Testosterone administration to elderly men increases skeletal muscle strength and protein synthesis

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Urban, Randall J., Yvonne H. Bodenburg, Charles Gilkison, Judy Foxworth, Andrew R. Coggan, Robert R. Wolfe, and Arny Ferrando. Testosterone administration to elderly men increases skeletal muscle strength and protein synthesis. Am. J. Physiol. 269 (Endocrinol. Metab. 32): E820–E826, 1995.—Aging men develop a significant loss of muscle strength that occurs in conjunction with a decline in serum testosterone concentrations. We investigated the effects of testosterone administration to six healthy men (67 ± 2 (SE) yr) on skeletal muscle protein synthesis, strength, and the intramuscular insulin-like growth factor 1 (IGF-I) system. Elderly men with serum testosterone concentrations of 480 ng/dl or less were given testosterone injections for 4 wk to produce serum concentrations equal to those of younger men. During testosterone administration muscle strength (isokinetic dynamometer) increased in both right and left hamstring and quadriceps muscles as did the fractional synthetic rate of muscle protein (stable-isotope infusion). Ribonuclease protection assays done on total RNA from muscle showed that testosterone administration increased mRNA concentrations of IGF-1 and decreased mRNA concentrations of insulin-like growth factor binding protein-4. We conclude that increasing testosterone concentrations in elderly men increases skeletal muscle protein synthesis and strength. This increase may be mediated by stimulation of the intramuscular IGF-I system.

**MEN LOSE MUSCLE**

and strength with aging regardless of their level of physical activity (25). This muscle weakness may lead to difficulties in daily activities, falls, and institutional care (26). This diminished muscle function in elderly men occurs in conjunction with a decrease in serum testosterone concentrations (22).

Studies show a correlation between muscle function and serum testosterone concentrations (1, 13). Serum testosterone concentrations in institutionalized elderly men with marked loss of muscle function are lower than their healthy counterparts (1). Serum concentrations of testosterone are correlated with training-induced strength increases in elderly people (13). Testosterone administration in elderly men (to increase serum testosterone concentrations to ranges comparable to young men) increases lean body mass (27) and muscular strength in the right hand (21).

Part of the response to testosterone may be mediated through the growth hormone-insulin-like growth factor I (IGF-I) system as testosterone administration in hypogonadal men increases the secretion of growth hormone (17), whereas administration to normal men increases serum concentrations of IGF-I (15). The anabolic effects of IGF-I in humans are well documented. Administration of recombinant IGF-I to humans increases whole body protein anabolism and ameliorates the catabolic effects of glucocorticoids (19). Recombinant IGF-I administration in young men increases forearm muscle protein synthesis (10). Moreover, IGF-I and insulin-like growth factor binding proteins (IGFBPs) are important regulators of muscle function. Skeletal muscle cells can produce IGF-I and have IGF-I receptors (8). Endogenous production of IGF-I is important in the growth response of skeletal myofibers to stretch (24). Hypertrophy of smooth muscle cells of rat bladder show an increase in IGFI-1 and IGFBP-2 mRNA concentrations (4). Therefore, evidence suggests that the skeletal muscle IGF system is involved in the synthesis of muscle protein and that testosterone will increase peripheral IGF-I concentrations. However, the relationship between testosterone administration and its subsequent effect on muscle protein synthesis and the local intramuscular IGF-I system is unknown.

In this study we increased testosterone concentrations in elderly men to ranges comparable with young men and examined effects on muscle strength and muscle protein synthesis. We also investigated whether testosterone affected molecular components of the intramuscular IGF-I system. We found that testosterone increased muscle strength and protein synthesis. Moreover, we found evidence of stimulation of the intramuscular IGF-I system, as evidenced by increased mRNA concentrations of IGF-I and decreased mRNA concentrations of IGFBP-4 in muscle.

**METHODS**

**Subjects.** Six healthy elderly men [67 ± 2 (SE) yr] volunteered for the study, which was approved by the Institutional Review Board at University of Texas Medical Branch (UTMB). Informed consent was obtained after the purpose and procedures were described. Inclusion criteria included being within 20% of ideal body weight (Metropolitan Life Insurance Table), prostate-specific antigen (PSA) ≤ 6.0 µg/l (3), serum total testosterone ≤ 480 ng/dl (1), serum low-density lipoprotein (LDL) ≤ 200 ng/dl (6), completion of a Bruce treadmill exercise test without significant findings of cardiovascular disease, and no medical illnesses causing disability. Exclusion criteria included a history of prostate cancer, severe coronary artery disease, knee replacement, or taking a blood anticoagulant, e.g., Coumadin. Because we wanted to determine the effects of testosterone administration on a nonexercising population, we excluded subjects engaged in regular exercise training (defined as 30 min of aerobic or resistance training activity ≥ 2 days/wk).

