 0.001$ ), and MuRF-1 expression was elevated in men after protein ingestion following 24 h recovery ( $P = 0.003$ ). These results demonstrate minor sex-based differences in signaling responses and no difference in the MPS response to resistance exercise in the fed state. Interestingly, our data demonstrate that exercise-induced increases in MPS are dissociated from postexercise testosterone and that stimulation of MPS occurs effectively with low systemic testosterone concentrations in women.

muscle protein synthesis; testosterone

THERE IS A PAUCITY of sex-based comparisons research on skeletal muscle protein turnover after feeding and exercise combined. To date, studies have reported basal postabsorptive rates of muscle protein synthesis that are similar (15, 21) or higher (18) in women compared with men. Dreyer and colleagues (13) reported similar increases between men and women in muscle protein synthesis and mTOR signaling in a 2-h period after exercise in the fasted state. Smith and colleagues (46) reported no major sex differences in rates of

muscle protein synthesis in response to hyperinsulinemic-hyperaminoacidemic clamps. However, there is a lack of data that has compared the combined effects of resistance exercise and dietary protein consumption on anabolic mechanisms in skeletal muscle of men and women. Here we superimpose the anabolic effect of whey protein (50, 52) on the anabolic effect of resistance exercise (7, 41) and thus make sex-based comparisons under conditions that would be expected to promote rates of muscle protein synthesis that are near a physiological maximum. Examining rates that are near maximal may provide the best chance of revealing sex-based differences in the ability to elevate muscle protein synthesis compared with the submaximal stimulus of feeding or exercise alone. Furthermore, to our knowledge, no study has made sex-based comparisons of muscle protein synthesis in the immediate and later (e.g., 24 h) stages of recovery after resistance exercise. Therefore, the primary aim of our study was to determine the impact of resistance exercise and protein feeding on rates of myofibrillar protein synthesis (MPS) and the molecular anabolic response during early (1–5 h) and late (24–28 h) recovery periods in men and women.

A secondary aim of this study was related to the potential impact of physiological testosterone to anabolic responses in men and women. Although evidence is conflicting (43, 53, 54) and there is a lack of direct evidence, it is frequently stated that short-term elevations in testosterone following a bout of heavy resistance exercise promote protein synthesis and hypertrophy (for review, see Ref. 26). Women have testosterone concentrations that are ~10- to 15-fold lower than men and do not experience significant elevations in postexercise testosterone compared with men (24, 25). This divergent postexercise testosterone has led to speculation that women may have an attenuated potential for resistance exercise-induced hypertrophy (44). Therefore, a secondary aim of our study was to test the hypothesis that low serum testosterone in women may attenuate anabolic responses after resistance exercise compared with men.

Herein, we quantify changes in phosphorylation of the mTOR signaling pathway to gain insight into cellular processes involved in regulating the anabolic response to resistance exercise (28, 36). Finally, as an [albeit imperfect (5)] complement to the anabolic markers that we measured, we quantified changes in the mRNA abundance of MuRF-1 and atrogin-1 to determine if there were sex-based differences in these ubiquitin ligase genes that have been shown to regulate myofibrillar proteolysis.

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Table 1. Participant characteristics

	Men (n = 8)	Women (n = 8)
Age, yr	20.0 ± 1.1	22.0 ± 1.8*
Height, m	1.80 ± 0.06	1.71 ± 0.07*
Weight, kg	77.0 ± 10.5	67.1 ± 5.6*
Body mass index, kg·m <sup>-2</sup>	24.3 ± 2.4	23.0 ± 1.9
Body fat %	15.6 ± 3.5	23.1 ± 4.1*
Thigh fat %	17.7 ± 3.8	28.6 ± 4.7*
Thigh lean mass, kg	9.2 ± 1.2	7.2 ± 0.8*
MVC (knee ext), Nm	233 ± 116	161 ± 36
Normalized strength, Nm · g lean mass <sup>-1</sup> · cm <sup>-1</sup>	1.14 ± 0.4	0.86 ± 0.38

Values are means ± SD. \*Significant difference from men,  $P < 0.05$ . Data were analyzed by unpaired *t*-tests. MVC, maximal voluntary contraction.

## METHODS

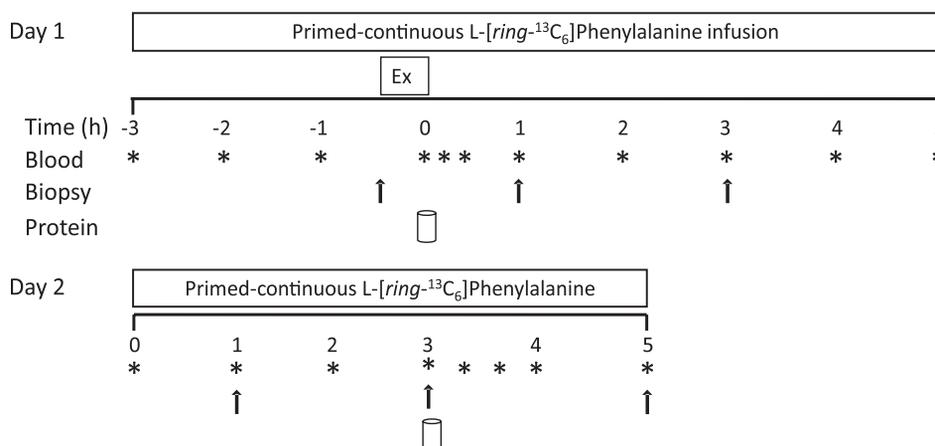
**Participants.** Eight men and eight women (Table 1), who were habitually engaging in two to five sessions of physical activity per week including no more than two lower body resistance training sessions, were recruited. Dual-energy x-ray absorptiometry (DEXA; QDR-4500A; Hologic, Waltham, MA) scans were taken of participants. The thigh was delineated as a region of interest and a ratio of isometric knee extension strength (Biodex System 3; Shirley, NY) to lean mass per unit length of the region of interest was calculated. This measure served as a marker of specific strength (strength per unit lean mass) and, together with a survey of physical activity habits, was used to match male and female participants. Equal numbers of women who were and who were not taking an oral contraceptive ( $n = 4$  on low dose combination of ethinyl estradiol and norgestimate) were recruited. It has been shown that menstrual cycle hormones do not affect rates of MPS at rest or after an acute bout of exercise (34). All participants gave written informed consent to a protocol approved by the Hamilton Health Sciences/Faculty of Health Sciences Research board and which was prepared in accordance with the Declaration of Helsinki and with current Canadian tri-council government funding agency guidelines for use of human participants in research [Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (10a); <http://www.pre.ethics.gc.ca/eng/policy-politique/initiatives/tcps2-eptc2/Default/>].

