Continuous testosterone administration prevents skeletal muscle atrophy and enhances resistance to fatigue in orchidectomized male mice

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Axell, Anna-Maree, Helen E. MacLean, David R. Plant, Leah J. Harcourt, Jennifer A. Davis, Mark Jimenez, David J. Handelsman, Gordon S. Lynch, and Jeffrey D. Zajac. Continuous testosterone administration prevents skeletal muscle atrophy and enhances resistance to fatigue in orchidectomized male mice. Am J Physiol Endocrinol Metab 291: E506–E516, 2006.—Androgens promote anabolism in skeletal muscle; however, effects on subsequent muscle function are less well defined because of a lack of reliable experimental models. We established a rigorous model of androgen withdrawal and administration in male mice and assessed androgen regulation of muscle mass, structure, and function. Adult C57Bl/6J male mice were orchidectomized (Orx) or sham-operated (Sham) and received 10 wk of continuous testosterone (T) or control treatment (C) via intraperitoneal implants. Mass, fiber cross-sectional area (CSA), and in vitro contractile function were assessed for fast-twitch extensor digitorum longus (EDL) and slow-twitch soleus (SOL) muscles. After 10 wk, Orx+C mice had reduced body weight gain (P < 0.05), seminal vesicle mass (P < 0.01), and levator ani muscle mass (P < 0.001) compared with Sham+C mice, and these effects were prevented with testosterone treatment. Orx+T mice had greater EDL (P < 0.01) and SOL (P < 0.01) muscle mass compared with Orx+C mice; however, median fiber CSA was not significantly altered in these muscles. EDL and SOL muscle force was greater in Sham+T compared with Orx+C mice (P < 0.05) in proportion to muscle mass. Unexpectedly, Orx+T mice had increased fatigue resistance of SOL muscle compared with Orx+C mice (P < 0.001). We used a rigorous model of androgen withdrawal and administration in male mice to demonstrate an essential role of androgens in the maintenance of muscle mass and force. In addition, we showed that testosterone treatment increases resistance to fatigue of slow- but not fast-twitch muscle.

In humans, there is clear evidence that physiological testosterone administration increases lean body mass in conditions of low circulating androgens. Testosterone replacement therapy has been effectively used to counteract loss of lean body mass in hypogonadal men (4, 11, 32), older men with normal or low serum testosterone (21, 54), and human immunodeficiency virus (HIV)-infected men with low serum testosterone (2). Similarly, muscle anabolism has been achieved in eugonadal states following supraphysiological administration to young, healthy men (3, 25) and in HIV-infected men with normal testosterone levels (20). However, although some studies have demonstrated enhanced muscle strength following androgen administration (3, 4, 21, 50, 64), others have failed to detect a significant functional effect of androgen therapy despite gains in muscle mass (7, 53, 54, 63).

Issues that still remain to be resolved from the human studies include the dose of testosterone and length of treatment required to increase muscle strength and whether changes in strength occur in a muscle-specific manner. Randomized controlled trials utilizing 20 wk of testosterone administration to subjects treated with a gonadotropin-releasing hormone agonist to suppress endogenous androgen production have demonstrated a dose-dependent response of lean body mass and leg strength to testosterone in both young (5) and elderly (6) men. In these studies, significant increases in muscle strength were only achieved in subjects that received either high-normal or supraphysiological doses of testosterone. Other studies have shown increased leg and arm/chest strength after 3 or 6 mo of physiological testosterone replacement in hypogonadal men (64), 10 wk of supraphysiological doses of testosterone in normal men (3), or 3 mo of treatment with the potent androgen oxymetholone in elderly men (49). In contrast, in other studies in hypogonadal men, in which physiological replacement doses of testosterone were used, no changes in muscle strength were observed over a 3-yr period despite increased lean body mass (53, 63). Furthermore, a 3-yr randomized controlled trial in elderly men, in which the testosterone dose used maintained serum testosterone within the normal range, also demonstrated no change in muscle strength despite increases in lean body mass (54). Other randomized controlled studies in older men, using low-dose testosterone treatment for 1 or 6 mo, with or without growth hormone treatment, also failed to show any effects of testosterone treatment alone on muscle strength (7, 10, 23). Another 3-yr randomized controlled study showed that...
elevated physiological levels had a significant increase in lean body mass and increased handgrip strength but no change in lower leg strength (42). Thus, although the data now clearly show that androgens can induce a dose-dependent increase in muscle mass and muscle strength in men, it also is apparent that androgen-dependent changes in muscle mass can occur without significant measurable effects on muscle strength. This may be due to the different methodologies used to quantitate strength or site of measurement (1-repetition leg press or chest press vs. leg flexion/extension or handgrip strength using an isokinetic dynamometer), may be because of muscle-specific actions of androgens, or may be because only high-normal or supraphysiological doses of androgens significantly increase muscle strength.

