*J Appl Physiol* 96: 1055–1062, 2004.
First published October 24, 2003; 10.1152/japplphysiol.00808.2003.

# Treatment with oxandrolone and the durability of effects in older men

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Submitted 31 July 2003; accepted in final form 17 October 2003

**Schroeder, E. Todd, Ling Zheng, Kevin E. Yarasheski, Dajun Qian, Yolanda Stewart, Carla Flores, Carmen Martinez, Michael Terk, and Fred R. Sattler.** Treatment with oxandrolone and the durability of effects in older men. *J Appl Physiol* 96: 1055–1062, 2004. First published October 24, 2003; 10.1152/japplphysiol.00808.2003.—We investigated the effects of the anabolic androgen, oxandrolone, on lean body mass (LBM), muscle size, fat, and maximum voluntary muscle strength, and we determined the durability of effects after treatment was stopped. Thirty-two healthy 60- to 87-yr-old men were randomized to receive 20 mg oxandrolone/day ($n = 20$) or placebo ($n = 12$) for 12 wk. Body composition [dual-energy X-ray absorptiometry (DEXA), magnetic resonance imaging, and $^2H_2O$ dilution] and muscle strength [1 repetition maximum (1 RM)] were evaluated at baseline and after 12 wk of treatment; body composition (DEXA) and 1-RM strength were then assessed 12 wk after treatment was discontinued (*week 24*). At *week 12*, oxandrolone increased LBM by 3.0 ± 1.5 kg ($P < 0.001$), total body water by 2.9 ± 3.7 kg ($P = 0.002$), and proximal thigh muscle area by 12.4 ± 8.4 cm$^2$ ($P < 0.001$); these increases were greater ($P < 0.003$) than in the placebo group. Oxandrolone increased 1-RM strength for leg press by 6.7 ± 6.4% ($P < 0.001$), leg flexion by 7.0 ± 7.8% ($P < 0.001$), chest press by 9.3 ± 6.7% ($P < 0.001$), and latissimus pull-down exercises by 5.1 ± 9.1% ($P = 0.02$); these increases were greater than placebo. Oxandrolone reduced total (−1.9 ± 1.0 kg) and trunk fat (−1.3 ± 0.6 kg; $P < 0.001$), and these decreases were greater ($P < 0.001$) than placebo. Twelve weeks after oxandrolone was discontinued (*week 24*), the increments in LBM and muscle strength were no longer different from baseline ($P > 0.15$). However, the decreases in total and trunk fat were sustained (−1.5 ± 1.8, $P = 0.001$ and −1.0 ± 1.1 kg, $P < 0.001$, respectively). Thus oxandrolone induced short-term improvements in LBM, muscle area, and strength, while reducing whole body and trunk adiposity. Anabolic improvements were lost 12 wk after discontinuing oxandrolone, whereas improvements in fat mass were largely sustained.

lean body mass; muscle mass; dual-energy X-ray absorptiometry; magnetic resonance imaging

ADVANCING AGE IS ASSOCIATED with a progressive loss of muscle mass (sarcopenia), skeletal muscle strength, and physical function (1, 2, 9, 14, 19). Sarcopenia increases the risk for frailty, falls, fractures, dependency, and depression (34, 36). Advancing age is also associated with increases in fat mass, particularly central adiposity, which increases the risk for insulin resistance, hypertension, dyslipidemia, and impaired fibrinolysis (metabolic syndrome) (37). The metabolic syndrome predisposes older persons to accelerated atherosclerosis and Type 2 diabetes.

The contribution of age-associated hormonal alterations to these adverse health consequences is unclear. Both cross-sectional (15, 28, 51) and longitudinal (17, 30) studies have shown that serum total and free concentrations of testosterone decline with advancing age in men. Testosterone regulates muscle and fat mass, but the relationship between gonadal hormone status and age-associated alterations in body composition, skeletal muscle strength, and metabolic disorders in older persons is uncertain. There is some evidence that bioavailable testosterone levels (free and the fraction loosely bound to albumin) correlate with skeletal muscle mass and muscle strength in different ethnic populations (3, 35).

Testosterone treatment in hypogonadal young men increases lean tissue (4, 7, 20, 45, 53, 54) and muscle strength (4, 54) and decreases fat mass (4, 20, 54). Despite evidence that supplemental testosterone increases myofibrillar protein synthesis rate in older men (11, 52), its effects on body composition and muscle function in these men are less clear (22, 31, 44, 46, 50, 51). In the largest studies, in which older relatively hypogonadal men received testosterone replacement for 1 and 3 yr, respectively, lean body mass (LBM) was only modestly increased (1.0 and 1.9 kg, respectively) (22, 46), and the effects on muscle strength were variable. Only three studies have shown increases in lower extremity maximum voluntary force (11, 22, 52). By contrast, in a controlled study of 108 older men randomized to receive placebo or testosterone (46), upper extremity grip strength and lower extremity isokinetic strength were unchanged with testosterone (50). Similarly, the effects of testosterone on fat mass have been variable with either no change or only modest reductions achieved (11, 21, 31, 46, 51, 52).

The variability in outcomes in older men may be related to the different delivery strategies for testosterone (intramuscular vs. transdermal delivery), dose (200 mg biweekly vs. 5 mg/day), change in testosterone levels in response to therapy, duration of treatment (4 wk vs. 3 yr), different methods to assess body composition [bioelectrical impedance analysis, dual-energy X-ray absorptiometry (DEXA), magnetic resonance imaging (MRI), hydrostatic weighing], as well as measures of muscle strength (handheld dynamometers, isokinetic dynamometers, free weights, or pneumatic resistance devices).

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Moreover, with one exception, these studies did not directly assess changes in muscle mass or muscle cross-sectional area (CSA).

Oxandrolone is a potent, oral anabolic androgen that is approved for the treatment of weight loss due to known medical or unexplained causes (43, 48). We evaluated whether the licensed dose of oxandrolone increases muscle mass and muscle strength and reduces body fat mass in older men at risk for sarcopenia and metabolic complications. Moreover, we followed these men for 12 wk after discontinuing oxandrolone to evaluate the durability of the alterations in body composition and muscle strength. We hypothesized that oxandrolone would increase LBM, muscle area, and muscle strength, and reduce whole body and central adiposity in older men, and that these benefits would not be fully sustained.

## METHODS

### *Study Design*

This was a single-center, investigator-initiated, double-blind, placebo-controlled investigation to determine the magnitude and durability of effects of a potent, convenient to administer anabolic androgen, oxandrolone (Oxandrin). The study was performed at the University of Southern California National Center for Research Resources-funded General Clinical Research Center, with the exception that skeletal muscle strength was assessed at the Clinical Exercise Research Center in the Department of Biokinesiology and Physical Therapy of the University. The study design and informed consent were approved and annually reviewed by the Institutional Review Board of the Los Angeles County-University of Southern California Medical Center.

### *Study Population*

Men ≥60 yr old were recruited from the Los Angeles communities surrounding the University of Southern California Health Sciences Campus. To be eligible for the study, subjects had to have a body mass index ≤35 kg/m$^2$, repeated resting blood pressure <180/95 mmHg, prostate-specific antigen (PSA) ≤4.1 ng/ml, serum hematocrit ≤50%, alanine aminotransferase (ALT) less than three times the upper limit of normal, and serum creatinine <2 mg/dl. Subjects with untreated endocrine abnormalities (e.g., diabetes, hypothyroidism), active inflammatory conditions, or cardiac problems (heart failure, myocardial infarction, or angina) in the proceeding 3 mo were excluded. An incremental treadmill exercise test with 12-lead electrocardiogram and blood pressure monitoring to achieve a heart rate ≥85% of age-predicted maximum was administered before resistance exercise testing to identify subjects at possible risk for exercise induced ischemia, abnormalities in cardiac rhythm, or abnormal blood pressure responses.

### *Study Interventions*

Eligible subjects were randomized in a 2:1 manner to receive either the licensed oral dose of oxandrolone (Oxandrin, Savient Pharmaceuticals, East Brunswick, NJ) of 20 mg/day (10 mg twice daily) or matching placebo for 12 wk. Twenty milligrams were chosen because this is the Food and Drug Administration-licensed dose for treatment of weight loss or inability to maintain normal body weight. Subjects returned for a follow-up evaluation at study *week 24* (12 wk after stopping study treatment). Adherence was monitored by tablet count at each study visit.

### *Safety Monitoring*

Complete blood counts, comprehensive chemistries with tests of renal and hepatic function, and PSA were measured at baseline and *weeks 6*, *12*, and *24*. Additionally, liver function tests were obtained at *weeks 3* and *9*.

### *Body Composition by DEXA*

Whole body DEXA scans (Hologic QDR-4500, version 7.2 software, Waltham, MA) were performed at baseline and *weeks 12* and *24* to quantify LBM and fat mass. One blinded, experienced technician (C. Flores) performed and analyzed the scans. The coefficient of variation (CV) for repeated measures was <1% for lean and fat mass.

### *Muscle CSA*

CSA of the dominant thigh muscles was assessed by using proton MRI at baseline and *week 12* (but not *week 24*). $^1$H-MRI was performed by using a 1.5-T GE Signa-LX scanner with the body coil used as both transmitter and receiver. Nine axial images of the thigh were acquired after obtaining a longitudinal relaxation time-weighted coronal scout image (relaxation time-weighted longitudinal repetition time/echo time 300/echo time) that was used to identify the exact anatomic location for the axial images. The slice thickness was 7.5 mm with a 1.5-mm gap. The field of view was 24 × 24 cm with a 254 × 128-pixel matrix. One signal average was used.

Thigh muscle CSA was measured at the junction of the proximal and middle third of the femur in the dominant leg, because greater relative increases in CSA of the proximal quadriceps have been reported after anabolic interventions (32). Pixels associated with intramuscular fat, bone, and major arteries, veins, and nerves were subtracted from the image (using Scion Image, version Beta 4.0.2 software, Scion). Muscle CSA was measured by setting a pixel intensity threshold value that distinguished fat from muscle pixels. This allowed adipose tissue to be differentiated from other more optically dense lean tissue (muscle, nerve, and blood vessels). Total thigh muscle CSA was calculated after area of the fat tissue was removed automatically and area of the femur, nerve tissue, and blood vessels were removed manually. The same investigator (E. T. Schroeder) blinded to treatment located the region of interest, set the threshold value and performed the image analyses. The CV for repeated measures of total thigh CSA was <1%.

### *Total Body Water*

Total body water (TBW) was determined at baseline and *week 12* by using $^2H_2O$ dilution. Subjects ingested $^2H_2O$ (Cambridge Isotopes Laboratory; 0.25 g/kg), and isotope dilution was estimated from plasma samples obtained at −15 min, 0, 3, and 4 h. Our laboratory has previously determined that steady-state $^2$H enrichment is achieved in plasma and maintained between 120 and 240 min (58). The dilution of tracer, corrected for the exchange of hydrogen with other body hydrogen pools (~4%), provides a measure of tracer dilution space, which is equivalent to TBW volume. Plasma samples were analyzed for $^2H_2O$ abundance using proton magnetic resonance spectroscopy and $d_9$-*tert*-butanol as an internal standard (interassay CV = 6.3%) (16). TBW was calculated from the average of the 3- and 4-h $^2$H enrichments in plasma water by using the following formula: TBW = dose (16/18 × g of $^2H_2O$)/deuterium enrichment, where TBW is expressed as $^2$H dilution space/1.04 (57).

### *Evaluation of Muscle Strength*

Maximal voluntary muscle strength was assessed by using the one-repetition maximum (1-RM) method (13) at baseline and *weeks 12* and *24*. The 1 RM was defined as the greatest resistance that could be moved through a defined range of motion with the use of proper technique. Before strength testing, subjects warmed up on a cycle ergometer or by walking for 5 min. Maximum voluntary strength was determined for the bilateral leg press, leg flexion, latissimus pulldown, and chest press exercises on Keiser A-300 pneumatic equipment (Keiser, Fresno, CA). The leg press and chest press machines

only displayed units of measure in newtons. The newton measurement of force cannot accurately be converted to kilograms, and therefore the strength data are reported in newtons for these two machines. To accommodate for familiarization and learning of the testing procedures, baseline strength was assessed twice within 1 wk before study therapy was initiated. The greatest 1 RM measured for each exercise during the two pretreatment testing sessions was used as the baseline value for maximal voluntary muscle strength. The technician was blinded to the subjects' treatment.

### *Nutritional Assessment*

Subjects recorded dietary intake on 3 consecutive days, including 2 weekdays and 1 weekend day in the week before baseline and *weeks 12* and *24*. Subjects were counseled that the days should be chosen to include usual activities and typical eating patterns. A licensed nutritionist (C. Martinez) reviewed all dietary entries with the subjects. This information was entered into the Nutritionist V software (First Data Bank, San Bruno, CA) and analyzed for total energy intake, macronutrients, and types of fat. Subjects were counseled not to change their routine dietary habits during the course of the study.

### *Measurement of Hormones and C-Reactive Protein*

Total testosterone concentration (ng/dl) was measured by the Los Angeles County-University of Southern California Medical Center Clinical Diagnostic Laboratory (Endocrinology Section) by using Diagnostic Products Coat-A-Count at baseline and *week 24*, 12 wk after the oxandrolone intervention was completed. This competitive radioimmunoassay uses a solid-phase polyclonal antibody. The CV for total testosterone was ≤7.7%. We did not measure testosterone levels at *week 12* because semisynthetic androgens, including oxandrolone, cross-react in these testosterone assays. Luteinizing hormone (LH) concentration (IU/ml) was measured by using a microparticle enzyme immunoassay (AxSYM; Abbott Diagnostics), at baseline and study *weeks 12* and *24*. The CV for LH was ≤4.9%.

To assess for evidence of inflammation, we evaluated the changes in ultrasensitive C-reactive protein (CRP) at the University of Southern California Pathology Reference Laboratory by using a latex particle enhanced immunoturbidimetric assay, distributed by Equal Diagnostics (Exton, PA) and manufactured by Kamiya Biochemical (Seattle, WA). The CV for CRP was ≤7.1%.

### *Statistical Considerations*

The study was conservatively powered at 80% to detect a difference in means between the oxandrolone and placebo group of 1.36 times the common SD, using a two-sample *t*-test with a Bonferroni-adjusted $P = 0.0008$, with 20 in oxandrolone group and 12 in placebo group. For total LBM by DEXA scanning, this sample size will be able to detect a mean difference of 2 kg, assuming the common SD is 1.47 kg. For the maximum voluntary skeletal muscle strength of the leg press exercise (which typically has the greatest variance of the exercises tested in this study), this sample size will be able to detect a mean relative difference of 6.8%, assuming the common standard deviation of 5.0%. Statistical analyses are presented in Tables 1–3 and the text as means ± SD.

For the main outcome variables, a two (oxandrolone and placebo group) by three (baseline, *week 12*, and *week 24*) repeated-measures ANOVA was used to statistically compare mean differences within subjects and between groups. Greenhouse-Geisser adjustment was used to justify the assumption of sphericity. When a significant group × time interaction was found, the changes from baseline to *week 12* and the changes from baseline to *week 24* between and within groups were compared by independent *t*-tests and paired *t*-tests, respectively. All post hoc tests were performed with Bonferroni adjustment for six possible comparisons. Baseline characteristic and the changes in safety evaluation from baseline to *week 12* were compared between oxandrolone and placebo groups by using an independent *t*-test. All statistical testing was performed at a two-sided 5% level of significance (0.83% for each post hoc *t*-test) by using Statistical Analysis System version 8.0 (SAS Institute, Cary, NC).

## RESULTS

### *Subjects*

Thirty-four eligible subjects were enrolled and randomized to either oxandrolone ($n = 22$) or placebo ($n = 12$). One subject randomized to receive oxandrolone elected not to participate after providing informed consent; however, he did not start the study drug. A second subject randomized to receive oxandrolone completed study therapy through *week 12* but did not return for follow-up at *week 24*. This subject could not be contacted until well after he missed the *week 24* evaluation; he indicated that he had not had adverse events but had been too busy to make his appointment. Therefore, 32 subjects completed all aspects of the study and were included in the final analysis. On the basis of tablet count, these subjects were adherent to their assigned treatment (94.0 ± 7.4% of all pills prescribed with no difference between the groups).

Baseline characteristics were similar in the two study groups (Table 1), except that serum PSA levels were greater ($P = 0.009$) in the oxandrolone group. Baseline energy, protein, carbohydrate, and fat intakes were similar between the two groups.

### *Changes in Body Composition*

*LBM.* There was a significant ($P < 0.001$) group × time interaction for total LBM. After 12 wk, LBM increased significantly ($P < 0.001$) in the oxandrolone group (3.0 ± 1.5 kg), and this increase in LBM was greater ($P < 0.001$) than the small change (0.0 ± 1.4 kg; $P = 0.91$) in the placebo group (Fig. 1). At *week 24*, LBM (56.5 ± 6.3 kg) had returned to baseline (56.0 ± 5.9 kg) in the oxandrolone group ($P = 0.15$).

Table 1. *Baseline characteristics of the study population*

| | Oxandrolone ($n = 20$) | Placebo ($n = 12$) | *P* Value* |
|---|---|---|---|
| Age, yr | 72.8±6.9 | 71.5±3.2 | 0.49 |
| DEXA weight, kg | 81.3±13.3 | 84.8±8.9 | 0.43 |
| DEXA LBM, kg | 56.5±5.6 | 58.3±5.9 | 0.47 |
| DEXA fat mass, kg | 23.5±7.7 | 23.7±4.4 | 0.51 |
| BMI, kg/m$^2$ | 27.5±3.5 | 29.1±2.9 | 0.20 |
| Caloric intake, kcal/kg | 25.8±6.3 | 25.6±4.5 | 0.87 |
| Intake of protein, g/kg | 1.2±0.4 | 1.1±0.1 | 0.97 |
| Intake of carbohydrate, g/kg | 3.0±0.6 | 3.2±0.7 | 0.47 |
| Intake of fat, g/kg | 1.0±0.3 | 1.0±0.3 | 0.94 |
| Hematocrit, % | 42.9±2.2 | 42.6±3.4 | 0.82 |
| Creatinine, mg/dl | 1.5±1.3 | 1.2±0.4 | 0.34 |
| Albumin, g/dl | 4.0±0.2 | 4.2±0.2 | 0.07 |
| ALT, U/l | 38.0±7.0 | 38.0±4.4 | 0.83 |
| Ultrasensitive CRP, mg/l | 1.4±1.0 | 2.3±2.7 | 0.21 |
| PSA, ng/ml | 2.4±1.1 | 1.3±0.8 | 0.009 |
| Total testosterone, μg/dl | 369±147 | 357±153 | 0.83 |
| Luteinizing hormone, U/l | 8.3±7.1 | 6.5±6.7 | 0.51 |
| Total cholesterol, mg/dl | 186±31 | 186±34 | 0.97 |

Values are means ± 1 SD; *n*, number of subjects. DEXA, dual-energy X-ray absorptiometry; LBM, lean body mass; BMI, body mass index; ALT, alanine aminotransferase; CRP, C-reactive protein; PSA, prostate-specific antigen. **P* value obtained by independent *t*-test.

In the placebo group, the change from baseline in LBM was not significant at either 12 or 24 wk.

*Thigh muscle CSA.* Oxandrolone increased the thigh muscle area (12.4 ± 8.4 $cm^2$, $P < 0.001$; Fig. 2), whereas placebo did not (1.4 ± 6.9 $cm^2$). After 12 wk, the increase in thigh muscle area was greater in the oxandrolone group than in the placebo group ($P = 0.002$). Thigh muscle area was not measured at *week 24*.

*TBW.* Oxandrolone increased TBW (2.9 ± 3.7 kg; $P = 0.002$), whereas placebo did not (−0.6 ± 2.8 kg; $P = 0.47$). After 12 wk, the increase in TBW tended to be greater in the oxandrolone group than in the placebo group ($P = 0.07$). TBW was not measured at *week 24*.

*Fat mass.* There was a significant ($P = 0.03$) group × time interaction for total fat mass. Oxandrolone reduced whole body fat mass (−1.9 ± 1.0 kg, $P < 0.001$; Fig. 3*A*) and trunk fat mass (−1.3 ± 0.6 kg, $P < 0.001$; Fig. 3*B*), whereas placebo did not (whole body = −0.2 ± 1.0 kg, $P = 0.58$; trunk = 0.0 ± 0.7 kg; $P = 0.87$). The decreases in whole body and trunk fat mass were greater in the oxandrolone group than in the placebo group ($P < 0.001$). After oxandrolone was discontinued (*week 24*), whole body and trunk fat were still less than baseline (−1.5 ± 1.8 kg, $P = 0.001$; −1.0 ± 1.1 kg, $P < 0.001$, respectively).

### *Changes in Maximal Voluntary Strength*

There was a significant group × time interaction for chest press ($P < 0.001$), leg press ($P = 0.009$), leg flexion ($P = 0.01$), and latissimus pull-down ($P = 0.04$). After 12 wk, the relative (Fig. 4) and absolute (Table 2) increases in maximal voluntary muscle strength were greater for subjects receiving oxandrolone. These increases were significantly different from the placebo group for leg press and chest press and approached significance for leg flexion and latissimus pull-down, even with our very conservative Bonferroni adjustment. For leg press, relative strength increased by 6.7 ± 6.4% ($P < 0.001$), for leg flexion by 7.0 ± 7.8% ($P < 0.001$), for chest press by 9.3 ± 6.7% ($P < 0.001$), and for latissimus pull-down by 5.1 ± 9.1% ($P = 0.02$, not significant with Bonferroni adjustment) in the group receiving oxandrolone (Fig. 4), whereas there were no significant changes in the placebo group. By *week 24*, the relative and absolute maximal voluntary strength were similar to baseline values in both the oxandrolone and placebo groups (Table 2, Fig. 4).

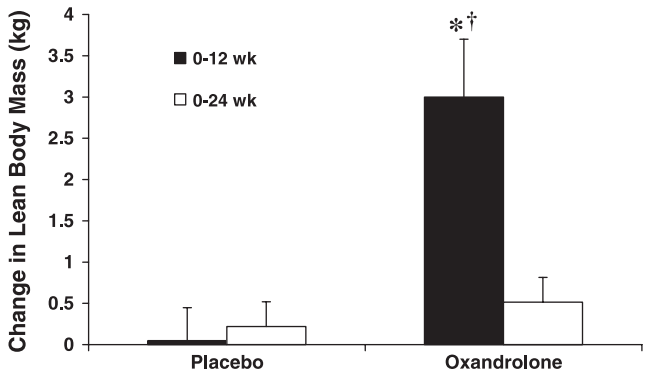


Fig. 1. Absolute change in lean body mass by dual-energy X-ray absorptiometry from baseline to study *week 12* (solid bars) and from baseline to study *week 24* (open bars) in the placebo ($n = 12$) and the oxandrolone ($n = 20$) study groups. Values are means ± SE. *Significant increase from baseline, $P < 0.001$. †Significant difference between study groups for change in lean body mass from 0 to 12 wk, $P < 0.001$.

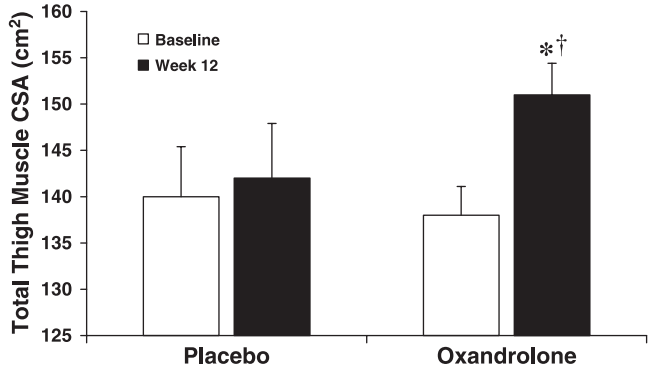


Fig. 2. Absolute measures of cross-sectional area (CSA) by magnetic resonance imaging at baseline (open bars) and study *week 12* (solid bars) in the placebo ($n = 12$) and the oxandrolone ($n = 20$) study groups. Values are means ± SE. *Significant increase from baseline $P < 0.001$. †Significant difference between study groups at *week 12*, $P < 0.01$.

### *Nutrition and Exercise*

Nutritional status, including total daily intake of energy, protein, carbohydrate, and fat, was not different within or

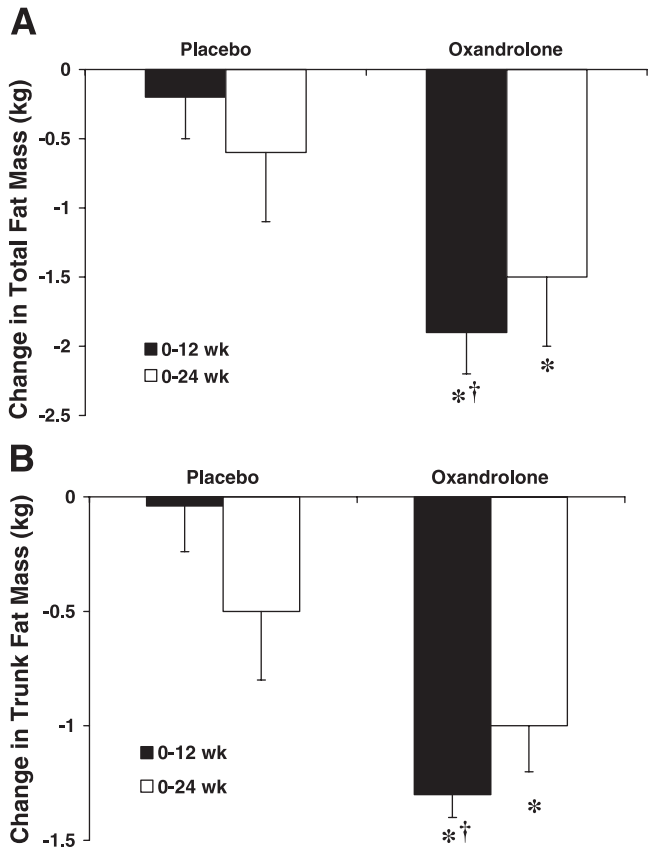


Fig. 3. Absolute change in total fat mass (*A*) and trunk fat (*B*) by dual-energy X-ray absorptiometry from baseline to study *week 12* (solid bars) and from baseline to study *week 24* (open bars) in the placebo ($n = 12$) and the oxandrolone ($n = 20$) study groups. Values are means ± SE. *Significant decrease from baseline, $P < 0.001$. †Significant difference between study groups for change in fat mass from 0 to 12 wk, $P < 0.001$.