**Experimental protocol.** Participants were familiarized with the exercises and strength tested at least 1 wk before the trial to determine the exercise load to be used during the trial. Participants consumed a controlled diet the day prior to the trial that was prepared according to an energy intake calculated from the Harris-Benedict equation (activity factor range = 1.4 to 1.55) that provided 15, 30, and 55% of energy from protein, fat, and carbohydrate, respectively. These ma-

cronutrient ratios were similar to those reported by the participants in 3-day diet logs. The infusion protocol is shown in Fig. 1. Participants arrived at the lab at 0600 after an overnight fast and had a catheter inserted into an antecubital vein. A baseline blood sample was drawn before a 0.9% saline drip was started to keep the catheter patent for repeated blood sampling. A second catheter was placed in the opposite arm for a primed continuous infusion ( $0.05 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ;  $2.0 \mu\text{mol}/\text{kg}$  prime) of L-[ring-<sup>13</sup>C<sub>6</sub>]phenylalanine (Cambridge Isotope Laboratories, Woburn, MA) that was passed through a 0.2- $\mu\text{m}$  filter. After ~3 h of tracer incorporation, a resting biopsy was taken from the vastus lateralis for the subsequent estimation of resting rates of MPS. After the biopsy, participants performed a bout of intense, high-volume lower body exercise consisting of 5 sets of 10 repetitions of leg press at ~90% of 10 repetition maximum and 3 sets of 12 repetitions of leg extension/leg curl supersets (1 set of each exercise, back-to-back, with no rest between sets) at ~90% of 12 RM (53, 54). Between-set rest intervals were 60 s. After exercise completion, participants consumed a drink containing 25 g of whey protein (Alacen 352, Fonterra, Palmerston North, New Zealand) and rested in a supine position for the remainder of the trial. Rather than normalizing the dose of the protein supplement to a given parameter (e.g., lean mass, body mass, body surface area, blood volume), we decided to simply administer an equal 25-g dose to both men and women. On the basis of a previous finding (37), we would expect that this dose would be sufficient to support maximal rates of protein synthesis in both sexes. Drinks were enriched to 4% with tracer according to a phenylalanine content of 3.5% in whey protein (10) to minimize disturbances in isotopic equilibrium. Further biopsies were obtained after 1, 3, and 5 h postexercise (*day 1*; see Fig. 1). After the last biopsy, participants were provided with a meal from their controlled diet and given the remainder of the food to consume over the course of the day. The following morning, participants arrived at the lab after an overnight fast and underwent a second primed, constant infusion (*day 2*) with muscle biopsies obtained 24, 26, and 28 h after the exercise bout performed on *day 1*. Muscle biopsies obtained on *day 2* were taken at 1 and 3 h after the start of the infusion to measure 24-h postexercise fasting MPS. Whey protein (25 g) was consumed after the second biopsy on *day 2* and a final biopsy was taken 2 h later to measure MPS in the fed state.

**Blood analyses.** Serum samples were analyzed for the concentrations of growth hormone, IGF-1, total testosterone, cortisol, and estradiol from time points acutely (pre to 60 min) and 24 h after exercise using solid-phase, two-site chemiluminescence immunometric assays (Immulite; Intermidico, Holliston, MA). The exercise-induced area under the hormone curve (AUC) was calculated by subtracting pre-exercise values (set as the baseline) from postexercise values and determining the AUC. Lactate and glucose concentrations were measured on whole blood deproteinized with perchloric acid and

Fig. 1. Schematic diagram of the infusion protocol. A resting fasted biopsy (vastus lateralis) was taken to determine resting fasted myofibrillar protein synthesis (MPS), after which a bout of bilateral lower body resistance exercise was performed. A drink containing 25 g of whey protein was ingested immediately after exercise (0 h). Blood draws were obtained periodically throughout the trial and muscle biopsies were taken at 1, 3, and 5 h postexercise. The next day, participants underwent biopsies at 24 and 26 h postexercise in the fasted state, were fed 25 g of protein at 26 h, and a final biopsy was obtained at 28 h.



neutralized with potassium bicarbonate, using an enzymatic-colorimetric assays (lactate: Pointe Scientific, Canton, MI; glucose: Stanbio Laboratory, Boerne, TX). Blood amino acid concentrations were analyzed by high-performance liquid chromatography as previously described (38). Plasma L-[ring-<sup>13</sup>C<sub>6</sub>]phenylalanine enrichments were determined as previously described (16).

**Calculations.** Because all participants were tracer-naïve, we calculated a resting fractional synthetic rate (FSR) from naturally abundant <sup>13</sup>C enrichments, determined from a baseline preinfusion plasma sample and a single biopsy taken following a period of tracer incorporation (10). This method assumes that the <sup>13</sup>C enrichment of a mixed plasma protein fraction reflects the <sup>13</sup>C enrichment of muscle protein (19). This method is appropriate because it has been shown (47) that obtaining the first (*t*<sub>1</sub>) biopsy immediately before the start of a primed constant infusion results in rates of muscle protein synthesis that are the same as rates that are calculated from a *t*<sub>1</sub> biopsy obtained 60 min after the start of the tracer infusion.