Therefore, the therapeutic use of androgens to alleviate muscle wasting and enhance physical strength requires further evaluation at both the physiological and molecular level. The mouse is a powerful tool to investigate physiological systems and underlying molecular mechanisms in vivo, and this potential has been further enhanced by the advent of genetically manipulated models (16). Despite the practical advantages of using small animal models to investigate physiological systems, current reports of androgen effects on muscle mass and function in animal models are contradictory. In particular, some studies in rodents have demonstrated significant muscle atrophy following orchidectomy (30), whereas others report no change in either fast-twitch (1) or slow-twitch muscle mass (13) in response to androgen withdrawal. Furthermore, androgen treatment ameliorates immobilization-induced muscle atrophy in some rat models (60) but not in others (67). Functional studies in rats have demonstrated significant correlations between androgen levels and muscle force (13) and fatigue properties (56); however, there are limited studies that focus particularly on androgen regulation of muscle function in the mouse. Early studies in mice showed that androgen withdrawal results in a significant reduction in muscle mass (30, 45) and maximum force production (30). On the other hand, another study failed to detect significant changes in muscle mass, force, or fatigue following high-dose continuous infusion of the testosterone derivative stanozolol (59). To date, no studies have simultaneously assessed the effect of orchidectomy and immediate continuous testosterone replacement on muscle mass and contractile function in a sedentary mouse model.

We have developed a rigorous in vivo mouse model to investigate the effect of continuous supraphysiological androgen administration on skeletal muscle mass and function following orchidectomy and to assess underlying changes in fiber size. The model was designed using orchidectomized and sham animals representing a range of androgen levels to determine the dose-response of skeletal muscle under sedentary conditions. We have tested the hypothesis that elevated androgen levels can sustain muscle mass and force-producing capacity in orchidectomized sedentary mice. In addition, we have studied the effects of androgens on regulation of muscle fatigue properties.

MATERIALS AND METHODS

Animals. Male C57BI/6J mice at 8 wk of age were randomly allocated into four treatment groups (n = 8 per group): sham + empty control implants (Sham+C), orchidectomy + empty control implants (Orx+C), sham + testosterone implants (Sham+T), and orchidectomy + testosterone implants (Orx+T). All operative procedures were performed under anesthesia administered by intraperitoneal injection of a 4 mg/ml solution of ketamine (Cvenet, Mansfield, Queensland, Australia) and xylazine (Cvenet; 100 μl/10 g body wt). All animals underwent orchidectomy or sham operation followed by immediate intraperitoneal insertion of three 1-cm testosterone-filled or empty implants for control. Implants were prepared from Silastic laboratory grade tubing (Dow Corning, Midland, MI) and sealed with Silastic adhesive (Dow Corning) as described previously (27). Each 1-cm testosterone-filled implant contained -11 ± 3 mg of crystalline testosterone (Sigma Chemical, St. Louis, MO). All animals underwent a 10-wk treatment period with food and water ad libitum before muscle analysis. Animals were housed in a conventional animal facility, and all experimentation was conducted in accordance with accepted standards of humane animal care as outlined by the National Health and Medical Research Council (NHMRC) and was approved by the Royal Melbourne Research Foundation Animal Ethics Committee (no. AEC201/023).

Total body weight and growth rate. Animals were weighed weekly over the 10-wk treatment period. Net total body weight gain was calculated from original and final weight measurements to an accuracy of 0.1 g. Body growth rate (g/wk) was determined by linear regression analysis of weekly body weight data over 10 wk for each animal.

Muscle excision and tissue harvest. At the end of the 10-wk treatment period, mice were anesthetized and the predominantly fast-twitch extensor digitorum longus (EDL) and slow-twitch soleus (SOL) muscles of the lower hindlimb were surgically excised for mass and in vitro function testing. Animals were then euthanized, and additional hindlimb muscles including the tibialis anterior (TA), plantaris (PLAN), and the levator ani muscle of the perineum [LA; also known as the pubocaudalis and iliocaudalis muscle group (91)] were excised and weighed for wet mass to an accuracy of 0.1 mg. EDL, SOL, and LA muscles were fixed at relaxed length in 4% paraformaldehyde for 24 h before histological processing. Additional tissues including the testis and seminal vesicles were excised and weighed for wet mass. In the data analysis, muscle mass was not corrected for body mass, because body mass was the same in all groups before treatment. Body mass is androgen dependent (40), and skeletal muscle mass contributes up to 40% of total body mass (37); therefore, expressing muscle mass relative to body mass after treatment would potentially mask androgen-dependent changes in muscle mass.