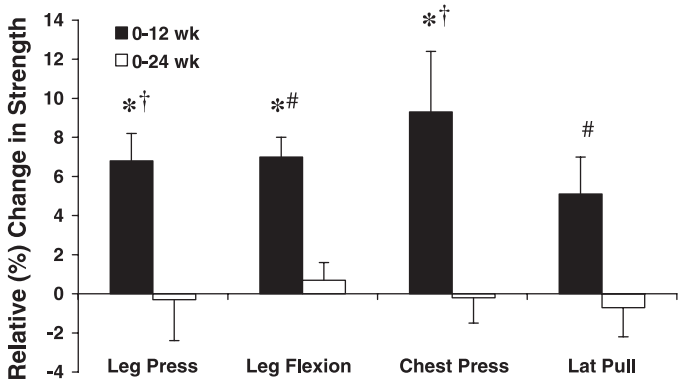


Fig. 4. Relative change in maximum voluntary muscle strength from baseline to study *week 12* (solid bars) and baseline to study *week 24* (open bars) in the oxandrolone ($n = 20$) study group only. Values are means ± SE. *Significant increase from baseline with Bonferroni adjustment, $P < 0.001$. †Significant difference between study groups at *week 12* with Bonferroni adjustment, $P < 0.001$. #Approaching significant increase from baseline for lat pull-down, and an approaching significant difference for leg flexion and lat pull-down between study groups at *week 12* with Bonferroni adjustment, $P < 0.001$.

between groups over the 24-wk course of the study ($P > 0.19$ by ANOVA for each; data not shown). Additionally, on entry into the study, subjects were instructed to maintain their habitual physical activity and not to engage in a new exercise routine during the course of the study. On the basis of self-report at each study evaluation, subjects did not alter their physical activity levels.

### *Safety Evaluation*

One serious adverse event occurred during the study. A subject randomized to oxandrolone developed hypotension (systolic blood pressure <90 mmHg) when his primary doctor modified the patient's antihypertensive medications at the subject's request. His systolic blood pressure had been in the 140- to 155-mmHg range before, and during the study and he desired tighter control. Study therapy was suspended for 3 wk while his antihypertensive medications were adjusted; study therapy was then resumed without problem.

There were no new symptoms or physical findings that could be ascribed to oxandrolone. After 12 wk, there were only modest changes in blood chemistry (Table 3). In the oxandrolone group, serum albumin and alkaline phosphatase levels decreased more than with placebo. The decline in albumin could have reflected the new onset of subclinical inflammation, but there was no change in ultrasensitive CRP levels at *week 12* (Table 3) or *week 24*. There were minimal increments in the liver transaminase levels that reached statistical significance, but ALT was only increased beyond the normal range in two subjects in whom it reached 71 and 99 U/l (≤1.5 times the upper limit of normal). Both subjects were asymptomatic without liver enlargement, and the ALT returned to normal in both at the *week 24* evaluation. Finally, there was a small but significant decrease in PSA in the oxandrolone group.

Table 3. *Change in safety measures after 12 wk of study therapy*

| | Oxandrolone | Placebo | *P* Value* |
|---|---|---|---|
| Hematocrit % | −2.9±2.2 | −2.9±1.5 | 0.95 |
| BUN, mg/dl | −1.0±3.6 | 2.0±4.8 | 0.06 |
| Albumin, g/dl | −0.6±0.2 | −0.3±0.2 | 0.003 |
| ALT, U/l | 15±18 | −1±5 | 0.001 |
| AST, U/l | 8±8 | −1±4 | 0.001 |
| Alkaline phosphatase, U/l | −24±13 | −7±12 | <0.001 |
| Total serum bilirubin, mg/dl | 0±0 | 0±0 | 0.93 |
| Ultrasensitive CRP, mg/l | 0.1±1.9 | 1.0±2.6 | 0.23 |
| PSA, ng/ml | −0.6±0.9 | 0.1±0.5 | 0.004 |
| Luteinizing hormone, U/l | −3.3±6.6 | −0.7±2.2 | 0.13 |
| Total cholesterol, mg/dl | 2±38 | −5±22 | 0.60 |

Values are means ± SD. BUN, blood urea nitrogen; AST, aspartate aminotransferase. **P* value obtained by independent *t*-test.

As described in METHODS, we only measured serum testosterone levels at baseline and *week 24*. Oxandrolone and placebo groups had similar baseline ($P = 0.28$; Table 1) and *week 24* testosterone levels (358 ± 119 ng/dl in the oxandrolone group and 421 ± 196 ng/dl; $P = 0.26$). There was a trend toward a greater decline in LH levels with oxandrolone, suggesting that oxandrolone treatment may have suppressed the hypothalamic-pituitary-gonadal axis.

## DISCUSSION

These findings demonstrated that a relatively brief course of treatment with a potent anabolic androgen in men over 60 yr of age increased LBM as well as upper and lower body maximal

Table 2. *Maximal voluntary skeletal muscle strength*

| | | | | *P* Value | |
|---|---|---|---|---|---|
| | *Week 0* | *Week 12* | *Week 24* | 0 vs. 12 | 0 vs. 24 |
| Leg press, N | | | | | |
| Oxandrolone | 1,245±132 | 1,357±189† | 1,266±191 | <0.001* | 0.81 |
| Placebo | 1,250±213 | 1,250±210 | 1,246±242 | 0.98 | 0.30 |
| Leg flexion, kg | | | | | |
| Oxandrolone | 69.6±9.1 | 74.4±10.6 | 70.5±8.8 | 0.002* | 0.58 |
| Placebo | 66.5±12.5 | 68.1±13.2 | 67.4±12.9 | 0.86 | 0.67 |
| Chest press, N | | | | | |
| Oxandrolone | 212±41 | 233±40† | 214.0±40.5 | <0.001* | 0.89 |
| Placebo | 216±44 | 213±49 | 198±43 | 0.69 | 0.43 |
| Latissimus pull-down, kg | | | | | |
| Oxandrolone | 52.8±9.9 | 55.5±11.0 | 52.4±10.3 | 0.02 | 0.48 |
| Placebo | 54.0±8.5 | 56.6±9.9 | 53.7±8.7 | 0.10 | 0.57 |

Values are means ± SD. **P* value significant at $P < 0.05$ with Bonferroni adjustment for within-group paired *t*-test. †*P* value significant at $P < 0.05$ with Bonferroni adjustment for between-group comparison on the change from baseline to *week 12*.

voluntary strength more than placebo. The increase of 3.0 ± 1.5 kg in LBM in this study is approximately twofold greater than the increase in LBM reported by other investigators using testosterone supplementation in older men (6, 21, 46, 51). The only other study of androgen therapy to achieve comparable increases in LBM (4.2 ± 0.6 kg) used a dose of testosterone enanthate adjusted to produce nadir levels in the upper normal range, suggesting that dosing was "supraphysiological" because nadir levels were tested 2 wk after a prior intramuscular dose (11). Moreover, subjects were treated for 24 wk compared with 12 wk in our study. These observations suggest that the formulation and potency of the androgen, dose, and duration of therapy may affect the changes in lean tissue achieved, which is in keeping with a recent dose ranging study of testosterone in younger men (5).

The significant increases in both upper and lower body maximal voluntary strength in subjects receiving oxandrolone are noteworthy. In the few studies assessing the effects of androgen supplementation in older men, muscle strength was not tested (51) or was evaluated with either handgrip (31, 44) or isokinetic dynamometry (46, 52), which may measure different mechanistic aspects of strength [reviewed in Storer et al. (47)]. Therefore, these evaluations may not be representative of true changes in maximal strength for larger muscle groups important for optimal physical function in older persons. Moreover, only one study demonstrated substantial increases in 1-RM strength in both upper body and lower body muscle groups, although neuromuscular learning may have contributed to the gains in strength with testosterone because multiple baseline trials of maximal strength were not assessed (11). However, older adults typically produce their best performance (highest force production) on the second or third 1-RM trial (12, 40). Thus, studies to assess the affects of anabolic interventions on maximal voluntary strength should test strength on at least two separate occasions before study therapy is initiated.

The increases in muscle strength and CSA in the oxandrolone group suggest that a major portion of the anabolic androgen-induced increase in LBM was due to increases in muscle protein mass, because strength is closely related to muscle size (27). Oxandrolone and testosterone exert their actions by enhancing the rate of mixed muscle (11, 52) and myofibrillar protein synthesis (7), and by reducing the rate of muscle protein breakdown (43). However, our $^2H_2O$ dilution measurements indicated a disproportionate increase in TBW (~2.9 kg) compared with the increase in DEXA-derived LBM (3 kg). If the entire increase in DEXA-derived LBM were protein, we would have anticipated only ~2.3-kg increase in TBW. Also, the rapid loss of LBM (~2.5 kg) after oxandrolone was discontinued suggests that tissue fluid was a component of the oxandrolone-induced increase in LBM. Future studies should measure muscle amino acid balance after androgen administration in elderly men at risk for physical frailty.

To our knowledge, this is the first study to determine the durability of the effects achieved with androgen therapy after the treatment was discontinued. We speculated that at least some portion of the gains in LBM and strength would be sustained 12 wk after treatment with oxandrolone. However, the fact that gains in both LBM and strength were largely lost within 12 wk after treatment was discontinued suggests that prolonged therapy with an anabolic androgen will be necessary to maintain and enhance increases in LBM and muscle strength. Other anabolic strategies with potentially better safety profiles such as resistance training, a potent stimulus for skeletal muscle protein synthesis in older persons (56), or specific androgen receptor modulators should be investigated for sustaining gains in muscle mass and strength during the aging process.

Another important and unique finding of this study was the oxandrolone-induced decrease in total and trunk fat that was largely sustained 12 wk after oxandrolone was stopped. In younger hypogonadal men, testosterone decreased total body and abdominal fat mass (4, 20, 54). However, it is not clear whether androgen therapy affects adipose tissue in eugonadal men. Bhasin et al. (4) reported no change in fat mass with replacement doses of 125 mg testosterone weekly over 4 mo in eugonadal, healthy men, although much higher supraphysiological doses reduced adipose tissue. Marin et al. (24) reported that low-dose androgen therapy reduced abdominal fat in middle-aged men with central obesity. However, the effects occurred primarily in subjects with low testosterone levels, which is consistent with observations that intra-abdominal fat is inversely correlated with free testosterone levels (42). Only five of our subjects had baseline total testosterone levels <270 ng/dl (lower limit of normal in our laboratory), but levels for the entire group were generally less than those of younger men. Whether the relative hypogonadism (compared with younger men) of our participants or the potency or structure of the synthetic androgen, oxandrolone, was primarily responsible for the reductions in whole body and trunk fat is uncertain.

These results do provide clarification as to whether metabolism of testosterone by aromatase to estradiol (~40%) is largely responsible for changes in fat mass when men are treated with testosterone (18). The fact that adipocytes contain estradiol receptors and the observation that estrogen receptor knockout mice have increased adipose tissue have suggested that estrogen is important in downregulating fat mass (8). However, oxandrolone is not aromatized to estrogen, suggesting that the favorable declines in adipose tissue observed in the present study were due to direct and specific actions of oxandrolone.

The discordant effects of oxandrolone on lean tissue and fat mass 12 wk after study therapy was discontinued were puzzling. According to 3-day food diaries and self-report of exercise activity, subjects did not change their dietary or habitual activity during the study. Thus the durability of the effects of oxandrolone on adipose but not lean tissue likely reflect the biological differences in these tissues and/or the effects of other concurrent regulators of metabolism. In a population prone to obesity, it is remarkable that 80% of the reduction in total and central fat mass after a relatively short period of androgen therapy (12 wk) were sustained for at least 3 mo after treatment was discontinued. The reductions in fat mass observed in obese middle-aged men have been associated with decreases in visceral adipose tissue, improvements in insulin sensitivity, and declines in cholesterol, triglycerides, and diastolic blood pressure (24, 25). These effects are consistent with the known effects of androgens to decrease lipoprotein lipase and upregulate β-adrenergic receptors on adipocytes, which would inhibit the accumulation of lipid and enhance the efflux of lipid from these cells in response to catecholamines (26, 38, 55). Further studies will be necessary

to assess whether the reductions in fat mass observed in our older men would be associated with beneficial measures of metabolism and health in an aging population.

A limitation of this study is that we assessed a 17-methylated androgen and not generic testosterone. Thus we cannot extrapolate our findings to a dose of testosterone. Although we did not demonstrate short-term adverse clinical effects with oxandrolone, evaluation of anabolic androgens, including testosterone, as potential treatments for sarcopenia, must be investigated in sufficiently powered studies of long-term treatment to demonstrate their safety for prostate and cardiovascular health.

In conclusion, substantial gains in LBM and muscle size were achieved safely with a relatively short course of therapy with an anabolic androgen in 60- to 87-yr-old men. Moreover, these changes were associated with significant gains in maximal voluntary strength in the large upper and lower body muscle groups, which are important for normal physical function in older persons. However, the benefits were lost within 12 wk after oxandrolone was discontinued, suggesting that prolonged androgen treatment would be needed to maintain these anabolic benefits. Thus the long-term safety and efficacy of androgen therapy in older men need to be established. In addition, whole body and trunk fat mass decreased significantly during therapy, and the effects were largely sustained after treatment was discontinued. Whether the reduction in central adiposity with androgen therapy has tangible health benefits is uncertain. These observations, therefore, raise several important questions that must be addressed before androgen therapy is widely prescribed as long-term therapy for sarcopenia in older individuals.

## ACKNOWLEDGMENTS

We thank the subjects who committed substantial time and efforts to make this study successful. We also appreciate the numerous helpful suggestions made by Colleen Azen, General Clinical Research Center (GCRC) statistician, and the work of Xianghong Chen, who performed the $^{2}H$ analyses in the Biomedical Mass Spectrometry Resource at Washington University Medical School [National Institutes of Health (NIH) National Center for Research Resources Grant RR-00954].

## GRANTS

Support was provided in part from NIH GCRC Grant MOI RR-00043 and by a grant-in-aid from Savient Pharmaceuticals.

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0021-972X/04/$15.00/0
*Printed in U.S.A.*

The Journal of Clinical Endocrinology & Metabolism 89(10):4863–4872

doi: 10.1210/jc.2004-0784

# Effects of Androgen Therapy on Adipose Tissue and Metabolism in Older Men

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**We investigated the effects of oxandrolone on regional fat compartments and markers of metabolism. Thirty-two 60- to 87-yr-old men (body mass index, 28.1 ± 3.4 kg/m$^2$) were randomized to oxandrolone (20 mg/d; n = 20) or matching placebo (n = 12) treatment for 12 wk. Oxandrolone reduced total (−1.8 ± 1.0 kg; $P < 0.001$), trunk (−1.2 ± 0.6 kg; $P < 0.001$), and appendicular (−0.6 ± 0.6 kg; $P < 0.001$) fat, as determined by dual energy x-ray absorptiometry. The changes in total and trunk fat were greater ($P < 0.001$) than the changes with placebo. By magnetic resonance imaging, visceral adipose tissue decreased (−20.9 ± 12 cm$^2$; $P < 0.001$), abdominal sc adipose tissue (SAT) declined (−10.7 ± 12.1 cm$^2$; $P = 0.043$), the ratio VAT/SAT declined from 0.57 ± 0.23 to 0.49 ± 0.19 ($P = 0.002$), and proximal and distal thigh SC fat declined [−8.3 ± 6.7 cm$^2$ ($P < 0.001$) and −2.2 ± 3.0 kg ($P = 0.004$), respectively]. Changes in proximal and distal thigh SC fat with oxandrolone were different than with placebo ($P = 0.018$ and $P = 0.059$). A marker of insulin sensitivity (quantitative insulin sensitivity check index) improved with oxandrolone by 0.0041 ± 0.0071 ($P = 0.018$) at study wk 12. Changes in total fat, abdominal SAT, and proximal extremity SC fat were correlated with changes in fasting insulin from baseline to study wk 12 ($r \geq 0.45$; $P < 0.05$). Losses of total fat and SAT were greater in men with baseline testosterone of 10.4 nmol/liter or less (≤ 300 ng/dl) than in those with higher levels [−2.5 ± 1.1 *vs.* −1.5 ± 0.8 kg ($P = 0.036$) and −24.1 ± 14.3 *vs.* −2.9 ± 21.3 cm$^2$ ($P = 0.03$), respectively]. Twelve weeks after discontinuing oxandrolone, 83% of the reductions in total, trunk, and extremity fat by dual energy x-ray absorptiometry scanning were sustained ($P < 0.02$). Androgen therapy, therefore, produced significant and durable reductions in regional abdominal and peripheral adipose tissue that were associated with improvements in estimates of insulin sensitivity. However, high-density lipoprotein cholesterol decreased by −0.49 ± 0.21 mmol/liter and directly measured low-density lipoprotein cholesterol increased by 0.57 ± 0.67 mmol/liter and non-high-density lipoprotein cholesterol increased by 0.54 ± 0.97 mmol/liter ($P < 0.03$ for each) during treatment with oxandrolone; these changes were largely reversible. Thus, therapy with an androgen that does not adversely affect lipids may be beneficial for some components of the metabolic syndrome in overweight older men with low testosterone levels. (*J Clin Endocrinol Metab* 89: 4863–4872, 2004)**

ADVANCING AGE IS associated with increasing risks for a number of serious medical disorders, including coronary artery disease, stroke, peripheral vascular complications, and diabetes mellitus. Impaired mobility, accumulation of central adiposity, and loss of muscle mass (sarcopenia) contribute to these risks, as do frailty, spontaneous and fall-related bone fractures, and loss of independence. With advancing age, increases in central adiposity, especially visceral adipose tissue (VAT), are associated with increased risk for hypertension, insulin resistance, abnormalities in serum lipids, and impaired fibrinolysis characteristic of the metabolic syndrome (1), also known as the insulin resistance syndrome. This constellation of abnormalities predisposes these older individuals to accelerated atherosclerosis and type 2 diabetes.

Abbreviations: BMI, Body mass index; CSA, cross-sectional area; CV, coefficient of variation; DEXA, dual energy x-ray absorptiometry; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistance; IMCL, intramyocellular lipid; IMF, intermuscular fat; LBM, lean body mass; LDL, low-density lipoprotein; Lp(a), lipoprotein a; MRI, magnetic resonance imaging; QUICKI, quantitative insulin sensitivity check index; SAT, sc adipose tissue; VAT, visceral adipose tissue.

**JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

Cross-sectional (2, 3) and longitudinal (4, 5) studies indicate that total and free levels of serum testosterone decline with advancing age in men. In healthy men, abdominal VAT quantified by computed tomography has been inversely associated with testosterone levels (6), and both natural and experimentally induced hypogonadism are associated with significant increases in fat mass (7, 8). Moreover, in middle-aged men with abdominal obesity and low levels of testosterone, replacement doses of androgen have resulted in significant decreases in VAT, increased lipid turnover in adipocytes, improvements in insulin sensitivity, and declines in blood pressure (9, 10). These observations are consistent with studies in hypogonadal young men, which showed that treatment with testosterone decreases fat mass (11–13).

However, the relationship between gonadal hormone status and age-associated increases in adipose tissue and the metabolic disorders frequently associated with central obesity in older individuals is less certain. Moreover, obesity is associated with decreases in SHBG, which may confound assessment of the relationship of change in testosterone levels and change in fat mass. The effects of testosterone on fat mass have been variable as testosterone treatment in older men have either shown no change (14–16) or only modest reductions in fat (17–19). The variability in the effects of

testosterone treatment in older men may be related to the different formulations of testosterone (im *vs.* transdermal delivery), dose (200 mg biweekly *vs.* 5 mg/d), baseline testosterone status, magnitude of change in testosterone levels in response to therapy, duration of treatment (weeks *vs.* years), and different methods used to assess body composition [*e.g.* magnetic resonance imaging (MRI), computed tomography scanning, dual energy x-ray absorptiometry (DEXA) scanning, hydrostatic weighing, or bioelectrical impedance analysis].

Based on the studies in younger men with low levels of testosterone and central obesity, we hypothesized that androgen therapy in older men would significantly decrease regional abdominal fat mass (visceral and sc fat stores) and appendicular (sc and intermuscular) adipose tissue as well as improve markers of the metabolic syndrome. To test this hypothesis, we conducted a substudy of a placebo-controlled investigation designed to evaluate the effects of treatment with a potent anabolic androgen for 3 months on lean body tissue, skeletal muscle mass, maximum voluntary muscle strength, and physical function (20). The study was unique in that it assessed the durability of effects 3 months after study therapy was discontinued. We, herein, describe the results of the substudy to test our hypothesis that androgen therapy would have beneficial effects on central and peripheral adipose tissue and that these changes would favorably affect systemic markers of the metabolic syndrome.

## Subjects and Methods

### *Study design*

This was an investigator-initiated, double-blind, placebo-controlled study with the primary objective to determine the magnitude and durability of effects of a potent, anabolic androgen, oxandrolone (Oxandrin) on measures of total and regional lean tissue, musculoskeletal muscle mass, and voluntary maximum muscle strength (20). This report describes the substudy to investigate the effects of these interventions on regional fat mass and markers of metabolism. The protocol and informed consent were approved and annually reviewed by the institutional review board of the Los Angeles County-University of Southern California Medical Center. Each study subject provided written informed consent before enrollment. The body composition and metabolic components of the study were performed at the University of Southern California General Clinical Research Center.

### *Study population*

Men at least 60 yr of age were recruited from the surrounding communities of southern California. To be eligible, subjects had to have a body mass index (BMI) of 35 kg/m$^2$ or less and have no untreated endocrine abnormalities (*e.g.* diabetes or hypothyroidism), active inflammatory conditions, uncontrolled hypertension, or cardiac problems in the prior 3 months. Blood tests for eligibility included a prostate-specific antigen level of 4.1 $\mu$g/liter or less and a hematocrit of 50% or less. Subjects performing or planning to initiate vigorous exercise were excluded, but casual walking was allowed.

### *Study interventions*

To provide subjects with increased opportunity to receive active study therapy, we used a 2:1 randomization schedule. Eligible participants were randomized to receive either the approved oral dose (10 mg twice daily) of oxandrolone (Oxandrin, Savient Pharmaceuticals, Inc., East Brunswick, NJ) or matching placebo for 12 wk. The dose of oxandrolone is the FDA-approved dose for treatment of weight loss or inability to maintain normal body weight. Adherence to study therapy was monitored by tablet counts at each study visit. Subjects were reevaluated 12 wk after completing the study therapy (*i.e.* at study wk 24) to assess the durability of effects.

### *Nutritional assessment*

Subjects were instructed to record dietary intake on 3 consecutive days, including 2 weekdays and 1 weekend day during the week before baseline as well as at study wk 12 and 24. Subjects were counseled that the days should be chosen to include usual activities and typical eating patterns. A licensed nutritionist (C.M.) reviewed dietary entries with the subjects. This information was entered into the Nutritionist V software (First Data Bank, San Bruno, CA) and analyzed for total energy intake, macronutrients, and types of fat. Subjects were counseled not to change their routine dietary habits during the course of the study.

### *Body composition by DEXA*

Whole body DEXA scans (QDR-4500, version 7.2 software, Hologic, Inc., Waltham, MA) were obtained at baseline and wk 12 and 24 to quantify lean body mass (LBM) and fat mass. One blinded, experienced technician (C.F.) performed and analyzed the scans. The coefficient of variation (CV) for repeated measures was less than 1% for lean and fat mass.

### *Body composition by MRI*

Cross-sectional area (CSA) of VAT and sc abdominal (SAT) fat were assessed using proton MRI ($^1$H-MRI) at baseline and study wk 12 (but not wk 24). $^1$H-MRI was performed using a 1.5 Tesla GE Signa-LX scanner with the body coil used as both transmitter and receiver. Single proximal and distal thigh slices were acquired after obtaining a T1-weighted coronal scout image (T1-weighted TR/TE 300/TE) that was used to demarcate the proximal and distal thigh by bisecting the femur into three segments. The mid of seven slices proximal and seven slices distal with a slice thickness of 7.5 mm and a 1.5-mm gap was analyzed for comparison. The junction of the proximal and middle third of the femur served as the proximal thigh, and the junction of the middle third and distal femur served as the distal thigh. The field of view was 24 $\times$ 24 cm with a 254 $\times$ 128-pixel matrix. One signal average was used.

Subcutaneous and visceral adipose CSA were measured by analyzing a single slice at the junction of the fourth and fifth lumbar vertebrae using specialized software (SliceOmatic version 4.2, TomoVision, Montréal, Canada). Because each pixel reflects a given density, regions of adipose tissue are segregated from other regions of tissue using the SliceOmatic Morpho mode of analysis. This allows the tissue compartments [sc fat, muscle, intermuscular fat (IMF), *etc.*] to be segmentalized based on signal amplitude by highlighting small regions of similar density pixels to determine adipose CSA. Subcutaneous and visceral adipose CSA were calculated after highlighting the respective adipose tissue regions with different colors. Similarly, CSA of thigh sc fat and IMF were calculated after highlighting the respective pixels with different colors. IMF was defined as high intensity pixels located between and within the thigh muscles, excluding the femur.

MRI scans were not performed at study wk 24. The same technician (M.O.), blinded to treatment, located the regions of interest and performed all image analyses. The CV for repeated measures of sc and visceral adipose CSA was less than 2%.

### *Serum lipid and lipoprotein concentrations*

Blood was collected after a 14-h overnight fast at baseline, study wk 12, and study wk 24 for plasma lipid determinations. Plasma was analyzed for total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides using the Ortho/Vitros DTII system (Ortho Diagnostics, Rochester, NY) in the University of Southern California General Clinical Research Center core laboratory. Plasma lipid concentrations in baseline, wk 12, and wk 24 samples for each subject were run in the same assay to eliminate the effects of interassay variation. The CVs for the three lipids were less than 4.5%, less than 4.4%, and less than 3.0%, respectively.

Low-density lipoprotein (LDL) cholesterol was measured directly with an enzymatic assay using photometric detection (Cobas Integra 400 System, Roche, Indianapolis, IN). The sensitivity of the assay was de-

fined as the change in the analytical response (ΔA) per unit change in analyte concentration at a path length of 1 cm. The sensitivity was 0.18 ΔA/mmol/liter LDL cholesterol. The precision of the assay was evaluated on the Integra 700 using two human serum pools and following the guidelines of the National Committee for Clinical Laboratory Standards manual EP5-T2. The level 1 CV was 1.9%, and the level 2 CV was 2.1%. Lipoprotein a [Lp(a)] was determined using an immunoassay, Macra Lp(a) (Wampole, Trinity Biotech, Jamestown, NY). The sensitivity of the test was 0.03 μmol/liter, and the CV was less than 10%.

