2-2015

Weekly Versus Monthly Testosterone Administration On Fast and Slow Skeletal Muscle Fibers in Older Adult Males

Robert Fitts
Marquette University, robert.fitts@marquette.edu

James R. Peters
Marquette University

E. Lichar Dillon
University of Texas Medical Branch at Galveston

William J. Durham
University of Texas Medical Branch at Galveston

Melinda Sheffield-Moore
University of Texas Medical Branch at Galveston

See next page for additional authors

Weekly Versus Monthly Testosterone Administration on Fast and Slow Skeletal Muscle Fibers in Older Adult Males

Robert H. Fitts
Department of Biological Sciences, Marquette University
Milwaukee, WI
James R. Peters
Department of Biological Sciences, Marquette University
Milwaukee, WI
E. Lichar Dillon
Department of Internal Medicine, University of Texas Medical Branch at Galveston
Galveston, TX
William J. Durham
Department of Internal Medicine, University of Texas Medical Branch at Galveston
Galveston, TX

Journal of Clinical Endocrinology and Metabolism, Vol 100, No. 2 (February 2015): pg. E223-E231. DOI.

Melinda Sheffield-Moore

Department of Internal Medicine, University of Texas Medical Branch at Galveston

Galveston, TX

Randall J. Urban

Department of Internal Medicine, University of Texas Medical Branch at Galveston

Galveston, TX

Abstract

Context: In older adults, loss of mobility due to sarcopenia is exacerbated in men with low serum T. T replacement therapy is known to increase muscle mass and strength, but the effect of weekly (WK) vs monthly (MO) administration on specific fiber types is unknown.

Objective: To determine the efficacy of WK vs MO T replacement on the size and functional capacity of individual fast and slow skeletal muscle fiber types.

Design, Setting, and Patients:

Subjects were randomized into a 5-month, double-blind, placebo-controlled trial. All subjects (ages, 61–71 y) were community-dwelling men who had T levels < 500 ng/dL.

Intervention: Subjects were dosed weekly for 5 months, receiving continuous T (WK, n = 5; 100 mg T enanthate, im injection), monthly cycled T (MO, n = 7; alternating months of T and placebo), or placebo (n = 7). Muscle biopsies of the vastus lateralis were obtained before and after treatment.

Main Outcome Measures: Main outcomes for individual slow and fast fibers included fiber diameter, peak force (P_0), rate of tension development, maximal shortening velocity, peak power, and Ca^{2+} sensitivity.

Results: Both treatments increased fiber diameter and peak power, with WK treatment 5-fold more effective than MO in increasing type I fiber P_0. WK effects on fiber diameter and force were 1.5-fold higher in slow fibers compared to fast fibers. In fast type II fibers, diameter and P_0 increased similarly between treatments. The increased power was entirely due to increased fiber size and force.

Conclusions: In conclusion, T replacement effects were fiber-type dependent, restricted to increases in cell size, P_0, and peak power, and dependent on the paradigm selected (WK vs MO).

Journal of Clinical Endocrinology and Metabolism, Vol 100, No. 2 (February 2015): pg. E223-E231. DOI.

The loss of muscle mass and strength with age can greatly limit the ability of older adults to maintain normal daily activities and physical function.[1,-4] This problem is exacerbated in hypogonadal men with low T.[5] T replacement therapy has been shown to protect against losses in muscle mass and strength by increasing muscle protein synthesis and decreasing protein degradation.[5,6] The gains observed with T treatment are both age- and dose-dependent, with greater effects typically seen in younger compared to older men.[5,7] Ferrando et al[6] found that chronic T administration increased lean body mass and strength in older men and, in the fasted state, stimulated reutilization of intracellular amino acids produced during protein breakdown. The observed decrease in protein breakdown in the T-treated group is important because an age-associated increase in skeletal muscle proteolysis is thought to contribute to the sarcopenia experienced by older adults.[8]

T administration, depending upon the dose and administration frequency, can be associated with adverse side effects including an increased hemoglobin and hematocrit and decreased high-density lipoprotein cholesterol.[9] Bhasin et al[9] studied the dose dependency of T therapy on gains in muscle strength and the severity of adverse effects and found both to increase with the dose. They concluded that the best trade-off was achieved with an intermediate T dose of 125 mg weekly injections.[9] Besides dose, the type of T administered and the duration of T administration may have implications on the functional responses in younger and older adults. The hormone-induced increase in the anabolic response may decrease with the duration of administration due to a return of the androgen receptor expression to pretreatment values.[10] To address both the adverse effects and diminishing anabolic response of prolonged T administration, Sheffield-Moore et al[11] studied a monthly cycled T regimen that used half the dose of the standard continuous therapy. They alternated months of T and placebo (PL) treatment for a 5-month period and compared the results with a group receiving standard continuous therapy. Despite declining serum T levels in the cycled months of PL injection, muscle strength, muscle protein synthesis, and lean body mass increased, and the percentage of body fat was reduced after 5 months of treatment in both groups. The rate of increase in lean body mass and extensor leg strength was less in the group receiving T in a cycled fashion, but both groups reached similar levels by 5 months.

Journal of Clinical Endocrinology and Metabolism, Vol 100, No. 2 (February 2015): pg. E223-E231. DOI.

To date, little is known about the specific effects of T replacement (either WK or MO) on the individual fast and slow fiber types of limb skeletal muscle. Sinha-Hikim et al[12] found significant increases in vastus lateralis volume and slow type I and fast type II fiber area, but only at serum T levels > 1000 ng/dL, which represent levels on the upper end of normal (or slightly above) and are much higher than seen in this study. Increases in leg and arm muscle strength suggest an improved fiber function, but the extent to which the adaptations are specific to a given fiber type are unknown. Additionally, the relative importance of changes in force and velocity in increasing peak power and whether or not there are changes in myofilament Ca^{2+} sensitivity that contribute to functional improvement are also unexplored. Thus, the goal of this study was to answer these questions using muscle biopsies obtained from the subject population previously reported on by Sheffield-Moore et al.[11]

Subjects and Methods

Ethical approval

This study was approved by the University of Texas Medical Branch (UTMB) and Marquette University Institutional Review Boards and complied with the Declaration of Helsinki. Written informed consent was obtained from all subjects.

Subjects

The subjects were a subset of a larger study of 24 individuals recruited by Sheffield-Moore et al.[11] From that subject population, we received muscle biopsies from 19 healthy, community-dwelling older men (ages, 60–85 y). In the case of the subjects not studied, there was insufficient sample size. All subjects had endogenous levels of serum total T in the lower half of the normal range (between 204 and 485 ng/dL). The recruitment process, preselection battery of tests, and exclusion criteria were exactly as described by Sheffield-Moore et al.[11]

Study design

Journal of Clinical Endocrinology and Metabolism, Vol 100, No. 2 (February 2015): pg. E223-E231. DOI.

The study design was as described previously and reviewed here.[11] We conducted a randomized, double-blinded, PL-controlled trial in older men to test whether T administered either continuously or cyclically improves contractile function of single slow and fast fibers isolated from the vastus lateralis muscle of older men. All men were randomized by the UTMB Investigational Drug Service pharmacist into the following groups: 1) weekly T (WK; n = 8; 100 mg T-enanthate im per week); 2) monthly T (MO; n = 8; alternating months of 100 mg T-enanthate and PL per week); or 3) PL (n = 8). Serum T levels were measured at the nadir before the next injection using the Bayer Advia Centaur immunoassay (Bayer Corp) as described in the Supplemental Data. The subset of subjects (ages, 61–71 y) for these data totaled 5, 7, and 7 for the WK, MO, and PL groups, respectively. Subjects were dosed weekly for 5 months by a research nurse at the UTMB Institute for Translational Science Clinical Research Center.[11]

Cell studies

Muscle biopsies

Before and at the end of the 5 months of T therapy, muscle biopsies (~100–200 mg) were taken from the vastus lateralis approximately 15–20 cm above the knee.[11] The portion used for the present experiment was immediately placed in cold skinning solution (composition in mm: 125 K propionate, 20 imidazole [pH 7.0], 2 EGTA, 4 ATP, and 1 $MgCl_2$, and 50% glycerol vol/vol), and shipped overnight at 4°C to Marquette University, where it was stored at −20°C. All of the fiber studies were conducted within 4 weeks of the biopsy.