Serum testosterone. After muscle function analysis (~45 min after initial anesthesia), mice were killed by cervical dislocation and blood was collected from the carotid artery. Serum testosterone was measured in orchidectomized mice by radioimmunoassay following organic extraction as described previously (51). The minimum detectable testosterone concentration of the assay at 95% confidence level is 0.1 nM, and the interassay coefficient of variance range is 3.1–7.5%.

Muscle contractile measurements. Contractile properties including maximum tetanic force (P0), specific force (sP0), fatigue, time to peak tension (TPT), and half-relaxation time (1/2RT) of the EDL and SOL muscles were assessed in vitro with the use of a force transducer according to methods described in detail previously (46). A frequency-force curve was established for EDL and SOL muscles after successive stimuli at 10–150 Hz and 5–120 Hz, respectively, with a 2-min rest between stimuli. P0 was determined from the plateau of the frequency-force relationship, after which the muscle was subjected to a 4-min stimulation protocol to induce muscle fatigue. Muscles were stimulated once every 5 s at optimum length, voltage, and frequency. P0 also was determined 5 min after the completion of the fatigue protocol as a measure of the recovery capacity of the muscle following fatigue. sP0 was calculated for each muscle by taking into account cross-sectional area (CSA) according to standard procedures described previously (46).
Muscle histology. Fixed EDL, SOL, and LA muscles were paraffin-embedded, and serial 5-μm transverse sections were cut through the mid-belly region of each muscle sample. Sections were stained with hematoxylin and eosin (H&E) to determine muscle CSA. Analysis was performed only on fibers in true cross section displaying normal polygonal morphology, excluding extracellular matrix. For fiber CSA, images were captured digitally and individual muscle fibers in cross section were traced on screen using Adobe Photoshop Elements 2.0. The areas of traced fibers were calculated using ImageJ software and were based on a calibrated pixel-to-actual size (μm) ratio. The median fiber CSA for each muscle was calculated from a minimum of 300 adjacent fibers per section and 4 animals per treatment group. All histological analyses were performed blinded to treatment group and assessed in random order with the use of coded sections by a single observer.

Statistical analyses. Statistical tests were performed using SPSS (version 11.0.2; SPSS, Chicago, IL). Variables were compared between groups with one-way ANOVA and Tukey’s post hoc test. Fiber CSA measurements for each muscle were not normally distributed (confirmed using the Kolmogorov-Smirnov test); thus the median fiber CSA per mouse was calculated and an average obtained for each treatment group. Differences in the average median fiber CSA between treatment groups were compared using one-way ANOVA and Tukey’s post hoc analyses. Analyses of weight gain over time and muscle fatigue curves were performed by comparing the slopes of linear regression lines to test for differences between treatment groups. Significance was set at \( P < 0.05 \). All values are expressed as means ± SE.

RESULTS

We have established a rigorous in vivo model of androgen withdrawal and administration with which to investigate androgen-dependent changes in skeletal muscle growth, structure, and function. Orchidectomized and sham-operated male mice were treated with testosterone or control implants for 10 wk. Human studies have shown a strict dose response of muscle mass and strength to androgen levels (5, 55). Therefore, we chose to have these four treatment groups representing a plateau in mass at the androgen levels achieved (5, 55). Thus, we chose to have these four treatment groups representing a range of androgen levels from low (orchidectomized) to physiological (sham) to supraphysiological (testosterone-treated sham), to determine the dose responsiveness of skeletal muscle mass and strength in mice.

Establishment of androgen withdrawal/administration model. Total body weight of animals randomly assigned to each group at the beginning of the study was not significantly different (Fig. 1). After 10 wk, orchidectomized control mice had restricted weight gain compared with sham control and testosterone-treated sham groups (Orx+C, 3.7 ± 0.6 g vs. Sham+C, 6.5 ± 0.8 g, \( P < 0.05 \); vs. Sham+T, 7.1 ± 0.7 g, \( P < 0.05 \)). Testosterone-treated orchidectomized mice gained weight at the same rate as sham control mice (Orx+T, 0.7 ± 0.1 g/wk vs. Sham+C, 0.7 ± 0.1 g/wk) and tended to be heavier than orchidectomized controls at completion of 10 wk of treatment (Orx+T, 28.2 ± 0.6 g vs. Orx+C, 24.7 ± 0.7 g, \( P = 0.06 \)). Results indicate that animals with depleted androgen levels had reduced weight gain over the 10-wk treatment period compared with mice with normal or administered androgen levels, and this was prevented with continuous testosterone administration.