### *Fasting blood sugar*

Blood was collected after a 14-h overnight fast in prechilled heparinized tubes. Plasma was removed and frozen within 10 min of collection. Glucose was measured by the glucose oxidase method (model 2300 STAT PLUS glucose analyzer, YSI, Inc., Yellow Springs, OH) with a CV of 2.5%.

### *Hormone concentrations*

The serum testosterone concentration was measured by the Los Angeles County-University of Southern California Medical Center Clinical Diagnostic Laboratory (endocrinology section) using Coat-A-Count (Diagnostic Products Corp., Los Angeles, CA). This competitive RIA uses a solid phase polyclonal antibody. The CV for total testosterone was 7.7% or less. The LH concentration (international units per milliliter) was measured using a microparticle enzyme immunoassay (AxSYM, Abbott Diagnostics, Chicago, IL). The CV for LH was 4.9% or less.

Insulin was measured by RIA (Linco Research, Inc., St. Charles, MO), which had less than 0.2% cross-reactivity with proinsulin and a CV of 3.2%. Glucose and insulin concentrations from baseline, 12-wk, and 24-wk tests for each subject were measured in the same assay to eliminate the effects of interassay variation.

### *Estimates of insulin resistance*

The homeostasis model assessment for insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI) were used to estimate insulin resistance. These estimates correlate with insulin sensitivity measured using the hyperinsulinemic euglycemic clamp (21, 22). HOMA-IR was calculated as $[(I_f) \times (G_f)]/22.5$, where $(I_f)$ is the fasting insulin level (microunits per milliliter) and $(G_f)$ is the fasting glucose level (millimoles per liter). QUICKI was calculated as $1/[\log (I_f) + \log (G_f)]$ (21).

### *Safety monitoring*

To assess the safety of the study therapy, blood was collected every 3 wk during treatment. Safety tests included complete blood counts, comprehensive chemistries with tests of renal and hepatic functions, and prostate-specific antigen. These tests were repeated at study wk 24.

### *Statistical considerations*

The study was conservatively powered at 80% to detect a difference in means between the oxandrolone and placebo groups of 1.36 times the common SD, using a two-sample *t* test with a Bonferroni-adjusted $P = 0.01$, with 20 in oxandrolone group and 10 in placebo group (20). The power calculation was based on the primary outcome, total LBM by DEXA scanning. As such, the sample size was selected to detect a mean difference of 2 kg, assuming the common SD is 1.5 kg. For the substudy, this sample size would provide a power of at least 85% to show a 1.0-kg difference in the change in trunk fat between the groups by DEXA scanning assuming a common SD of 0.8 kg and a 25-$cm^2$ difference in the change in abdominal VAT by MRI assuming a common SD of change of 20 $cm^2$.

For the main outcome variables, a two (oxandrolone and placebo group) by three (baseline, wk 12, and wk 24) repeated measure ANOVA was used to statistically compare mean differences within subjects and between groups. Greenhouse-Geisser adjustment was used to justify the assumption of sphericity. When a significant group by time interaction was found, the changes from baseline to wk 12 and the changes from baseline to wk 24 between and within groups were compared by independent *t* tests and paired *t* tests, respectively. All *post hoc* tests were performed with Bonferroni adjustment for six possible comparisons. Baseline characteristics and the changes in safety evaluation from baseline to wk 12 were compared between oxandrolone and placebo group using an independent *t* test.

The change in fat measured from baseline to wk 12 by DEXA and MRI was also compared between low testosterone (≤10.4 nmol/liter; ≤300 ng/dl) and normal testosterone (>10.4 nmol/liter; >300 ng/dl) using both independent *t* tests and Wilcoxon rank-sum tests. The significant between-strata differences observed in independent *t* tests were also demonstrated in Wilcoxon rank-sum tests. Based on the distribution of data, the associations between the change in body fat from baseline to study wk 12 compared with baseline characteristics were tested with Spearman's correlation coefficients. Associations between the change in fat and changes in metabolic markers from baseline to study wk 12 were tested with Pearson's correlation coefficients.

All statistical testing was performed with a two-sided 5% level of significance (0.83% for each *post hoc t* test) using Statistical Analysis System version 8.0 (SAS Institute, Inc., Cary, NC). Statistical analyses are presented in the tables and text as the mean ± 1 SD.

## Results

### *Subjects (Table 1)*

Thirty-two subjects were randomized to receive either oxandrolone (n = 20) or placebo (n = 12). As reported previously (20), the baseline characteristics of the two treatment groups were similar, including indirect measures of body composition (weight and BMI), markers of inflammation (albumin and C-reactive protein), and thyroid and hypothalamic-pituitary-gonadal functions (Table 1). Two subjects (one assigned to each group) did not return for the wk 24 evaluation; thus, there were 19 subjects in the oxandrolone group and 11 in the placebo group at the last evaluation.

### *BMI*

The average BMI of the total population of 32 subjects was 28.1 ± 3.4 $kg/m^2$. Of these, 20 subjects had a BMI in the overweight range of 25–29.9 $kg/m^2$, and seven others had a BMI of 30 $kg/m^2$ or more, indicating obesity. Thus, 27 (84%) of 32 subjects were either overweight or obese.

**TABLE 1.** Baseline characteristics of the study population

| | Oxandrolone | Placebo | *P* value[a] |
|---|---|---|---|
| n | 20 | 12 | |
| Age (yr) | 72.8 ± 6.9 | 71.5 ± 3.2 | 0.49 |
| Weight (kg) | 81.3 ± 13.3 | 84.8 ± 8.9 | 0.43 |
| BMI ($kg/m^2$) | 27.5 ± 3.5 | 29.1 ± 2.9 | 0.20 |
| Hemoglobin (mmol/liter) | 2.3 ± 0.1 | 2.3 ± .1 | 0.76 |
| Creatinine (mmol/liter) | 0.13 ± 0.16 | 0.12 ± 0.04 | 0.34 |
| Albumin (μmol/liter) | 606 ± 30 | 636 ± 30 | 0.07 |
| ALT (U/liter) | 38 ± 7.0 | 38 ± 4.4 | 0.83 |
| Ultrasensitive CRP (μg/liter) | 1.4 ± 1.00 | 2.3 ± 2.7 | 0.21 |
| TSH (U/liter) | 1.86 ± 1.31 | 1.97 ± 0.63 | 0.79 |
| Total testosterone (nmol/liter) | 12.8 ± 5.1 | 12.4 ± 5.3 | 0.83 |
| LH (U/liter) | 8.3 ± 7.1 | 6.5 ± 6.7 | 0.51 |

Values are the mean ± 1 SD. ALT, Alanine aminotransferase; CRP, C-reactive protein. For conventional metric units, divide hemoglobin by 0.1550 for g/dl, creatinine by 0.8840 for mg/dl, albumin by 151.5 for g/dl, and total testosterone by 0.0345 for ng/dl.

[a] *P* value was obtained by independent *t* test.

*Nutrition and exercise (Table 2)*

During the course of the study, there were no significant changes in total caloric or macronutrient intake when analyzed by ANOVA with week as the repeated measure for study group using Greenhouse-Geisser adjustment (Table 2). Additionally, upon entry into the study, subjects were instructed to maintain their habitual physical activity and not to engage in a new exercise program during the course of the study. Based on self-report at each study evaluation, subjects did not alter their physical activity levels.

*Body composition (Table 3 and Figs. 1 and 2)*

*Changes in fat mass by DEXA scanning.* There was a significant ($P = 0.03$) group by time (baseline, study wk 12, and study wk 24) interaction for total fat mass. At study wk 12, oxandrolone reduced whole body fat mass ($-1.8 \pm 1.0$ kg; $P < 0.001$) and trunk fat mass ($-1.2 \pm 0.6$ kg; $P < 0.001$), whereas placebo did not [whole body, $-0.2 \pm 1.0$ kg ($P = 0.58$); trunk, $0.0 \pm 0.7$ kg ($P = 0.87$); Table 3]. The decreases in whole body and trunk fat mass were greater in the oxandrolone group than the changes in the placebo group ($P < 0.001$). Seven

**TABLE 2.** Measures of dietary intake

| | Oxandrolone | | | Placebo | | |
|---|---|---|---|---|---|---|
| | Wk 0 | Wk 12 | Wk 24 | Wk 0 | Wk 12 | Wk 24 |
| Total calories (kcal/kg·d) | 25.9 ± 6.3 | 25.3 ± 7.2 | 26.2 ± 8.3 | 25.2 ± 4.6 | 23.0 ± 2.6 | 22.6 ± 4.9 |
| % Protein | 19 ± 5.4 | 20 ± 5.8 | 20 ± 7 | 18 ± 1 | 19 ± 2 | 18 ± 4 |
| % Carbohydrate | 47 ± 9.9 | 46 ± 11 | 47 ± 12 | 50 ± 12 | 47 ± 12 | 48 ± 16 |
| % Total fat | 34 ± 11.8 | 34 ± 13 | 32 ± 12 | 32 ± 8 | 33 ± 8 | 33 ± 10 |
| % Saturated fat | 11 ± 3.6 | 11 ± 4.3 | 10 ± 4 | 11 ± 3 | 12 ± 6 | 10 ± 3 |

*P* values for each dietary parameter using two-way ANOVA with week as repeated measure for treatment group × week with Greenhouse-Geisser adjustment were all greater than 0.20.

**TABLE 3.** Measures of regional abdominal and appendicular adipose tissue

| | Wk 0 | Wk 12 | Wk 24 | *P* value | |
|---|---|---|---|---|---|
| | | | | 0 *vs.* 12 | 0 *vs.* 24 |
| DEXA | | | | | |
| Total fat (kg) | | | | | |
| Oxandrolone | 23.5 ± 7.7 | 21.7 ± 7.4[a] | 22.0 ± 7.3 | <0.001 | 0.001 |
| Placebo | 23.7 ± 4.4 | 23.5 ± 4.3 | 22.9 ± 5.6 | 0.58 | 0.29 |
| Trunk fat (kg) | | | | | |
| Oxandrolone | 13.7 ± 4.2 | 12.4 ± 4.1[a] | 12.7 ± 4.1 | <0.001 | 0.001 |
| Placebo | 13.2 ± 2.4 | 13.2 ± 2.4 | 12.6 ± 3.4 | 0.87 | 0.17 |
| Appendicular fat (kg; 4 extremities) | | | | | |
| Oxandrolone | 8.9 ± 3.6 | 8.3 ± 3.5[b] | 8.2 ± 3.5 | <0.001 | 0.014 |
| Placebo | 9.5 ± 2.2 | 9.3 ± 2.2 | 9.3 ± 2.5 | 0.22 | 0.70 |
| MRI | | | | | |
| VAT ($cm^2$) | | | | | |
| Oxandrolone (n = 20) | 136.2 ± 53.3 | 115.3 ± 51.8 | | <0.001 | |
| Placebo (n = 11) | 157.6 ± 47.5 | 146.9 ± 53.9 | | 0.30 | |
| SAT ($cm^2$) | | | | | |
| Oxandrolone | 255.1 ± 92.7 | 244.4 ± 93.7 | | 0.04 | |
| Placebo | 260.8 ± 63.6 | 260.7 ± 60.4 | | 0.98 | |
| VAT/SAT abdominal ratio | | | | | |
| Oxandrolone | 0.57 ± 0.23 | 0.49 ± 0.19 | | 0.002 | |
| Placebo | 0.62 ± 0.19 | 0.58 ± 0.21 | | 0.18 | |
| MRI | | | | | |
| Proximal sc thigh fat ($cm^2$) | | | | | |
| Oxandrolone | 98.3 ± 41.7 | 90.0 ± 38.7[c] | | <0.001 | |
| Placebo | 104.9 ± 25.4 | 104.1 ± 27.5 | | 0.79 | |
| Distal sc thigh fat ($cm^2$) | | | | | |
| Oxandrolone | 41.8 ± 18.3 | 39.6 ± 16.4[d] | | 0.004 | |
| Placebo | 47.5 ± 11.3 | 51.5 ± 17.9 | | 0.36 | |
| Proximal IMF ($cm^2$) | | | | | |
| Oxandrolone | 25.6 ± 6.2 | 24.5 ± 5.2 | | 0.43 | |
| Placebo | 29.5 ± 8.6 | 27.4 ± 9.9 | | 0.32 | |
| Distal IMF ($cm^2$) | | | | | |
| Oxandrolone | 32.5 ± 20.5 | 26.1 ± 6.6 | | 0.22 | |
| Placebo | 26.7 ± 9.4 | 26.8 ± 8.8 | | 0.98 | |

*P* values are shown for between-group comparison of the change from study wk 0 (baseline) to study wk 12.
[a] $P < 0.001$.
[b] $P = 0.08$.
[c] $P = 0.018$.
[d] $P = 0.059$.

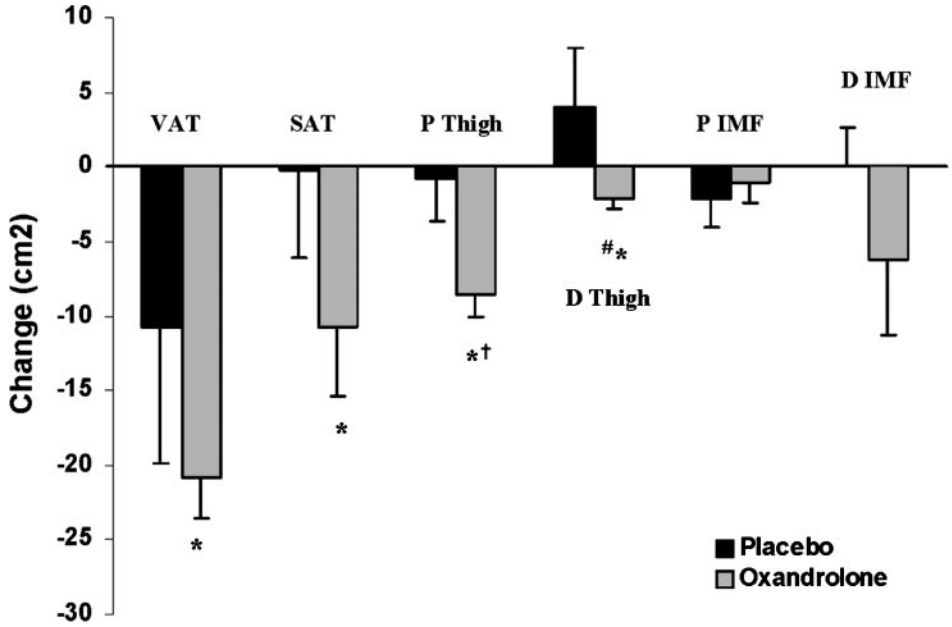


FIG. 1. Absolute change in fat mass, determined by MRI, between baseline and study wk 12 for VAT, SAT of the abdomen, and SAT of the proximal and distal thigh (P Thigh and D Thigh, respectively) and the IMF of the proximal and distal thigh (P IMF and D IMF, respectively). The whiskers are 1 SE. *, Significant ($P < 0.001$) within-group difference from baseline; †, significant ($P = 0.018$) difference in the change between study groups; #, near-significant difference ($P = 0.059$) in the change for the study groups.

subjects with baseline testosterone levels of 10.4 nmol/liter or less lost a total of $-2.5 \pm 1.1$ kg, whereas the 13 subjects with baseline testosterone levels greater than 10.4 nmol/liter lost only $-1.5 \pm 0.8$ kg during treatment with oxandrolone ($P = 0.036$, by Wilcoxon rank-sum test). Moreover, the change in trunk fat at wk 12 was correlated ($r = 0.5$; $P = 0.02$) with baseline testosterone. At study wk 12, there was also a significant ($P < 0.001$) decrease in total appendicular fat ($-0.6 \pm 0.6$ kg), but this difference was not statistically different ($P = 0.08$) from the decrease in appendicular fat ($-0.2 \pm 0.6$; $P = 0.22$) in the group receiving placebo.

Twelve weeks after discontinuing study treatment (wk 24), whole body, trunk, and appendicular fat, determined by DEXA scanning, were still less than baseline [$-1.5 \pm 1.7$ kg ($P = 0.001$), $-1.0 \pm 1.1$ kg ($P = 0.001$), and $-0.5 \pm 0.8$ ($P = 0.014$), respectively] for the group receiving oxandrolone. However, differences were not statistically different ($P > 0.10$) from the small changes in total, trunk, and appendicular fat ($-0.6 \pm 1.6$, $-0.5 \pm 1.2$, and $-0.5 \pm 0.8$ kg, respectively) in the placebo group.

*Change in fat by MRI scanning.* At study wk 12, VAT significantly ($P < 0.001$) declined by $-20.9 \pm 12$ cm$^2$, whereas the change in the placebo group ($-10.7 \pm 32$ cm$^2$) was not significant ($P = 0.30$; Table 3 and Fig. 1). The difference in change between the groups was not significant ($P = 0.22$). Similarly, abdominal SAT declined significantly ($P = 0.043$) by $-10.7 \pm 12.1$ cm$^2$ with oxandrolone, but only $-0.2 \pm 20.4$ with placebo ($P = 0.98$); the difference in change between the groups was not significant ($P = 0.20$). The improvement in abdominal SAT with oxandrolone was greater ($P = 0.03$) in subjects with baseline testosterone levels of 10.4 nmol/liter or less compared with those with levels greater than 10.4 nmol/liter ($-24.1 \pm 14.3$ *vs.* $-2.9 \pm 21.3$ cm$^2$, respectively). Finally, the VAT/SAT ratio declined significantly ($P = 0.002$) from $0.57 \pm 0.23$ to $0.49 \pm 0.19$ after 12 wk in the oxandrolone group. However, the change from $0.62 \pm 0.19$ to $0.58 \pm 0.21$ in the placebo group was not significant ($P = 0.18$), nor was the difference in change between the groups ($P = 0.11$).

The group receiving oxandrolone also experienced significant ($P < 0.001$) decreases of $-8.3 \pm 6.7$ cm$^2$ in proximal sc thigh fat, whereas the change in the placebo group of $-0.8 \pm 9.8$ cm$^2$ was not significant ($P = 0.79$), but the difference in the changes in these two groups was significant ($P = 0.018$). The changes in distal sc thigh fat were smaller, but significant ($P = 0.004$), with oxandrolone ($-2.2 \pm 3.0$ cm$^2$); this difference was not quite significantly different ($P = 0.059$) from the nonsignificant change ($P = 0.36$) in the placebo group of $4.0 \pm 13.8$ cm$^2$. The changes in proximal thigh IMF of $-1.1 \pm 5.9$ cm$^2$ and $-2.1 \pm 6.8$ cm$^2$ in the oxandrolone and placebo groups, respectively, were not significant ($P = 0.43$ and $P = 0.32$). Similarly, the changes in distal thigh IMF of $-6.4 \pm 22.5$ and $0.1 \pm 8.9$ cm$^2$ in the oxandrolone and placebo groups, respectively, were not significant ($P = 0.22$ and $P = 0.98$). These small changes in IMF were not different between the groups ($P > 0.40$).

### *Glucose metabolism and estimates of insulin resistance (Table 4)*

There were no significant group by study week interactions (two-way ANOVA with Greenhouse-Geisser adjustment) for fasting blood sugar ($P = 0.26$), fasting insulin ($P = 0.41$), HOMA-IR ($P = 0.53$), or QUICKI ($P = 0.10$). Similarly, there were no significant within-group changes ($P > 0.05$) for HOMA-IR. However, there was a significant ($P = 0.018$) increase in QUICKI ($0.0041 \pm 0.0071$) at study wk 12 for the oxandrolone group (Table 4). The increase in QUICKI (from wk 0 to wk 12) was greater in the oxandrolone group than in the placebo group ($P = 0.045$).

### *Lipid metabolism (Table 4)*

There were no significant group by study week interactions (two-way ANOVA with Greenhouse-Geisser adjust-

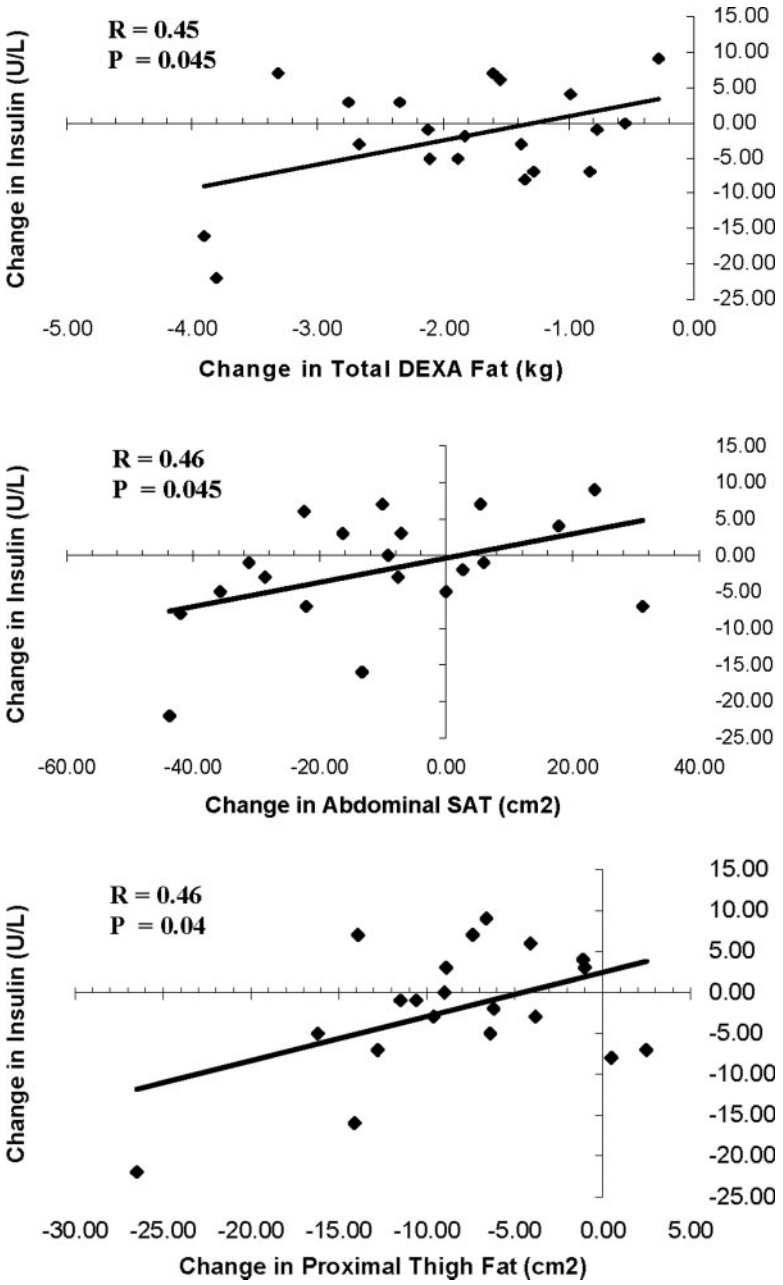


FIG. 2. Change in fasting insulin related (Pearson correlation coefficients) to change in total fat determined by DEXA (*top panel*), change in abdominal SAT determined by MRI (*middle panel*), and change in proximal sc thigh fat determined by MRI (*lower panel*) from baseline to study wk 12 for subjects receiving oxandrolone (n = 20).

ment) for total cholesterol ($P = 0.75$), LDL cholesterol ($P = 0.06$), non-HDL cholesterol ($P = 0.20$), or fasting triglycerides ($P = 0.058$). There were significant group by study week interactions for HDL cholesterol ($P < 0.001$) and Lp(a) ($P = 0.006$). At study wk 12, HDL cholesterol decreased ($-0.49 \pm 0.21$ mmol/liter) significantly ($P < 0.001$) in the oxandrolone group. HDL cholesterol decreased modestly with placebo ($-0.07 \pm 0.12$ mmol/liter) at wk 12; thus, the difference in change between the two groups was significant ($P < 0.001$). The effects of oxandrolone on HDL cholesterol were not sustained, and there was a rebound to levels greater than baseline at study wk 24 (Table 4).

At study wk 12, directly measured LDL cholesterol increased by $0.57 \pm 0.67$ mmol/liter in the oxandrolone group ($P = 0.001$) and remained greater than baseline at study wk 24 ($0.41 \pm 0.57$ mmol/liter; $P = 0.004$). The increase in LDL chcholesterol from study wk 0 to wk 12 was greater in the oxandrolone group than in the placebo group ($P = 0.008$). Non-HDL cholesterol increased significantly ($P = 0.022$) during treatment with oxandrolone by $0.54 \pm 0.97$ mmol/liter, which was significantly different ($P = 0.03$) than the small nonsignificant decrease in non-HDL cholesterol with placebo ($-0.06 \pm 0.47$ mmol/liter) at study wk 12. The increase in non-HDL cholesterol of $0.39 \pm 0.70$ mmol/liter at wk 24 in the oxandrolone group remained significant ($P = 0.02$), but the change was not different ($P = 0.45$) from the nonsignificant change with placebo ($P = 0.52$).