Solutions and single-fiber isolation procedures

The relaxing (pCa 9.0, where pCa is the negative log of the Ca^{2+} concentration) and activating (pCa 4.5) solutions and fiber isolation procedure were as previously described.[13,–15] The total and free concentrations of each metal, ligand, and metal ligand complex in the solutions were calculated using an iterative computer program with stability constants adjusted for temperature, pH, and ionic strength.[16,17]

On the day of an experiment, a single fiber segment was isolated from the muscle bundle, submerged in relaxing solution, and mounted between a force transducer and a position motor as previously described.[13,14] The fiber was viewed at ×800, sarcomere length was adjusted to 2.5 μm, and fiber length (FL) and diameter were determined.[15] Fiber cross-sectional area (CSA) was calculated from the diameter.[18]

Determination of peak force (P_0), rate of tension development (k_{tr}), maximal unloaded shortening velocity (V_0), and force-velocity relationship

Fibers were maximally activated in pCa 4.5 solution (15°C), and peak force (P_0) was recorded. Fiber V_0 (FL s^{-1}) was measured by the slack test.[14]

In slow fibers, we determined the rate constant for force redevelopment (k_{tr}) by generating a rapid slack (20% of FL) and re-extension of a fully activated fiber.[19,20] The redevelopment of tension was fit by a single exponential, and k_{tr} was determined.

The force-velocity relationship was determined from a series of isotonic releases as described previously.[13] V_{max} was defined as the intercept of the force-velocity curve with the velocity axis, and peak absolute power was calculated from P_0, V_{max}, and a/P_0 where the latter parameter specifies the curvature of the force-velocity relation.[13] For graphical purposes, composite force-velocity-power relationships were plotted using the mean parameters.[13]

Determination of the pCa-force relationship and fiber stiffness

A separate group of fibers was used to determine the pCa-force relationship, which reflects the myofibril Ca^{2+} sensitivity. These experiments were conducted exactly as described by Widrick et al.[13] Hill plots were used to determine the minimal Ca^{2+} required for force development (activation threshold) and that required for 50% activation (pCa_{50}).[13] To determine fiber stiffness, fibers were vibrated first in relaxing solution (pCa 9) and then at the peak of the pCa 4.5 activation with a sinusoidal length change of 0.05% of FL and 1.5

Journal of Clinical Endocrinology and Metabolism, Vol 100, No. 2 (February 2015): pg. E223-E231. DOI.

kHz.[15] Changes in length (Δlength) and force (Δforce) were used to calculate peak elastic modulus (E_0) as follows: {E_0 = [Δforce in activating solution − Δforce in relaxing solution/(Δlength)] (FL/CSA)}.

Fiber type identification

After the contractile measurements, fibers were solubilized in 10 μL of 1% SDS sample buffer and stored at −80°C. Fibers were identified as fast type II or slow type I based on their myosin heavy chain isoform determined from 5% PAGE.[14,21]

Statistical analysis

Pre- and post-treatment means were analyzed using a two-tailed *t* test. Statistical significance was accepted at $P < .05$.

Results

Cell diameter and P_0

The effects of WK and MO T therapy on fiber diameter, P_0, and V_0 for slow type I and fast type II fibers are shown in Table 1. Before treatment, the MO group had significantly smaller fiber diameters for both slow type I and fast type II fiber than the PL group. The WK and MO treatments had differential effects, with WK significantly increasing type I fiber diameter and MO significantly increasing type II fiber diameter (Table 1). Although not significant, there was a trend ($P < .1$) for MO to increase type I fiber diameter. The PL group showed a decreased type II fiber diameter, which resulted in an increased specific force (Table 1).

Table 1. Type I and II Fiber Diameter, P_0, and V_0 With Weekly and Monthly T

VL Muscle		Diameter, μm	P_0, mN	P_0, kN/m^2	V_0, FL/s
Type I					
PL					
	Pre	101 ± 2 (54)	0.89 ± 0.04 (53)	111 ± 3 (53)	0.71 ± 0.04 (53)
	Post	99 ± 2 (75)	0.88 ± 0.04 (71)	112 ± 3 (71)	0.66 ± 0.02 (73)
	% Difference	−3	−1	1	−7
Weekly					
	Pre	102 ± 2 (60)	0.87 ± 0.03 (58)	106 ± 2 (58)	0.66 ± 0.03 (58)
	Post	118 ± 4 (48)[a]	1.25 ± 0.08 (48)[a]	111 ± 2 (48)	0.64 ± 0.02 (48)

Journal of Clinical Endocrinology and Metabolism, Vol 100, No. 2 (February 2015): pg. E223-E231. DOI.

VL Muscle			Diameter, μm	P_0, mN	P_0, kN/m^2	V_0, FL/s
		% Difference	16	44	4	−3
	Monthly					
		Pre	90 ± 2 (70)[b]	0.76 ± 0.02 (70)[b]	118 ± 2 (70)	0.66 ± 0.02 (70)
		Post	94 ± 2 (66)[c]	0.82 ± 0.03 (65)[a]	116 ± 2 (65)	0.68 ± 0.02 (65)
		% Difference	5	8	−2	3
Type II						
	PL					
		Pre	96 ± 2 (71)	0.89 ± 0.04 (67)	128 ± 4 (67)	2.96 ± 0.14 (66)
		Post	86 ± 2 (60)[a]	0.83 ± 0.03 (60)	143 ± 4 (60)[a]	2.72 ± 0.21 (59)
		% Difference	−10	−7	11	−8
	Weekly					
		Pre	96 ± 3 (22)	0.98 ± 0.07 (21)	130 ± 5 (21)	2.79 ± 0.25 (21)
		Post	106 ± 4 (44)	1.26 ± 0.09 (44)[a]	141 ± 3 (44)[a]	2.47 ± 0.11 (44)
		% Difference	10	29	9	−11
	Monthly					
		Pre	85 ± 2 (49)[b]	0.81 ± 0.03 (46)	144 ± 3 (46)[b]	2.44 ± 0.08 (46)[b]
		Post	93 ± 2 (39)[a]	0.99 ± 0.03 (37)[a]	144 ± 3 (37)	2.51 ± 0.14 (37)
		% Difference	9	22	0	3

Abbreviation: VL, vastus lateralis muscle. Values represent means ± SE with the number of fibers studied in parentheses. The weekly group received T weekly for 5 months; the monthly group received T weekly during months 1, 3, and 5.
[a]Significant difference ($P < .05$) between pre and post mean for a given group.
[b]Significant difference ($P < .05$) between PL pre mean and treatment group pre mean.
[c]Significant difference ($P < .10$) between pre and post mean for a given group.

For the type I fiber, both treatments increased the absolute P_0 (millinewtons [mN]), but the magnitude of the effect was considerably greater with WK (44% increase) vs MO (8% increase) (Table 1). The increased type I fiber force appears primarily to be due to the increase in fiber size because the specific force (kilonewtons [kN]/m^2) was not altered by either treatment. Similar results were observed for the type II fiber, except the percentage increase in absolute P_0 was of the same magnitude with both treatments, and specific force significantly increased with WK (Table 1).