Seminal vesicle mass was measured because it is a sensitive biomarker of androgen action (69). Orchidectomy caused a significant decrease in seminal vesicle mass compared with sham controls (\( P < 0.01 \); Fig. 2A), reflecting the expected marked decline in androgen action. Testosterone treatment abolished the orchidectomy-induced loss of seminal vesicle mass, indicating that continual testosterone delivery via intraperitoneal implants was maintained for up to 10 wk postimplantation. In addition, seminal vesicle mass was an average of 2.5 times greater in testosterone-treated sham and testosterone-treated orchidectomized animals compared with sham controls (\( P < 0.001 \)), confirming a supraphysiological level of androgen action in testosterone-treated mice.

To demonstrate the known myotrophic effects of orchidectomy and testosterone treatment, we examined the mass of the LA muscle, an androgen-responsive skeletal muscle of the perineum (19). Orchidectomized mice had significantly decreased LA muscle mass (Fig. 2B) compared with sham controls (\( P < 0.001 \)), and this decrease in mass was prevented by testosterone treatment. Unlike the seminal vesicles, there was no additional increase in LA muscle mass in testosterone-treated sham mice compared with sham controls, possibly representing a plateau in mass at the androgen levels achieved with three implants. Sham mice receiving testosterone treatment also demonstrated a significant reduction in testsis mass compared with sham controls (\( P < 0.05 \); Table 1). It is likely that the observed testicular atrophy is indicative of suppressed endogenous androgen production due to negative feedback regulation via the hypothalamic-pituitary-gonadal axis by exogenous testosterone (58).

To further confirm that the testosterone implants were functional, we measured serum testosterone levels in orchidectomized mice from blood taken at the completion of muscle function analysis (~45 min after initial anesthesia). The efficacy of testosterone treatment was evaluated in implanted orchidectomized mice only, because endogenous testosterone levels in intact sham mice are known to fluctuate greatly under stress of surgery and anesthesia (33, 41). As expected, testosterone levels in orchidectomized control mice were low, and treatment with testosterone implants increased serum testosterone levels 50-fold (\( P < 0.01 \); Table 1). These results confirm the efficacy of intraperitoneal implants to deliver consistent,
sustainable levels of testosterone for up to 10 wk in orchidectomized mice.

These results established the androgen responsiveness of the mouse model of androgen withdrawal and continuous administration, showing predictable biological effects on classic androgen-responsive tissues. In particular, the range of seminal vesicle mass values obtained in the different groups indicated that androgen levels encompassing low, physiological, and supraphysiological levels were achieved.

Muscle mass is androgen dependent. Using this model, we investigated the effect of different androgen levels on the mass of hindlimb skeletal muscles. To differentiate potential androgen actions on muscles of different fiber type proportions, we examined the EDL, PLAN, and TA muscles, which comprise predominantly fast-twitch fibers, and the SOL muscle, comprising a high proportion of slow-twitch fibers. Orchidectomy significantly decreased the mass of all fast-twitch muscles by up to 17% compared with sham controls (EDL: Orx+C, 9.2 ± 0.3 mg vs. Sham+C, 11 ± 0.4 mg, P < 0.05; PLAN: Orx+C, 15.2 ± 0.4 mg vs. Sham+C, 18.0 ± 0.7 mg, P < 0.05; TA: Orx+C, 39.6 ± 1.2 mg vs. Sham+C, 47.7 ± 1.7 mg, P < 0.05; Fig. 3). The orchidectomy-induced decline in fast-twitch muscle mass was prevented by testosterone administration, given that treated mice displayed up to 18% greater mass than orchidectomized controls (EDL: Orx+C, 9.2 ± 0.3 mg vs. Orx+T, 10.9 ± 0.3 mg, P < 0.01; PLAN: Orx+C, 15.2 ± 0.4 mg vs. Orx+T, 17.7 ± 0.6 mg, P < 0.01; TA: Orx+C, 39.6 ± 1.2 mg vs. Orx+T, 45.3 ± 1.3 mg, P < 0.05). For the slow-twitch SOL muscle, orchidectomized animals also had significantly reduced mass compared with testosterone-treated orchidectomized and testosterone-treated sham animals (Orx+C, 7.3 ± 0.2 mg vs. Orx+T, 9.3 ± 0.4 mg, P < 0.01; vs. Sham+T, 9.5 ± 0.4 mg, P < 0.01), with SOL mass of testosterone-treated orchidectomized and sham mice 27 and 30% higher, respectively, than orchidectomized controls.