At study wk 12, Lp(a) declined by $252 \pm 283$ $\mu$mol/liter in the oxandrolone group ($P = 0.001$), whereas there was a small increase of $16 \pm 56$ $\mu$mol/liter in the placebo group

**TABLE 4.** Change in metabolic markers

| | Wk 0 | Wk 12 | Wk 24 | *P* value | |
|---|---|---|---|---|---|
| | | | | 0–12 wk | 0–24 wk |
| Fasting blood sugar (mmol/liter) | | | | | |
| Oxandrolone | 5.3 ± 0.7 | 4.8 ± 2.1 | 5.9 ± 3.2 | 0.39 | 0.39 |
| Placebo | 6.5 ± 3.5 | 6.1 ± 2.9 | 6.1 ± 2.7 | 0.08 | 0.14 |
| Fasting insulin (μU/ml) | | | | | |
| Oxandrolone | 20.3 ± 10.7 | 18.2 ± 9.0 | 19.4 ± 8.4 | 0.25 | 0.66 |
| Placebo | 17.0 ± 6.1 | 18.4 ± 6.8 | 19.4 ± 8.3 | 0.22 | 0.12 |
| HOMA-IR | | | | | |
| Oxandrolone | 4.67 ± 2.42 | 3.99 ± 2.99 | 5.14 ± 3.70 | 0.17 | 0.48 |
| Placebo | 5.26 ± 4.30 | 5.22 ± 3.98 | 5.52 ± 4.22 | 0.93 | 0.43 |
| QUICKI | | | | | |
| Oxandrolone | 0.134 ± 0.008 | 0.139 ± 0.009[a] | 0.134 ± 0.008 | 0.018 | 0.54 |
| Placebo | 0.135 ± 0.011 | 0.134 ± 0.010 | 0.134 ± 0.010 | 0.60 | 0.29 |
| Total cholesterol (mmol/liter) | | | | | |
| Oxandrolone | 4.8 ± 0.8 | 4.9 ± 1.1 | 5.4 ± 1.1 | 0.85 | 0.005 |
| Placebo | 4.8 ± 0.9 | 4.7 ± 0.8 | 5.0 ± 0.8 | 0.46 | 0.26 |
| Fasting triglycerides (mmol/liter) | | | | | |
| Oxandrolone | 1.1 ± 0.5 | 1.0 ± 0.4[b] | 1.3 ± 0.5 | 0.12 | 0.11 |
| Placebo | 1.0 ± 0.6 | 1.2 ± 0.9 | 1.1 ± 0.5 | 0.22 | 0.77 |
| HDL cholesterol (mmol/liter) | | | | | |
| Oxandrolone | 1.0 ± 0.3 | 0.5 ± 0.2[a] | 1.2 ± 0.4 | <0.001 | 0.009 |
| Placebo | 1.2 ± 0.3 | 1.1 ± 0.3 | 1.3 ± 0.3 | 0.08 | 0.005 |
| LDL cholesterol direct (mmol/liter) | | | | | |
| Oxandrolone | 2.9 ± 0.6 | 3.4 ± 0.9[a] | 3.3 ± 0.8 | 0.001 | 0.004 |
| Placebo | 2.8 ± 0.6 | 2.7 ± 0.5 | 2.8 ± 0.5 | 0.67 | 0.66 |
| Non-HDL cholesterol (mmol/liter) | | | | | |
| Oxandrolone | 3.8 ± 0.7 | 4.3 ± 1.0[a] | 4.2 ± 1.0 | 0.02 | 0.02 |
| Placebo | 3.7 ± 0.8 | 3.6 ± 0.6 | 3.7 ± 0.7 | 0.68 | 0.52 |
| Lp(a) (μmol/liter) | | | | | |
| Oxandrolone | 0.36 ± 0.40 | 0.05 ± 0.02[a] | 0.14 ± 0.46 | 0.001 | 0.10 |
| Placebo | 0.31 ± 0.25 | 0.33 ± 0.25 | 0.29 ± 0.21 | 0.35 | 0.46 |
| Ultrasensitive CRP (μg/liter) | | | | | |
| Oxandrolone | 2.3 ± 2.7 | 2.3 ± 2.9 | 1.9 ± 1.8 | 0.90 | 0.24 |
| Placebo | 1.5 ± 1.0 | 2.6 ± 2.9 | 1.5 ± 0.7 | 0.20 | 0.83 |

For conventional metric units divide: fasting blood sugar by 0.05551 for mg/dl; total, LDL, HDL, and non-HDL cholesterol by 0.02586 for mg/dl; fasting triglycerides by 0.01129 for mg/dl; and LP(a) by 0.0357 for mg/dl. CRP, C-reactive protein.

[a] Change from study wk 0 to 12 was significantly different from placebo group, $P < 0.05$.

[b] Change from study wk 0 to 12 differed from placebo group, $P = 0.053$.

($P = 0.35$). The decrease in Lp(a) from study wk 0 to wk 12 was greater in the oxandrolone group than in the placebo group ($P < 0.001$).

*Associations of hormone levels and changes in fat (Fig. 2)*

Three different estimates of insulin sensitivity, namely, fasting insulin, HOMA-IR, and QUICKI at baseline, were related (Spearman correlation coefficients of −0.53, −0.49, and 0.49, respectively) to change in total fat determined by DEXA scanning from baseline to study wk 12 ($P = 0.016$, 0.03, and 0.03, respectively) for the group receiving oxandrolone. Similarly, these baseline markers of insulin sensitivity were related ($r = -0.45$, −0.49, and 0.49, respectively) to the change in trunk fat determined by DEXA during the 12 wk of study therapy ($P = 0.048$, 0.03, and 0.03, respectively). Importantly, the change in fasting insulin was correlated (Pearson correlation coefficients) with the change in total fat determined by DEXA ($r = 0.45$; $P = 0.045$), as was the change in abdominal SAT and proximal thigh sc fat by MRI and fasting insulin ($r = 0.46$; $P = 0.045$ and $r = 0.46$; $P = 0.04$, respectively) from baseline to study wk 12 in the oxandrolone group (Fig. 2). The small changes in estimates of insulin sensitivity were not related to the small, nonsignificant changes in various total or regional measures of body fat with placebo ($P > 0.05$).

*Safety evaluation*

During the study there were no new symptoms or physical findings that could be ascribed to oxandrolone (20). In addition, there were no significant changes or differences in prostate symptoms, prostate-specific antigen levels, or hematocrit during therapy with oxandrolone and placebo (20).

## Discussion

The findings of this study indicate that treatment with an anabolic androgen may produce substantial reductions in central and appendicular fat mass in older, largely overweight or obese men at risk for the metabolic syndrome. To our knowledge, this is the first study of androgen therapy in an aging population to quantify regional changes in sc and deep adipose tissue using both DEXA scanning and MRI. Although worsening of the atherogenic lipid profile with oxandrolone would preclude prolonged use of this agent in older subjects at risk for cardiovascular complications, the study provides important new information about the poten-

tial effects of androgen therapy in older subjects with increased fat stores.

Three prior studies of testosterone therapy in older men reported reductions of total fat by up to 3.0 kg (18, 19, 23). In the largest of these studies, involving 108 subjects treated for 3 yr, there was no reduction in trunk fat determined by DEXA scanning (23). In our study, DEXA scanning showed significant decreases in central fat during treatment with oxandrolone. Moreover, by MRI there were significant reductions in both abdominal VAT and SAT with oxandrolone. These effects on abdominal fat are consistent with the known effects of androgens to decrease lipoprotein lipase and up-regulate $\beta$-adrenergic receptors on adipocytes to inhibit the accumulation of lipid and enhance the efflux of lipid from these cells in response to catecholamines (10, 24, 25). Importantly, the amount of VAT has been directly correlated with insulin resistance (26, 27) and various markers of atherosclerosis (28, 29), and abdominal SAT has been positively correlated to the acute insulin response (27). In our study VAT decreased more than abdominal SAT with oxandrolone treatment, as reflected by the significant between-group decrease in the VAT/SAT ratio, which is consistent with the larger reductions in VAT than SAT during diet-induced weight loss (30). These improvements in abdominal fat would be expected to result in favorable effects on risks for cardiovascular complications in older subjects.

DEXA scanning also revealed significant reductions in appendicular (extremity) fat. Similarly, MRI of the dominant leg showed significant reductions in sc fat in both the proximal and the distal thigh. Although several studies have suggested that sc extremity fat is directly related to insulin resistance (31, 32), others suggest that intramyocellular lipid (IMCL) is the most important peripheral site of fat relative to insulin sensitivity (33, 34). However, accurately determining IMCL requires proton or carbon MR spectroscopy, which to date has not been performed in older subjects treated with androgens. Because evidence suggests that IMF may also be correlated with insulin sensitivity in type 2 diabetes (32), we examined IMF at the distal and the proximal thigh using MRI. Oxandrolone had no appreciable effect on IMF, but the lack of change may have been due to the small volume of IMF in the cross-sectional area at only two landmarks of the thigh and may require measurement of the entire thigh muscle volume.

To our knowledge, this is also the first study to determine the durability of effects achieved with androgen therapy after treatment was discontinued. Unlike the effects on LBM and skeletal muscle strength, which generally returned to baseline (20), the reductions in both central and peripheral fat mass were largely (>80%) sustained 3 months after treatment with oxandrolone was discontinued. Our nutritionists carefully assessed dietary intake with 3-d food diaries and self-report of exercise activity during the 24 wk of the study. Because there were no significant changes in consumption of daily total calories or specific macronutrients, and habitual activity did not change, this suggests that the observed alterations in body composition and metabolic markers were related to the effects of the androgen treatment. We speculate that the durability of the effects of oxandrolone on adipose tissue, but not lean mass, may reflect biological differences in these tissues, such as density or saturation of ligands on androgen receptors, differences in regulation of androgen-responsive corepressors or coactivators, or the effects of other concurrent regulators of metabolism, such as glucocorticoid activity, that might be affected by androgen therapy.

Although testosterone therapy decreases fat mass in young hypogonadal men (35, 36), Bhasin *et al.* (37) reported no change in VAT or abdominal SAT by MRI even with supraphysiological doses of testosterone (300 and 600 mg weekly) over 4 months in healthy, lean, young men, raising the question of whether supplemental androgen has the potential to reduce abdominal fat in eugonadal lean men. In obese, middle-aged men, androgen therapy has been associated with decreases in VAT, improvements in insulin sensitivity, and declines in cholesterol, triglycerides, and diastolic blood pressure, but the effects were largely limited to men with low pretreatment testosterone levels (10, 38).

In our study 84% of the subjects were either overweight or obese, and the loss of total body fat was significantly greater in subjects with baseline testosterone levels of 10.4 nmol/liter or less ($\leq$300 ng/dl) compared with those with higher levels ($-2.5 \pm 1.1$ *vs.* $-1.5 \pm 0.8$ kg, respectively; $P = 0.036$). Similarly, decreases in abdominal SAT were significantly greater in subjects with baseline levels of testosterone of 10.4 nmol/liter or less ($-24.1 \pm 14.3$ *vs.* $-2.9 \pm 21.3$ cm$^2$; $P = 0.03$). Thus, in older overweight or obese men with hypogonadal or age-related low normal levels of testosterone, central adipose tissue may be more sensitive to the lipolytic actions of androgen than in eugonadal, lean, younger subjects.

Androgens appear to directly affect pleuripotent stems cells by promoting the commitment of mesenchymal precursor cells to the myogenic lineage while down-regulating adipogenic differentiation and the transformation of preadipocytes into mature fat cells (39). The durability of the reductions in the metabolically active abdominal VAT and SAT tissues observed in our study is consistent with the hypothesis that subjects had a decrease in total adipocytes (40), because the simple loss of lipid induced by lipolysis during androgen therapy would be expected to reaccumulate under conditions of constant diet and exercise once treatment is discontinued.

The masses of central (abdominal VAT and SAT) and appendicular (sc, IMF, and IMCL) adipose tissue have been related to insulin resistance and dyslipidemia (41, 42). In castrated rats, pharmacological doses of androgen produce insulin resistance, as assessed with the euglycemic clamp (43), whereas treatment with physiological doses of androgen improves insulin sensitivity using the hyperinsulinemic euglycemic clamp in middle-aged men with abdominal obesity and low normal testosterone levels (9, 10). We, therefore, determined whether changes in central and peripheral fat would affect markers related to enhanced risk for diabetes and cardiovascular complications during treatment with a potent androgen. There were no significant changes in fasting glucose, insulin, or HOMA-IR.

However, QUICKI improved significantly after 12 wk of oxandrolone therapy, and the changes were significantly different from those occurring with placebo. Moreover, the changes in total fat determined by DEXA as well as in abdominal SAT and proximal thigh sc fat determined by MRI

were correlated with the change in fasting insulin from baseline to study wk 12. Together, these results suggest that the improvements in adipose tissue stores with androgen therapy have the potential to favorably affect insulin-carbohydrate metabolism in older men who are overweight or obese as they do in younger men with low testosterone levels and central obesity.

Free fatty acids entering the portal circulation from VAT are hypothesized to be important in the pathogenesis of dyslipidemia as well as the development of hepatic insulin resistance (44, 45). Despite significant reductions in VAT in our subjects, HDL and LDL cholesterol concentrations worsened during the 12 wk of therapy with oxandrolone. These changes are in keeping with the known effects of 17-alkylated steroids to reduce HDL cholesterol, presumably through induction of hepatic lipase (46), and evidence that the hepatic lipase gene is closely linked with HDL cholesterol levels (47). The effects of anabolic androgens on LDL cholesterol have been variable, with some studies showing increases, others relatively little change, and one study even showed improvements in LDL particle morphology (48), although dose, duration of therapy, and type of agent varied (reviewed in Ref. 49). Regardless, reductions in intraabdominal fat could not overcome the direct adverse effects of oxandrolone on lipid metabolism. Thus, 17-methyl-substituted androgens would not be suitable for therapy in older men with relatively low testosterone levels and abdominal obesity.

As demonstrated with other androgens (48, 50, 51), there were sizable reductions in plasma Lp(a) with oxandrolone, a potentially beneficial effect because elevation of Lp(a) has been highly correlated with measures of atherosclerosis (52, 53). However, it is uncertain whether improvements in Lp(a) have lipid protective effects, although decreasing Lp(a) with hormone replacement therapy in women with elevated pretreatment levels of Lp(a) resulted in less subsequent coronary heart disease events (54).

There were several limitations of this study. Because we assessed the effects of a 17-alkylated androgen, changes in fat mass and metabolic markers cannot be extrapolated to a dose of testosterone. Although the favorable reductions in total and trunk fat masses by DEXA and sc extremity fat determined by MRI after treatment were significantly greater in the oxandrolone group than in the placebo group, the between-group changes in appendicular fat determined by DEXA ($P = 0.08$) and in abdominal VAT and SAT fat as measured by MRI were not significant ($P = 0.22$ and $P = 0.20$, respectively). However, the significant within-group changes in appendicular fat determined by DEXA and abdominal fat determined by MRI were consistent with the significant between-group changes in extremity sc fat with MRI and changes in trunk fat with DEXA, respectively. In addition, the fact that the changes in abdominal SAT determined by MRI correlated with improvements in fasting insulin during treatment provides additional biological plausibility to the observed within-group-only changes in abdominal fat determined by MRI in the oxandrolone group. Finally, evidence that oxandrolone improved one static estimate of insulin sensitivity (QUICKI) should be interpreted cautiously and confirmed with dynamic tests of insulin sensitivity (*e.g.* hyperinsulinemic euglycemic clamp or frequently sampled iv glucose tolerance test).

We were unable to demonstrate short-term adverse clinical effects with oxandrolone, other than the expected changes in atherogenic lipids. However, the use of any anabolic androgen, including testosterone, as a potential treatment for increased adipose tissue or sarcopenia in older subjects must be investigated in sufficiently powered, long-term treatment studies to demonstrate the safety of these agents for prostate and cardiovascular health, even though a body of data already exists suggesting that replacement doses of testosterone should be safe in an aging population (reviewed in Ref. 55). The results of our study provide new information about the biological effects of androgen therapy on regional fat mass that could favorably affect measures of metabolism and cardiovascular health in an aging population. Moreover, the observation that these changes were largely sustained for 3 months after treatment was discontinued should be explored to determine the mechanisms by which androgens may cause fat loss in older individuals.

## Acknowledgments

We gratefully acknowledge the volunteer subjects who committed substantial time and effort to make this study successful. We also gratefully acknowledge Dr. Kevin Yarasheski and his laboratory for assistance with specimen analysis, and Dr. Yarasheski's helpful suggestions about the writing of the manuscript.

Received April 28, 2004. Accepted July 8, 2004.

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This work was supported in part by NIH Grant GCRC MOI-RR-00043 and a grant-in-aid from Savient Pharmaceuticals, Inc. The body composition and metabolic components of the study were performed at the University of Southern California National Center for Research Resources-funded General Clinical Research Center.

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**JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

*Journal of Gerontology: MEDICAL SCIENCES*
2005, Vol. 60A, No. 12, 1586–1592


# Six-Week Improvements in Muscle Mass and Strength During Androgen Therapy in Older Men

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***Background.*** The purpose of our study was to assess the early effects of a potent anabolic androgen on muscle mass and strength, lower extremity power, and functional performance in older men.

***Methods.*** Thirty-two men 72 ± 6 years of age were randomized to receive oxandrolone (10 mg twice daily) or matching placebo in a 2:1 manner for 12 weeks. Total and appendicular lean body mass (LBM) were assessed by dual-energy x-ray absorptiometry (DEXA). Lower extremity muscle volume was determined by magnetic resonance imaging to validate DEXA changes.

***Results.*** Total LBM increased by 2.7 ± 1.6 kg after 6 weeks with oxandrolone ($p < .001$), which was greater ($p < .001$) than the decline in LBM (–0.5 ± 0.9 kg) with placebo. Appendicular LBM increased by 1.2 ± 0.9 kg after just 6 weeks with oxandrolone ($p < .001$), which was greater ($p < .001$) than the decline in LBM (–0.4 ± 0.5 kg) with placebo. These changes were >90% of the gains in total and appendicular LBM (3.0 ± 1.5 kg and 1.3 ± 0.9 kg, respectively) after 12 weeks. Total thigh and hamstring muscle volume increased by 111 ± 29 $mm^3$ ($p = .001$) and 75 ± 18 $mm^3$ ($p = .001$), respectively, after 12 weeks. Maximal strength increased for the leg press 6.3 ± 5.6% ($p = .003$), leg curl 6.7 ± 8.6% ($p = .01$), chest press 6.9 ± 6.5% ($p = .001$), and latissimus pull-down 4.8 ± 6.3% ($p = .009$) with oxandrolone after 6 weeks; these increases were different than those with placebo ($p < .001$) and were 93%, 96%, 74%, and 94% of the respective gains at week 12. There were no improvements in functional measures.

***Conclusion.*** Treatment with a potent anabolic androgen may produce significant increases in muscle mass and strength after only 6 weeks in healthy older men. However, such treatment did not improve leg muscle power or walking speed.

ADVANCING age is associated with a progressive loss of muscle mass (sarcopenia), skeletal muscle strength, and physical function (1–3). Sarcopenia increases the risk for frailty, falls, fractures, dependency, and depression (4,5). The contribution of age-associated hormonal alterations to these adverse health consequences is unclear. Both cross-sectional (6,7) and longitudinal (8,9) studies have shown that serum total and free concentrations of testosterone, an important regulator of net myofibrillar protein anabolic balance, decline with advancing age in men. Evidence, albeit limited, suggests that bioavailable testosterone levels correlate with skeletal muscle mass and muscle strength in different ethnic populations (10,11), but the relationship between gonadal hormone status and age-associated alterations in body composition, skeletal muscle strength, and physical function in older persons remains uncertain.

Testosterone treatment in hypogonadal young men increases lean tissue (12–14) and muscle strength (12,13). In the largest studies, in which relatively hypogonadal older men received testosterone replacement for 1 and 3 years, respectively, lean body mass (LBM) was only modestly increased (1.0 and 1.9 kg, respectively), and there were no improvements in skeletal muscle strength. Furthermore, smaller studies conducted for shorter periods of time in similar aged men showed comparably modest changes in body composition (i.e., increases in total LBM of <2 kg) and no consistent improvements in strength of the major muscle groups (15–19). Whereas, in one study of relatively hypogonadal men older than 60 years, testosterone treatment produced significant increases in upper and lower body maximal voluntary strength, but doses of testosterone were largely supraphysiologic (20). Different doses of testosterone (200 mg biweekly vs 5 mg/day), variable duration of treatment (3 months to 3 years), different delivery strategies for testosterone (intramuscular vs transdermal), different methods to assess body composition (bioelectrical impedance analysis, dual-energy x-ray absorptiometry [DEXA], magnetic resonance imaging [MRI], hydrostatic weighing), and variable methods to measure muscle strength (handheld dynamometers, isokinetic dynamometers, or weight machines) likely contributed to the lack of consistent findings in the aforementioned studies of older men.

We previously reported that 12 weeks of treatment with oxandrolone, a potent anabolic androgen, significantly increased LBM and muscle strength in older men. These gains were almost entirely lost 12 weeks after discontinuing treatment (21), suggesting that prolonged therapy with an anabolic androgen would be necessary to sustain these benefits. However, the long-term safety of androgen supplementation for the prostate and heart has not been established. We, therefore, sought to determine if benefits could be achieved earlier than the 3–4 months, a common duration of treatment in many androgen supplementation studies (22). We speculated that if short-term benefits could be achieved, other potentially safer treatments such as resistance exercise could then be implemented to sustain or

even augment the early changes achieved with androgen treatment. We believe that this is the first study to report significant improvements in muscle mass and strength in both the upper and lower extremities as early as 6 weeks after treatment was initiated.

## Methods

### *Study Design*

This was a single center, investigator initiated, double blind, placebo-controlled investigation to determine the 6-week effects of a potent, convenient to administer anabolic androgen, oxandrolone (Oxandrin; Savient Pharmaceuticals, Inc., East Brunswick, NJ). The study was performed at the University of Southern California, National Center for Research Resources-funded General Clinical Research Center with the exception that skeletal muscle strength was assessed in the Clinical Exercise Research Center in the Department of Biokinesiology and Physical Therapy of the University. The study design and informed consent were approved and annually reviewed by the Institutional Review Board of the Los Angeles County–University of Southern California Medical Center.

### *Study Population*

Men 60–87 (mean 72 $\pm$ 6) years of age were recruited from the Los Angeles communities surrounding the University of Southern California Health Sciences Campus. To be eligible, men had to have a body mass index (BMI) $\leq$ 35 kg/m$^2$ and have no untreated endocrine abnormalities (e.g., diabetes, hypothyroidism), active inflammatory conditions, uncontrolled hypertension, or active cardiac problems. Blood tests for eligibility included a prostate specific antigen (PSA) $\leq$4.1 μg/L and hematocrit $\leq$50%. Men doing or planning to initiate vigorous exercise were excluded, but regular walking programs were allowed. Further details of the study population have been described previously (23).

### *Study Interventions*

Eligible participants were randomized in a 2:1 manner to receive either oxandrolone (Oxandrin) at a dose of 20 mg/day (10 mg twice daily) or matching placebo for 12 weeks. Twenty milligrams was chosen because this is the FDA-approved dose for treatment of weight loss or inability to maintain normal body weight. Adherence was monitored by tablet count at each study visit.

### *Safety Monitoring*

Complete blood counts, comprehensive chemistries with tests of renal and hepatic function, and PSA were measured at baseline and study weeks 6 and 12. Additionally, liver function tests were obtained at study weeks 3 and 9. We did not measure testosterone levels at study week 6 or 12 because semisynthetic androgens, including oxandrolone, cross-react in testosterone assays.

### *Body Composition by DEXA*

Whole-body DEXA scans (Hologic QDR-4500, version 7.2 software; Waltham, MA) were performed at baseline and study weeks 6 and 12 to quantify LBM and fat mass. One blinded, experienced technician (CF) performed and analyzed the scans. The coefficient of variation for repeated measures was $<$1% for lean and fat mass.

### *Muscle Volume*

Volume of the dominant thigh muscles was assessed using proton MRI at baseline and week 12. $^1$H-MRI was performed using a 1.5 Tesla GE Signa-LX scanner (Philips ACS II; Shelton, CT) with the body coil used as both transmitter and receiver. Nine axial images of the thigh were acquired after obtaining a T1-weighted coronal scout image (T1-weighted TR/TE 300/TE) that was used to identify the exact anatomical location for the axial images. The slice thickness was 7.5 mm with a 1.5-mm gap. The field of view was 24 $\times$ 24 cm with a 254 $\times$ 128 pixel matrix. One signal average was used.

Pixels associated with intramuscular fat, bone, and major arteries, veins, and nerves were subtracted from the image by using specialized software (SliceOmatic version 4.2; TomoVision, Montreal, Canada) previously validated (24). Because each pixel reflects a given density, regions of muscle tissue are segregated from other regions of tissue using the SliceOmatic Morpho mode of analysis. This segregation allows the tissue compartments (muscle, subcutaneous fat, intermuscular fat) to be segmentalized based on signal amplitude by highlighting small regions of similar density pixels to determine muscle cross-sectional area (CSA). The thigh musculature was calculated after highlighting the respective tissue regions with different colors. Thigh muscle volume was then automatically calculated using the SliceOmatic Morpho mode following analysis of serial slices for CSA. The same investigator (AFV) blinded to treatment located the region of interest, set the threshold value, and performed the image analyses. The coefficient of variation for repeated measures of total thigh CSA was $<$1%.

### *Evaluation of Muscle Strength*

Maximal voluntary muscle strength was assessed using the one-repetition maximum (1-RM) method (25) at baseline and weeks 6 and 12. The 1-RM was defined as the greatest resistance that could be moved through a defined range of motion using proper technique. Prior to strength testing, participants warmed up on a cycle ergometer or by walking for 5 minutes. Maximum voluntary strength was determined for the bilateral leg press, leg flexion, latissimus (lat) pull-down, and chest press exercises on Keiser A-300 pneumatic equipment (Keiser Corp., Fresno, CA). The leg press and chest press machines displayed units of measure in Newtons only. The Newton measurement of force cannot accurately be converted to kilograms; therefore, the strength data are reported in Newtons for these two machines. To accommodate for familiarization and learning of the testing procedures, baseline strength was assessed twice within 1 week prior to initiating study therapy. The greatest 1-RM measured for each exercise during the two pretreatment testing sessions was used as the baseline value for maximal voluntary muscle strength. The exercise technician was blinded to the participants' treatment.

*Evaluation of Muscle Power and Function*

Unilateral leg extension power (Watts) was determined using the Bassey Power Rig (University of Nottingham, Nottingham, U.K.) and has been described elsewhere (26). Leg extension power measured with the Bassey Power Rig has been highly correlated with lower extremity physical function in older adults (27). Additionally, lower extremity power was assessed using the Margaria stair-climb test (28). Lastly, lower extremity muscular endurance was determined by having the participants walk and/or jog as quickly as possible for 400 meters (29).

*Statistical Considerations*

The details of power calculation based on the primary outcome, total LBM by DEXA scanning, have been described elsewhere (21). Data were presented in the tables and text as the mean ± 1 standard deviation (*SD*). For the outcome variables of interest in this study, a two (oxandrolone vs placebo group) by three (baseline, week 6, and week 12) repeated-measures analysis of variance was used to compare mean differences within participants and between groups. Greenhouse–Geisser adjustment was used to justify the assumption of sphericity. When a significant Group × Time interaction was found, the changes from baseline to week 6 and the changes from baseline to week 12 between and within groups were compared by independent *t* tests and paired *t* tests, respectively. All post hoc tests were performed with Bonferroni adjustment for six possible comparisons. All statistical testing was performed with a two-sided 5% level of significance (0.83% for each post hoc *t* test) using Statistical Analysis System version 8.0 (SAS Institute, Inc., Cary, NC).