V_0, k_{tr}, and peak power

For both fiber types, the V_0 was unaltered by WK or MO (Table 1), and the type I fiber k_{tr} was also unaffected by the treatments, with mean values ranging from 1.25 ± 0.03 to 1.29 ± 0.03^{-s} for the three groups. Although there was no change in V_0, type I fiber peak power significantly increased in the WK and MO groups (Figure 1 and

Supplemental Table 1), and like force, the increase was greater with WK (41 vs 13%). For the type II fiber, there was a trend ($P < .1$) for both treatments to increase peak power (Figure 1 and Supplemental Table 1). The curvature of the force-velocity relationship was unaltered by the treatments, except for type II fibers where WK significantly ($P < .05$) reduced the curvature as reflected by an increased a/P_0 ratio (0.072 ± 0.004 vs 0.081 ± 0.004). There were no changes in relative peak power (Supplemental Table 1), suggesting that the increase in absolute peak power is primarily due to a T-induced increase in fiber size. Figure 1 shows that power increased with both treatment paradigms across the entire force-power spectrum from very light to heavy loads, and across this range the increase was greater with WK compared to MO.

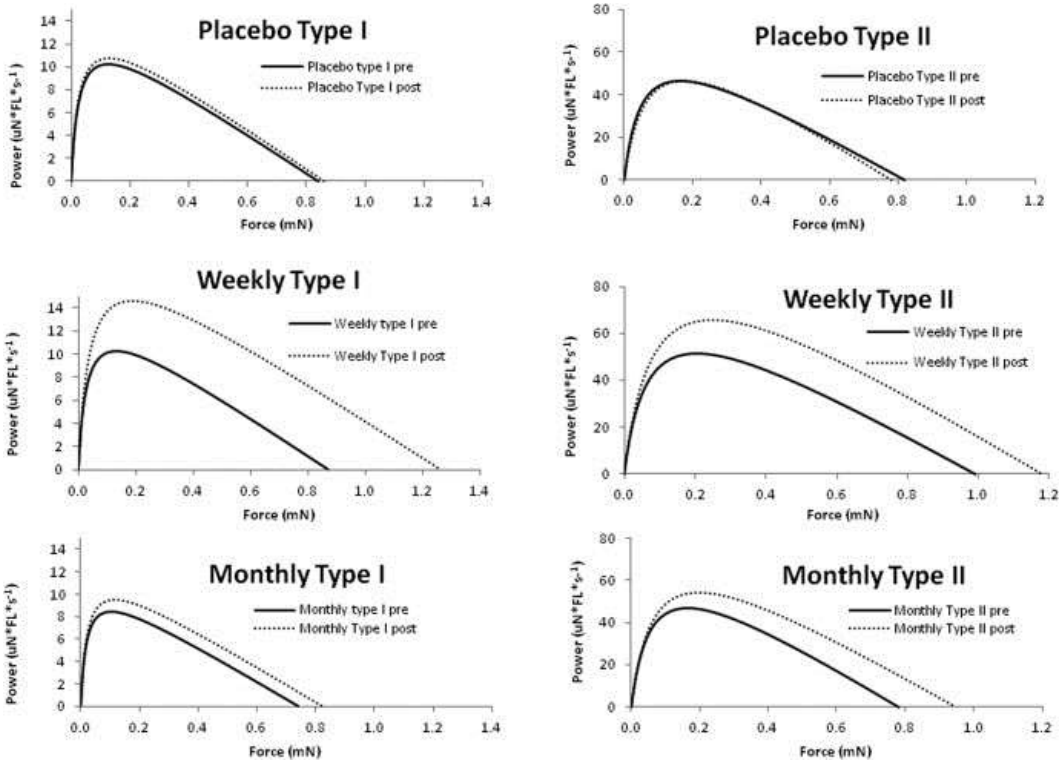


Figure 1. Force-power relationships for PL and treatment groups. Continuous lines represent the pretreatment and dashed lines represent the post-treatment force-power relationships for type I and type II fibers for PL and weekly and monthly treatment groups.

Journal of Clinical Endocrinology and Metabolism, Vol 100, No. 2 (February 2015): pg. E223-E231. DOI.

E_0 and the P_0/E_0 ratio

The E_0, a reflection of the number of attached cross-bridges per CSA, was not significantly altered by either paradigm in type I (Figure 2A) or type II fibers. For both groups combined, the mean stiffness (E_0) was 2.19 ± 0.04 (n = 160) and 1.83 ± 0.04 (n = 44) for type I and II fibers, respectively. The lack of change in E_0 with treatment suggests that neither WK nor MO affected the number of cross-bridges per CSA. The P_0/E_0 ratio, an indicator of the force per cross-bridge, was also unaltered except for the type I fibers where WK significantly

Journal of Clinical Endocrinology and Metabolism, Vol 100, No. 2 (February 2015): pg. E223-E231. DOI.

increased the ratio (Figure 2B).

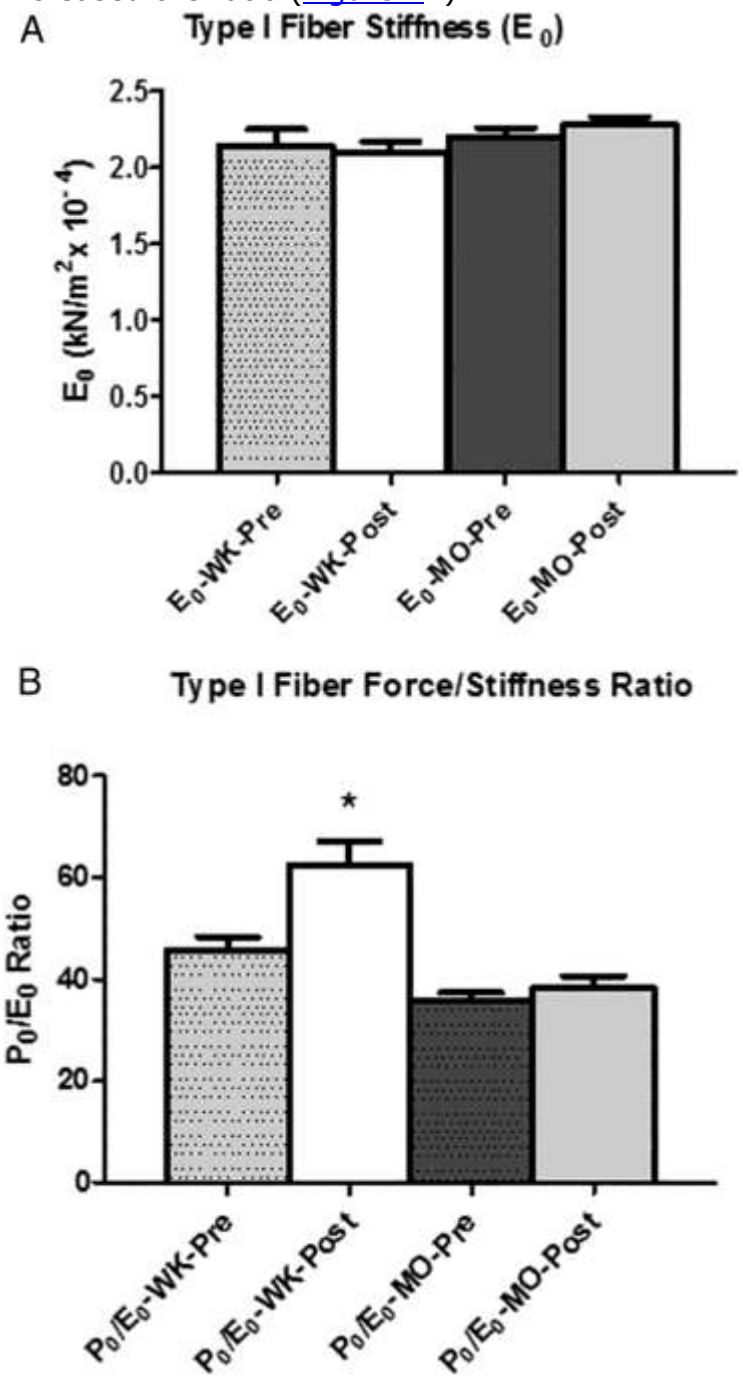


Figure 2. Stiffness and force stiffness ratio. A, Mean type I fiber stiffness before (E_0-WK-Pre) and after (E_0-WK-Post) weekly T treatment, and before (E_0-MO-Pre) and after (E_0-MO-Post) monthly T treatment. B, Mean force/stiffness ratio before (P_0/E_0-WK-Pre) and after (P_0/E_0-WK-Post) weekly T treatment, and before (P_0/E0-MO-Pre) and after (P_0/E_0-MO-Post) monthly T treatment. *, Significant difference ($P < .05$) between pre and post mean for a given group.