These data indicate that both fast- and slow-twitch muscles display androgen-dependent changes in mass, with the slow-twitch muscle particularly responsive to androgen withdrawal and administration. Ten weeks of continuous testosterone administration prevented orchidectomy-induced muscle atrophy and maintained mass at levels comparable to or higher than those of sham control mice for both fast- and slow-twitch muscles.

### Table 1. Effect of intraperitoneal testosterone implants on testis mass and serum testosterone levels

<table>
<thead>
<tr>
<th>Testis Mass, mg</th>
<th>Serum Testosterone, nM</th>
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<tbody>
<tr>
<td>Sham + C</td>
<td>106.9 ± 9.3</td>
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<tr>
<td>Orx + C</td>
<td>ND</td>
</tr>
<tr>
<td>Sham + T</td>
<td>79.5 ± 3.2*</td>
</tr>
<tr>
<td>Orx + T</td>
<td>56.2 ± 13.6†</td>
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Testis mass and serum testosterone (T) levels of orchidectomized (Orx) and sham-operated (sham) mice after 10 wk of testosterone or control (C) treatment. Values are means ± SE; n ≥ 4 per group. *P < 0.05 vs. Sham + C; †P < 0.01 vs. Orx + C (testis mass, Student’s t-test; serum T, 1-way ANOVA and Tukey’s post hoc test). ND, not determined. (serum testosterone not assayed in Sham + C mice because of suppressive effects of stress and anesthesia on endogenous testosterone levels; testes not present in Orx mice).
Androgens affect muscle force in proportion to mass. To determine the effects of altered androgen levels on skeletal muscle function, we tested fast-twitch EDL and slow-twitch SOL skeletal muscles in vitro for contractile function. Physiological measurements of $P_o$, $sP_o$, fatigue, $1/2RT$, TPT, and frequency-force relationships were determined by in vitro electrical stimulation of isolated EDL and SOL muscles from all mice. $P_o$ is a measure of the maximum force-producing capacity of muscles (13). This was determined using a frequency-force curve generated for each muscle by applying increasing electrical stimuli until a plateau of maximum force was reached (data not shown). The effect of androgens on EDL muscle force mirrored the changes observed for muscle mass (Fig. 4A), with orchidectomized control animals showing lower maximum force production than sham controls ($P = 0.06$) and testosterone-treated sham animals demonstrating 25% greater $P_o$ than orchidectomized controls ($P < 0.01$; Fig. 4B). Similarly to the EDL muscle, SOL muscles of testosterone-treated sham animals produced significantly greater force than orchidectomized controls ($P < 0.05$; Fig. 5B) and displayed overall trends similar to those observed for SOL mass (Fig. 5A). These data show that the maximum force generated by both fast- and slow-twitch muscles is regulated by testosterone, with a dose-dependent relationship between force and testosterone levels.

To determine whether the observed testosterone-induced increments in EDL and SOL force were directly related to the muscle mass, we calculated $sP_o$ by expressing force in relation to muscle CSA (Table 2). $sP_o$ therefore provides a measure of muscle quality and identifies changes in intrinsic contractile properties of muscle beyond that accountable by mass (55). In both EDL and SOL muscles, $sP_o$ remained unchanged across all treatment groups. This indicates that intrinsic muscle contractile ability is not affected by androgen withdrawal or testosterone administration, and altered muscle force is predominantly due to changes in mass.

Androgens affect muscle fatigue in muscle type-specific manner. Muscle fatigue was measured as the percentage reduction of initial maximum force capacity after 4 min of successive electrical stimulation. Fast-twitch EDL muscle force of control shams was reduced to 18% of the initial maximum force following the fatigue protocol, and this reduction was not affected by androgen withdrawal or administration (Fig. 4C). As expected, the slow-twitch SOL muscle was more resistant to fatigue than the fast-twitch EDL muscle, with sham controls showing a reduction to 36% of maximum force capacity following the fatigue protocol (Fig. 5C). There was no significant difference in SOL muscle fatigue properties of orchidectomized control mice compared with sham controls. However, testosterone-treated orchidectomized mice had significantly enhanced resistance to fatigue compared with both orchidectomized and sham controls (Orx + T, $-12.4 \pm 0.8\%$ $P_o$/min vs. Orx + C, $-18.7 \pm 0.7\%$ $P_o$/min, $P < 0.001$; vs. Sham + C, $-16.4 \pm 0.9\%$ $P_o$/min, $P < 0.05$). These results suggest that testosterone treatment increases fatigue resistance in slow-twitch SOL muscle.