Table 1. Baseline Characteristics of the Study Population

| Characteristic | Oxandrolone ($N = 20$) | Placebo ($N = 12$) | *p* Value* |
|---|---|---|---|
| Age, y | 72.8 ± 6.9 | 71.5 ± 3.2 | .49† |
| Laboratory values | | | |
| Hemoglobin g/dL | 15.1 ± 0.9 | 15.1 ± 0.5 | .76 |
| Creatinine, mg/dL | 1.5 ± 1.3 | 1.2 ± 0.4 | .34 |
| Albumin, g/dL | 4.0 ± 0.2 | 4.2 ± 0.2 | .07 |
| ALT, U/L | 38 ± 7 | 38 ± 4 | .83 |
| Ultrasensitive CRP, mg/L | 1.4 ± 1.0 | 2.3 ± 2.7 | .21 |
| Thyroid stimulating hormone, U/L | 1.86 ± 1.31 | 1.97 ± 0.63 | .79 |
| Total testosterone, ng/dL | 369 ± 147 | 357 ± 153 | .83 |
| Luteinizing hormone, U/L | 8.3 ± 7.1 | 6.5 ± 6.7 | .51 |
| PSA, ng/ml | 2.4 ± 1.1 | 1.3 ± 0.8 | .009 |
| Total cholesterol, mg/dL | 187 ± 30 | 186 ± 34 | .91 |
| HDL cholesterol, mg/dL | 40 ± 10 | 45 ± 11 | .18 |
| LDL cholesterol, mg/dL | 126 ± 28 | 123 ± 31 | .75 |
| Body composition | | | |
| Weight, kg | 81.3 ± 13.3 | 84.8 ± 8.9 | .43 |
| BMI, kg/m$^2$ | 27.5 ± 3.5 | 29.1 ± 2.9 | .20 |
| Total lean body mass, kg | 56.5 ± 5.6 | 58.3 ± 5.9 | .39 |
| Appendicular lean body mass, kg | 25.0 ± 2.8 | 24.5 ± 2.4 | .54 |
| Total thigh muscle volume, mm$^3$ | 1394 ± 168 | 1496 ± 215 | .17 |
| Maximum muscle strength and power | | | |
| Chest press, Newtons | 212 ± 41 | 215 ± 44 | .82 |
| Latissimus pull-down, kg | 54 ± 9 | 55 ± 10 | .78 |
| Leg press, Newtons | 1245 ± 132 | 1250 ± 213 | .95 |
| Leg curl, kg | 70 ± 9 | 28 ± 13 | .42 |
| Bassey leg power, Watts | 180 ± 40 | 212 ± 65 | .10 |

*Note*: Values are means ± 1 standard deviation.

*$p$ value obtained by independent $t$ test.

†Unequal variance.

ALT = alanine aminotransferase; CRP = C-reactive protein; BMI = body mass index; PSA = prostate-specific antigen; high-density lipoprotein; LDL = low-density lipoprotein.

## Results

*Participants*

Thirty-two participants, 20 receiving oxandrolone and 12 receiving placebo, completed the study and were included in this analysis (21). Although the mean age was similar between groups (Table 1), the distribution in age resulted in unequal variance with a range of 60–87 years in the oxandrolone group and 67–78 years in the placebo group. On the basis of tablet count, these participants were adherent to their assigned treatment (94 ± 7.4% of all pills prescribed were ingested with no difference between the groups). Baseline characteristics for laboratory values, body composition, and maximum skeletal muscle strength and power were similar in the two study groups (Table 1). In particular, baseline total testosterone was 369 ± 147 ng/dL and 357 ± 153 ng/dL in the groups receiving oxandrolone and placebo, respectively. Albumin tended to be lower in the oxandrolone treatment group but the difference did not reach significance ($p = .07$). Additionally, baseline energy, protein, carbohydrate, and fat intakes were similar between the two groups (23).

*Repeated-Measures Analysis of Variance*

A significant Group × Time interaction was found for total LBM ($p < .001$), appendicular LBM ($p < .001$), chest press ($p = .001$), lat pull-down, leg press, leg curl, and leg extension power ($p < .02$ for each). Therefore, post hoc tests were performed for each outcome variable of interest (Table 2).

*Changes in Body Composition*

*Lean body mass.*—Total LBM increased significantly ($p < .001$) in the oxandrolone group (2.7 ± 1.6 kg) after 6 weeks; this increase was greater ($p < .001$) than the small decline in LBM (–0.5 ± 0.9 kg) in the placebo group (Table 2; Figure 1A). By study week 12, total LBM in the oxandrolone group increased only an additional 10% to 3.0 ± 1.5 kg; this change was significantly different than baseline ($p < .001$) and different than the 12-week change (0.1 ± 1.5 kg) in the placebo group (Table 2; Figure 1A) (21). Similar to total LBM, 90% of the gains in appendicular LBM were achieved by study week 6. Appendicular LBM increased significantly ($p < .001$) in the oxandrolone group (1.2 ± 0.9 kg) after 6 weeks of therapy; this increase was greater ($p < .001$) than the small decline in LBM (–0.4 ± 0.5) in the placebo group (Table 2; Figure 1B). By study week 12, appendicular LBM in the oxandrolone group increased only 9% to 1.3 ± 0.9 kg, which was significantly different from baseline ($p < .001$); this increase was greater

Table 2. Absolute Change in Body Composition, Muscle Strength, and Power

| Variable | Oxandrolone ($N = 20$) | Placebo ($N = 12$) | $p$ Value* |
|---|---|---|---|
| Total lean body mass, kg | | | |
| Change at week 6 | 2.7 ± 1.6† | −0.5 ± 0.9 | <.001 |
| Change at week 12 | 3.0 ± 1.5† | 0.1 ± 1.5 | <.001 |
| Appendicular lean body mass, kg | | | |
| Change at week 6 | 1.2 ± 0.9† | −0.4 ± 0.5 | <.001 |
| Change at week 12 | 1.3 ± 0.9† | −0.9 ± 0.5 | <.001 |
| Total thigh muscle volume, $mm^3$ | | | |
| Change at week 6 | NT | NT | NT |
| Change at week 12 | 111 ± 29† | −47 ± 48 | .006 |
| Chest press, Newtons | | | |
| Change at week 6 | 13 ± 13† | −2 ± 14 | .006 |
| Change at week 12 | 19 ± 14† | −2 ± 12 | <.001 |
| Latissimus pull-down, kg | | | |
| Change at week 6 | 2.6 ± 3.9† | −0.8 ± 2.6 | .006 |
| Change at week 12 | 2.6 ± 4.9† | −1.5 ± 2.9 | .006 |
| Leg press, Newtons | | | |
| Change at week 6 | 75 ± 63† | 5 ± 45 | .001 |
| Change at week 12 | 81 ± 78† | 1 ± 65 | .005 |
| Leg curl, kg | | | |
| Change at week 6 | 4.3 ± 5.3† | −0.4 ± 2.7 | .003 |
| Change at week 12 | 4.8 ± 5.7† | 0.2 ± 3.1 | .007 |
| Bassey leg extension power, Watts | | | |
| Change at week 6 | 3 ± 24 | −13 ± 14 | .23 |
| Change at week 12 | 10 ± 27 | −13 ± 12 | .12 |

*Note*: *Significant difference between groups by independent $t$ test.

†Significant within-group change compared to baseline $p < .05$ by paired $t$ test.

NT = not tested.

($p < .001$) than the 12-week change (–0.9 ± 0.5 kg) in the placebo group.

*Thigh muscle volume.*—Total thigh muscle volume in the oxandrolone group increased significantly (111 ± 29 $mm^3$; $p = .001$) from baseline to study week 12; this increase was greater ($p = .006$) than the change (–47 ± 48 $mm^3$) in the placebo group (Table 2, Figure 2). Similarly, in the oxandrolone group, hamstring muscle volume increased significantly (75 ± 18 $mm^3$; $p = .001$) from baseline to study week 12. This increase was greater ($p = .009$) than the change (1 ± 13 $mm^3$) in the placebo group (Figure 2). Quadriceps muscle volume did not significantly change in either the oxandrolone or placebo group ($p > .05$ for both).

### *Changes in Maximal Voluntary Strength*

After 6 and 12 weeks, the absolute (Table 2) and relative (Figure 3A and B) increases in maximal voluntary muscle strength were greater for participants receiving oxandrolone. These increases were significantly different from the placebo group for chest press, lat pull-down, leg press, and leg curl exercises and for the assessment of leg extension power (Table 2). The increases in strength by study week 6 were greater than 90% of the gains achieved by study week 12 with the exception of the chest press

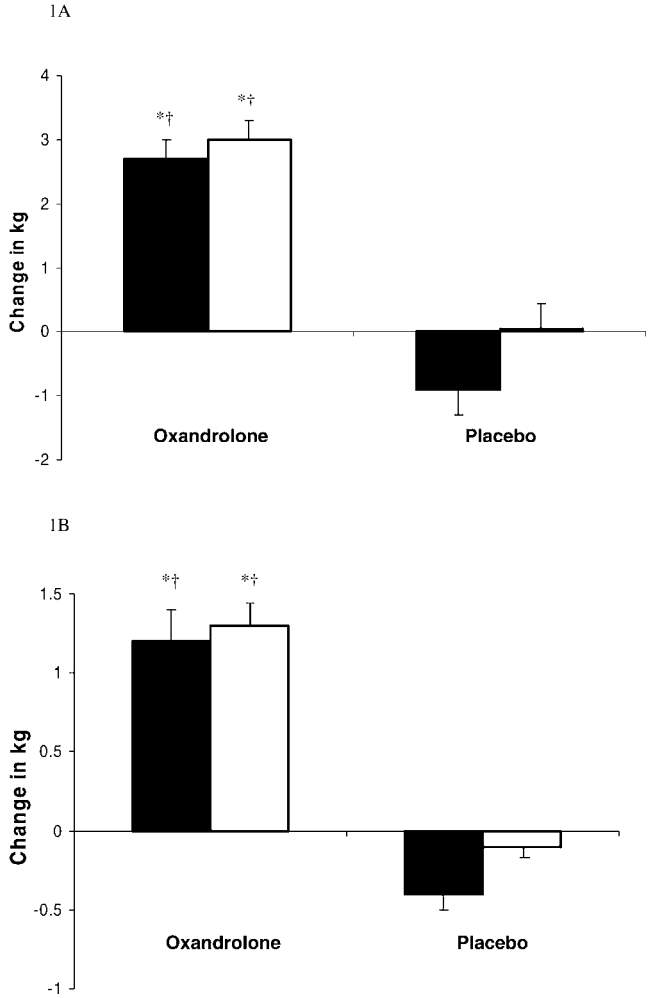


Figure 1. Absolute change in total lean mass (**A**) and appendicular lean mass (**B**) by dual-energy x-ray absorptiometry. Filled bars represent change from baseline to study week 6; open bars represent change from baseline to study week 12. The whiskers are one standard error. *Significant ($p < .001$) within-group difference from baseline. †Significant ($p < .001$) difference in the change between study groups.

exercise that reached approximately 75% of the gains in strength achieved by study week 12. For the leg press at study week 6, the relative strength increased by 6.3 ± 5.6% ($p = .003$), for leg curl by 6.7 ± 8.6% ($p = .01$), for chest press by 6.9 ± 6.5% ($p = .001$), and for lat pull-down by 4.8 ± 6.3% ($p = .009$) in the group receiving oxandrolone. By study week 12, the relative strength for leg press increased by 6.8 ± 6.4% ($p = .005$), for leg curl by 7.0 ± 7.8% ($p = .012$), for chest press by 9.3 ± 6.7% ($p < .001$), and for lat pull-down by 5.1 ± 9.1% ($p = .013$) in the group receiving oxandrolone (Figure 3A and B). Thus, the increases in maximum voluntary strength at study week 6 were 93%, 96%, 74%, and 94% of the respective gains for leg press, leg curl, chest press, and lat pull-down at study week 12.

### *Lower Extremity Muscle Function*

Leg extension power assessed using the Bassey Power Rig did not significantly improve (3 ± 24 and −13 ± 14

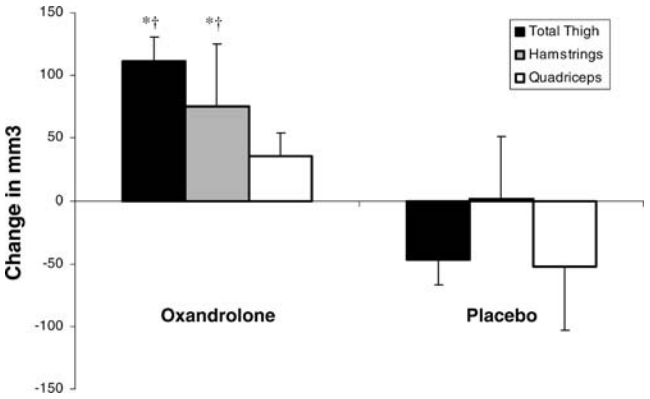


Figure 2. Absolute change in muscle volume by magnetic resonance imaging for the total thigh (black bars), hamstrings (gray bars), and quadriceps (white bars) muscle groups. The whiskers are one standard error. *Significant ($p = .001$) within-group difference from baseline. †Significant ($p = .006$) difference in the change between study groups.

Watts) by study week 6 in either the oxandrolone or placebo group, respectively (Table 2). Furthermore, by study week 12 the increases (10 ± 27 Watts) in leg extension power in the group receiving oxandrolone was not different from the loss (–13 ± 12 Watts) in the placebo group. Lower extremity power determined by the Margaria stair-climb test and the 400 meter walk and/or jog test did not demonstrate significant improvements ($p > .05$) nor was there a significant difference between groups (data not shown).

*Safety Evaluation*

There were no serious adverse events that could be attributed to oxandrolone. However, one serious adverse event occurred during the study when a participant randomized to oxandrolone developed hypotension after his primary doctor modified the patient's antihypertensive medications at the participant's request (21); this participant resumed study therapy without further problems. Although serum albumin and alkaline phosphatase levels decreased more with oxandrolone than with placebo, there was no change in ultrasensitive C-reactive protein levels at week 12, suggesting that inflammation was not more common in the oxandrolone group. There were minimal increments in the liver transaminase levels that reached statistical significance, but alanine aminotransferase was only increased beyond the normal range in two participants where it reached 71 and 99 U/L (≤1.5 times the upper limit of normal) (21). Both participants were asymptomatic without liver enlargement, and the alanine aminotransferase returned to normal in both shortly after study therapy was discontinued.

Total cholesterol and triglyceride levels did not change by study week 12 in either group. However, at study week 6, low-density lipoprotein (LDL) cholesterol increased (31 ± 41 mg/dL; $p = .02$) and by study week 12 was 23 ± 37 mg/dL greater ($p = .06$) than were baseline values in the oxandrolone group but returned to baseline values 12 weeks after study therapy was discontinued (23). For high-density lipoprotein (HDL) cholesterol, levels decreased (20 ± 7 mg/dL; $p < .001$) at study week 6 and by study week 12, remained

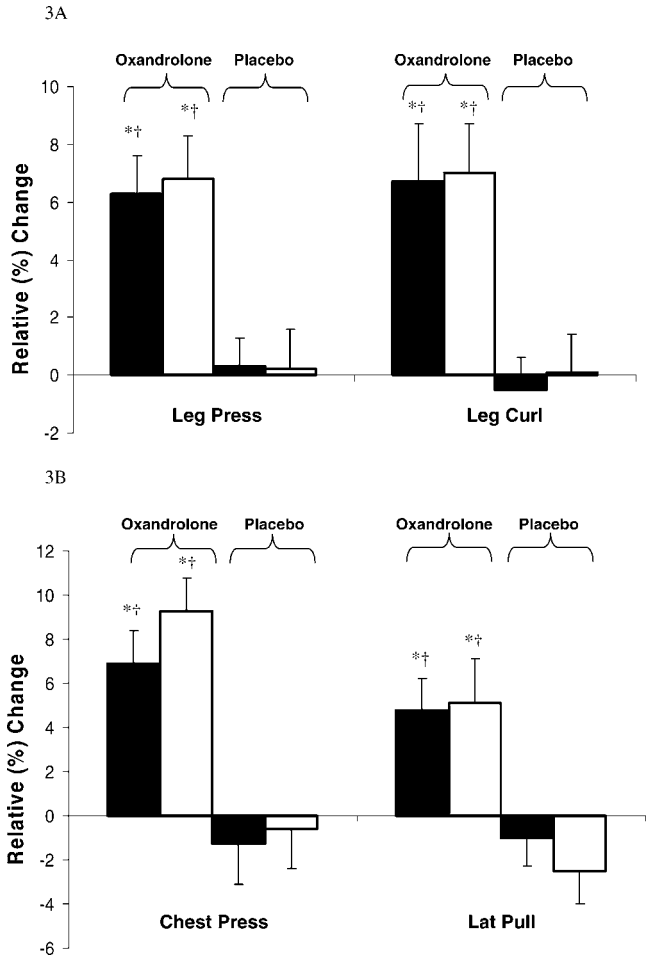


Figure 3. Relative (%) change in lower (**A**) and upper (**B**) extremity voluntary maximum muscle strength. Filled bars represent change from baseline to study week 6; open bars represent change from baseline to study week 12. The whiskers are one standard error. *Significant ($p < .001$) within-group difference from baseline. †Significant ($p < .001$) difference in the change between study groups.

decreased (19 ± 8 mg/dL, $p < .001$) in the oxandrolone group. The effects of oxandrolone on HDL cholesterol were not sustained, and 3 months following treatment there was a rebound to levels greater than baseline (23).

Hematocrit levels did not increase in either group. The PSA measures did not change significantly by study week 6; however, by study week 12 there was a small (–0.6 ± 0.9 ng/ml) but significant ($p = .03$) decrease in the PSA in the oxandrolone group.

## Discussion

Testosterone replacement therapy in older men, even when given for more than a year, has resulted in only modest increases in total lean tissue of 1–2 kg (22), and the increases in muscle strength have been modest at best and inconsistent (15,16,30,31). Whereas, treatment with oxandrolone, a potent oral androgen, produced a robust 3-kg increase in total LBM along with significant increases in appendicular LBM, muscle volume, and maximal voluntary strength of the major

muscle groups of the upper and lower body after just 12 weeks of treatment. The only other study in which comparable changes in lean tissue and consistent improvements in the major muscle groups were achieved used largely supraphysiologic doses of testosterone for 6 months (20). Likewise, the FDA-approved dose of oxandrolone (20 mg/day dose) appeared to be supraphysiologic because endogenous production of luteinizing hormone was suppressed. Thus, the magnitude of accretion in myofibrillar protein and benefits for skeletal muscle strength may be related to dose or potency of the anabolic androgen used for treatment.

The most important finding of this study was that greater than 90% of the gains in total LBM, appendicular lean tissue, and skeletal muscle strength were achieved by study week 6. Indeed, total LBM increased by 2.7 ± 1.6 kg at study week 6 and only increased an additional 0.3 ± 0.1 kg by study week 12. Appendicular LBM by DEXA, an indirect measure of muscle mass, increased by 1.2 ± 0.9 kg at study week 6, which was greater than 90% of the gain at study week 12, namely 1.3 ± 0.9 kg. Similarly, the increases in maximum voluntary strength at study week 6 were 93%, 96%, 74%, and 94% of the respective gains for leg press, leg curl, chest press, and lat pull-down at study week 12. These increases in maximal voluntary strength of the upper and lower body appendicular muscles suggest that functionally important improvements may be attained with a relatively short course of therapy using a potent androgen. These observations are consistent with the findings of Bhasin and colleagues, who showed that changes in body composition and skeletal muscle strength are proportional to the dose of testosterone administered for 20 weeks to younger men (12).

We believe that the significant improvements in maximal skeletal muscle strength corroborate our findings of increased total and appendicular LBM by DEXA scanning and that these findings are not merely the result of hydration from the androgen treatment (21). Furthermore, our findings of increased thigh muscle volume determined by serial MRI CSA slices support the contention that increases in muscle mass with androgen supplementation are responsible for the gains in strength because CSA is proportional to muscle strength (32) and we have previously shown that increases in muscle CSA are proportional to increases in strength (33). Of note, there were greater absolute changes in hamstrings compared to quadriceps muscle volume probably due to the quadriceps muscle compartment being larger and more likely recruited during normal physical activity. The fact that the quadriceps muscle group is more often used for habitual daily activities may explain why this muscle group did not respond as well to the oxandrolone treatment. It is possible that smaller muscle groups, such as the hamstrings, which are not recruited as often for typical patterns of movement or activity, have a lower threshold for stimulus and respond better to androgen therapy.

Increases in LBM in older men at risk for sarcopenia and frailty is of limited value unless meaningful improvements in skeletal muscle strength, power, and physical function can be demonstrated. We tested muscle strength for various upper and lower body muscle groups as well as leg extension power, stair-climb power, and time to walk and/or jog 400 meters. Although the majority of strength gains were achieved by study week 6, the modest yet statistically significant 7% average improvement in relative strength for lower extremity muscle groups did not translate into improvements in lower extremity leg extension power, stair-climb power, or the ability to walk and/or jog 400 meters, which has been associated with changes in physical function (26,27). The most likely explanation for the absence of improvements in measures of lower extremity performance may have related to the functional status of the population tested. Our participants were healthy, active, ambulatory community-dwelling older men. It is possible that similar treatment of sedentary, frail individuals may have resulted in substantive improvement in physical function but this is largely speculation. In addition, our study was not powered to show changes in physical function.

That more than 90% of the gains in muscle mass and strength were achieved in just 6 weeks could be beneficial to individuals with physical limitations, frailty, or catabolic illness and associated muscle wasting because the long-term safety of androgen supplementation for cardiovascular and prostatic health is unknown. In addition, short-term treatment with potent anabolic androgens may ''jumpstart'' the anabolic process for improving muscle mass and skeletal muscle strength until these individuals are capable of engaging in resistance exercises, a potent stimulus for myofibrillar muscle protein synthesis and proven means to significantly increase muscle quality (33,34) and physical function even in nonagenarians (35).

There are several limitations of this study. First, oxandrolone unlike testosterone is 5-alpha reduced and nonaromitizable, and blood levels for this drug are not available in clinical laboratories. This makes it difficult to determine dose–response effects or compare timing and magnitude of outcomes measured in our study to other trials using testosterone or testosterone conjugates. Second, it is unclear whether treatment for longer than 12 weeks would have resulted in further gains in LBM and skeletal muscle strength that would have clinical or functional significance as there appears to be a threshold for androgen effects as there are limited benefits of treatment with testosterone when therapy is prolonged beyond 3 months (15,31). Third, whether the effects of treatment in a more impaired population of frail individuals or those with underlying catabolic disease would be similar is unknown. Lastly, there are additional challenges of conducting clinical trials in older adults, such as impaired nutritional status, catabolic effects of various comorbidities, and the physical limitations associated with frailty that complicate studying hormone replacement therapy.

Lastly, the safety and efficacy of long-term androgen therapy has yet to be established in older persons. Because 17-alkylated androgens, such as oxandrolone, reduce HDL cholesterol and may elevate LDL cholesterol as occurred in this study, oxandrolone should not be used for otherwise healthy persons with increased risk of cardiovascular disease. However, it is possible that short-term treatment may minimize the cardiovascular and prostatic disease risks that could occur with long-term androgen therapy until definitive safety studies have been completed as advised by the Institutes of Medicine (36). Furthermore, when life expectancy is short, as may occur in older persons with

advanced cancer, severe obstructive lung disease, or cardiac cachexia, risk–benefit considerations may be different and a brief course of treatment with a potent anabolic androgen may produce significant increases in muscle mass, also an important source of amino acids for synthesis of white blood cells and proteins to combat infection and cancer cells (37). Thus, the observation that as little as 6 weeks of treatment with a potent androgen improves muscle mass serves to justify further studies in older populations.

Acknowledgments

Support was provided in part from the National Institutes of Health GCRC MOI RR00043 and by a grant-in-aid from Savient Pharmaceuticals Inc.

We gratefully acknowledge the volunteer participants who committed substantial time and efforts to make this study successful. We also gratefully acknowledge Dr. Kevin Yarasheski and his laboratory for assistance with specimen analysis.

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*Received February 2, 2005*
*Accepted May 4, 2005*
*Decision Editor: Luigi Ferrucci, MD, PhD*

# Resistance Exercise and Supraphysiologic Androgen Therapy in Eugonadal Men With HIV-Related Weight Loss

## A Randomized Controlled Trial

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**Context** Repletion of lean body mass (LBM) that patients lose in human immunodeficiency virus (HIV) infection has proved difficult. In healthy, HIV-seronegative men, synergy between progressive resistance exercise (PRE) and very high-dose testosterone therapy has been reported for gains in LBM and muscle strength.

**Objective** To determine whether a moderately supraphysiologic androgen regimen, including an anabolic steroid, would improve LBM and strength gains of PRE in HIV-infected men with prior weight loss and whether protease inhibitor antiretroviral therapy prevents lean tissue anabolism.

**Design** Double-blind, randomized, placebo-controlled trial; post hoc analysis for effect of HIV-protease inhibitor therapy conducted from January to October 1997.

**Setting** Referral center in San Francisco, Calif.

**Patients** Volunteer sample of 24 eugonadal men with HIV-associated weight loss (mean, 9% body weight loss), recruited from an AIDS clinic and by referral and by advertisement.

**Intervention** For 8 weeks, all subjects received supervised PRE with physiologic intramuscular testosterone replacement (100 mg/wk) to suppress endogenous testosterone production. Randomization was between an anabolic steroid, oxandrolone, 20 mg/d, and placebo.

**Main Outcome Measures** Lean body mass, nitrogen balance (10-day metabolic ward measurements), body weight, muscle strength, and androgen status.

**Results** Twenty-two subjects completed the study (11 per group). Both groups showed significant nitrogen retention and increases in LBM, weight, and strength. The mean (SD) gains were significantly greater in the oxandrolone group than in the placebo group (5.6 [2.1] vs 3.8 [1.8] g of nitrogen per day [$P = .05$]; 6.9 [1.7] vs 3.8 [2.9] kg of LBM [$P = .005$]; greater strength gains for various upper and lower body muscle groups by maximum weight lifted [$P = .02$-$.05$] and dynamometry [$P = .01$-$.05$]). The mean (SD) high-density lipoprotein cholesterol level declined 0.25 (0.14) mmol/L (9.8 [5.4] mg/dL) significantly in the oxandrolone group ($P < .001$ compared with placebo). Results were similar whether or not patients were taking protease inhibitors. One subject in the oxandrolone group discontinued the study because of elevated liver function test results.