Journal of Clinical Endocrinology and Metabolism, Vol 100, No. 2 (February 2015): pg. E223-E231. DOI.

Calcium sensitivity

The WK and MO treatments had no effect on calcium sensitivity of the contractile filaments, as reflected by no change in the Ca^{2+} required to initiate contraction (activation threshold) or that needed for one-half maximal activation (pCa_{50}). Additionally, the slope of the force-pCa relationship (Hill plot) below (N_2) and above (N_1) one-half maximal activation was unaltered (Supplemental Table 2).

Comparison of T administration to fiber size and force outcomes

From Table 1, it is clear that the primary effect of T therapy administered either continuously or in a cycled fashion was to increase fiber size and absolute force. To further assess this aspect, we graphed the relationship between fiber diameter and peak absolute force for each individual for type I (Figures 3 and 4) and type II (Supplemental Figures 1 and 2) fibers. As is often seen in the clinical realm, some men respond to a given dose and others do not, thus requiring dose adjustment to manage symptoms. As such, in this study, where dose adjustment was not performed as done clinically, certain men responded to their administration paradigm (WK or MO), and others did not. If subjects showed a significant increase in fiber force in both slow and fast fibers, they were classified as responders (Tables 2 and 3, Figures 3 and 4 and Supplemental Figures 1 and 2). With WK therapy, subjects WK 1 and WK 2 both showed an increase in type I and type II fiber diameter and force (post-treatment fibers all shifted to the right and up on the plot; Figure 3 and Supplemental Figure 1). WK 3 was a responder who showed an increased force with no change in fiber diameter (Figure 3). With MO treatment, subjects MO 1 and MO 2 (Figure 4 and Supplemental Figure 2) responded similarly to subjects WK 1 and WK 2. Also, with MO therapy we observed two subjects (MO 3 and MO 4) who were partial responders showing significant increases in fast type II fiber P_0 but no change in the slow type I fiber type.

Journal of Clinical Endocrinology and Metabolism, Vol 100, No. 2 (February 2015): pg. E223-E231. DOI.

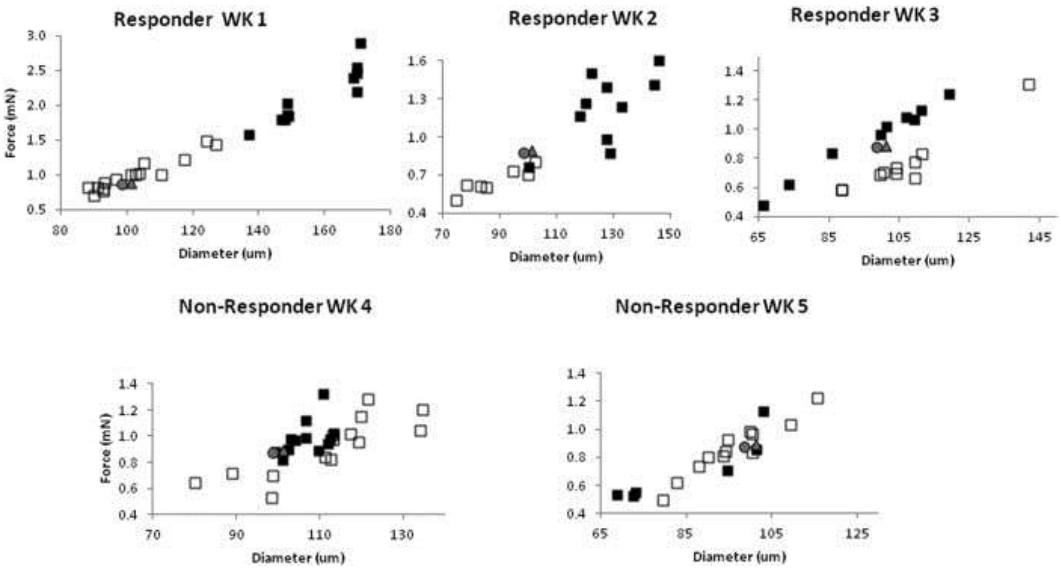


Figure 3. Relationship between type I fiber diameter and peak Ca^{2+}-activated force (P_0) for weekly T (WK) treatment. Each symbol represents the results of a single vastus lateralis type I fiber for each subject where open squares are pretreatment fibers and filled squares are post-treatment fibers. The gray shaded triangle and gray shaded circle are the mean pre- and post-treatment values for the PL group, respectively.

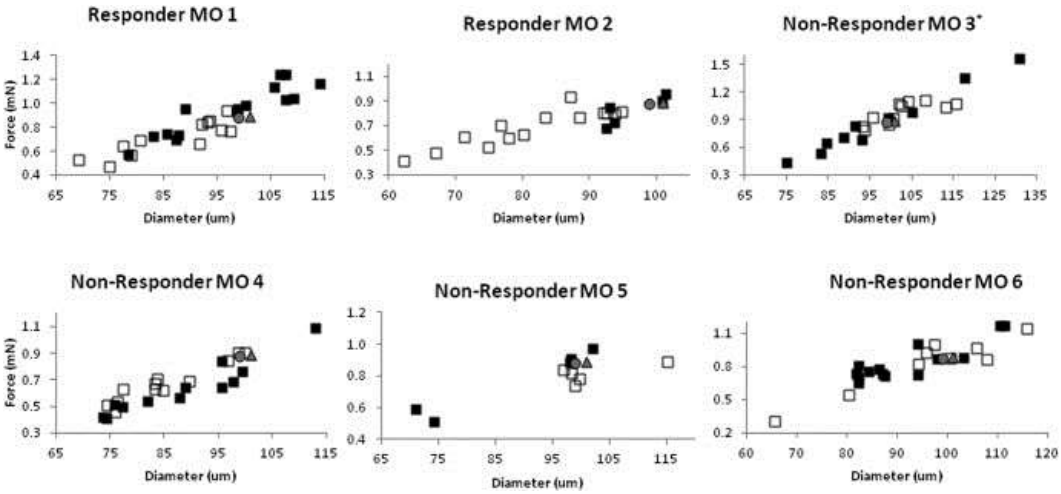


Figure 4. Relationship between type I fiber diameter and peak Ca^{2+}-activated force (P_0) for monthly T (MO) treatment. Each symbol represents the results of a single vastus lateralis type I fiber for each subject where open squares are pretreatment fibers and filled squares are post-treatment fibers. The gray shaded triangle and gray shaded circle are the mean pre- and post-treatment values for the PL group, respectively. The asterisk indicates that subject MO 3, although a nonresponder for the slow type I fiber, was a responder for the fast type II fiber.

Journal of Clinical Endocrinology and Metabolism, Vol 100, No. 2 (February 2015): pg. E223-E231. DOI.