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Experimental protocol. The studies were performed on the General Clinical Research Center (GCRC) at UTMB. After initial screening, subjects were instructed to complete a 5-day dietary record to determine average daily caloric and protein intake before the first GCRC admission. After admission to the GCRC, subjects were tested for peak oxygen consumption during cycle ergometer exercise (V̇O₂peak) and had anthropometric measurements of the thigh taken. Subjects then practiced on use of the Cybex II isokinetic dynamometer for strength and endurance testing. Dinner was provided so as not to deviate from the previous 5-day dietary record. Subjects began fasting at 2000. At 2000 an intravenous line was inserted into an antecubital vein, and blood was withdrawn every hour until 0800 the next morning. These 12 samples were used to measure growth hormone (GH) concentrations as an estimate of integrated GH secretion. The sample at 0800 was also used to measure total testosterone, IGF-1, blood lipids, and estradiol. The next morning, subjects were tested on the isokinetic dynamometer for lower-extremity strength and endurance. Subjects then returned to the GCRC for stable-isotope infusion. The antecubital catheter previously used for the 12-h blood sampling was also used for infusion of the stable isotope (ring-\(^{13}\)C\(_6\))phenylalanine. Another catheter was inserted in a retrograde manner into a hand vein for sampling of "arterialized" blood, via the heated-hand technique (5). Background blood samples were drawn, and a primed (2 \(\mu\)mol/kg) constant (0.05 \(\mu\)mol/kg \(\cdot\)min\(^{-1}\)) infusion of (ring-\(^{13}\)C\(_4\))phenylalanine was started.

Artificial blood samples were drawn at 1 h and every 20 min from 3 to 4 h. Muscle biopsies (80–100 mg) of the vastus lateralis were performed at 1 and 4 h according to the Bergström technique (7). Fractional synthetic rate (FSR) of skeletal muscle protein was determined from the protein-bound \(^{13}\)C (7).

After the infusion protocol was completed, subjects were given an intramuscular injection of 100 mg of testosterone enanthate and discharged from the GCRC. Subjects returned weekly for intramuscular injections of testosterone. Serum total testosterone concentrations were measured every week before the next injection. The amount of testosterone injected was adjusted to maintain serum total testosterone concentration between 500 and 1,000 ng/dl (based on weekly testosterone concentration before the next injection). Daily activity logs were recorded for 24 h several days before starting the study and the last week of the study.

After 4 wk of treatment, subjects were readmitted to the GCRC, and the studies were repeated as outlined for the first admission. This admission occurred 6 days after the last testosterone injection.

Stable-isotope measurements. Plasma and muscle analysis for (ring-\(^{13}\)C\(_6\))phenylalanine was performed as previously described (29). Briefly, amino acids were separated from plasma by ion-exchange chromatography and analyzed. The tert-butylmethylsilyl derivative was prepared by standard methods (29). Gas chromatography-mass spectrometry (GC-MS) using electron impact ionization and selected ion monitoring was used to analyze the tracer derivatives.

Muscle biopsies were analyzed for intracellular and protein-bound phenylalanine enrichment. Each sample was homogenized, proteins precipitated, and the supernatant collected. The pooled supernatant was processed as the plasma samples described above. The remaining pellet of muscle tissue was dried, and hydrolyzed in 6 N HCl. Phenylalanine was isolated and purified by liquid chromatography and then combusted at 700°C in a vacuum. The purified phenylalanine-derived CO\(_2\) was analyzed by isotope ratio mass spectrometry (IRMS).

The FSR of muscle protein was calculated as

\[
FSR (\%/h) = \frac{(E_t - E_i) \cdot 1.5}{
\frac{E_m}{1} \cdot 100}
\]

where \(E_t\) is the protein-bound enrichment (\(^{13}\)C/\(^{12}\)C) from the first biopsy at \(t_0\) (isotopic equilibrium, 1 h), \(E_i\) is the protein-bound enrichment (\(^{13}\)C/\(^{12}\)C) from the biopsy at \(t_1\) (4 h), \(t\) is the incorporation time (\(t_1 - t_0\), h), and \(E_m\) is the average free tissue precursor enrichment ((ring-\(^{13}\)C\(_4\))phenylalanine) over the time of label incorporation. The intracellular free phenylalanine enrichment was used as the precursor enrichment. The numerator is multiplied by 1.5 to normalize the measures between the IRMS and GC-MS.