**Conclusions** A moderately supraphysiologic androgen regimen that included an anabolic steroid, oxandrolone, substantially increased the lean tissue accrual and strength gains from PRE, compared with physiologic testosterone replacement alone, in eugonadal men with HIV-associated weight loss. Protease inhibitors did not prevent lean tissue anabolism.

*JAMA. 1999;281:1282-1290* www.jama.com

THE PRIMARY AIM OF THERAPY IN wasting syndromes is to restore lean tissue.[1,2] The use of alimentation or appetite stimulants in wasting due to human immunodeficiency virus (HIV) has, however, resulted in fat deposition with little lean tissue gains.[3-6] Administration of HIV-protease inhibitors to patients with acquired immunodeficiency syndrome (AIDS) also results in weight gain, but most of the weight gained is body fat.[7-10] Alterations in the metabolic or endocrine milieu,[11,12] inadequate exercise, or other factors may be responsible for disproportionate fat vs lean body mass (LBM) gains in HIV infection. Recombinant growth hormone (rGH)[13,14] and androgen replacement therapy in men with low or borderline low serum testosterone concentrations[15,16] are effective in restoring LBM in men with HIV infection.

**See also pp 1275 and 1326.**

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The high cost of rGH has limited its use in clinical practice; however, many men with HIV-related weight loss are eugonadal. The use of androgens has not proved effective in the latter group. The optimal strategy for increasing LBM in eugonadal men with HIV-associated weight loss remains uncertain.[17]

Bhasin et al[18] performed an important study documenting the interaction between progressive resistance exercise (PRE) and very high intramuscular dosages of testosterone (600 mg/wk, or 6 times the usual replacement dosage) in healthy, eugonadal men. The combined intervention resulted in significantly greater increases in LBM, muscle size, and strength than either intervention alone. However, the long-term safety and behavioral consequences of testosterone at dosages as high as 600 mg/wk are unknown.

Based on these results in healthy men,[18] we performed a randomized, placebo-controlled trial among men with HIV infection. The prospectively defined hypotheses were, first, that a supraphysiologic androgen regimen would increase the LBM and strength gains from PRE in eugonadal men with HIV-associated weight loss and, second, that this interaction would not require extremely high doses of androgens. A subgroup analysis was also included addressing whether protease inhibitor antiretroviral therapy prevents lean tissue anabolic response in HIV-infected men.

## METHODS

### Experimental Design

The design was a prospective, randomized, placebo-controlled trial to compare supervised PRE plus physiologic testosterone replacement (placebo) with the same regimen combined with supplementation with an anabolic steroid, oxandrolone, at a dose that is approved and is well tolerated over the long-term.[19-21] All subjects received intramuscular injections of testosterone enanthate (100 mg/wk). Those in the placebo group took placebo tablets and those in the oxandrolone group took oxandrolone tablets 20 mg/d (both tablets were provided by Bio-Technology General Corporation, Iselin, NJ) (FIGURE 1). Oxandrolone and placebo tablets were identical in appearance, taste, and texture. The supervised PRE program was held 3 times a week. Individual treatment group assignments were based on a random number–generated sequence generated by an independent study monitor (Bio-Technology General Corporation), which was double-blinded to all study personnel, including exercise trainers. The assignment was executed independently by study personnel (A.S.) in San Francisco, Calif. The subjects were stratified post hoc for use of protease inhibitors. The code was held by the independent study monitor who remained anonymous to all study personnel. The envelope containing the randomization code was delivered to the principal investigator and the code was broken in San Francisco with the study personnel present. All data analyses and statistical comparisons were completed before the code was broken.

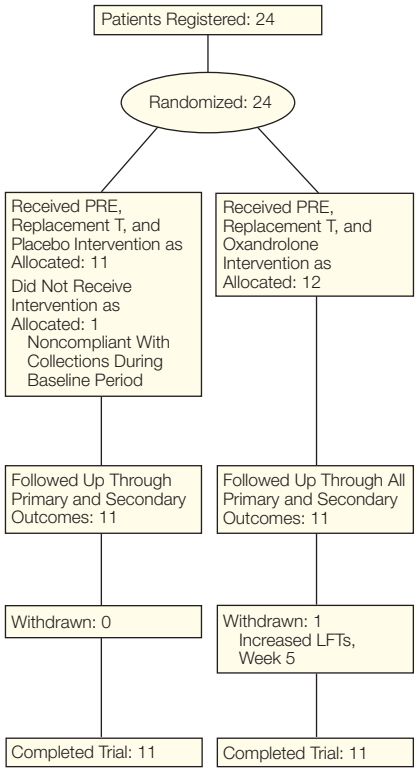
The therapeutic trial lasted 8 weeks (Figure 1). Two 10-day inpatient admissions to a metabolic research unit (MRU) were carried out to assess nitrogen balance and measures of metabolism. The first MRU admission began 10 days prior to treatment (days –10 to 0), and the second between days 21 and 30 of treatment.

**Subjects.** Twenty-four men who acquired HIV or AIDS through homosexual transmission were recruited from the AIDS-wasting clinic at San Francisco General Hospital, through referrals, and through advertisements at a San Francisco food bank. The protocol was approved by the committees on human research of the University of California, San Francisco, University of California, Berkeley, and the US Department of Agriculture. Informed consent was obtained for all procedures.

**Inclusion Criteria.** Patients were included if they (1) were HIV-seropositive; (2) had experienced at least a 5% weight loss during the preceding 2 years; (3) were clinically stable with no active opportunistic infections and weight stable during the preceding 3 months; (4) were eugonadal (serum total testosterone concentration of 7.8-31.2 nmol/L [225-900 ng/dL]); (5) had maintained a stable antiretroviral regimen for at least 3 months; (6) were not currently or previously participating in PRE or aerobic exercise; and (7) could comply with protocol and give informed consent.

**Exclusion Criteria** Patients were excluded if they had (1) used testosterone or other androgens in the 3 months preceding the study; (2) used medications or dietary supplements known to alter nutritional status including marinol, megestrol acetate, rGH, thalidomide, pentoxifylline, glucocorticoids, or dehydroepiandrosterone in the 3 preceding months; (3) used investigational agents; (4) had severe diarrhea

**Figure 1.** Progress of Patients Through Randomized Controlled Trial




T indicates testosterone enanthate, 100 mg intramuscularly per week; PRE, progressive resistance exercise; and LFT, liver function test results.

**Table 1.** Baseline Characteristics of Subjects Who Completed the Metabolic Research Unit Phase of the Study*

| | Placebo (n = 11) | Oxandrolone (n = 11) |
|---|---|---|
| Age, y | 40 (8) | 42 (7) |
| Serum total testosterone levels, nmol/L [ng/dL] | 22.7 (11.2) [655 (322)] | 20.9 (6.7) [603 (192)] |
| CD4 cells, $\times 10^9$/L | 0.337 (0.236) | 0.234 (0.097) |
| Plasma viral load, $\log_{10}$ copies/mL | 4.9 (5.3) | 3.9 (4.3) |
| Weight, kg | 73.3 (14.7) | 68.8 (9.6) |
| Percentage of usual body weight | 91 (4) | 92 (2) |
| Body mass index, kg/m$^2$ | 23.1 (3.5) | 22.3 (2.5) |

*Data are presented as mean (SD). No group measures were significantly different by unpaired *t* test at baseline. All patients participated in progressive resistance exercise.

(≥3 loose bowel movements per day), chewing or swallowing difficulties, oropharyngeal pain, or inadequate access to food; and (5) had comorbid medical conditions or abnormalities in screening laboratory test results (blood cell count, chemistry profile).

Thirteen of 24 subjects were taking HIV-protease inhibitor antiretroviral agents in combination with nucleoside and/or nonnucleoside reverse transcriptase inhibitors. Other patient characteristics are shown in TABLE 1. There were no significant differences between assignment groups for any potential prognostic variables (eg, age, weight, prior weight loss, CD4 cell counts, viral load, serum testosterone levels).

## Metabolic Ward Protocol

Subjects were confined to the MRU of the Western Human Nutrition Research Center in San Francisco for both 10-day inpatient periods. Energy requirements were estimated using the Harris-Benedict equation with a physical activity level of 1.6.[22] Food was provided to match these requirements. Meals were under strict supervision and subjects were required to eat all food provided. Food not eaten was presented at the next meal. During the baseline MRU study, exercise level was sustained through 2 chaperoned walks of 1 km daily. No other exercise was permitted. Weight remained stable to within 2% of starting weight, or dietary alterations were made. For the follow-up MRU admission, the energy requirements were calculated based on readmission weight; food was adjusted during the first 4 days in response to reports of hunger (increments of 418 kJ/d).

During the free-living periods, subjects returned to the study site weekly to receive medication and testosterone injections.

**Exercise Protocol.** The major muscle groups were worked according to a defined protocol individually tailored to each subject's exercise capacity, based on the 1-repetition maximum (1-RM) measured at baseline.[23] Each subject was assigned to a personal trainer who was present at every exercise session. Three exercise trainers participated in the study. The protocol involved three, 1-hour training sessions of resistance exercise per week on nonconsecutive days, alternating between upper and lower body workouts, consisting of 6 upper body exercises and 3 lower-body exercises performed on standard weight-stack isotonic exercise equipment. Three sets of each exercise were performed during a session; each set consisted of 10 repetitions of the exercise at approximately 80% of the subject's 1-RM. Reassessment of 1-RM was performed at week 4, and the weights were adjusted accordingly. All subjects were able to progress appropriately during the study. No subjects complained about the exercise intensity or dropped out because the exercise was too difficult.

**Nitrogen Balance.** Twenty-four-hour urine and stool collections were carried out each day in the MRU. Nitrogen balance assessment began on the fourth day of each 10-day inpatient phase to allow initial equilibration.

Total urinary nitrogen was analyzed by combustion[24] (LECO nitrogen determinator, FP-428 Corporation, St Joseph, Mich). Daily urinary creatinine levels were analyzed by spectrophotometric assay (Roche Diagnostic Systems, Somerville, NJ).[25] Stool aliquots were homogenized, lyophilized, crushed, dried, and analyzed for nitrogen content using the LECO analyzer. The SD of repeated measurements of 24-hour nitrogen output in this MRU is less than 0.5 g/d (M.V.L., J.K. unpublished data, April 1997).

Diet composition for both MRU admissions was the same. The mean (SD) protein intake was 1.47 (0.0) g/kg per day (16.1% [0.4%] of dietary energy); 53.4% (0.8%) of dietary intake was from carbohydrate, and 30.7% (0.3%) from fat. The nitrogen content of the diet was verified by combustion. This protein intake is within the range of recommended dietary intake for wasted patients and is the same as we have used previously.[15]

**Stable Isotope/Mass Spectrometric Studies of de Novo Lipogenesis.** De novo lipogenesis was measured by mass isotopomer distribution analysis.[26-28] A constant intravenous infusion of sodium [1-$^{13}$C]acetate (99% atom enriched, Isotec Inc, Miamisburg, Ohio) at 5.2 mmol/h was performed from 2 AM to 6 PM. Subjects fasted from 8 PM until 9 AM, then ate ad libitum.

Very low-density lipoprotein was isolated from plasma by ultracentrifugation and transesterified for analysis by gas chromatography-mass spectrometry.[26] The isotopic enrichment of the intrahepatic acetyl-coenzyme A precursor pool and the contribution from de novo lipogenesis to very low-density lipoprotein palmitate were calculated by mass isotopomer distribution analysis.[26,27]

**Weight, Height, and Body Composition.** Each morning before breakfast subjects were weighed. Body composition was measured by dual-energy x-

ray absorptiometry (DEXA; Model DPX, Lunar, Madison, Wis). The reproducibility of DEXA for repeated measurements of body composition in the same individual is better than 0.5% (M.V.L., unpublished data, May 1997).

**Resting Energy Expenditure (REE).** Resting energy expenditure was measured by indirect calorimetry using a Deltatrac metabolic monitor (SensorMedics, Yorba Linda, Calif) in the canopy mode for 30 minutes shortly after awakening.

**Muscle Strength Testing.** *One-Repetition Maximum Testing.* One-repetition maximum testing was carried out with the same exercise equipment used for training. Subjects were given instruction and an opportunity to practice during a trial session.

***Isokinetic Dynamometer Testing.*** Strength and endurance were tested by an isokinetic dynamometer (Cybex 6000, Ronkonkoma, NY). Cybex testing was chosen to minimize the effects of neuromuscular learning on measurement outcome since the subjects' training regimen did not involve the Cybex. Right quadriceps and shoulder muscle strength were assessed by measurement of peak torque (maximal force) during 3 complete repetitions of flexion and extension at a constant angular velocity of 60° per second.

**Serum Gonadal Hormones and Urine Androgen Screening.** Serum gonadal hormone levels were measured by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, Calif). In addition, liquid chromatography–mass spectrometry–mass spectrometry and gas chromatography–mass spectrometry were used to screen urine samples at baseline and week 8 for metabolites of oxandrolone and other widely available testosterone analogs (nandrolone, danazol, stanozolol, methyltestosterone, and fluoxymesterone) as a check of compliance.[29,30] The urine testosterone to epitestosterone ratio was also measured as an index of exogenous testosterone administration.[29,30]

**Quality of Life Measurements.** A portion of the Medical Outcomes Study–HIV Specific Questionnaire[31] was administered before and after intervention.

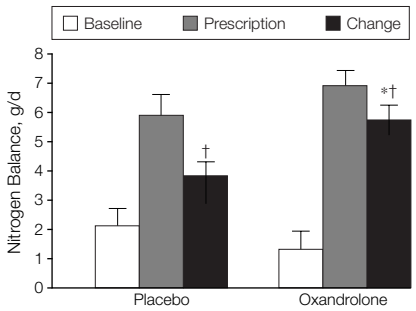
**Blood Chemistries.** Routine blood chemistries, CD4 lymphocyte count, and measurement of serum HIV viral load were carried out by SmithKline-Beecham Laboratories (San Francisco, Calif).

**Open-Label Phase.** A 12-week open-label phase was offered to subjects who completed the placebo-controlled study, during which time testosterone, oxandrolone, and supervised PRE continued to be provided. DEXA scans were performed at the conclusion of the 12 weeks. Reassessment of 1-RM was performed every 4 weeks and the weights were adjusted accordingly.

### Statistical Analysis

Results are expressed as mean (SD) unless otherwise indicated. Statistical significance was determined using Statview computer software (Abacus Concepts, Berkeley, Calif). A significance level of .05 was used. Unpaired 2-tailed *t* tests were used to assess differences between groups at baseline. Repeated measures analysis of variance was used to compare treatment effects over time, with a group factor (treatment) and a trial factor (time). When a significant treatment by time interaction was observed, follow-up comparison was performed using the Tukey Studentized range test at a procedurewise rate of 0.05. Correlations were performed using the Pearson product moment. Analyses were performed on study completers, not on an intention-to-treat basis. The primary outcome measures were nitrogen retention, body composition changes, and muscle strength. Secondary outcome measures were gonadal hormone concentrations, REE, and de novo lipogenesis. The sample size of 12 was calculated to detect a standardized effect size of 0.9 (for effect within each group) and 1.2 (for comparison of effect between groups) for change in LBM, using (1) an estimated SD of between 1.0 and 2.0 kg LBM for the response to effective anabolic therapies in HIV-associated wasting,[14,15] and (2) the uncertain biologic significance of LBM changes less than about 1.0 to 1.5 kg in magnitude. Accordingly, n = 12 per group was selected to detect differences in LBM of 2 kg between groups at $P = .05$, with 80% power.

**Figure 2.** Nitrogen Retention Following Treatment




Data are presented as mean (SD) grams of nitrogen retained per day. The asterisk indicates significantly different change between groups by repeated measures analysis of variance ($P<.05$); the dagger, significantly different change from baseline by the Tukey follow-up procedure ($P<.05$). The coefficient of variation in the creatinine levels, which were measured every 24 hours, for the study group was 17.8% (5.3%), which is within the published acceptable range[25] and which indicates satisfactory completeness of daily urine collection.

## RESULTS

### Subject Completion

Of the 24 subjects enrolled, 23 completed both inpatient studies, with 22 completing the 8-week study (Figure 1). One subject from the placebo group was disqualified from the study for noncompliance with sample collections during the first inpatient phase. Another subject in the oxandrolone group discontinued at week 5 because of elevated liver function test results. Seventeen of the 22 subjects entered the open-label phase of the study; all 17 completed the 12-week follow-up.

### Nitrogen Balance

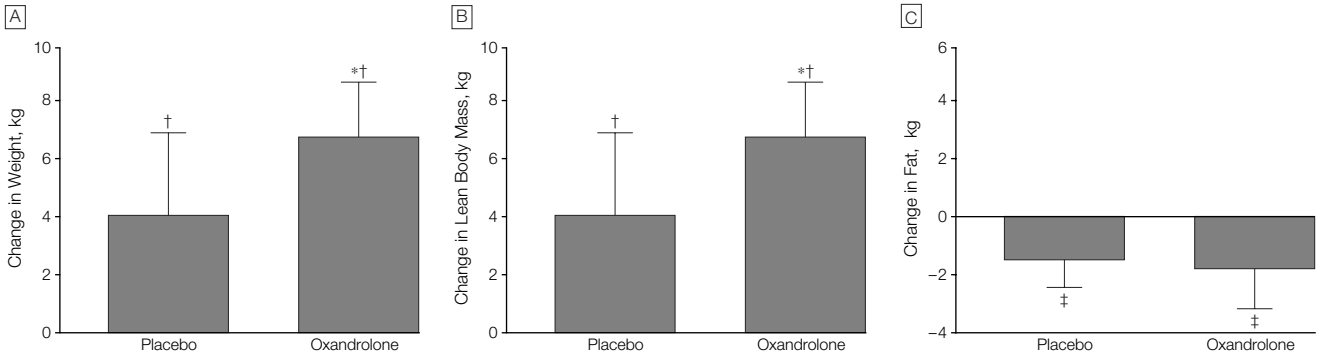
There was a significantly greater cumulative nitrogen retention observed in the oxandrolone group compared with the placebo group (5.6 [2.1] g/d vs 3.8 [1.8] g/d). The change from baseline was significant for both groups (FIGURE 2). All 22 subjects showed an increase in nitrogen retention. There were no differences between the 2

groups for baseline nitrogen balance. Five of the 22 subjects had slightly negative nitrogen balance at baseline, 2 in the placebo group and 3 in the oxandrolone group. Assuming that each gram of retained nitrogen represents 32 g of LBM,[31] the predicted LBM gains are 0.9 (0.4) kg/wk in the placebo group and 1.3 (0.5) kg/wk in the oxandrolone group. Use of protease inhibitors had no effect on nitrogen retention.

### Weight and Body Composition

There was significant weight gain in both groups ($P<.05$ for time effect vs baseline); the mean (SD) gains were significantly greater in the oxandrolone group than in the placebo group(6.7 [2.0] kg vs 4.2 [2.8] kg; $P=.03$)(FIGURE 3, A). Increases in LBM were significant in both groups relative to baseline ($P<<.05$ for time effect), with a significantly greater increase in the oxandrolone group than in the placebo group (6.9 [1.7] kg vs 3.8 [2.9] kg; $P<=.005$) (Figure 3, B). Regional distribution of accrued LBM by DEXA was not significantly different between the groups. The percentages of total LBM gain by region for those in the oxandrolone group were arms, 20.4% (1.9%); legs, 34.4% (2.3%); and trunk, 45.2% (3.3%). For those in the placebo group, it was arms, 21.2% (8.7%); legs, 21.3% (7.0%); and trunk 57.5% (7.0%).

**Figure 3.** Change in Body Weight and Body Composition by Dual-Energy X-ray Absorptiometry at Week 8




Data are presented as mean (SD). The asterisk indicates significantly different change between the groups by repeated measures analysis of variance ($P<.05$); dagger, significantly different change from baseline by the Tukey test follow-up procedure ($P<.05$); and double dagger, significant change from baseline in both groups ($P<.05$), which is not significantly different between the groups by repeated measures analysis of variance.

**Table 2.** Exercise Capacity*

| | Placebo | | | Oxandrolone | | |
|---|---|---|---|---|---|---|
| | Baseline | 8 Week | Change | Baseline | 8 Week | Change |
| | | | 1 Repetition, Maximum lbs | | | |
| Chest press | 138 (38) | 159 (36) | 21 (31)‡ | 143.0 (40) | 190.0 (54) | 47 (25)†‡ |
| Shoulder press | 76.5 (39) | 90.5 (36) | 14.0 (16)§ | 61 (15) | 84 (16) | 23 (12)§ |
| Biceps pull | 41.7 (16) | 48.1 (18) | 6.4 (6)‡ | 36.0 (10) | 50.0 (15) | 14 (9)†‡ |
| Triceps push | 57.0 (13) | 66.3 (15) | 9.3 (7)‡ | 59.1 (10) | 77 (13) | 17 (11)†‡ |
| Leg press | 186 (75) | 232 (80) | 46 (18)‡ | 177 (25) | 241 (38) | 64 (35)†‡ |
| Leg extension | 129 (69) | 168.8 (76) | 39.8 (27)§ | 126 (30) | 173 (46) | 47 (31)§ |
| | | | Cybex Shoulder Strength, ft-16 | | | |
| Shoulder strength | | | | | | |
| Flexion, PT 60°/s | 37.2 (8.3) | 37.7 (9.0) | 0.5 (4.4) | 34.3 (5.2) | 39.1 (8.1) | 4.8 (4.8)†‡ |
| Extension, PT 60°/s | 50.5 (9.6) | 54.7 (12.4) | 4.2 (5.4) | 49.4 (8.6) | 60.6 (12.9) | 11.2 (6.2)†‡ |
| Flexion, TW | 84.0 (17.9) | 81.5 (17.6) | −2.5 (12.1) | 76.6 (13.6) | 83.0 (21.9) | 6.4 (14.8)‡ |
| Extension, TW | 126.0 (26.6) | 125.5 (25.3) | −0.5 (13.2) | 120.0 (23.3) | 132.7 (27.5) | 12.7 (18.6)‡ |
| Knee strength | | | | | | |
| Flexion, PT 60°/s | 72.1 (20.2) | 75.4 (17.7) | 3.3 (10.7)§ | 67.8 (10.0) | 77.3 (14.6) | 9.5 (9.9)§ |
| Extension, PT 60°/s | 104.4 (34.5) | 111.5 (28.4) | 7.1 (16.2)§ | 106.7 (18.0) | 120.5 (26.5) | 13.8 (17.4) |
| Flexion, TW | 83.5 (22.4) | 84.2 (20.3) | 0.7 (10.9) | 83.3 (8.9) | 85.7 (17.3) | 2.4 (19.6) |
| Extension, TW | 107.7 (32.5) | 111.8 (26.7) | 4.1 (20.5) | 115.8 (13.7) | 122.8 (28.7) | 7.0 (23.8) |

*Data are presented as mean (SD). All patients received testosterone and participated progressive resistance exercise. PT indicated peak torque; TW, total work.
†Significantly different change between groups by repeated measures analysis of variance ($P<.05$).
‡Significantly different change from baseline by Tukey test follow-up ($P<0.5$).
§Significant change from baseline in both groups by repeated measures analysis of variance.

The rate of LBM gain for those in the oxandrolone group was 0.9 (0.2) kg/wk, and for those in the placebo group, it was 0.5 (0.4) kg/wk. There were no differences in weight, LBM, or fat changes between subjects taking and those not taking protease inhibitors. The correlation between the change in nitrogen balance and the change in LBM was significant ($P<.05$, $r^2 = 0.46$).

A statistically significant decrease in fat occurred in both groups at week 8 ($P = .005$), which was not different between groups (oxandrolone, 1.7 [2.8] kg; placebo, 1.6 [1.9] kg). A significant increase in bone mineral content was also observed in both groups ($P<.001$ for time effect), which was not different between groups (oxandrolone, 105 [101] g; placebo, 80 [83] g).

**Resting Energy Expenditure.** Baseline REE was not significantly different between groups. For the placebo group it was 7414 (874) kJ/d (1772 [209] kcal/d), and for the oxandrolone group it was 6916 (1004) kJ/d (1653 [240] kcal/d), which was 106% (14%) of the values predicted. After the treatment phase, there was a significant increase in REE in the oxandrolone group compared with the placebo group (1213 [1004] kJ/d [290 {240} kcal/d] vs 377 [753] kJ/d [90 {180} kcal/d]; $P = .03$). When expressed per kilogram of LBM, the difference in REE between groups was no longer significant.

**One-Repetition Maximum Testing.** Improvements in strength from baseline were observed for all upper and lower body muscle groups in the oxandrolone and the placebo groups ($P<.05$) (TABLE 2). The increase in the oxandrolone group was significantly greater than in the placebo group for chest press ($P = .04$), biceps pull ($P = .04$), triceps push ($P = .05$), and leg press ($P = .02$). There were no differences between subjects taking and those not taking protease inhibitors.

## Cybex Testing

Significant improvements from baseline were also seen in force of flexion, extension, and total work measured by dynamometer testing of both the shoulder and knee muscles in both groups (Table 2). The changes in shoulder strength were significantly greater in the oxandrolone group than in the placebo group for measures of both flexion ($P = .04$) and extension ($P = .01$). The changes in lower body (knee) strength were not significantly different between groups. There were no differences between subjects taking and not taking protease inhibitors.

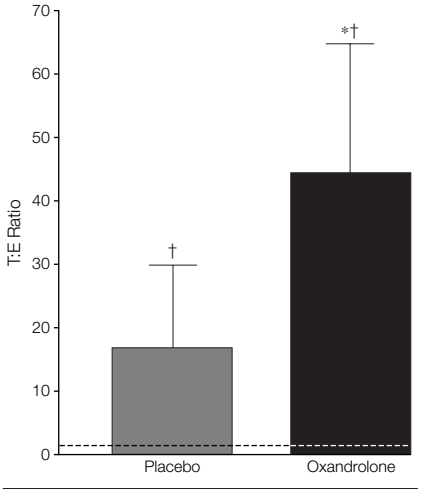
**Table 3.** Change in Serum Hormone Status After Treatment*

| Measurement | Placebo | Oxandrolone |
|---|---|---|
| Total testosterone levels, nmol/L [ng/dL] | | |
| After treatment | 19.0 (3.2) [548 (92)] | 16.9 (4.2) [486 (122)] |
| Change from baseline values | –3.7 (3.3) [–108 (95)] | 4.1 (5.1) [–117 (148)] |
| Luteinizing hormone, IU/L | | |
| After treatment | 0.5 (0.2)† | 0.1 (0.1)† |
| Change from baseline values | –1.8 (0.5) | –3.0 (0.5) |
| Follicle-stimulating hormone, IU/L | | |
| After treatment | 1.2 (0.4)† | 0.1 (0.1)† |
| Change from baseline values | –3.7 (1.1) | –4.7 (1.1) |

*The posttreatment value was measured at the 7-day nadir after the weekly 100-mg intramuscular testosterone injection. Data are presented as mean (SEM). All patients received testosterone and participated in progressive resistance exercise.
†Significant change from baseline for the 2 groups ($P<.05$), which was not significantly different between the groups by repeated measures analysis of variance. Normal ranges for serum total testosterone levels are 7.8 to 31.2 nmol/L [225-900 ng/dL]; luteinizing hormone, 0.4 to 5.7 IU/L; and follicle-stimulating hormone, 1.1 to 13.5 IU/L.