Table 2. Age, Leg Strength, and Plasma T for PL, MO, and WK Groups

Groups	Age, y	Leg Extension Strength, kg		Leg Curl Strength, kg		T, ng/dL	
		Pre	Post	Pre	Post	Pre	Post
PL	65 ± 3	70 ± 21	74 ± 13	46 ± 6	48 ± 7	344 ± 85	343 ± 73
Monthly (MO)	72 ± 8	73 ± 15	85 ± 19	44 ± 7	50 ± 11[a]	357 ± 103	665 ± 25[a,+]
Weekly (WK)	73 ± 8	66 ± 16	83 ± 17[a]	40 ± 9	51 ± 12[a]	341 ± 85	659 ± 282[a,+]

Values are expressed as means ± SD. WK group received 100 mg T-enanthate weekly throughout the 5-month study; MO group received 100 mg T-enanthate weekly in alternating months.
[a]Post value significantly different from pre value, $P \leq .05$.
[+]Post value significantly different from PL post value, $P < .05$.

Table 3. Leg Strength, Type I Fiber P_0, and Plasma T of Individual Subjects

Individuals	Leg Extension Strength, kg		Type I Fiber P_0, mN		T, ng/dL	
	Pre	Post	Pre	Post	Pre Ave 1–5 Mo	Highest
WK 1 (Responder)	79.4	86.2	1.01	2.12[a]	261 503	553
WK 2 (Responder)	41.2	60.8	0.65	1.22[a]	204 613	818
WK 3 (Responder)	72.6	81.6	0.76	0.93[a]	356 595	782
WK 4 (NonRes)	77.1	86.2	0.92	0.98	401 894	952
WK 5 (NonRes)	74.8	97.5	0.86	0.71	280 386	492
MO 1 (Responder)	77.1	81.6	0.71	0.94[a]	277 365	584
MO 2 (Responder)	74.8	72.6	0.69	0.96[a]	485 473	669
MO 3 (PartRes)	54.4	61.2	0.98	0.86	260 674	985
MO 4 (PartRes)	77.1	-	0.68	0.63	275 460	682
MO 5 (NonRes)	104.3	113.4	0.81	0.77	409 459	598
MO 6 (NonRes)	61.2	93.0	0.83	0.83	456 500	642
MO 7 (NonRes)	68.0	68.0	0.62	0.64	241 384	617
PL 1	90.7	74.8	1.03	1.05	379 382	463
PL 2	56.7	63.5	-	0.77	255 253	318
PL 3	113.4	95.2	0.92	1.24[a]	428 388	527
PL 4	59.0	59.0	1.01	0.73[a]	444 387	444
PL 5	68.0	81.6	1.08	0.63[a]	417 423	470
PL 6	63.5	68.0	0.72	0.65	270 275	331
PL 7	59.0	81.6	0.56	0.48	226 206	226

Journal of Clinical Endocrinology and Metabolism, Vol 100, No. 2 (February 2015): pg. E223-E231. DOI.

Abbreviations: NonRes, non-responder; PartRes, partial responder to T treatments; WK, received 100 mg T-enanthate weekly throughout the 5-month study; MO, received 100 mg T-enanthate weekly in alternating months; Ave 1–5 Mo, the mean T over the study calculated from each months' T.
[a]Post value significantly different from pre value, $P \leq .05$.

Table 2 shows the average leg strength (extension and flexion) as well as serum T levels before and after T therapy for all 24 subjects. Before treatment, all subjects participating in this study (7 PL, 5 WK, and 7 MO) had circulating total T concentrations in the lower half of the normal range (333 ± 21 ng/dL), and the mean values stayed low in the PL group (284 ± 24 ng/dL) and cycled up and down in the MO group with a 5-month value of 474 ± 38 ng/dL, whereas in the WK group, T increased in the first month and remained high with 598 ± 84 ng/dL at 5 months. Individual changes in T for the WK, MO, and PL subjects are shown in Table 2. The subjects with the greatest cellular response in the WK group started with low serum T that increased with treatment to above 500 ng/dL for both the average and peak values (subjects WK 1 and WK 2; Table 2). Non-responders either failed to show an increase in serum T above 500 ng/dL (subject WK 5) or had relatively high pretreatment T levels (subject WK 4).

Discussion

The use of T to treat adult males with serum T levels in the lower half of the normal range has been shown to improve muscle strength, but the ideal treatment paradigm and the selective effects on fast and slow fiber types are unknown.[10,–12] A novel finding of this study is that P_0 and peak power of the slow type I and fast type II fibers significantly increase in response to both continuous weekly administration of T for 5 months (WK) and monthly administration (MO) where T is administered weekly in months 1, 3, and 5 with PL injections in months 2 and 4.

Fiber type differences with T therapy were observed with the WK paradigm to be 5-fold more effective than MO in increasing the slow type I fiber P_0, whereas with the fast type II fiber P_0 increases were similar between treatments. The higher sensitivity of the type I fiber to T therapy agrees with the observation of Sinha-Hikim et al,[12] who reported type I fiber area to increase in response to T supplements at levels less than that required for type II fibers.

Journal of Clinical Endocrinology and Metabolism, Vol 100, No. 2 (February 2015): pg. E223-E231. DOI.

Interestingly, subjects that responded well to WK treatment showed significant increases in fiber diameter and P_0 in slow and fast fibers, whereas with the MO paradigm, in addition to responders with adaptations in both fiber types, we observed partial responders with significant functional changes in fast but not slow fibers. This likely resulted from the lower response of slow fibers to MO treatment (Table 1). Our results demonstrate that slow fibers respond better to a continuous cellular exposure to T, whereas with fast fibers a threshold level of T is required, but not a continuous exposure. The increased effectiveness of WK over MO treatment for the type I fiber might not have been due to the pattern of administration, but rather to the different cumulative T dosage over the 5 months. An explanation for this fiber type difference is not readily apparent, but it might result from differences in receptor sensitivity and/or half-life of the cellular responses to T.[10] To our knowledge, fiber type differences in T receptor sensitivity or response to hormone have not been studied.

Our data suggest that the cellular improvements in P_0 and peak power with T replacement were largely dependent upon the increase in fiber size. Thus, for the most part (an exception being the effect of WK on fast fibers, and for subject WK 3 both fiber types), there was no increase in relative force (kN/m^2). As expected, the PL group, with the exception of a reduced type II fiber diameter and specific force, showed no change in slow or fast fiber function. The fast fiber-specific force increased due to the fiber atrophy. Although the cause of the fiber atrophy in the PL group is unknown, it could be a result of changes with aging over the 5-month period.[22] Fiber stiffness (E_0), an indicator of the number of cross-bridges, did not change with either WK or MO treatment. However, the P_0/E_0 ratio did significantly increase with WK in the slow type I fiber, which suggests an increase in the force per cross-bridge in this fiber. This adaptation should result in an increased relative force that increased, but not significantly. Thus, we conclude that any increase in force per cross-bridge with T treatment was relatively unimportant compared to the force increase due to the larger fiber size.

Importantly, there were no changes in any kinetic parameter because neither V_0 nor the k_{tr} was altered by T replacement. The Ca^{2+} sensitivity of activation, as reflected by the activation threshold, pCa_{50}, and slope of the Ca^{2+}-force relationship (Supplemental Table 2), was

Journal of Clinical Endocrinology and Metabolism, Vol 100, No. 2 (February 2015): pg. E223-E231. DOI.

unaltered as well. Additionally, there was no change in the curvature of the force-velocity relationship (a/P_0 ratio) that could have increased peak power. These results suggest that the T therapy did not alter the kinetics of the cross-bridge cycle and that the observed increases in power (Figure 1) can be entirely attributed to the increased force.