Rates of myofibrillar protein synthesis were calculated using the standard precursor-product method:  $FSR (\%/h) = (E_{2b} - E_{1b}) / E_p \times t^{-1} \times 100$ , where *E*<sub>2b</sub> and *E*<sub>1b</sub> are the bound protein enrichments from muscle at time 2 (*E*<sub>2b</sub>) and plasma proteins or the previous muscle biopsy at time 1 (i.e., baseline; *E*<sub>1b</sub>), *E*<sub>p</sub> is the precursor L-[ring-<sup>13</sup>C<sub>6</sub>]phenylalanine enrichment and *t* is the tracer incorporation time.

**Gene expression.** A small piece (~10–15 mg) of frozen wet muscle tissue was soaked overnight in RNAlater-ICE (Applied Biosystems, Foster City, CA) at –20°C. Tissue was homogenized in 500 μl TRIzol Reagent (Invitrogen, Carlsbad, CA), and isolated RNA was eluted through a spin cartridge according to manufacturer instructions (PureLink RNA Minikit, Invitrogen, Foster City, CA). Extracted RNA was quantified using a QUANT-iT analyzer kit (Invitrogen, cat. no. Q32852) according to the manufacturer's directions and then diluted appropriately with nuclease-free water to yield a total volume of 20 μl. First-strand complementary DNA (cDNA) synthesis was performed using commercially available TaqMan Reverse Transcription Reagents (Invitrogen, Melbourne, Victoria, Australia) on a BioRad thermal cycler (BioRad, Gladesville, Australia). Serial dilutions (100, 10, 1, 0.1, 0.01 ng) of template RNA (AMBION; cat. no. AM7982) were included for calculation of a standard curve for real-time quantitative RT-PCR. Quantification of mRNA (in duplicate) was performed on a 72-well Roto-Gene 3000 Centrifugal Real-Time Cycler (Corbett Research, Mortlake, NSW, Australia). Taqman FAM-labeled primer/probes for MuRF-1, Atrogin-1, and androgen receptor were used in a final reaction volume of 20 μl. The amplification calculated by Rotor-Gene software was specific and efficient (1.05 ± 0.05) for all genes. Threshold cycle data were analyzed using the 2<sup>–ΔΔ</sup> C<sub>T</sub>

method (31), with genes of interest expressed as fold-changes from baseline and normalized to housekeeping gene GAPDH expression.

**Western blot analysis.** A piece of frozen wet muscle (~50 mg) was homogenized by hand with a Dounce glass homogenizer on ice in a buffer (10 μl/mg, pH 7.4) containing (in mM): 10 Tris (pH 7.2), 250 sucrose, 10 NaCl, 3 MgCl<sub>2</sub>, 1 dithiothreitol, 1 EGTA, 1 EDTA, 0.3% vol/vol Triton-X 100, and protease/phosphatase inhibitor cocktail tablets (Complete Protease Inhibitor Mini-Tabs, Roche, Indianapolis, IN; PhosSTOP, Roche Applied Science, Mannheim, Germany). The homogenate was transferred to an Eppendorf tube and spun at 15,000 g for 10 min at 4°C. The supernatant was removed for protein concentration determination using a standard protein assay kit (Pierce Biotechnology, Rockford, IL) and subsequent Western blotting analysis. Cell lysates were prepared in Laemmli sample buffer (30) and were loaded (40 μg protein) on 10% sodium dodecyl sulfate polyacrylamide gels for separation by electrophoresis. Proteins were transferred to polyvinylidene fluoride membranes, blocked for 1 h in 5% (wt/vol) non-fat milk prepared in Tris-buffered saline with Tween (TBST; 10 mM Tris-HCl, 100 mM NaCl, and 0.02% Tween 20). Membranes were incubated in primary antibody (1:1,000) overnight at 4°C: phospho-Akt<sup>Ser473</sup> (#9271), phospho-mTOR<sup>Ser2448</sup> (#2971), phospho-p70S6K<sup>Thr389</sup> (#9205), and total androgen receptor (#3202S) were from Cell Signaling Technology (Danvers, MA). Androgen receptor molecular weight (110 kDa) was confirmed using an LNCaP cell lysate as a positive control. Membranes were then washed in TBST and incubated in secondary antibody (1:2,000) for 1 h at room temperature before washing and detection by chemiluminescence imaging (SuperSignal Chemiluminescent Substrate, Pierce Biotechnology, Rockford, IL; FluorChem, Alpha Innotech, Santa Clara, CA). Blots were quantified by assessing peak density using Image J software (National Institutes of Health). All time points for each participant were run on the same gel, and data are expressed relative to α-tubulin (1:2,000, T6074, Sigma-Aldrich, St. Louis, MO).

**Protein-bound enrichments.** Myofibrillar proteins were extracted from frozen wet muscle (~50 mg) as previously described (38). The resulting protein pellet was hydrolyzed overnight 6 M HCl at 110°C, and the free amino acids were purified using cation-exchange chromatography (Dowex 50WX8–200 resin Sigma-Aldrich) and converted to their *N*-acetyl-*n*-propyl ester derivatives for analysis by gas chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS; Hewlett Packard 6890; IRMS model Delta Plus XP, Thermo Finnigan, Waltham, MA).

**Statistics.** Anthropometry and AUC measures were analyzed by unpaired *t*-tests. Blood analytes, MPS, protein and gene expression were analyzed (separate analyses performed for days 1 and 2) by two-factor (sex and time) ANOVA with Tukey post hoc tests where

Table 2. Blood glucose, insulin, and amino acid concentrations in the rested fasted state and after resistance exercise plus ingestion of 25 g of whey protein (day 1)

Time (min):	Rested Fasted		Postexercise				
	–60	0	15	30	60	90	120
Glucose, mmol/l							
Women	4.5 ± 0.3	5.2 ± 0.6*	5.2 ± 0.8*	4.6 ± 0.4	4.3 ± 0.2	4.0 ± 0.3	4.3 ± 0.2
Men	4.5 ± 0.4	5.3 ± 0.6*	5.2 ± 0.7*	5.1 ± 0.8	4.7 ± 0.1	4.5 ± 0.5	4.5 ± 0.5
Insulin, μIU/ml							
Women	3.5 ± 2.1	6.3 ± 3.6	9.9 ± 6.0	17.8 ± 11.5*	19.9 ± 13.2*	8.4 ± 5.4	7.5 ± 5.6
Men	5.1 ± 3.7	5.0 ± 2.4	9.4 ± 7.9	13.8 ± 11.0*	19.2 ± 7.5*	13.0 ± 4.2	9.9 ± 3.0
Total AA, μmol/l							
Women	2169 ± 468	2092 ± 456	—	2425 ± 854	3202 ± 746*	2702 ± 597*	2577 ± 560
Men	1967 ± 444	2340 ± 200	—	2488 ± 306	2994 ± 417*	2712 ± 535*	2691 ± 337