**Serum Gonadal Hormone Concentrations and Urine Screening for Androgens.** The endogenous gonadal axis was suppressed in both groups compared with baseline, with significant decreases in luteinizing hormone ($P<.001$) and follicle-stimulating hormone levels ($P<.001$), but there were no differences between groups (TABLE 3). Serum total testosterone levels were within the normal range and were not significantly different between groups or from baseline. All subjects' urine tested negative for all anabolic steroids other than oxandrolone at baseline and during the treatment period. Oxandrolone was undetectable in all subjects at baseline and in the placebo group during treatment but was present in all subjects in the oxandrolone group during treatment. The testosterone to epitestosterone ratio was similar to published normal values (median, 1.1)[30] in both groups at baseline (oxandrolone, 1.4 [1.4]; placebo, 1.1 [1.1]), and increased significantly from baseline in both groups ($P<.05$ for time effect). The significantly greater increase in testosterone to epitestosterone ratio in the oxandrolone group compared with the placebo group (44.0 [25.0] vs 16.7 [12.8], after treatment; $P = .002$) (FIGURE 4) suggests that residual endogenous androgen synthesis in the presence of testosterone replacement alone was more completely suppressed by the addition of oxandrolone.

**Figure 4.** Change in Testosterone-Epitestosterone (T:E) Ratio Following Treatment at Week 4




The asterisk indicates significantly different change between groups by repeated measures analysis of variance ($P = .002$); dagger, significantly different change from baseline by the Tukey test follow-up procedure ($P<.05$); and dotted line, T:E ratio.

**Stable Isotope/Mass Spectrometric Measurement of de Novo Lipogenesis.** Baseline de novo lipogenesis was elevated in both groups, compared with

**Table 4.** Change in Blood Parameters*

| Measurements | Placebo | Oxandrolone |
|---|---|---|
| CD4 cell count, $\times 10^9$/L | | |
| After treatment | 0.310 (0.260) | 0.234 (0.108) |
| Change from baseline values | −0.028 (0.087) | 0.0 (0.057) |
| High-density lipoprotein cholesterol, mmol/L [mg/dL] | | |
| After treatment | 0.89 (0.46) [34.2 (17.8)] | 0.44 (1.1) [16.9 (4.1)]† |
| Change from baseline values | −0.02 (0.11) [−0.7 (4.4)] | −0.25 (0.14) [−9.8 (5.4)]‡ |
| Total cholesterol, mmol/L [mg/dL] | | |
| After treatment | 4.5 (1.1) [173 (42)] | 4.5 (1.6) [175 (60)] |
| Change from baseline values | −0.06 (0.50) [−2.4 (19.4)] | 1.1 (0.80) [−4.3 (30.9)] |

*Data are presented as mean (SD). All patients received testosterone and participated in progressive resistance exercise.
†Significantly different change from baseline between groups ($P<.05$).
‡Significantly different from baseline ($P<.05$).

age and weight-matched HIV-seronegative controls (after eating, 7.9% [0.8%] in combined groups at baseline vs 3.0% [0.3%] in healthy controls; $P<.05$) and increased significantly from baseline in both the oxandrolone and placebo groups, after treatment (13.9% [2.1%] vs 15.2% [1.8%]) ($P<.001$ for time effect); there were no significant differences between groups.

### Quality of Life Measurements

No change was observed for overall health or energy/fatigue domains,[31] although there were significant increases in the physical function domain ($P = .001$ for time effect).

**Blood Chemistries.** There were no significant changes in CD4 cell counts during the study (TABLE 4). Viral load decreased nonsignificantly in both groups (oxandrolone, 3.9 [4.3] to 3.7 [4.0] $\log_{10}$ copies/mL; placebo, 4.9 [5.3] to 4.8 [5.1] $\log_{10}$ copies/mL). There was a statistically significant decrease in high-density lipoprotein cholesterol (HDL-C) and increase in the total cholesterol–HDL-C ratio in the oxandrolone group, but there was no change in either parameter in the placebo group ($P<.001$ between groups).

**Adverse Effects.** Two subjects in the oxandrolone group had elevations in liver function test results, which led to 1 subject's discontinuing medication before the end of the 8-week study. Both of these patients were also receiving protease inhibitors. Mood swings were reported in 8 subjects, 5 in the oxandrolone group and 3 in the placebo group. In the oxandrolone group, 4 subjects experienced anxiety and 1 reported nausea. Finally, 4 subjects, 2 in each group, reported an increase in libido during the study.

**Open-Label Phase.** The group as a whole continued to gain LBM over 12 weeks (1.0 [0.6] kg), with loss of fat (−0.9 [0.6] kg) ($P<.05$ for both vs preopen label). When stratified by preceding study arm, subjects who were oxandrolone-naive had significantly greater gains in LBM (1.8 [0.5] kg) than subjects who previously had taken oxandrolone (0.4 [0.6] kg; $P<.05$).

## COMMENT

Perhaps the most important finding of this study is that extremely high dosages of androgens were not required for a significant beneficial interaction with PRE in men with HIV-related weight loss. In their study, Bhasin et al[18] gave intramuscular testosterone at 600 mg/wk. We gave a physiologic replacement dosage of intramuscular testosterone (100 mg/wk) plus an oral anabolic steroid, oxandrolone, at a dosage of 20 mg/d, previously shown to be well tolerated for long-term use in humans.[19-21] There is no simple way to compare relative potencies of different testosterone analogs[17,32]; our intent was not to establish the androgen dose-response curve for synergy with PRE but to test the efficacy of a dose and form that has been given safely over the long-term to patients, eg, with alcoholic hepatitis.[19-21] In contrast, the safety and behavioral consequences of extremely high doses of testosterone[18] have not been established.

Several independent measures confirmed that LBM gains represent functional lean tissue. Strength was markedly improved; nitrogen retention was substantial and correlated with accrual of LBM; and REE increased. These complementary findings strengthen the external validity of the conclusion that lean tissue anabolism was significantly improved. Because the precision of measures such as DEXA and nitrogen balance is extremely good, the central issue of interpretation in studies attempting to alter body composition relates more to external validity (ie, biological meaning of measured changes) than to internal validity (ie, precision and accuracy of the measurements).

Comparison of these results with nutritional and anabolic therapies reported previously in AIDS patients is instructive (TABLE 5). The LBM gains and nitrogen retention in members of the oxandrolone group in the current study are considerably greater than with previously reported therapies in HIV infection or cancer cachexia.[33] The remarkable increases observed in LBM and strength in the oxandrolone group obviate the need to consider massive doses of androgens or anabolic steroids for the treatment of weight loss in HIV-infected men, in our view.

Moreover, the use of protease inhibitor therapy did not affect the gains in lean tissue or muscle strength, based on our post hoc analysis. This is an important point because weight gain after initiation of protease inhibitor treatment represents predominantly body fat.[7-10] Although our post hoc analysis must be interpreted with caution, the use of protease inhibitors did not prevent substantial gains in LBM. Finally, it is interesting to compare these results in men with HIV infection and prior weight loss with results previously reported by Bhasin et al[18] using high-dose testosterone with PRE and

placebo with PRE in healthy men. We observed a 7-kg LBM increase in the oxandrolone group and 4 kg in the placebo group compared with the report of Bhasin et al[18] of 6 kg and 2 kg of fat-free mass, respectively, in HIV-seronegative men. Strength improvements were also comparable. (Lean body mass and fat-free mass differ operationally by the mode of measurement [DEXA and underwater weighing, respectively], but gains in either parameter represent metabolically active, nonfat tissue in this setting.)

Certain design features of this study should be noted. We confirmed compliance and excluded exogenous anabolic steroid use by monitoring urine and blood.[29,30] The exercise regimens were supervised and strictly controlled. The intervention was blinded to all study participants, including the exercise trainers. Finally, both the placebo and the oxandrolone groups received a physiologic replacement dose of testosterone. This last feature was included for several reasons: (1) to make hormonal status more comparable between groups, by suppressing endogenous testosterone production[17,34]; (2) to ensure that borderline hypogonadism[11,15] was not present in either group; and (3) to avoid the possibility of inducing hypothalamic hypogonadism secondary to the exercise program, as has been reported in other clinical settings.[35,36]

The exercise regimen was well tolerated. Although overtraining can suppress immune function,[37] we found no evidence of worsening immunologic or virologic status (Table 4). We did observe significantly elevated de novo lipogenesis after PRE in both groups. We speculate that this reflects the systemic effects of cytokine release induced by muscle damage,[38,39] but we have no direct evidence to support this hypothesis. The lipid profile deteriorated in the oxandrolone group (Table 4), including substantially reduced HDL-C concentrations. Other 17a-methylated androgens also reduce HDL-C concentrations.[40] This effect on plasma lipid levels could be important in HIV-infected patients, in view of lipid abnormalities associated with HIV infection[12,41] that can be exacerbated by HIV-protease inhibitors.[7] One subject in the oxandrolone group was forced to discontinue the study because of elevation of liver enzyme levels. Other adverse effects were modest.

**Table 5.** Comparison of Therapeutic Regimens for HIV–Related Weight Loss*

| Source, y | Nutritional or Anabolic Therapy | Nitrogen Retention, g/d | Rate of Change in Body Composition, kg/wk | |
|---|---|---|---|---|
| | | | LBM† | Weight |
| Von Roenn et al,[5] 1994; and Oster et al,[6] 1994 | Megestrol acetate | . . . | 0.00-0.15 | 0.45 |
| Kotler et al,[3] 1990 | Parenteral nutrition | . . . | 0 | 0.30 |
| Mulligan et al,[13] 1993; and Schambelan et al,[14] 1996 | rGH | 4.0 | 0.25 | 0.13 |
| Strawford et al,[15] 1998 | Nandrolone decanoate (hypogonadal)‡ | 3.7 | 0.25 | 0.41 |
| Current study | PRE | 3.8 | 0.48 | 0.53 |
| Current study | PRE and oxandrolone | 5.6 | 0.86 | 0.84 |

*HIV indicates human immunodeficiency virus; ellipses, information not available; rGH, recombinant human growth hormone; and PRE, progressive resistance exercise.
†Lean body mass (LBM) was determined by dual-energy x-ray absorptiometry.
‡Hypogonadal indicates treatment of men with borderline levels of testosterone (lowest quartile of testosterone serum levels).

The subjects in this study had experienced on average 8% to 9% weight loss and were currently weight stable. Weight loss of more than 5% is associated with reduced survival and higher rates of opportunistic infections.[42] Moreover, the goal for patients like these is often to increase strength and exercise capacity. Therefore, we believe that it is reasonable to consider HIV-seropositive patients with this degree of weight loss for a regimen similar to that used in our study, even if their weight is currently stable.

This study was not designed to differentiate between the possible anabolic roles played by the components provided to both study groups (eg, the exercise regimen, replacement dosage of testosterone, diet, or personal attention received through participation). The study was designed to address whether the addition of 20 mg/d of oxandrolone improves the anabolic and functional response to a regimen of PRE and physiologic testosterone replacement. These results answer this question definitively but do not reveal which factors were responsible for gains in the placebo group. Grinspoon et al[16] showed that administration of testosterone at replacement dosages in frankly hypogonadal men with HIV-related weight loss increases LBM; Strawford et al[15] demonstrated that nandrolone administration in borderline hypogonadal men also increases LBM. Neither of these studies were performed in eugonadal men, however, and neither involved exercise training. It will be important in future studies to assess the independent role of specific components.

In conclusion, the combination of PRE with a moderately supraphysiologic androgen regimen that included an anabolic steroid, oxandrolone, resulted in significantly greater increases in lean tissue and muscle strength than PRE with physiologic testosterone replacement alone in eugonadal, HIV-infected men with prior weight loss. The use of protease inhibitor therapy did not affect the lean tissue response.

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Medical Pharmacology, University of California, Los Angeles (Dr Catlin).
**Funding/Support:** This work was supported in part by grants DK-40995 from the National Institutes of Health, Bethesda, Md, and Bio-Technology General Corporation, Iselin, NJ (Dr Hellerstein), and from a sport consortium of the National Collegiate Athletic Association, the United States Olympic Committee, and the National Football League (Dr Catlin).
**Acknowledgment:** We wish to thank Mark Rincon, Casey Bass, and Allan Moore, the exercise trainers for this study, for their invaluable help; Tom Celentano and Molly Ramsey for their help with recruitment; Boro Starcevic and Emma Di Stefano for developing the mass spectrometric oxandrolone assay; and the nursing and laboratory staff at the Western Human Nutrition Research Center, Presidio of San Francisco, Calif.

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0021-972X/99/$03.00/0
The Journal of Clinical Endocrinology & Metabolism

Vol. 84, No. 8
*Printed in U.S.A.*

# Short-Term Oxandrolone Administration Stimulates Net Muscle Protein Synthesis in Young Men*

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**ABSTRACT**

Short term administration of testosterone stimulates net protein synthesis in healthy men. We investigated whether oxandrolone [Oxandrin (OX)], a synthetic analog of testosterone, would improve net muscle protein synthesis and transport of amino acids across the leg. Six healthy men [22 ± 1 (±SE) yr] were studied in the postabsorptive state before and after 5 days of oral OX (15 mg/day). Muscle protein synthesis and breakdown were determined by a three-compartment model using stable isotopic data obtained from femoral arterio-venous sampling and muscle biopsy. The precursor-product method was used to determine muscle protein fractional synthetic rates. Fractional breakdown rates were also directly calculated. Total messenger ribonucleic acid (mRNA) concentrations of skeletal muscle insulin-like growth factor I and androgen receptor (AR) were determined using RT-PCR. Model-derived muscle protein synthesis increased from 53.5 ± 3 to 68.3 ± 5 (mean ± SE) nmol/min·100 mL/leg ($P < 0.05$), whereas protein breakdown was unchanged. Inward transport of amino acids remained unchanged with OX, whereas outward transport decreased ($P < 0.05$). The fractional synthetic rate increased 44% ($P < 0.05$) after OX administration, with no change in fractional breakdown rate. Therefore, the net balance between synthesis and breakdown became more positive with both methodologies ($P < 0.05$) and was not different from zero. Further, RT-PCR showed that OX administration significantly increased mRNA concentrations of skeletal muscle AR without changing insulin-like growth factor I mRNA concentrations. We conclude that short term OX administration stimulated an increase in skeletal muscle protein synthesis and improved intracellular reutilization of amino acids. The mechanism for this stimulation may be related to an OX-induced increase in AR expression in skeletal muscle. (*J Clin Endocrinol Metab* **84:** 2705–2711, 1999)

ATHLETES have long used anabolic agents for improving lean muscle mass and strength. However, clinicians have only recently recognized the benefits of anabolic agents for patients with trauma- and disease-related muscle wasting. Recently, several clinical studies demonstrated the positive benefits of testosterone (T) administration to various patient populations. In particular, hypogonadal men benefit from T replacement therapy via enhanced skeletal muscle mass (1–3), increased bone density (2), and increased protein synthesis (1). Likewise, elderly men receiving T replacement therapy have increased lean body mass (4), strength (5), and protein synthesis (5) along with decreased bone resorption (4). Moreover, changes in body composition, including a loss in lean body mass, are highly correlated with androgen levels in hypogonadal men with the acquired immunodeficiency syndrome (AIDS) wasting myopathy (6).

We recently showed that T enanthate (TE), administered im to healthy young men, increased net protein synthesis and reutilization of intracellular amino acids in skeletal muscle (7). In addition, several other studies have found T administration to increase muscle protein synthesis (1, 5, 8), although these studies failed to measure protein breakdown. One of the major limitations of previous studies of fractional synthetic rate (FSR) is that no estimation of protein breakdown could be made simultaneously. Consequently, the traditional approach to the study of muscle protein kinetics (*i.e.* FSR) provided no information on the net balance between synthesis and breakdown. Therefore, our laboratory developed a new method for measuring fractional protein breakdown that is independent of the arterio-venous (A-V) model (9).

Although natural androgens such as T clearly stimulate muscle protein synthesis, they also possess androgenic or virilizing effects. Often this limits the clinician's use of these androgens to specific patient populations such as hypogonadal men. However, efforts have been made to find alternative anabolic agents that can be used in women and children suffering from muscle-wasting diseases or trauma. Oxandrolone [Oxandrin (OX) Bio-Technology General, Iselin, NJ], a synthetic analog of T, is an oral anabolic steroid currently used as an adjunctive therapy to promote weight gain in patients after surgery, chronic infections, and severe trauma. OX improved weight gain in patients experiencing AIDS-wasting myopathy (10) as well as in convalescing burn

Received March 3, 1999. Revision received May 4, 1999. Accepted May 11, 1999.

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* This work was conducted at the General Clinical Research Center at the University of Texas Medical Branch (Galveston, TX). Funding was provided by NIH Grants M01–00073 and 5-R01-DK-38010–11 (to R.R.W.) and NIH Grant AG-11000 (to R.J.U.).

patients (30–50% total body surface area burns) (11). In addition, OX is used by clinicians to treat children with growth disorders such as Turner's syndrome and constitutional delay of growth and puberty (12, 13). A recent pilot study in boys with Duchenne muscular dystrophy found that OX, given at a dose of 0.1 mg/kg·day, improved muscle strength over a 3-month period (14). Given that OX is administered orally, as opposed to im as with TE, its ease of administration makes it attractive to clinicians and patients alike. Further, OX is purported to have a much greater anabolic potential than T, with fewer of the androgenic effects. However, no studies have demonstrated whether OX, similar to TE, promotes the stimulation of protein synthesis in skeletal muscle.

Hence, we investigated whether OX, a purported anabolic agent, improves net muscle protein synthesis and transport of amino acids in fasted young men. The present study was designed to mimic the 5-day TE study in normal males previously discussed (7). We sought to evaluate the short term (5-day) effects a moderate dose (15 mg/day) of OX on the incorporation of amino acids into muscle proteins using an established protein kinetic model (15, 16). We further examined the effect of OX on the messenger ribonucleic acid (mRNA) concentrations of skeletal muscle insulin-like growth factor I (IGF-I) and androgen receptors (AR).

## Subjects and Methods

### *Subjects*

Six healthy men [age, 22 ± 3 (±SD) yr; weight, 77 ± 13 kg; height, 178 ± 7 cm] were studied before and after taking a daily dose of oral OX (15 mg/day) for 5 days. All subjects gave informed written consent according to the guidelines established by the institutional review board at the University of Texas Medical Branch (Galveston, TX). Subject eligibility was assessed by performing a medical screening, which included an electrocardiogram, blood count, plasma electrolytes, blood glucose concentration, and liver and renal function tests. Subjects presenting with heart or liver disease, hypo- or hypercoagulation disorders, vascular diseases, hypertension, diabetes, or an allergy to iodides were excluded from participation.

### *Experimental protocol*

All isotope infusion studies were performed at the General Clinical Research Center at the University of Texas Medical Branch. Subjects were admitted the night before each study and were fasted from 2200 h until completion of the 5-h isotope infusion study. At approximately 0630 h the following morning (day 0), a 20-gauge polyethylene catheter (Insyte-W, Becton Dickinson and Co., Sandy, UT) was inserted into the antecubital vein of one arm for the infusion of amino acids. A second 20-gauge polyethylene catheter was placed in the contralateral wrist for blood sampling for measurement of systemic indocyanine green (ICG). A heating pad was placed around the arm and wrist to maintain a temperature of about 65 C during blood flow measurements.

At 0700 h on days 0 and 5, baseline blood samples were drawn for the analysis of background amino acid enrichment, ICG concentration, and peak T and OX concentrations. A primed continuous infusion of labeled phenylalanine was initiated at the following infusion rate (IR) and priming dose (PD): L-[ring-$^2H_5$]phenylalanine, IR = 0.05 μmol/kg·min, PD = 2 μmol/kg. At approximately 0730 h, a 3-Fr 8-cm polyethylene Cook catheter (Bloomington, IN) was placed under local anesthesia into the femoral artery and vein. Femoral catheters were required for A-V blood sampling and infusion of ICG (artery) for determination of leg blood flow.

Biopsies of the vastus lateralis were obtained at 2 h, 4 h 30 min, and 5 h of tracer infusion using a 5-mm Bergström needle as previously described (16). Tissue was immediately frozen in liquid nitrogen and stored at −80 C until analysis. After the 2-h biopsy, a primed (2 μmol/kg) continuous infusion of L-[$^{15}$N]phenylalanine was initiated and maintained until 4 h (Fig. 1). Arterial and intracellular L-[$^{15}$N]phenylalanine enrichments at plateau and again after decay were obtained for purposes of determining the fractional breakdown rate (FBR). The 4 h 30 min and 5 h biopsies were used to determine FBR. The fractional synthetic rate (FSR) of skeletal muscle protein was determined by the incorporation of L-[ring-$^2H_5$]phenylalanine into protein from 2–5 h.

A-V blood samples were obtained at 20-min intervals from 4–5 h to determine amino acid kinetics. Fifteen minutes before the sampling hour, a continuous infusion (IR = 0.5 mg/min) of ICG was initiated and allowed to reach systemic equilibrium (10–15 min) for purposes of measuring leg blood flow. Subsequent blood sampling was performed simultaneously from the femoral vein and heated wrist vein throughout the sampling hour. To avoid disrupting blood flow measurements, all A-V blood samples for amino acid kinetics were obtained after taking the blood flow measures, and the ICG was stopped. ICG was restarted and allowed to run uninterrupted for approximately 10–15 min before the next blood flow measurement.

At the end of the 5-h infusion study, subjects were fed, and all peripheral and femoral catheters were removed. Beginning at 2100 h on day 0, all subjects were given 15 mg OX (BTG Pharmaceuticals Co., Iselin, NJ), orally, for 5 days. On day 3, subjects returned to the General Clinical Research Center at 0700 h for venous blood sampling to determine total T and OX concentrations. On day 5, the above experimental protocol was repeated.

### *Analytical methods*

*Blood.* The concentrations of unlabeled and labeled phenylalanine were determined by gas chromatography-mass spectrometry (GC-MS) as previously described (16). Briefly, A-V blood samples were collected in preweighed tubes containing 15% sulfosalicylic acid. A known internal standard (100 μL/mL blood) was added and thoroughly mixed. The composition of this standard mixture was 50.3 μmol/L L-[ring-$^{13}C_6$]phenylalanine. After reweighing the tubes to determine the final blood volume, tubes were centrifuged, and the supernatant was collected and stored at −20 C until analysis. Blood amino acids were separated using cation exchange chromatography (16) and enrichments of the internal standard, and infused tracers were determined on their *tert*-butyldimethylsilyl (*t*-BDMS) derivatives (17). Using the GC-MS, the isotopic enrichment of free amino acids in blood was determined by positive chemical ionization and selected ion monitoring (model 5973, Hewlett-Packard Co., Palo Alto, CA). Finally, leg blood flow was determined spectrophotometrically by measuring serum ICG concentration at λ = 805 nm.

*Muscle.* Muscle samples were weighed, and protein was precipitated with 500 μL 14% perchloric acid. A known internal standard solution (2 μL/mg muscle tissue) was added to measure the intracellular concentrations of phenylalanine. The solution contained 2.4 μmol/L L-[ring-$^{13}C_6$]phenylalanine. The supernatant was collected after homogenization of the tissue and centrifugation. This procedure was repeated three times. The pooled supernatant with muscle amino acids was separated

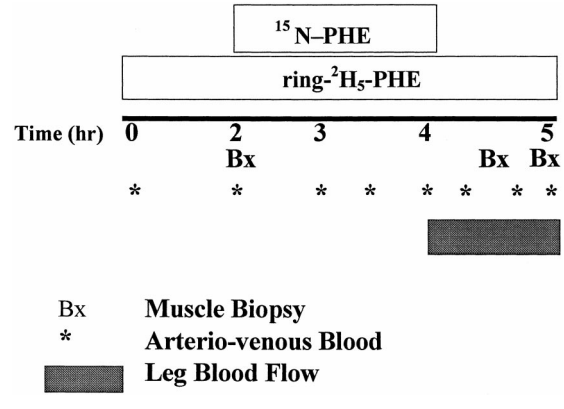


FIG. 1. Stable isotope infusion protocol. ring-$^2H_5$-PHE, L-[ring-$^2H_5$]phenylalanine; $^{15}$N-PHE, L-[$^{15}$N]phenylalanine.

using cation exchange chromatography (16). Intracellular amino acid enrichments and concentrations were determined on their *tert*-butylmethylsilyl derivatives (17) using GC-MS in the electron impact mode. Intracellular enrichment was determined by correction for extracellular fluid based on the chloride method (18). The remaining pellet was washed several times with 0.9% saline and again with absolute ethanol, dried at 50 C overnight, and hydrolyzed in 6 N HCl at 110 C for 24 h. The hydrolysate was then passed over a cation exchange column in the same manner as the blood was processed. Samples were analyzed for phenylalanine enrichment by GC-MS (model 8000, MD 800, Fisons Instruments, Manchester, UK) using chemical ionization and the standard curve approach (19).