An explanation for individual differences in response to T replacement therapy is not known and may involve factors not studied here. One possibility is that the initial serum T level and the extent of its increase with therapy are important to whether or not therapy produces significant cell responses. Although our data support this hypothesis (Table 2), future studies powered to address this question are needed. The variability in subject response to T therapy could also result from monthly variations in serum T levels achieved in response to the 100-mg monthly dose. This seems unlikely because for all of the WK subjects there was very little variation around their 1- to 5-month average.[11] Because there were no data on T therapy effects on single fibers, the study was powered based on the known treatment effects on leg extension strength. Significant increases with treatment in some of the subjects (responders) suggest that the study was adequately powered. Nonetheless, the possibility exists that we may have observed more responders if the number of observations per subject was increased.

Although it is not clinically practical to base a T therapeutic regimen on the fiber type distribution of the individual, we believe these data suggest that similar whole-muscle functional benefits occur in response to cycled and standard continuous T administration by virtue of the similarity in the cellular-level responses observed in type II fibers. Older men generally have significant muscle atrophy with selective loss of the fast type II fiber,[22] which is considerably more powerful than type I fibers. To prevent large losses in peak power with aging, it is important to reduce atrophy and loss of function in the type II fiber. In that regard, the MO paradigm is equally as effective as the WK paradigm with one-half the dose of T.

In conclusion, our work demonstrates for the first time that the improvements in skeletal muscle function in older men after T replacement therapy are fiber-type dependent and restricted to increases in cell size, P_0, and peak power, and that the degree of

Journal of Clinical Endocrinology and Metabolism, Vol 100, No. 2 (February 2015): pg. E223-E231. DOI.

change in each fiber type depends on the treatment paradigm selected weekly vs monthly. Specifically, we found that monthly T administration increased type II fiber function equally to weekly administration. Because type II fibers show the greatest atrophy and are most often lost with aging, the monthly paradigm of T administration affords equal gains in fiber power with half the dose of T as weekly administration. Overall, these results suggest that improvements in single fiber function in skeletal muscle are possible with both standard weekly T administration and a monthly paradigm. Future studies are needed to determine the mechanisms involved, such as the role of IGF-1, in mediating the functional changes in slow and fast fiber function.

Acknowledgments

The authors acknowledge the help of Patti Colloton, in the analysis of the data and figure development; and Cassie Nelson, in careful reading of an earlier version of this manuscript. We also acknowledge the help of Charlie Gilkison for helping manage the study patients.

Author Contributions: R.H.F. conducted the cell experiments, analyzed the data, and wrote the manuscript. J.R.P. conducted the cell experiments and assisted with data analysis. M.S.-M., E.L.D., and W.J.D. recruited and managed the subjects, conducted in vivo muscle analyses and the muscle biopsies, and revised the manuscript. The conception, design, and interpretation of the experiments were done by R.J.U., M.S.-M., and R.H.F.

This study was supported by National Institutes of Health Grants AG022023 (to R.J.U.) and CA127971 (to M.S.-M.) and the Moody Endowment (to R.J.U.). This study was conducted with the support of the Institute for Translational Sciences at the University of Texas Medical Branch in Galveston, supported in part by a Clinical and Translational Science Award (UL1TR0000071) from the National Center for Advancing Translational Sciences, National Institutes of Health.

Disclosure Summary: The authors have nothing to disclose.

Footnotes

Abbreviations:

CSA cross-sectional area
E_0 peak elastic modulus
FL fiber length
kN kilonewton
k_{tr} rate of tension development
mN millinewton
MO monthly
P_0 peak force
pCa negative log of the Ca^{2+} concentration
PL placebo
V_0 maximal unloaded shortening velocity
WK weekly.

References

[1] Brown M, Sinacore DR, Host HH. The relationship of strength to function in the older adult. *J Gerontol A Biol Sci Med Sci*. 1995;50(Spec no):55–59.

[2] Frontera WR, Hughes VA, Krivickas LS, Kim SK, Foldvari M, Roubenoff R. Strength training in older women: Early and late changes in whole muscle and single cells. *Muscle Nerve*. 2003;28(5):601–608.

[3] Kent-Braun JA, Ng AV, Doyle JW, Towse TF. Human skeletal muscle responses vary with age and gender during fatigue due to incremental isometric exercise. *J Appl Physiol*. 2002;93(5):1813–1823.

[4] Janssen I, Heymsfield SB, Wang ZM, Ross R. Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr. *J Appl Physiol.* 2000;89(1):81–88.

[5] Brodsky IG, Balagopal P, Nair KS. Effects of testosterone replacement on muscle mass and muscle protein synthesis in hypogonadal men–a clinical research center study. *J Clin Endocrinol Metab*. 1996;81(10):3469–3475.

[6] Ferrando AA, Sheffield-Moore M, Paddon-Jones D, Wolfe RR, Urban RJ. Differential anabolic effects of testosterone and amino acid feeding in older men. *J Clin Endocrinol Metab*. 2003;88(1):358–362.

[7] Tenover JS. Effects of testosterone supplementation in the aging male. *J Clin Endocrinol Metab*. 1992;75(4):1092–1098.

[8] Yarasheski KE. Exercise, aging, and muscle protein metabolism. *J Gerontol A Biol Sci Med Sci.* 2003;58(10):M918–M922.

Journal of Clinical Endocrinology and Metabolism, Vol 100, No. 2 (February 2015): pg. E223-E231. DOI.

[9] Bhasin S, Woodhouse L, Casaburi R, et al. Older men are as responsive as young men to the anabolic effects of graded doses of testosterone on the skeletal muscle. *J Clin Endocrinol Metab*. 2005;90(2):678–688.

[10] Ferrando AA, Sheffield-Moore M, Yeckel CW, et al. Testosterone administration to older men improves muscle function: molecular and physiological mechanisms. *Am J Physiol Endocrinol Metab*. 2002;282(3):E601–E607.

[11] Sheffield-Moore M, Dillon EL, Casperson SL, et al. A randomized pilot study of monthly cycled testosterone replacement or continuous testosterone replacement versus placebo in older men. *J Clin Endocrinol Metab*. 2011;96(11):E1831–E1837.

[12] Sinha-Hikim I, Artaza J, Woodhouse L, et al. Testosterone-induced increase in muscle size in healthy young men is associated with muscle fiber hypertrophy. *Am J Physiol Endocrinol Metab*. 2002;283(1):E154–E164.

[13] Widrick JJ, Norenberg KM, Romatowski JG, et al. Force-velocity-power and force-pCa relationships of human soleus fibers after 17 days of bed rest. *J Appl Physiol*. 1998;85(5):1949–1956.

[14] Widrick JJ, Romatowski JG, Bain JL, et al. Effect of 17 days of bed rest on peak isometric force and unloaded shortening velocity of human soleus fibers. *Am J Physiol*. 1997;273:C1690–C1699.

[15] Fitts RH, Romatowski JG, Peters JR, Paddon-Jones D, Wolfe RR, Ferrando AA. The deleterious effects of bed rest on human skeletal muscle fibers are exacerbated by hypercortisolemia and ameliorated by dietary supplementation. *Am J Physiol Cell Physiol*. 2007;293(1):C313–C320.

[16] Godt RE, Lindley BD. Influence of temperature upon contractile activation and isometric force production in mechanically skinned muscle fibers of the frog. *J Gen Physiol*. 1982;80(2):279–297.

[17] Fabiato A, Fabiato F. Calculator programs for computing the composition of the solutions containing multiple metals and ligands used for experiments in skinned muscle cells. *J Physiol (Paris).* 1979;75(5):463–505.

[18] Metzger JM, Moss RL. Shortening velocity in skinned single muscle fibers. Influence of filament lattice spacing. *Biophys J.* 1987;52(1):127–131.

[19] Metzger JM, Moss RL. pH modulation of the kinetics of a Ca2(+)-sensitive cross-bridge state transition in mammalian single skeletal muscle fibres. *J Physiol*. 1990;428:751–764.