Values are means ± SD (*n* = 8 in each group). Sex × time interactions for glucose, insulin, and total amino acids, respectively: *P* = 0.69, 0.96, 0.84. Main effects for time (all *P* < 0.001); \*values are significantly different from pre-exercise, men and women combined, *P* < 0.05. Total AA, summed total amino acid concentrations (Asp, Ser, Asn, Glu, Gly, Gln, His, Tau, Thr, Arg, Ala, Pro, Tyr, Cys, Val, Met, Ile, Leu, Lys, Lys, Phe). Drink comprised 25 g of whey protein isolate.

significant ( $P \leq 0.05$ ) main effects or interactions were indicated. In the figures, significant main effects are described in the legends and symbols indicate between-sex differences that are the result of a significant post hoc test. Statistical analyses were performed using SigmaStat version 3.1 software (Systat Software, Point Richmond, CA). All data are expressed as means  $\pm$  SD.

## RESULTS

**Participant characteristics.** Participant characteristics are shown in Table 1. Women had a greater percentage of total body and thigh fat than men and had a lower lean body mass and thigh lean mass (all  $P < 0.01$ ). Body mass index was not different between men and women ( $24.3 \pm 2.4$  vs.  $23.0 \pm 1.9$ , respectively;  $P = 0.25$ ). The ratio of isometric strength to thigh lean mass (which was normalized to femur length) was not

different between men and women ( $1.14 \pm 0.4$  vs.  $0.86 \pm 0.38$ ,  $P = 0.37$ ).

**Blood metabolites and serum hormones.** Blood glucose concentration was elevated above resting concentrations immediately and 15 min after exercise (Table 2, main effect for time,  $P < 0.001$ ); glucose concentrations were unchanged on day 2 (data not shown). Plasma insulin concentrations were elevated 30 and 60 min after protein ingestion on both day 1 (Table 2, main effect for time,  $P < 0.001$ ) and day 2 (data not shown). Blood amino acids were elevated similarly in men and women at 60 and 90 min after the 25-g protein drink ( $P < 0.001$ ; sex  $\times$  time interaction,  $P = 0.84$ ). Blood lactate concentrations increased from 0.6 mM in both men and women to 10.7 mM in men and to 7.1 mM in women immediately postexercise (sex  $\times$  time interaction,  $P < 0.001$ ), resulting in