Androgens do not affect contraction speed of fast- or slow-twitch muscles. EDL and SOL muscle contraction speed was assessed by measurement of $1/2RT$ and TPT. There were no significant differences in $1/2RT$ or TPT between treatment groups for either EDL or SOL muscles (Table 2), indicating no effect of androgens on contraction kinetics.
Androgen-dependent changes in muscle mass correlate with changes in fiber size. To determine the structural changes underlying alterations in muscle mass and function, we analyzed EDL, SOL, and LA muscles for fiber CSA. Median LA muscle fiber CSA was decreased by 70% after orchidectomy in control mice compared with sham (P < 0.001) (Fig. 6A). Testosterone treatment in orchidectomized mice prevented LA fiber atrophy and maintained median fiber area at sham control levels, significantly greater than orchidectomized controls (P < 0.001). When LA fiber area was plotted against muscle mass for each mouse, there was a significant correlation between the androgen-dependent changes in median fiber area and changes in mean muscle mass (LA: r = 0.8, P < 0.001; Fig. 6A). In contrast to that for LA muscle, median fast-twitch EDL and slow-twitch SOL muscle CSA was not significantly different between treatment groups because of large variability between samples (Fig. 6, B and C, respectively). However, despite no significant difference in median fiber CSA between treatment groups for the EDL muscle, there was a significant correlation between androgen-induced changes in median fiber area and changes in mean muscle mass (EDL: r = 0.5, P < 0.05; Fig. 6B). These results demonstrate that the absence of androgens causes marked atrophy of muscle fibers in the highly androgen-sensitive LA muscle, and this effect is prevented with testosterone treatment. In addition, the strong correlation between LA fiber area and muscle mass suggests that changes in fiber size directly contribute to altered muscle mass. Given the variability in fiber CSA, particularly in the EDL and SOL muscles, the current study would have the power to detect only large changes in area, of ~60 and 35% for the EDL and SOL, respectively (power ≥ 0.8). Therefore, compared with the LA muscle, changes in EDL and SOL muscle fiber areas that contribute to changes in muscle mass may be too modest to detect using the current methodology.

DISCUSSION

In the present study, we have demonstrated a crucial role for testosterone in the preservation of skeletal muscle mass, structure, and contractile function in male mice, using a rigorous in vivo model of androgen withdrawal and administration. We
also observed an unexpected muscle type-specific effect of androgens on muscle fatigue.

The level of circulating androgens required to maintain muscle mass or promote anabolism in humans or animals is currently not known. Therefore, to be confident of the sensitivity of our model, it was essential to first demonstrate androgen effects on known responsive markers. In line with previous studies (38, 45, 57, 62), androgen withdrawal in our model resulted in a significant reduction in total body weight gain, seminal vesicle mass, and LA muscle mass compared with sham and testosterone-treated animals. There was a clear pattern of response observed for the mass of seminal vesicles and LA muscle across the treatment groups. Testosterone treatment prevented orchidectomy-induced atrophy of seminal vesicles and the LA muscle and increased seminal vesicle mass ~2.5-fold higher than that of the sham controls, confirming the consistent delivery of androgens via intraperitoneal implants over 10 wk, as previously reported (36). The efficacy of testosterone delivery by implants also was confirmed by serum testosterone analysis in orchidectomized mice. The experimental design of our protocol did not allow us to obtain blood for testosterone assays until the end of a 45-min surgical procedure, making it impossible to obtain physiologically meaningful measures of endogenous testosterone levels in control mice, given the known effects of stress and anesthesia on testosterone in mice (33, 41). Moreover, our group (40) and others (17)