[20] Fitzsimons DP, Patel JR, Campbell KS, Moss RL. Cooperative mechanisms in the activation dependence of the rate of force development in rabbit skinned skeletal muscle fibers. *J Gen Physiol*. 2001;117(2):133–148.

[21] Nelson CR, Fitts RH. Effects of low cell pH and elevated inorganic phosphate on the pCa-force relationship in single muscle fibers at near-physiological temperatures. *Am J Physiol Cell Physiol*. 2014;306(7):C670–C678.

Journal of Clinical Endocrinology and Metabolism, Vol 100, No. 2 (February 2015): pg. E223-E231. DOI.

[22] Fitts RH. Effects of regular exercise training on skeletal muscle contractile function. *Am J Phys Med Rehabil*. 2003;82(4):320–331.

Corresponding author.
Address all correspondence and requests for reprints to: Robert H. Fitts, Department of Biological Sciences, Marquette University, 530 North 15th Street, PO Box 1881, Milwaukee, WI 53233. E-mail: Robert.fitts@marquette.edu

Journal of Clinical Endocrinology and Metabolism, Vol 100, No. 2 (February 2015): pg. E223-E231. DOI.

Supplemental Methods

Testosterone assay. Serum testosterone was determined using The ADVIA Centaur Testosterone assay exactly as described by the manufacturer().It is a competitive immunoassay using direct chemiluminescent technology. Testosterone in the patient sample competes with acridinium ester- labeled testosterone in the Lite Reagent for a limited amount of polyclonal rabbit anti testosterone antibody bound to monoclonal mouse anti-rabbit antibody, which is coupled to paramagnetic particles in the solid phase. The assay uses testosterone releasing agent to release bound testosterone from the endogenous binding proteins in the sample. The system automatically performs the following steps: Dispenses 15 µL of sample and 50 µL of releasing agent into a cuvette; Washes the reagent probe with 100 µL of probe wash; Dispenses 50 µL of Lite reagent and 300 µL of solid phase and incubates for 5.0 minutes at 37°C; Separates, aspirates, and washes the cuvettes with reagent water; Dispenses 300 µL each of acid reagent and base reagent to initiate the chemiluminescent reaction.

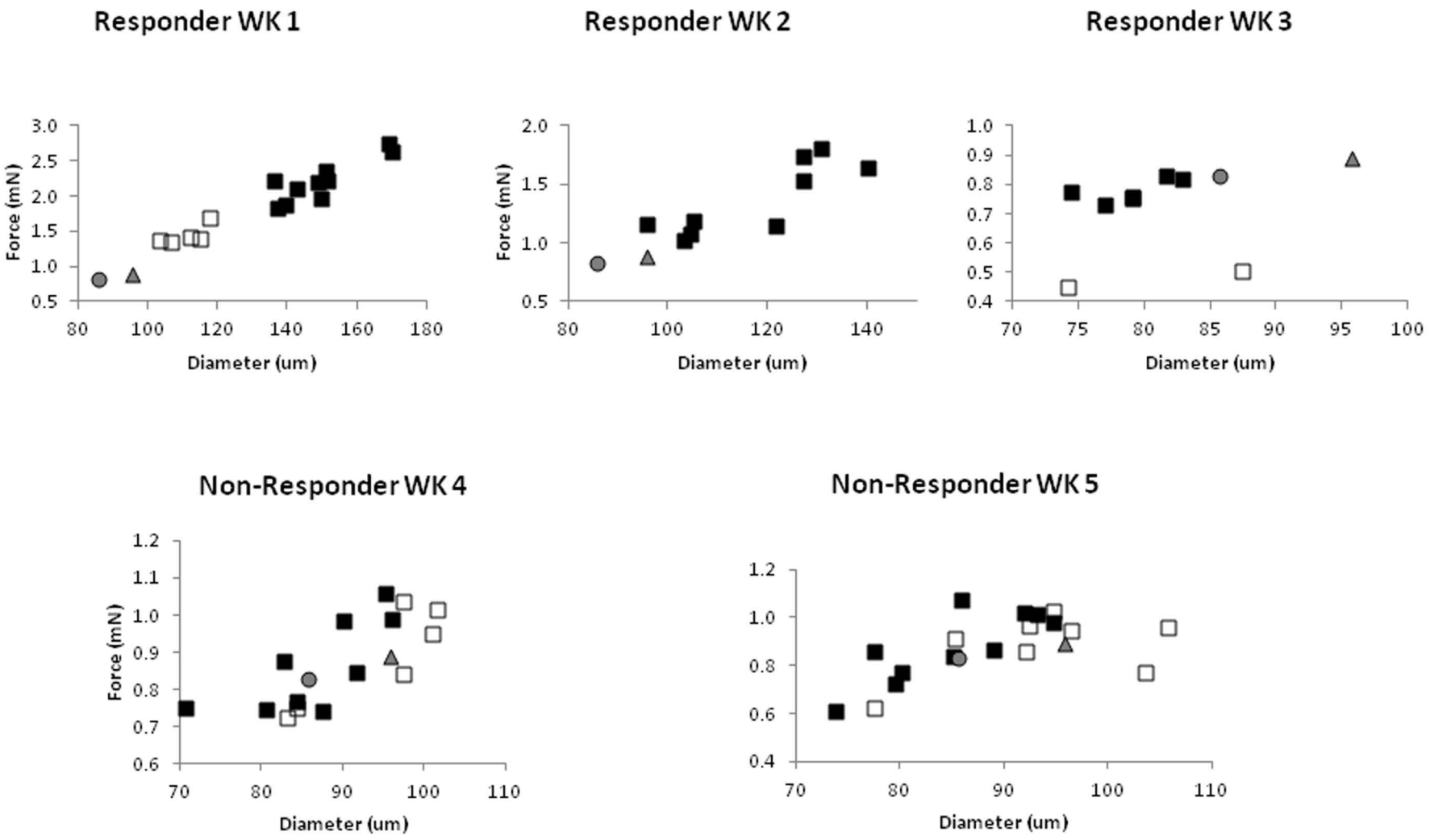
Supplemental Figure 1: Relationship between type II fiber diameter and peak Ca^{2+}-activated force (P_0) for weekly testosterone (WK) treatment

Each symbol represents the results of a single vastus lateralis type II fiber for each subject where open squares are pre-treatment fibers and filled squares are post-treatment fibers. For subject WK 2 no type II pre-treatment fibers were studied. The gray shaded triangle and gray shaded circle are the mean pre- and post-treatment values for the placebo group, respectively.

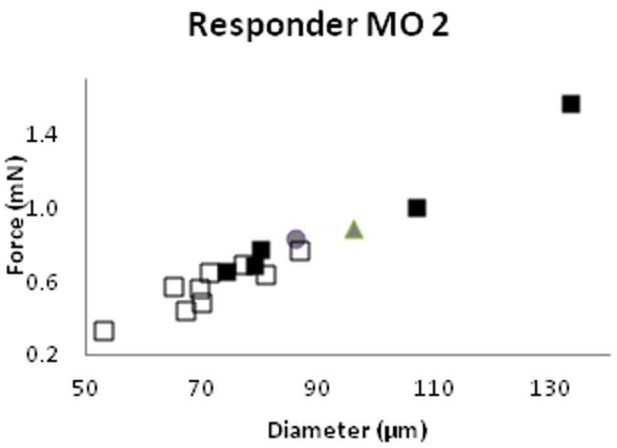
Supplemental Figure 2: Relationship between type II fiber diameter and peak Ca^{2+}-activated force (P_0) for monthly testosterone (MO) treatment

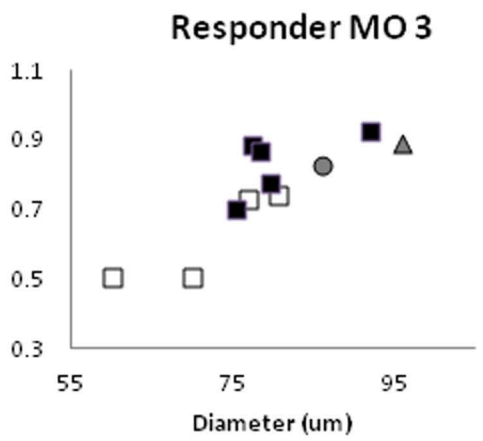
Each symbol represents the results of a single vastus lateralis type II fiber for each subject where open squares are pre-treatment fibers and filled squares are post-treatment fibers. The gray shaded triangle and gray shaded circle are the mean pre- and post-treatment values for the placebo group, respectively. Subject MO 1 is not shown as no post-treatment fast fibers were isolated from this sample.