Total RNA isolation and ribonuclease protection assays. Approximately 75 mg of muscle tissue were available for the isolation of total RNA. Biopsy samples were first blotted free of extraneous blood, placed immediately in liquid nitrogen, and then transferred to a -70°C freezer. Total RNA was isolated from the sample using RNAzol B (Tel-Test, Friendswood, TX). The frozen biopsy sample was placed in 2 ml of RNAzol B and homogenized with a Polytron PCU-2 homogenizer. The sample was then clarified with chloroform, precipitated, and washed according to the RNAzol B protocol. A 75-mg muscle biopsy tissue sample yielded ~40 μg of total RNA.

The ribonuclease (RNase) protection assay has been previously described in detail (28). The protection assay was used with human cDNA clones for IGF-I, IGF-I receptor, myogenin, IGFBP-2, -3, and -4. The Ribobrope Gemini II Core System (Promega, Madison, WI) was used with 200–300 ng of the plasmid. T\(_0\) or T\(_1\) RNA polymerases are used to generate an anti-sense [\(^{32}\)P]UTP-labeled RNA probe, depending on the configuration of the plasmid. After addition of an RNase inhibitor (Ribonucleoside Vanadyl Complex, Bethesda Research Laboratories, Bethesda, MD), the template DNA was digested with deoxyribonuclease (DNase, 1 μg/μl). The RNA was precipitated and mixed with 250,000 counts/min of the probe, hybridized, and digested with RNase A and RNase T\(_1\). After digestion of RNase with proteinase K followed by phenol chloroform extraction and ethanol precipitation, the sample underwent electrophoresis on an 8% polyacrylamide, 0.5X tris(hydroxymethyl)aminomethane-borate gel for 3 h at 60 W. The RNA-protected bands were detected using a PhosphorImager (model 425E, Molecular Dynamics, Sunnyvale, CA) with an overnight exposure. Band intensities were measured using the ImageQuant analysis program accompanying the PhosphorImager.

Hormone assays. Serum IGF-I concentrations were measured by radioimmunoassay by Specialty Laboratories (Santa Monica, CA). GH concentrations were measured using an immunoradiometric assay (Nichols, San Juan Capistrano, CA). Serum total testosterone, PSA, lipids, and estradiol were measured in radioimmunoassays by National Health Laboratories (Houston, TX).

Anthropometric measurements. Anthropometric measurements were done on the thigh, using measurement locations as previously described (16). These measurements were done by the same investigator to reduce error. Height and thigh circumference were measured, and muscle volume was calculated as previously described (16).

Muscle strength and endurance. Leg muscle strength and endurance were measured on a Cybex II isokinetic dynamometer. Muscle strength was defined as the average of the total area under the torque curve for each repetition (work/repetition) of five repetitions at 60°/s. Muscular endurance...
TESTOSTERONE ADMINISTRATION IN ELDERLY MEN

Results

Hormone and metabolic parameters. Serum testosterone concentrations were measured (at ~0800) every week before administration of the next injection. Figure 1 shows the testosterone concentrations for each subject over the study period. Adjustments were necessary in the concentration of testosterone in two of the subjects as indicated in Fig. 1. These adjustments were made to keep the testosterone concentrations in the normal range for men. All subjects remained within normal testosterone ranges during hormone administration.

Hormone and metabolic parameters showed a significant increase in concentrations of estradiol (P < 0.03) and PSA (P < 0.04) with testosterone administration. There was a significant decrease in high-density lipoprotein (HDL) concentrations (P < 0.03), but all other parameters did not change during the 1 mo of testosterone administration. These data are summarized in Table 1.

Muscle function analysis. Lower-extremity muscle function was studied as a predictor of subsequent disability in the elderly (12). There was an increase in work per repetition (an assessment of strength) in the right (P < 0.04) and left (P < 0.04) hamstring and right (P < 0.05) and left (P < 0.02) quadriceps after testosterone administration (Fig. 2). However,

Table 1. Hormone and metabolic parameters before and after testosterone administration

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Basal</th>
<th>Testosterone</th>
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<tbody>
<tr>
<td>PSA, ng/ml</td>
<td>1.7 ± 0.6</td>
<td>2.1 ± 0.7*</td>
</tr>
<tr>
<td>IGF-I, ng/ml</td>
<td>141 ± 27</td>
<td>163 ± 23</td>
</tr>
<tr>
<td>Estradiol, pg/ml</td>
<td>17 ± 2</td>
<td>36 ± 6*</td>
</tr>
<tr>
<td>GH, ng/ml</td>
<td>0.9 ± 0.2</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>186 ± 14</td>
<td>171 ± 11</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>54 ± 8</td>
<td>45 ± 6*</td>
</tr>
<tr>
<td>LDL, mg/dl</td>
<td>115 ± 11</td>
<td>107 ± 10</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 subjects. PSA, prostate-specific antigen; IGF-I, insulin-like growth factor I; GH, growth hormone; HDL, and LDL, high- and low-density lipoprotein. *P < 0.05 as determined by paired t-test.
there was no increase in muscle endurance during testosterone administration (Fig. 3). 