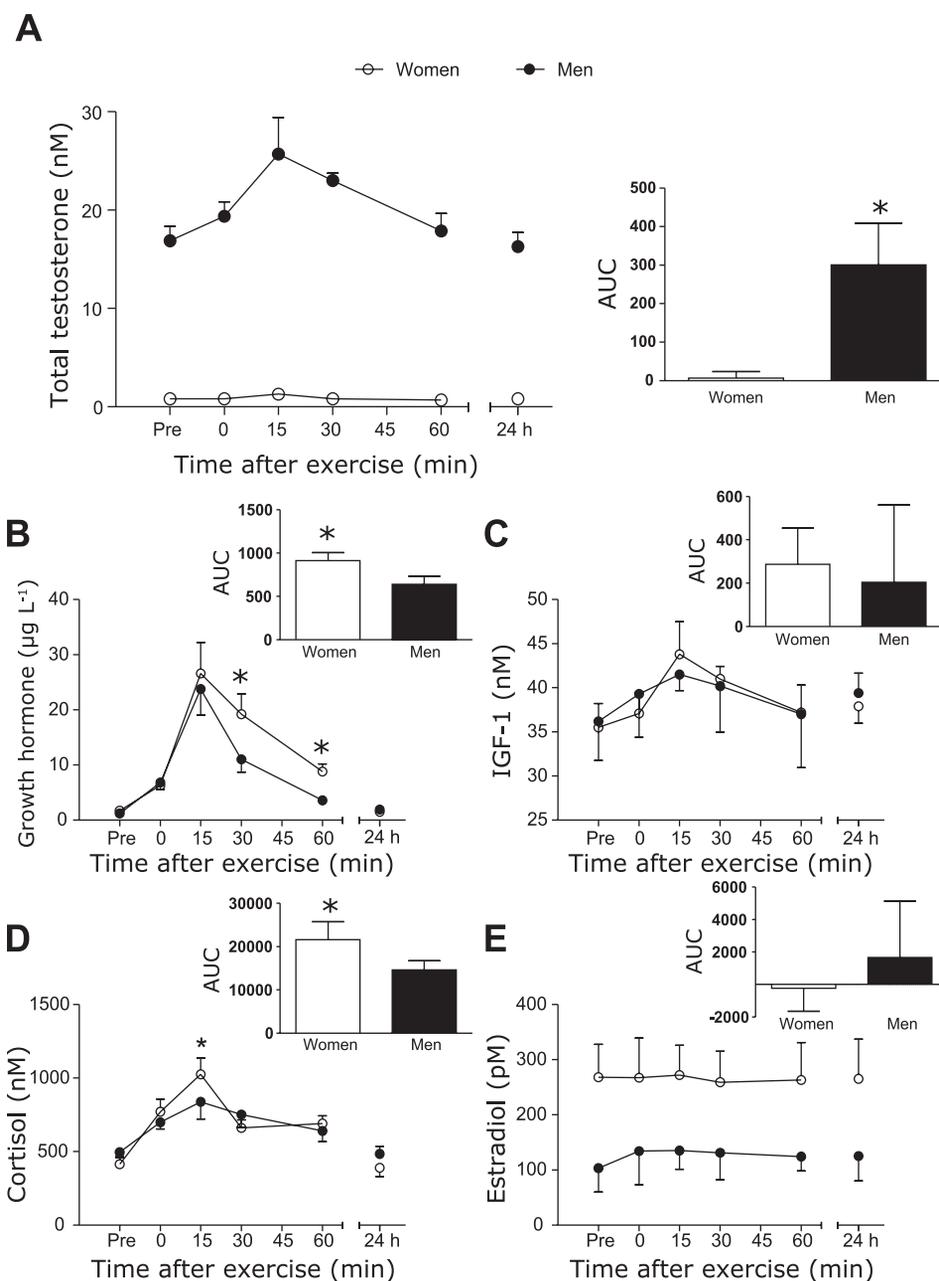


Fig. 2. Serum postexercise testosterone (A), growth hormone (GH) (B), IGF-1 (C), cortisol (D), and estradiol (E) concentrations in men and women. Insets are net areas under the curve where pre-exercise (Pre) = 0; \*significant difference between sexes,  $P < 0.001$ . \*Significant difference between sexes at the same time point,  $P < 0.01$ . Values are means  $\pm$  SD,  $n = 8$  in each group.

a greater area under the curve in men ( $P = 0.02$ ). Serum concentrations of total testosterone, growth hormone, IGF-1, cortisol, and estradiol are shown in Fig. 2 A, B, C, D, and E, respectively. Fifteen minute postexercise, testosterone increased from 17 to 26 nM in men and from 0.8 to 1.3 nM in women (sex  $\times$  time interaction,  $P < 0.001$ ); this resulted in an exercise-induced area under the testosterone curve that was  $\sim 45$ -fold greater in men ( $P < 0.001$ ). Postexercise growth hormone concentration peaked similarly in men and women at 15 min, with a more prolonged elevation in women such that concentrations were greater in women at 30 and 60 min (both  $P < 0.01$ ; sex  $\times$  time interaction,  $P < 0.001$ ), resulting in a greater AUC ( $P < 0.001$ ). IGF-1 was similarly elevated in men and women (time effect,  $P < 0.001$ ; sex  $\times$  time interaction,  $P = 0.55$ ). Cortisol concentration peaked at a higher concentration 15 min post-exercise in women compared with men (sex  $\times$  time interaction,  $P < 0.001$ ), resulting in a greater AUC in women ( $P < 0.001$ ). Estradiol concentrations were higher at all time points in women and remained unchanged in the acute post-exercise period (sex effect,  $P < 0.001$ ; sex  $\times$  time interaction,  $P = 0.60$ ).

**Myofibrillar protein synthesis.** Plasma phenylalanine tracer-to-tracee ratios during fasted, 1- to 3-h, 3- to 5-h, 24- to 26-h, and 26- to 28-h periods, respectively, for men and women (respectively) were 0.052 and 0.051, 0.057 and 0.054, 0.61 and 0.061, 0.071 and 0.068, 0.053 and 0.062, 0.059 and 0.060. Basal rates of MPS were similar between men ( $0.021 \pm 0.007\%/h$ ) and women ( $0.020 \pm 0.008$ ). MPS was elevated acutely in men and women at 1–3 h ( $P = 0.018$ ) and 3–5 h ( $P = 0.021$ ) after exercise and protein consumption (Fig. 3A) and at 26–28 h in the fed state ( $P < 0.001$ ). There was a trend for a sex  $\times$  time interaction for MPS acutely after exercise ( $P = 0.087$ ) that appeared to be attributable not to the magnitude of the response but more to subtle temporal differences (Fig. 3A); MPS rates calculated across the aggregate 1- to 5-h time period were not different between sexes (*inset* of Fig. 3A).

**Cell signaling.** Akt<sup>Ser473</sup> phosphorylation increased at 1 h ( $P < 0.001$ , main effect for time; Fig. 4A) and to a greater extent in men (sex  $\times$  time interaction,  $P = 0.018$ ). Phosphorylation of mTOR<sup>Ser2448</sup> was increased at 1, 3, and 5 h ( $P < 0.001$ ; Fig. 4B); there was a main effect for sex (men  $>$  women,  $P = 0.003$ ). Phosphorylation of mTOR<sup>Ser2448</sup> was elevated similarly between sexes after next-day protein feeding,  $\sim 26$  h after the exercise bout (sex  $\times$  time interaction,  $P = 0.49$ ; main effect for time, 28  $>$  26 h,  $P = 0.006$ , data not shown). Phosphorylation of p70S6K1<sup>Thr389</sup> increased at 1, 3, and 5 h (all  $P < 0.001$ ; sex  $\times$  time interaction,  $P = 0.13$ ; Fig. 4C) and there was a significant interaction with next-day feeding (28  $>$  26 h in women only, sex  $\times$  time interaction,  $P = 0.016$ ; data not shown). Androgen receptor content was greater overall in men ( $P = 0.049$ ) but there was no significant interaction ( $P = 0.47$ ; Fig. 4D).

**Gene expression.** MuRF-1 mRNA was not different from rest in men and women 1 and 5 h after exercise (sex  $\times$  time interaction,  $P = 0.58$ ; Fig. 5A); MuRF-1 increased at 28 h in men only (sex  $\times$  time interaction,  $P = 0.003$ ). Atrogin-1 mRNA decreased irrespective of sex at 5 h compared with rest ( $P < 0.001$ ) 1 and 5 h ( $P = 0.016$ ) (sex  $\times$  time interaction,  $P = 0.98$ ; Fig. 5B); Atrogin-1 increased at 28 h (time effect,  $P = 0.002$ ; Fig. 5B) with a trend ( $P = 0.09$ ) toward a greater increase in men. Androgen receptor mRNA was not different