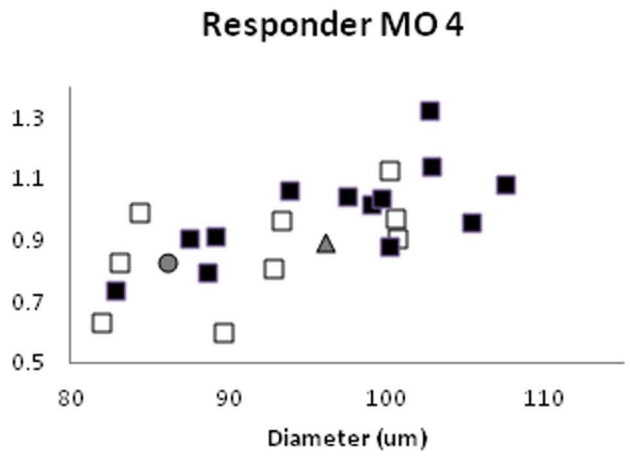
Supplemental Figure 1. Weekly Hormone: Type II Fibers

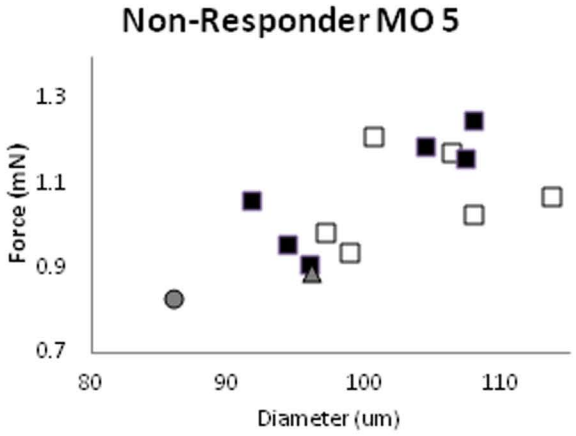


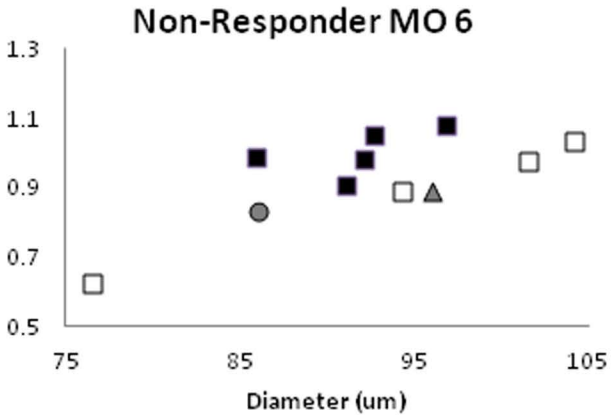
Supplemental Figure 2. Monthly Hormone: Type II Fibers

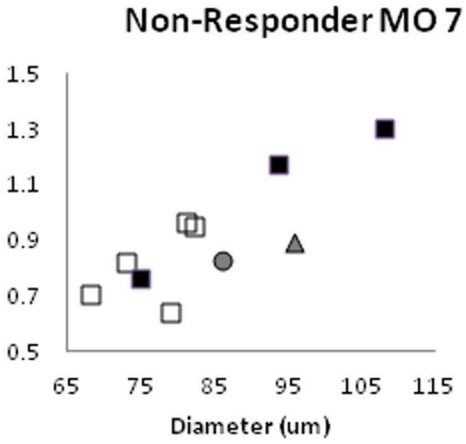












Supplemental Table 1. Type I and II fiber peak power with weekly and monthly TE

	Absolute Peak Power		Relative Peak Power	
Muscle	$uN*FL*s^{-1}$	$uN*FL*s^{-1}$	$kN*m^{-2}*FL*s^{-1}$	$kN*m^{-2}*FL*s^{-1}$
VL	**Type I**	**Type II**	**Type I**	**Type II**
Placebo				
Pre	10.18 ± 0.53 (47)	46.18 ± 2.71 (48)	1.31 ± 0.03 (47)	6.99 ± 0.34 (48)
Post	10.72 ± 0.65 (53)	45.53 ± 3.10 (37)	1.39 ± 0.05 (53)	8.28 ± 0.63 (37)†
% difference	5	-1	6	18
Weekly				
Pre	10.40 ± 0.46 (49)	51.23 ± 4.29 (16)	1.25 ± 0.05 (49)	6.51 ± 0.37 (16)
Post	14.62 ± 1.20 (44)**	65.99 ± 4.77 (41)†	1.25 ± 0.03 (44)	7.60 ± 0.44 (41)
% difference	41	29	0	17
Monthly				
Pre	8.43 ± 0.26 (68)*	47.12 ± 3.24 (35)	1.33 ± 0.04 (68)	8.41± 0.50 (35)*
Post	9.53 ± 0.39 (58)**	54.22 ± 2.36 (27)†	1.36 ± 0.04 (58)	8.31 ± 0.52 (27)
% difference	13	15	2	-1

Values represent means ± SE; VL = vastus lateralis muscle; FL = fiber length; Weekly group received testosterone (TE) weekly for five months; Monthly group received TE weekly during months 1, 3 and 5.

**Significant difference ($p < 0.05$) between pre and post means for a given group

*Significant difference ($p < 0.05$) between placebo pre means and subject pre means

†Significantly different ($p < 0.10$) between pre and post mean for a given group

Supplemental Table 2. Calcium sensitivity of slow type I and fast type II fibers

Condition	Activation Threshold		pCa_{50}		N_1		N_2	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Weekly (WK)								
Type I	6.58 ± 0.02	6.57 ± 0.01	5.72 ± 0.01	5.70 ± 0.01	2.10 ± 0.09	2.14 ± 0.07	2.84 ± 0.06	2.80 ± 0.06
Type II	6.46 ± 0.06	6.53 ± 0.03	5.92 ± 0.04	5.86 ± 0.01	1.85 ± 0.20	2.13 ± 0.11	4.68 ± 0.72	3.89 ± 0.21
Monthly (MO)								
Type I	6.53 ± 0.01	6.59 ± 0.02	5.87 ± 0.05	5.79 ± 0.02	1.85 ± 0.07	2.00 ± 0.07	3.32 ± 0.07	3.04 ± 0.08
Type II	6.54 ± 0.10	6.55 ± 0.09	5.90 ± 0.01	5.92 ± 0.01	1.91 ± 0.11	1.92 ± 0.09	4.34 ± 0.55	5.36 ± 0.85

All data are pCa units (-log of Ca^{2+} concentration) expressed as means ± SE; WK type I fibers pre treatment n = 30, post n = 37; WK type II fibers pre treatment n= 7, post n = 15; MO type I fibers pre treatment n = 50, post n = 43; MO type II fibers pre treatment n = 7, post n = 14; Activation Threshold is Ca^{2+} that just elicits force, pCa_{50} equals –log of Ca^{2+} concentration that elicits 50% of peak tension; N_1 and N_2 are slope of Hill plot for values greater than and less than half-maximal activation, respectively.