\( \text{V}_2 \text{peak} \) did not change after testosterone administration: basal 21.3 ± 2.3 vs. testosterone 21.5 ± 2.7 ml·min\(^{-1}\)·kg\(^{-1}\). By anthropometric measurement, there was no change in thigh muscle volume before or after testosterone administration: basal 6.8 ± 4 vs. testosterone 7.0 ± 5 liters. Finally, comparison of logs of daily activity in the subjects were not different by inspection or subject interview during testosterone administration.

**Stable-isotope studies.** There was an approximately twofold increase \((P < 0.04)\) in FSR in the six men after testosterone administration (Fig. 4). The FSR was calculated as described in METHODS.

**IGF-I system mRNA concentrations from muscle biopsies.** RNase protection assays were used to determine the mRNA concentrations of myogenin, IGF-I, IGF-I receptor, and IGF binding proteins in skeletal muscle. These probes were first used in protection assay on human cadaveric skeletal muscle total RNA to verify the assay. Human myogenin was tested because it is important in the development of myoblasts into muscle fibers and can be stimulated by IGF-I in vitro (9). There was not a detectable signal for this clone in skeletal muscle total RNA. The signal for IGFBP-2 required 40 \( \mu \)g of total RNA for detection and therefore was not used due to sample limitation. Concentrations of mRNA were detected for IGF-I, IGF-I receptor, IGFBP-3, and IGFBP-4. However, because of the limited amounts of total RNA from the muscle biopsies, testing was focused on IGFBP-4 and IGF-I. IGF-I receptor mRNA concentrations were measured (before and after testosterone administration) in five subjects and IGFBP-3 mRNA concentrations in four subjects. There was no change in either of these components of the IGF-I system during testosterone administration. The results from these probes are shown in Table 2.

**DISCUSSION**

The results of this study demonstrate that short-term testosterone administration in elderly men (to increase testosterone concentrations to ranges similar to these of young men) increases skeletal muscle strength and protein synthesis. The subjects in the study were healthy elderly men with total testosterone concentrations of 480 ng/dl or less. We did not measure free testosterone or sex hormone-binding globulin (SHBG) concentrations in this study. SHBG concentrations increase in elderly men, and SHBG concentrations are positively correlated with total testosterone concentrations and negatively correlated with percentages of free testosterone \((18)\).

Testosterone administration caused an ~twofold increase in mRNA concentrations of IGF-I in the six elderly subjects \((P < 0.03)\). Figure 5 shows a representative image as generated by the PhosphorImager and results from the six subjects. Testosterone administration also caused ~twofold decrease in mRNA concentrations of IGFBP-4 \((P < 0.05)\). Figure 6 shows representative images obtained from the PhosphorImager for IGFBP-4 and results from the six subjects.

**Table 2. Messenger RNA concentrations of components of the intramuscular IGF-I system**

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Testosterone</th>
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<tbody>
<tr>
<td>Myogenin</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>IGFBP-2</td>
<td>MD</td>
<td>MD</td>
</tr>
<tr>
<td>IGFBP-3</td>
<td>247 ± 32( \text{SE} )</td>
<td>150 ± 22</td>
</tr>
<tr>
<td>IGF-I receptor</td>
<td>182 ± 10( \text{SE} )</td>
<td>164 ± 27</td>
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Values are means ± SE, \( n = 4 \) for IGFBP-3 and 5 for IGF-I receptor; ND, none detected in total RNA from human cadaver; MD, minimally detected in total RNA from human cadaver; \( \text{SE} \) arbitrary units from pixels measured by the PhosphorImager on total RNA from muscle biopsy samples from study subjects.
Trations were comparably low in healthy, elderly men (1), indicating that SHBG effects on the circulating bound-free ratio of testosterone are not greater that the decrease in hormone production that occurs with aging. Eighty-five percent of healthy, elderly men have total testosterone concentrations below 480 ng/dl and can be classified as relatively hypogonadal compared with their younger counterparts (1). All men lose muscle size and strength with aging (25), so that a diminished testosterone concentration is just one of multiple factors causing a loss of muscle strength. Nevertheless, our results indicate that testosterone administration can improve skeletal muscle strength in the elderly population with relative hypogonadism.