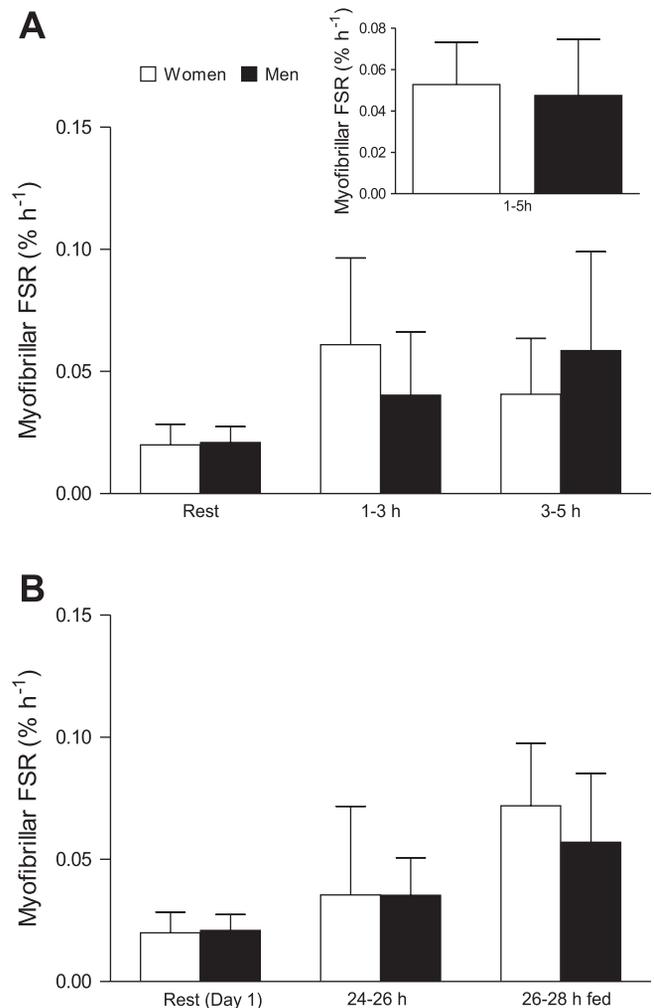


Fig. 3. Myofibrillar fractional synthetic rates (FSR) in the rested fasted state, 1–3 and 3–5 h after resistance exercise and protein ingestion (A). Myofibrillar FSR 24–26 h postexercise and 26–28 h postexercise after 25 g of protein ingested at 26 h (B). *Inset* in A is the aggregate FSR from 1–5 h calculated from enrichments obtained from biopsies taken at 1 and 5 h. 1–3 h and 3–5 h  $>$  rest,  $P < 0.001$ ; sex  $\times$  time interaction,  $P = 0.087$ . The rested fasted rate from day 1 A is simply redrawn in B for comparisons sake. 26–28 h  $>$  24–26 h,  $P < 0.001$ ; sex  $\times$  time interaction,  $P = 0.56$ . Values are means  $\pm$  SD,  $n = 8$  in each group.

from rest in men and women at 1 and 5 h after exercise (sex  $\times$  time interaction,  $P = 0.38$ ; Fig. 5C) and was increased at 28 h compared with 26 h (time effect,  $P < 0.022$ ; sex  $\times$  time interaction,  $P = 0.16$ ; Fig. 5C).

## DISCUSSION

The present study reports similar rates of MPS and anabolic signaling responses between men and women after resistance exercise and bolus protein ingestion early (1–5 h) and late (24–28 h) in postexercise recovery. The increase in MPS at 1–5 h was similar between men and women and is in support of previous findings showing comparable changes in men and women with feeding and exercise individually (13, 46). The sustained increase in MPS in the 3- to 5-h recovery period observed, a time when amino acids had returned to basal concentrations, is in agreement with our previous observations (38, 51). This observation indicates that the resistance exercise