![Fig. 6. Muscle fiber cross-sectional area (CSA) and the relationship with muscle mass of Orx and Sham mice after 10 wk of testosterone (T) or control (C) treatment. A: levator ani muscle. B: EDL muscle. C: SOL muscle. Box plots (left) indicate the median values with 25 and 75 percentiles (±SD) per treatment group (n = 3 per group). Scatter plots (right) indicate relationship between muscle mass and fiber CSA for mice from each treatment group. Sham+C; Orx+C; Sham+T; Orx+T. *P < 0.001 vs. Sham+C, Sham+T, and Orx+C (1-way ANOVA and Tukey’s post hoc test). The r value represents the Pearson correlation coefficient.]
have found testosterone levels to be highly variable in mice. Therefore, we used the mass of androgen-responsive tissues as a measure of the overall effect of the 10-wk androgen withdrawal/treatment regime. The fact that seminal vesicle mass was significantly increased in both testosterone-treated groups compared with sham controls strongly suggests that supraphysiological levels of testosterone were maintained over the 10-wk period. Together, these data confirm the androgen sensitivity of our model and show that orchidectomy-induced reduced body weight gain and tissue atrophy are prevented with continual testosterone administration via intraperitoneal implants.

Our study demonstrated a clear relationship between muscle mass and androgen levels. The mass of the fast-twitch EDL was lowest in the orchidectomized control animals and was 18% higher in the testosterone-treated orchidectomized mice and 25% higher in the testosterone-treated sham animals. Although the mass of the slow-twitch SOL muscle was not statistically significantly decreased in orchidectomized mice compared with sham control animals ($P = 0.09$), the mean mass was lowest in the orchidectomized control animals and was 27% higher in the testosterone-treated orchidectomized mice and 30% higher in the testosterone-treated sham animals. These data suggest the mass of fast-twitch muscles in adult male mice is more sensitive to androgen withdrawal than slow-twitch muscle mass, but the slow-twitch SOL mass may be more sensitive to androgen administration. Our findings are in agreement with an earlier study showing significant atrophy of the fast-twitch TA, biceps brachii, and EDL muscles, but not the slow-twitch SOL muscle 17 wk postorchidectomy in male mice (45). The molecular basis for the differential sensitivity of muscles to androgens is not completely understood. The density of the androgen receptor (AR) varies among different muscles (31), with greater numbers of AR-expressing myonuclei observed in the highly androgen-responsive LA muscle compared with the EDL (38), and this may explain intermuscular differences in androgen sensitivity. In addition, the basic differences between slow- and fast-twitch muscles, including their different contractile proteins (43), metabolic activity (44), suite of genes expressed (14), and intrinsic activity (28), may contribute to their differential androgen responses.

Supraphysiological testosterone levels in our model maintained mass of fast-twitch muscles from orchidectomized mice within the normal range. For the slow-twitch SOL muscle, there was no statistically significant difference in mass from sham and testosterone-treated sham mice. However, the fact that there was a significantly higher mass in the testosterone-treated sham mice compared with the orchidectomized group but no significant difference between sham control mice and orchidectomized mice suggests that the elevated androgen levels in the testosterone-treated sham mice elicited an anabolic response. It is possible that higher doses of testosterone in this model would result in statistically significant increases in muscle mass; however, this is beyond the scope of the current study.

Few reports exist that clearly demonstrate the effect of androgens on muscle contractile function in mice. In the current study we have unequivocally demonstrated that androgen-induced increases in muscle mass resulted in a proportional increase in maximum force. In contrast, it has been shown previously that continuous infusion of the testosterone derivative stanozolol did not alter muscle mass or contractile properties of EDL or SOL muscle of male sedentary mice after 3 or 6 wk of treatment, despite significant changes in androgen-responsive LA muscle mass (59). These conflicting data may reflect the different sensitivity of muscles to various androgen metabolites. Furthermore, treatment duration has been reported to be a critical factor in evaluating the effect of androgens on muscle, with a study demonstrating hypertrophy of rabbit TA muscle after 12 wk, but not 4 wk, of treatment with nandrolone decanoate (47). Similarly, we observed significant androgen-induced changes in muscle mass after 10 wk of testosterone treatment, but no changes were detected after 4 wk of treatment (data not shown).

The androgen effects on force displayed a similar pattern to that observed for muscle mass, because animals with higher androgen levels tended to have greater muscle force capacity than those with low androgen levels for both fast- and slow-twitch muscles. These results indicate that the androgen-induced effects on muscle force are tightly coupled to, and primarily caused by, changes in muscle mass. Our findings are in agreement with previous studies in humans, which show that androgen-induced changes in maximum force capacity are proportional to muscle mass and that specific force is not regulated by androgens (55). In contrast, a previous study showed that nandrolone decanoate increases specific force of the rabbit TA muscle after 12 wk of treatment (47). Furthermore, orchidectomy has been shown to reduce contractile force in both fast- and slow-twitch muscles of rats despite no change in muscle mass, thus representing a decline in specific force (13). It is possible that the shorter treatment period of 4 wk used by the latter study was not sufficient to induce significant muscle atrophy but may still have resulted in functional deficits due to contractile protein degradation.