Testosterone increases skeletal muscle protein synthesis in young men (ages 19–40) given pharmacological concentrations of testosterone for 12 wk (11). In the present study, physiological testosterone concentrations increased muscle protein synthesis in elderly men. Muscle protein turnover is comprised of both muscle protein synthesis and muscle protein degradation. Therefore, testosterone administration caused an increase in muscle protein turnover and strength without an increase in muscle size. Moreover, testosterone administration increased muscle strength without the benefit of an exercise regimen. This suggests that synthesis of new structural proteins and/or preferential breakdown of older, less functional structural proteins can enhance muscle strength. Long-term studies with testosterone administration without exercise are needed to determine whether muscle size will also increase over time.

Long-term studies are needed to determine the risks of testosterone administration, because PSA concentrations showed a small but significant increase in this study, consistent with an earlier report (27). PSA is a normal product of prostatic epithelial cells, and an increase in its concentration is indicative of stimulation of prostatic tissue (27). Whether this stimulation will worsen benign prostatic hypertrophy or increase the risk of prostate cancer in elderly men will need to be determined. HDL concentrations were also decreased with testosterone administration (but still above 35 mg/dl), whereas estradiol concentrations were increased. Long-term studies will also determine whether the net effect of these deleterious and beneficial changes in lipid profiles will cause any cardiovascular risk to elderly men.

This study also investigated the effects of testosterone on the intramuscular IGF-I system. Previous studies have shown that testosterone administration will stimulate the peripheral GH-IGF-I system in men (15, 17),...
but none has investigated testosterone effects on the intramuscular IGF-I system. The paradigm of this study (testosterone administration for only 1 mo) did not show any change in the peripheral GH-IGF-I system, but did find significant changes in molecular components of the intramuscular IGF-I system. These results are consistent with a recent study where administration of GH to elderly men to raise peripheral concentrations of IGF-I did not enhance muscle strength and anabolism caused by resistance exercise (30). Therefore, our results imply that changes in the intramuscular IGF-I system do not correlate with changes in peripheral IGF-I concentrations. Further evidence for the importance of local tissue IGF-I concentrations can be found with the increase in intramuscular IGF-I mRNA concentrations during smooth muscle hypertrophy in Sprague-Dawley rats (4).

Several possibilities exist for the increase in IGF-I mRNA concentrations after testosterone administration. Testosterone may directly stimulate protein synthesis in fully mature myocytes and hence cause an increase in the autocrine expression of IGF-I. In support of this concept, in vitro studies in differentiated skeletal muscle cells show that the exogenous stimulus of stretch stimulates autocrine secretion of IGF-I (24). Testosterone could also directly increase the number of myocytes, resulting in increased muscle protein synthesis and IGF-I mRNA concentration. Although there was no measure of cellularity in this study, the finding of a reduced concentration of IGFBP-4 mRNA is indicative of differential regulation of the IGF-I system during testosterone administration.

Increases in local IGF-I concentrations could increase the rate of muscle protein turnover by effects on either synthesis or degradation. IGF-I stimulates myoblasts in vitro to express myogenin, which mediates the differentiation of myoblasts to myotubes (9). Therefore, IGF-I could increase muscle protein synthesis by stimulating the differentiation of satellite cells into mature myocytes, thereby increasing muscle cellularity and protein synthesis. We did not detect any expression of myogenin in the samples of fully differentiated muscle, but this does not preclude IGF-I effects on myogenin expression in satellite cells. Moreover, IGF-I could reduce muscle protein degradation and thereby influence net muscle protein synthesis. However, muscle degradation was not determined in this study.

Finally, this study also investigated IGFBP mRNA concentrations during testosterone administration. Messenger RNA concentrations were determined in muscle for IGFBP-2, -3, and -4. We did not investigate expression of IGFBP-5 and -6, both of which have effects on myoblast differentiation (2, 23). We did find a reduction in expression of IGFBP-4. IGFBP-4 inhibits the mitogenic action of IGF-I (20). Therefore, the decrease in the production of this binding protein as implied by the decreased mRNA concentrations would enhance the availability of free IGF-I in skeletal muscle.

In conclusion, testosterone administration to elderly men increases skeletal muscle strength and protein synthesis. Results suggest involvement of the intramuscular IGF-I system in these effects. An increase in skeletal muscle strength in the elderly decreases the number of falls, allows for independent living, and improves the quality of life for these individuals (12, 26). Therefore, the physiological administration of testosterone in elderly men, similar to the paradigm of estrogen replacement in menopausal women, may have significant beneficial effects on muscle strength.

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