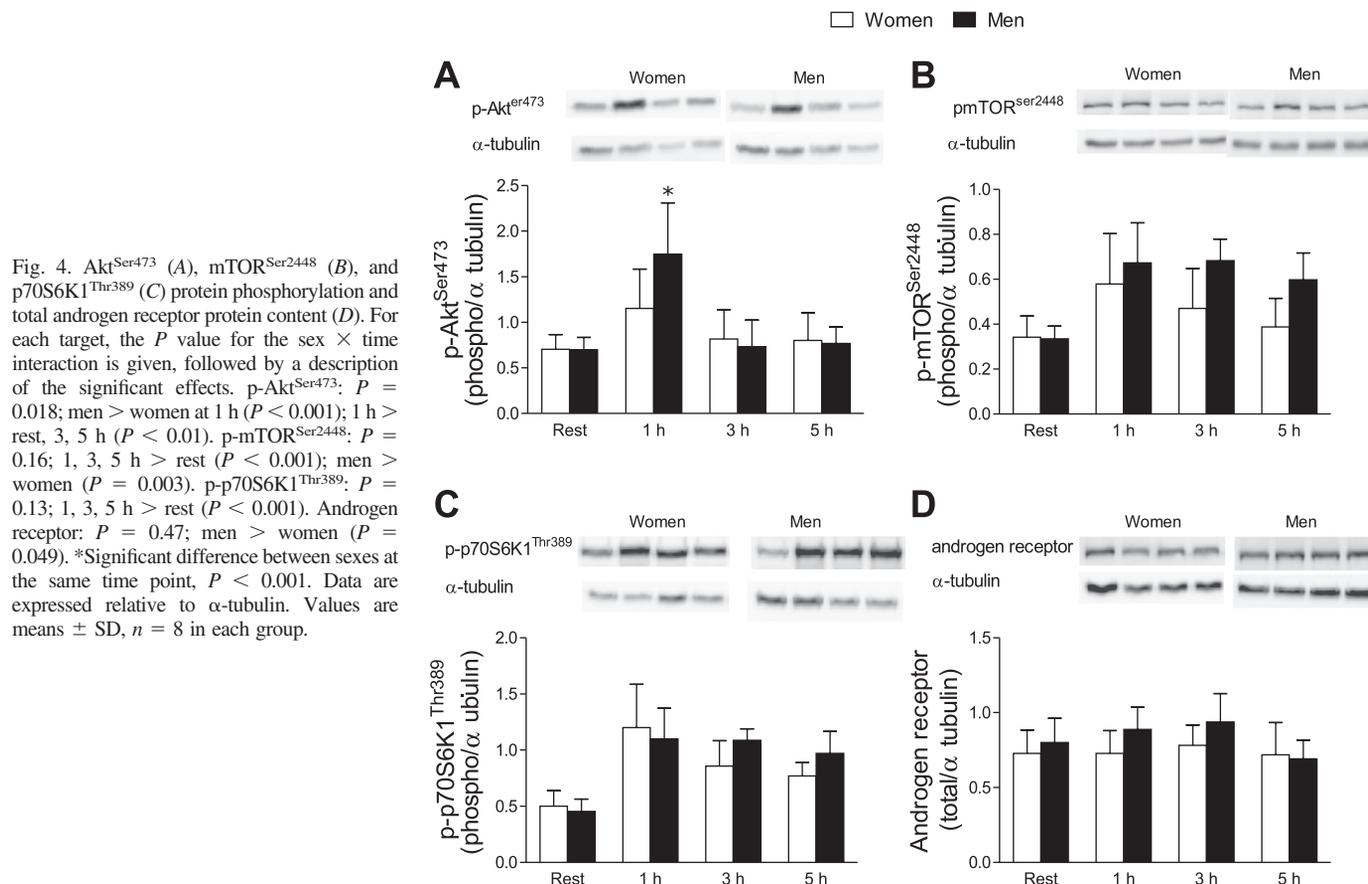


Fig. 4. Akt<sup>Ser473</sup> (A), mTOR<sup>Ser2448</sup> (B), and p70S6K1<sup>Thr389</sup> (C) protein phosphorylation and total androgen receptor protein content (D). For each target, the *P* value for the sex  $\times$  time interaction is given, followed by a description of the significant effects. p-Akt<sup>Ser473</sup>: *P* = 0.018; men > women at 1 h (*P* < 0.001); 1 h > rest, 3, 5 h (*P* < 0.01). p-mTOR<sup>Ser2448</sup>: *P* = 0.16; 1, 3, 5 h > rest (*P* < 0.001); men > women (*P* = 0.003). p-p70S6K1<sup>Thr389</sup>: *P* = 0.13; 1, 3, 5 h > rest (*P* < 0.001). Androgen receptor: *P* = 0.47; men > women (*P* = 0.049). \*Significant difference between sexes at the same time point, *P* < 0.001. Data are expressed relative to  $\alpha$ -tubulin. Values are means  $\pm$  SD, *n* = 8 in each group.

stimulus, not feeding, is responsible for sustained increases in fractional synthetic rate (4, 9).

The phosphorylation profiles of the signaling proteins we measured were also broadly similar between men and women (Fig. 4). However, we found that mTOR<sup>Ser2448</sup> was phosphorylated to a greater extent in men during the acute recovery period after resistance exercise, which is contrast to the equal increase between sexes previously reported (13). Despite these differences in mTOR<sup>Ser2448</sup> phosphorylation, both studies report similar (in magnitude and between sexes) increases in p70S6K1 phosphorylation acutely after resistance exercise. This may indicate that the level of mTOR activation in women was sufficient to result in downstream effects on p70S6K1, changes in the phosphorylation status of which may better indicate mTOR activation than phosphorylation changes at the particular Serine<sup>2448</sup> site (45). The reason(s) for differences in mTOR phosphorylation are unclear; both groups were fed identical amounts of protein, had similar aminoacidemia and insulin responses (Table 2), and performed the exercise bout at the same relative intensity. Men have a greater percentage type IIA fiber area on average (48) and mTOR has been reported to be localized in type IIA fibers (40), which could potentially account for some of the differences observed. Nevertheless, on the basis of the small differences in muscle protein synthesis between fiber types (12, 35) this would not be expected to have a major influence.

The optimal approach to capture mechanistic insight from changes in the signaling of individual proteins remains elusive and it has been demonstrated that there is often a disconnect

between signaling and muscle protein synthesis in both magnitude (17) and time course (2). In light of this disconnect, it is worth recognizing that the phosphorylation of signaling proteins is a temporal snapshot of the propagated signal for translation initiation. It is also unclear if there is a minimum threshold signal required to initiate and completely activate or turn on translation. If there is such a threshold then it seems plausible that greater phosphorylation above such a threshold would be unlikely to further amplify the signal/lead to increased rates of translation. Nonetheless, it has been demonstrated that the elevations in protein synthesis that are mediated by amino acids (11) and contraction (14) are dependent on mTOR activation, which regulates translation initiation (22).

In addition to sex-based comparisons of anabolic indices we compared the mRNA response of genes that regulate muscle protein breakdown. The ubiquitin ligases MuRF-1 and atrogin-1 are associated with skeletal muscle atrophy (8) but their specific role in muscle remodeling and exercise-induced adaptation is yet to be defined. Our data are consistent with other studies (23, 32) in reporting an attenuation in atrogin-1 expression after acute resistance exercise, a decrease that we found was similar in men and women. Collectively, the present and previous (32) data are in agreement with the notion that, despite being thought to perform similar roles in ubiquitin-proteasome-mediated protein breakdown, MuRF1 and atrogin-1 can be differentially regulated after acute resistance exercise and may function independently (39). Surprisingly, there was an increase in atrogin-1 and MuRF-1 expression with protein feeding  $\sim$ 24 h after exercise. No study has made direct measures of muscle