Postnatal muscle growth is facilitated by fiber hypertrophy (15). In the present study, androgen-dependent changes in LA and EDL muscle mass were associated with changes in fiber size. Our data indicate that androgen-induced growth of the LA and EDL muscle is facilitated by muscle fiber hypertrophy and that loss of muscle mass following orchidectomy is due to fiber atrophy. In addition, the fact that increased muscle mass was accompanied by a parallel increase in maximum force suggests that androgen-induced increases in muscle mass arise through increased synthesis of contractile elements, rather than changes in noncontractile protein synthesis as suggested by previous reports (56).

Muscle fatigue is characterized by a decrease in force production and a slower contraction speed (65). Female muscles have been shown to be more fatigue resistant and to recover faster from exercise-induced fatigue than male muscles in human studies (22, 26). Surprisingly, our study demonstrated that the slow-twitch SOL muscle of testosterone-treated orchidectomized mice had increased resistance to muscle fatigue compared with sham and orchidectomized controls despite a trend of increased force production. A previous study has shown that rats treated with nandrolone decanoate had increased fatigue resistance of the fast-twitch plantaris muscle (56). Similarly, resistance to fatigue of the EDL muscles was improved in sedentary female rats after 5–6 wk of treatment with nandrolone phenylpropionate (18). In another study, EDL and SOL muscle fatigue indexes were not significantly altered.
after 3 or 6 wk of continuous infusion of stanozolol in male sedentary mice; however, postfatigue recovery of the soleus muscle was enhanced after 3 wk of treatment (59). Although the mechanism of androgen regulation of muscle fatigue remains unclear, androgens have been shown to modify mitochondrial size (48) and metabolic enzyme activity (34), indicating a possible role in oxidative metabolism. In addition, an effect of androgens on calcium flux and sarcoplasmic reticulum function has been previously identified (8), suggesting a site for androgen regulation of muscle function and fatigue. Our findings suggest a novel role for androgens in control of fatigue resistance of slow-twitch muscles in male mice.

The androgen-dependent effects on muscle mass and function observed in our model mostly likely arise from a combination of direct actions of androgens on skeletal muscle and indirect actions on other target tissues. Androgens act directly on myogenic cells (15, 52), and it is likely that a significant portion of the anabolic androgen action occurs directly in skeletal muscle. However, indirect actions contributing to changes in muscle mass and function could include modulation of the growth hormone/insulin-like growth factor-1 axis (61), actions on motor neurons (68), or effects arising due to androgen-dependent changes in spontaneous activity (12).

Although androgens act predominantly directly through the AR (35), some effects are also elicited via the estrogen receptor (ER) through aromatization of testosterone to estradiol (39). It is possible that the supraphysiological androgen levels observed in our model could lead to elevated estradiol levels, and androgen effects on muscle fatigue may be elicited via an ER-mediated pathway. A recent study using ERβ knockout mice demonstrated that muscle fatigue and subsequent recovery are regulated by an ER-mediated pathway (24). The increased fatigue resistance of SOL muscles following testosterone treatment in our study could potentially be due to increased aromatization to estrogen either locally or systemically. It is possible that a balance between androgen and estrogen levels may enhance muscle mass and strength while preserving resistance to fatigue. Further investigation is required to delineate between AR- and ER-mediated effects of androgens, possibly with the use of nonaromatizable agents such as 5α-dihydrotestosterone or nandrolone. In addition, the use of AR knockout mice (40) would be beneficial to identify the potential direct mechanisms of androgen action on skeletal muscle mass and function.

In summary, we have demonstrated that androgens are essential for the maintenance of fast- and slow-twitch muscle mass, structure, and strength and that supraphysiologically testedosterone administration to orchidectomized mice can prevent orchidectomy-associated muscle atrophy. Our results have shown that androgens alter muscle fatigue in a muscle type-specific manner. Rigorously characterized models such as the one described in this study are essential for the further investigation of the anabolic actions of androgens in muscle. Moreover, the use of genetically modified mice will further delineate key pathways, forming a crucial step in the development of targeted therapeutic strategies for treatment of muscle wasting disorders.

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