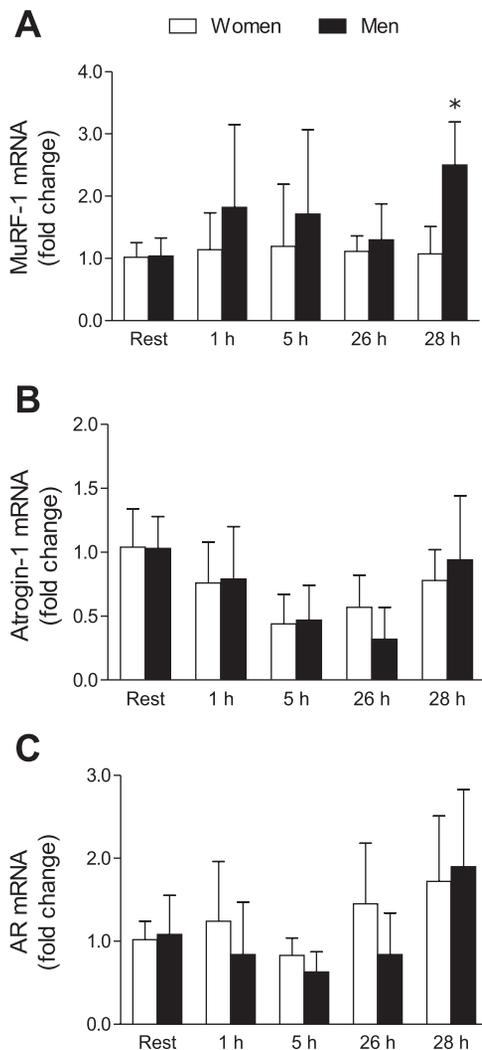


Fig. 5. MuRF-1 (A), atrogin-1 (B), and androgen receptor (C) gene expression in the rested fasted state; 1, 5, and 26 h after resistance exercise plus 25 g of protein; and at 28 h (second 25 g protein bolus given at 26 h). Sex  $\times$  time interactions and significant post hoc effects (respectively) are as follows. MuRF-1: *day 1*,  $P = 0.58$ ; *day 2*,  $P = 0.003$ , men  $>$  women at 28 h ( $P < 0.001$ ). Atrogin-1: *day 1*,  $P = 0.98$ , time effect  $P = 0.001$ , rest and 1 h  $>$  5 h ( $P = 0.001$  and  $0.016$ , respectively); *day 2*,  $P = 0.092$ , time effect 28 h  $>$  26 h,  $P = 0.002$ . Androgen receptor: *day 1*,  $P = 0.38$ ; *day 2*,  $P = 0.16$ , time effect 28 h  $>$  26 h,  $P = 0.022$ . Threshold cycle data were analyzed using  $2^{-\Delta\Delta C_T}$  method (31) with glyceraldehyde-3-phosphate dehydrogenase as a house-keeping gene. Values are means  $\pm$  SD,  $n = 8$  in each group.

protein breakdown under circumstances similar to the present study, making it somewhat difficult to place the finding in context. Whether the provision of exogenous amino acids permits the muscle to return to a state of increased remodeling (we know that MPS is elevated), including increased breakdown, after an overnight fast requires further investigation. We recognize that there is a lack of data that links gene-based markers of proteolysis to an actual phenotypic change. Reasons for elevated MuRF-1 mRNA in men compared with women with next-day protein feeding are not clear, although it is interesting to note that estradiol supplementation in men attenuates exercise-induced muscle inflammation (33) and that MuRF-1 is responsive to local inflammation (39). However, inflammation was not measured in the present study and how

female sex steroids affect sex-based differences in postexercise muscle inflammation (42, 49) and muscle protein breakdown is presently unclear.

Our finding of increased androgen receptor mRNA abundance at 28 h into recovery after resistance exercise adds to studies showing increases 48 h postexercise (6, 20, 56). Androgen receptor expression, which is reported to be affected by feeding (27) as well as mechanical load (6), did not appear to be affected by differences in acute postexercise testosterone concentration between men and women.

A secondary aim of this study was to examine the potential impact of sex-based differences in post-exercise testosterone. We report that despite dramatic (45-fold) differences in exercise-induced serum testosterone, men and women had similar increases in MPS after resistance exercise and protein ingestion. Our data are in contrast to the theory that the absence of a transient acute hypertestosteronemia in response to resistance exercise in women compared with men limits their potential for hypertrophy (44). Although not causative, the clear dissociation of the postexercise rise in MPS and systemic testosterone suggests that the anabolic response to resistance exercise does not rely on postexercise hypertestosteronemia and can occur effectively despite low systemic testosterone in women. Our present and previous (53–55) work suggests that intramuscular mechanisms are predominantly driving the synthesis of myofibrillar proteins after resistance exercise and that exercise-induced testosterone is not required for this process. Whereas our sex-based model suggests postexercise testosterone is not a prime mediator of postexercise MPS, the potential role of intramuscular androgen-related events [testosterone and dihydrotestosterone can be synthesized in peripheral, non-gonadal tissue (1, 29, 57)] is unclear. Thus testosterone concentrations in systemic circulation may not be a good indication of the potential impact of androgens on muscle tissue (29).

In conclusion, we report similar increases in rates of MPS between men and women after resistance exercise with protein ingestion during the early (1–5 h) and late (24 h) recovery period. Interestingly, the postexercise testosterone responses in women, which were 45-fold lower than men, did not appear to attenuate increases in MPS or p70S6K1 phosphorylation from occurring. Indeed, the clear dissociation between the testosterone response and the MPS response that we observed, suggests that postexercise testosterone is not likely a predominant driver of MPS. Further studies that measure intramuscular androgen intracrinology and receptor activity are necessary to elucidate the potential role for physiological androgen-related mechanisms in regulating skeletal muscle mass in men and women.

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## DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

## AUTHOR CONTRIBUTIONS

Author contributions: D.W.W., N.A.B., and S.M.P. conception and design of research; D.W.W., N.A.B., T.A.C.-V., C.J.M., S.K.B., and S.M.P. performed experiments; D.W.W., N.A.B., D.M.C., V.G.C., and S.M.P. analyzed data; D.W.W., N.A.B., D.M.C., J.A.H., V.G.C., and S.M.P. interpreted results of experiments; D.W.W. prepared figures; D.W.W. drafted manuscript; D.W.W., N.A.B., T.A.C.-V., D.M.C., C.J.M., S.K.B., J.A.H., V.G.C., and S.M.P. edited and revised manuscript; D.W.W., N.A.B., T.A.C.-V., D.M.C., C.J.M., S.K.B., J.A.H., V.G.C., and S.M.P. approved final version of manuscript.

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