*Hormone assays.* The concentration of total T was measured in serum with a commercial RIA kit (Diagnostic Products, Los Angeles, CA). Free or bioavailable T was measured by equilibrium dialysis [Mayo Medical Laboratories (Rochester, MN), Quest Diagnostics, Inc., Nichols Institute (San Juan Capistrano, CA)]. Serum OX concentrations were measured by University of California-Los Angeles Olympic Analytical Laboratory. Briefly, a liquid-liquid extraction was performed by adding 50 $\mu$L internal standard [16,16,17-$^3$H]T ($d^3$T; 12 $\mu$g/mL; MSD Isotopes, Montreal, Que.), 1 mL 50% saturated sodium acetate buffer (0.5 mol/L; pH 5.5), and ethyl ether (5 mL) to 0.5 mL plasma. After vortexing (10 min) and centrifugation (15 min at 2000 rpm), the ethyl ether layer was dried under nitrogen at room temperature and reconstituted in 200 $\mu$L methanol for high performance liquid chromatography analysis. Liquid chromatography was performed on a Shimadzu system (Shimadzu, Columbia, MD) equipped with a Hypersil BDS $C_{18}$, 50 × 2-mm column (Keystone Scientific, Inc., Bellefonte, PA) and Hypersil BDS $C_{18}$, 20 × 2-mm precolumn, operated at a flow rate of 400 $\mu$L/min. The injection volume was 5 $\mu$L. The gradient was methanol-water (1:1) for 1 min, methanol-water (9:1) for the next 1 min, a 1-min hold, and a return to the starting condition in 0.5 min. MS analyses were performed on a triple quadrupole Perkin Elmer Corp.-Sciex API 300 (Norwalk, CT) equipped with an APCI interface. The nebulizer temperature was optimized for maximum sensitivity at 350 C. Positive ions (m+1) for OX (307.2; Searle Pharmaceutical, Chicago, IL) and $d^3$T (292.2) were admitted into the second quadrupole for collision-induced dissociation. Product ions 289.2 and 97.0 were monitored and used to quantitate OX and $d^3$T, respectively. Concentrations were determined by reference to a six-point calibration curve.

*Total RNA isolation and qualitative RT-PCR.* Total RNA was isolated from muscle biopsy samples (50–75 mg) using RNAzol B (Tel-Test, Inc., Friendswood, TX). Two micrograms of total RNA were then converted to DNA using a Reverse Transcription System (Promega Corp., Madison, WI). The DNA (5 $\mu$L) was then subjected to PCR in the presence of the appropriate primers. The products of the PCR were run on Southern gel, and amplified DNA products were sized by DNA ladder. Southern blots were then made and hybridized to oligonucleotides of the DNA fragment. Glyceraldehyde phosphate dehydrogenase (GAP) was coamplified in each sample as an internal control. For the AR, the downstream primer was included in the reverse transcriptase reaction. The primers and hybridization oligonucleotides for the IGF-I and AR are as follows: IGF-I: sense, 5′-AAATCAGCAGTCTTGGAACC-3′; antisense, 5′ CTTCTGGGTCTTGGGCATGT 3′; oligonucleotide, 5′-CAAGCCCACAGGGTATGGCTCCAGCAGT-3′; AR: sense, 5′-GATGCTCTACTTCGCCCCTGA-3′; antisense, 5′-CCCAGCAAATAGAATTCCATGAC-3′; oligonucleotide, 5′-CTGGGTGTGGAAATAGATG-3′; and GAP: sense, 5′-GGTATCGTGGAAGGACTCAT-3′; antisense, 5′-TCCACCACCCTGTTGCTGTA-3′; oligonucleotide, 5′-GTGGGTGTCGCTGTTGAAGT-3′.

Southern blot band densities were measured using the ImageQuant analysis program (Molecular Dynamics, Inc., Sunnyvale, CA).

## *Calculations*

*Kinetic model.* The kinetics of intracellular free amino acids have been described previously (16). However, we will briefly detail the kinetic parameters that make up the three-pool model of leg amino acid kinetics (Fig. 2).

The femoral artery delivers ($F_{in}$) amino acids to the leg, whereas amino acids leave via the femoral vein ($F_{out}$). These amino acids can, therefore, move intercompartmentally between the artery (A), vein (V), and muscle (M). Inward amino acid transport from A to M ($F_{M,A}$) and outward amino transport from M to V ($F_{V,M}$) occur via the femoral artery and vein, respectively. Thus, inward ($F_{in}$) and outward ($F_{out}$) tissue transport were calculated as follows:

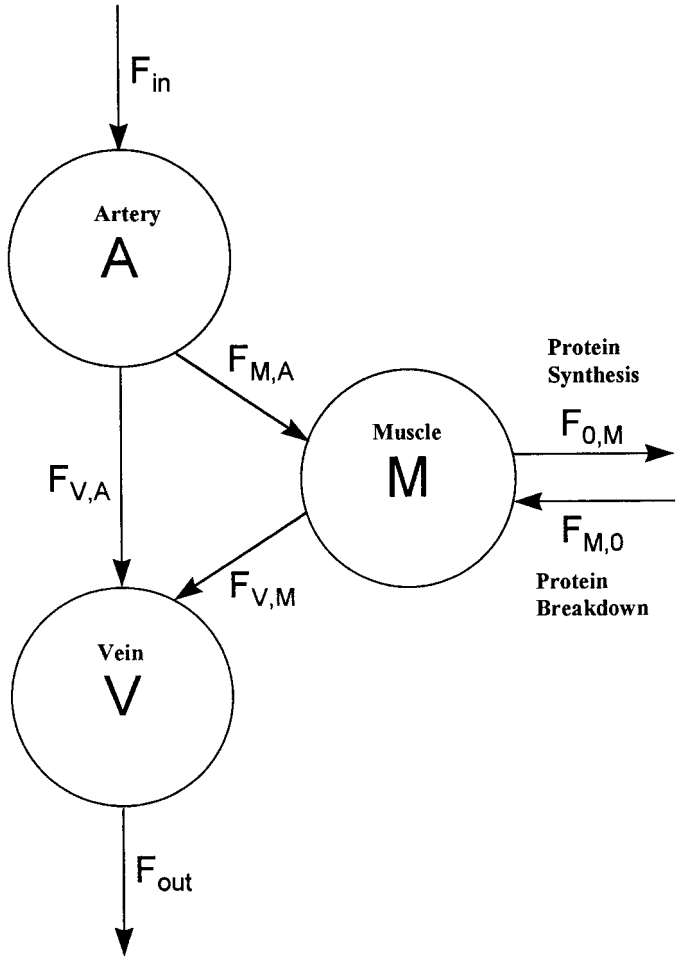


FIG. 2. Three-compartmental model of leg amino acid kinetics. Free amino acid pools in femoral artery (A), femoral vein (V), and muscle (M) are connected by *arrows*, indicating unidirectional amino acid flow between each compartment. Amino acids enter the leg via femoral artery ($F_{in}$) and leave via femoral vein ($F_{out}$). $F_{V,A}$ is the direct flow from artery to vein of the amino acids that do not enter the intracellular fluid. $F_{M,A}$ and $F_{V,M}$ are the inward and outward transport from the artery to the muscle and from the muscle to the vein, respectively. $F_{M,O}$ is the intracellular amino acid appearance from proteolysis for phenylalanine. $F_{O,M}$ is the rate of disappearance of intracellular amino acids for protein synthesis for phenylalanine.

$$F_{in} = C_A \times BF \quad (I)$$

$$F_{out} = C_V \times BF \quad (II)$$

$$F_{M,A} = \{[(E_M - E_V)/(E_A - E_M)] \times C_V + C_A\} \times BF \quad (III)$$

$$F_{V,M} = \{[(E_M - E_V)/(E_A - E_M)] \times C_V + C_V\} \times BF \quad (IV)$$

where $C_A$ and $C_V$, and $E_A$ and $E_V$ are amino acid concentrations and tracer enrichments in the femoral artery and vein, and $E_M$ is enrichment in the muscle. Leg blood flow is represented by BF. Amino acids that bypass the muscle via the femoral artery can be calculated by either of the following expressions:

$$F_{V,A} = F_{in} - F_{M,A} \quad (V)$$

$$F_{V,A} = F_{out} - F_{V,M} \quad (VI)$$

The model also enables the calculation of the rate of intracellular appearance ($F_{M,O}$) of amino acids from protein breakdown and the rate of amino acid utilization ($F_{O,M}$) for protein synthesis. Amino acid appearance and utilization are calculated by the following formulas, respectively:

$$F_{M,O} = F_{M,A} \times (E_A/E_M - 1) \quad (VII)$$

$$F_{O,M} = (C_A \times E_A - C_V \times E_V) \times BF/E_M \quad (VIII)$$

The following expression represents the total rate of appearance ($Ra_M$) of the intracellular amino acids, which is a function of protein breakdown ($F_{M,O}$) and inward tissue transport ($F_{M,A}$).

$$Ra_M = F_{M,O} + F_{M,A} \quad (IX)$$

*Protein synthesis efficiency (PSE).* Using phenylalanine, we calculated the relative efficiency of protein synthesis as follows:

$$PSE = F_{O,M}/(F_{M,A} + F_{M,O}) \quad (X)$$

PSE is defined as the fraction of the intracellular amino acid rate of appearance that is incorporated into muscle proteins, taking into account that phenylalanine is not oxidized in the muscle. Therefore, $F_{O,M}$ represents the amount of amino acid incorporated in the muscle proteins.

*FSR.* Using the traditional precursor-product method, we determined the FSR of muscle proteins by measuring the rate of phenylalanine tracer incorporation into protein and the enrichment of the intracellular pool as the precursor

$$FSR = [(E_{p2} - E_{p1})/(E_M \times t)] \times 60 \times 100 \quad (XI)$$

where $E_{p1}$ and $E_{p2}$ are the enrichments of the protein-bound L-[*ring*-$^2H_5$]phenylalanine at the 2 and 5 h sampling points. Average intracellular L-[ring-$^2H_5$]phenylalanine enrichment is $E_M$, whereas time in minutes is represented by *t*. To express FSR as a percentage per h, the expression is then multiplied by the factors 60 (minutes per h) and 100, respectively.

*FBR.* We will briefly discuss the new method for measuring fractional protein breakdown, which has been derived and described in detail previously (9). Further, the FBR method has recently been validated in a report from this laboratory (20). This method employs a variation of the traditional precursor-product method for determining FSR. In this case, the product is free intracellular amino acids, and the precursors are arterial blood and tissue protein.

The FBR technique involves stopping enrichment after reaching an isotopic equilibrium of L-[$^{15}$N]phenylalanine and determining the rate of decay of the intracellular amino acid enrichment. The rate of decay of the free intracellular enrichment is determined by the arterial decay (which continues to provide a certain amount of label to the intracellular pool as well as unlabeled amino acids) and the FBR (which provides the rest of the unlabeled amino acids). Because FBR is constant at physiological steady state, and the decay curves in the arterial and intracellular pools are measurable, FBR is measurable. The following equation is used for the calculation of FBR:

$$FBR = \frac{E_F(t_2) - E_F(t_1)}{p\int_{t_1}^{t_2} E_A(t)dt - (1+p)\int_{t_1}^{t_2} E_F(t)dt} \times \frac{T}{Q_F} \quad (XII)$$

where $P = E_F/(E_A - E_F)$ at isotope plateau, $E_A(t)$ and $E_F(t)$ are the arterial and intracellular enrichments, and $T/Q_F$ is the ratio of bound to unbound amino acid in the tissue sample.

Without the variables P and $T/Q_F$ in the above equation, the equation is simply the traditional precursor-product equation. The traditional precursor-product equation assumes that the product is only derived from one precursor. However, when determining FBR, the product has two sources, plasma amino acids and protein-bound amino acids. These two sources are therefore represented by the variable P. P is equal to the ratio of protein breakdown to transport of amino acids into the cell and is calculated by determining the dilution of amino acid enrichment between plasma and the intracellular space at isotopic steady state.

The factor $T/Q_F$ is necessary to make the units of FBR comparable to those of FSR, such that the units of FBR are rate of protein breakdown divided by the bound amino acid pool size. The traditional precursor-product equation calculates the rate of conversion of precursor to product divided by the product pool size. However, with FBR, the rate of protein breakdown is divided by the free intracellular amino acid pool size. Finally, in this FBR model, as well as in the kinetic determination of the rate of appearance from protein breakdown ($F_{M,O}$) and disappearance to protein synthesis ($F_{O,M}$), an assumption must be made that the label is not recycled from protein breakdown back to the free intracellular pool. This is reasonable, given the low enrichment of the muscle pool, compared to the free intracellular enrichment at isotopic equilibrium. This leaves arterial blood as the only source of tracer for the free intracellular pool. In contrast, unlabeled amino acids from both arterial blood and protein breakdown contribute to the free intracellular pool.

*Statistical analysis.* Comparisons between basal and treatment conditions were performed using paired *t* tests. Statistical significance was established at $P \leq 0.05$. Data are presented as the mean ± SE.

## Results

As depicted in Table 1, arterial steady state was achieved during the sampling hour (240–300 min) of both the control period and after 5 days of OX administration. However, arterial enrichments were significantly higher after OX treatment (Table 1; $P < 0.05$). Due to noncompliance with medications by one subject, all data presented include only the results from five subjects.

Serum OX concentrations on day 3 (1.9 ± 0.4 ng/dL) and day 5 (2.2 ± 0.3 ng/dL) of OX administration, measured 10 h after each evening's oral dose (2100 h), remained steady. However, by 18 h posttreatment on day 5, serum OX levels were markedly reduced (0.48 ± 0.06 ng/dL; $P < 0.01$) compared to day 3 or day 5 10-h values. Total serum T concentrations were within normal physiological range on day 0 (449 ± 35 ng/dL) and day 3 (441 ± 44 ng/dL) of OX treatment. However, by day 5, total serum T concentrations were significantly reduced (282 ± 45 ng/dL; $P < 0.05$) below day 0 and day 3 values (Fig. 3). Serum free T concentrations were within normal physiological range on days 0, 3, and 5. However, by day 5, serum free T concentrations were significantly reduced (98 ± 10 pg/mL; $P < 0.001$) below day 0 (121 ± 12 pg/mL) and day 3 (126 ± 9 pg/mL) values. Hence, the total androgen concentration (T + OX) was reduced in parallel to the reduction in T (Fig. 3).

FSR increased from 0.057 ± 0.004% to 0.082 ± 0.008%/h after 5 days of OX administration (Fig. 4; $P < 0.05$), whereas FBR remained unchanged (0.138 ± 0.005% *vs.* 0.118 ± 0.0008%/h; $P = 0.40$). Whole body appearance of phenylalanine decreased from 0.80 ± 0.03 to 0.75 ± 0.03 μmol/kg·min after OX treatment ($P < 0.05$).

The model-derived parameters of leg muscle free amino acid kinetics of the five subjects in the control period and after 5 days of OX are shown in Table 2. OX treatment had no effect on amino acid delivery to ($F_{in}$) or the release of labeled phenylalanine from the leg ($F_{out}$). Moreover, the inward transport rate ($F_{M,A}$) of phenylalanine remained unchanged. However, 5 days of OX administration resulted in a significant reduction in the rate of outward

**TABLE 1.** Femoral artery free amino acid enrichments

| Min of infusion | Phenylalanine | |
|---|---|---|
| | Control (tracer/tracee) | Oxandrolone (tracer/tracee) |
| 240 | 0.0646 ± 0.0035 | 0.0759 ± 0.0032[a] |
| 260 | 0.0640 ± 0.0030 | 0.0722 ± 0.0016[a] |
| 280 | 0.0647 ± 0.0038 | 0.0672 ± 0.0025[a] |
| 300 | 0.0638 ± 0.0017 | 0.0715 ± 0.0022[a] |

Data are the mean ± SE and are expressed as the tracer to tracee ratio. All tracer values during control and oxandrolone infusions are at steady state across infusion time by ANOVA. Samples from 240–300 min of isotope infusion were used for model calculations. Mean tracer to tracee ratios are significantly greater after 5 days of oxandrolone administration by paired *t* test.

[a] $P < 0.05$.

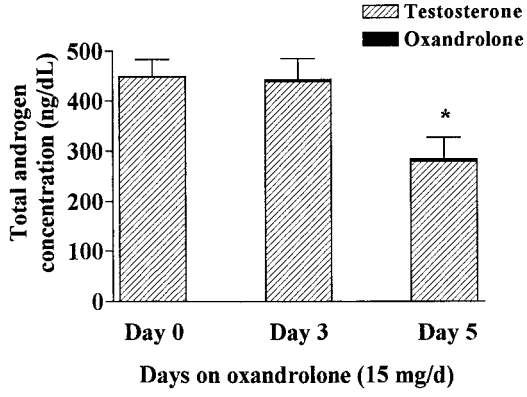


FIG. 3. Total androgen concentration. Total serum T (*hatched portion*) and OX (*black portion*) concentrations in five young men on days 0, 3, and 5. *, T decreased significantly from days 0 and 3 to day 5 ($P < 0.05$).

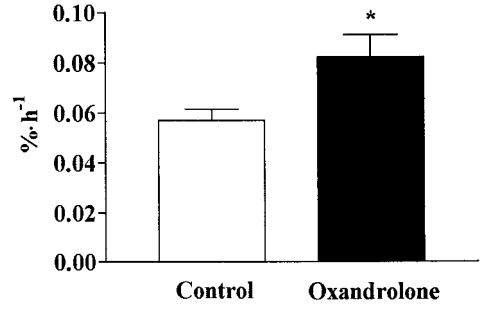


FIG. 4. Muscle protein FSR. Muscle protein FSR in five young men during the postabsorptive state both before (control, *open bar*) and after (OX, *black bar*) OX administration. *, Five days of OX administration increased the synthesis rate of muscle proteins by approximately 44% ($P < 0.05$).

**TABLE 2.** Effect of oxandrolone treatment on leg muscle free amino acid kinetics in young men

| Kinetic parameter | Phenylalanine | |
|---|---|---|
| | Control | Oxandrolone |
| $F_{in}$ (arterial delivery) | 263 ± 46 | 227 ± 42 |
| $F_{out}$ (venous outflow) | 293 ± 46 | 228 ± 45 |
| $F_{M,A}$ (inward transport) | 144 ± 17 | 124 ± 25 |
| $F_{V,M}$ (outward transport) | 175 ± 15 | 125 ± 29[a] |
| $F_{V,A}$ (functional shunting) | 118 ± 35 | 102 ± 17 |
| $F_{M,O}$ (protein breakdown) | 83 ± 8 | 69 ± 8 |
| $F_{O,M}$ (protein synthesis) | 53 ± 3 | 68 ± 5[a] |
| $Ra_M$ (intracellular appearance) | 228 ± 16 | 193 ± 32 |
| NB (net balance) | −30 ± 6 | −1 ± 4[a] |

Data are the mean ± SE and are expressed as nanomoles per min/100 mL leg.

[a] Significantly different after 5 days of oxandrolone administration, $P < 0.05$.

transport of phenylalanine ($F_{V,M}$; $P < 0.05$). The intracellular rate of appearance of phenylalanine ($F_{M,O}$), an index of proteolysis, did not change after OX administration (Table 2). However, consistent with the direct incorporation data, the rate of intracellular utilization of phenylalanine for protein synthesis ($F_{O,M}$) increased significantly after OX treatment (Table 2; $P < 0.05$). As a result of the increase in $F_{O,M}$ and the lack of change in $F_{M,O}$, net balance (NB) shifted from a net negative output (−30 ± 6) during the fasted control period to an approximate zero balance (−1 ± 4) during an overnight fast after 5 days of OX (Table 2; $P < 0.05$). Protein synthesis efficiency increased significantly from control to OX periods (24 ± 0.03% *vs.* 39 ± 0.07%; $P < 0.05$). Finally, OX had no effect on leg blood flow.

OX administration significantly increased mRNA concentrations of skeletal muscle AR without changing IGF-I mRNA concentrations. Figure 5 shows a representative autoradiogram of a Southern blot hybridization for skeletal muscle AR and a graph of the densitometry data from all five subjects. IGF-I mRNA concentrations were not significantly increased after 5 days of OX treatment (control, 2.3 ± 0.6; OX, 3.1 ± 0.5). GAP concentration did not change. The data are presented as a ratio of the AR band density over the GAP band density.

## Discussion

We examined the response of muscle protein kinetics to OX administration in normal young men. We demonstrated that a moderate dose of OX, given over 5 days, stimulated muscle protein anabolism in young men. Further, we demonstrated for the first time in humans an increase in skeletal muscle ARs after anabolic intervention. Muscle anabolism during OX treatment occurred by stimulation of protein synthesis, as protein breakdown was unchanged. Moreover, a significant decrease in model-derived outward transport ($F_{V,M}$) along with the calculated increase in protein synthetic efficiency indicate increased intracellular reutilization of amino acids. Taken together, these results demonstrate the mechanism of OX's anabolic properties in fasted skeletal muscle.

A recent study from our laboratory (7) demonstrated that 5 days after a single im injection of TE (200 mg), FSR and model-derived protein synthesis increased 2-fold, with no change in FBR. Further, in agreement with the present findings, Ferrando *et al.* (7) demonstrated an increased utilization of intracellular amino acids by showing a strong relationship between protein breakdown and protein synthesis. Although our kinetic data strongly support these findings, the magnitude of the synthetic response with OX was not as great as that with TE. With OX, we found 44% and 28% increases in FSR and model-derived protein synthesis ($F_{O,M}$), respec-

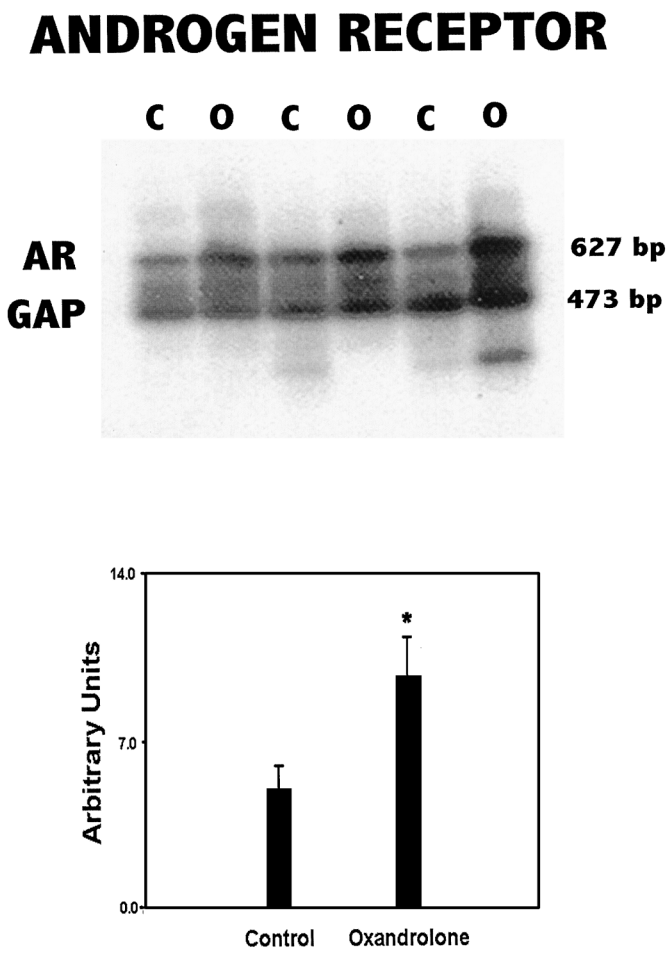


FIG. 5. AR mRNA concentrations. *Top*, Representative autoradiogram of a Southern blot hybridization of RT-PCR product from the total RNA of muscle biopsies from three subjects who were treated with OX (15 mg/day for 5 days). GAP was used as an internal control. C, Control; O, OX. *Bottom*, Graph of the mean ± SE from all five subjects. The data are expressed as arbitrary units calculated as the ratio of the band densities of AR over the band densities of GAP (internal control). Statistical significance was determined by paired *t* test (*, $P \leq 0.05$).

tively. Several important factors may account for these differences.

In our previous study, the total T concentration increased to twice the physiological norm 2 days after TE injection (2094 ± 561 ng/dL). Further, T was still in the upper physiological range (953 ± 283 ng/dL) by day 5 and was statistically different from preinjection T concentration (425 ± 99 ng/dL) (7). In contrast, the magnitude of response we found in serum OX concentration was much less than that reported with TE. For example, total serum OX, as measured in the morning 10 h after oral ingestion, was consistent on days 3 and 5, whereas total serum and free T concentrations declined significantly from days 0 and 3 to day 5. Viewed in combination, total serum androgen levels with OX treatment were far below those obtained with TE. Although the total androgen exposure to the skeletal muscle with OX may have been considerably less than that we found previously with TE, an increase in protein synthesis was nonetheless observed. This suggests that OX may exert a greater anabolic influence on skeletal muscle than TE, thereby overcoming the decrease in the T concentration.

Further evidence indicates that the method of administration and metabolism of the anabolic agent may account for the magnitude of difference in protein synthesis with OX compared to TE. For example, im TE injections are administered in a lipid base such that they can be stored in adipose tissue and released slowly, giving a sustained duration of action. After im injection of 200 mg TE, serum T levels rise and can reach the supraphysiological range within 24 h of administration. Over a period of several weeks, these levels gradually decline to hypogonadal levels (21). In the present study, serum OX levels on day 5 were 2.19 ng/dL 10 h after oral administration. However, by 18 h, serum OX levels fell to 0.48 ng/dL, representing a 78% reduction in serum OX in only 8 h. Because of this rapid decline in OX blood levels, it may be warranted to administer OX twice a day to maintain higher sustained blood levels of total androgen, possibly further enhancing its anabolic effect on skeletal muscle.

Moreover, OX's potent protein synthetic response was sufficient to ameliorate the net amino acid efflux and protein catabolism associated with an overnight fast. Given that most trauma and burn patients are acutely hypercatabolic, and most cancer and AIDS patients are chronically catabolic, the ability to reverse the inevitable losses in lean body mass using an oral anabolic agent has considerable clinical implications. However, the timeframe needed for protein accretion to occur in these patient groups is not known. At a minimum, efficient reutilization of intracellular amino acids is necessary for continued maintenance of the metabolic state (7, 22). We know that during the fasted state, protein breakdown is normally much higher than protein synthesis (16, 22). Despite being fasted overnight, all subjects had an increased reutilization of amino acids as outward transport ($F_{V,M}$) decreased 28% after OX treatment. We further showed a 65% increase in the efficiency of protein synthesis with OX. In combination, this could lead to an accrual of lean body mass in the fed state.

Androgens induce their specific response via the AR, which, in turn, regulates the transcription of androgen-responsive target genes. Although we know that accumulation of DNA is essential for muscle growth, the mechanisms of androgen-induced DNA accretion in skeletal muscle are unclear. AR (23) and AR mRNA (24) have been detected in human skeletal muscle. However, to date there are no human studies that have examined the response of skeletal muscle ARs to androgen exposure. Moreover, it has been suggested that prior cellular exposure to androgens may somehow prime these cells for the action of secondary agents such as IGF-I. Therefore, a secondary objective of this study was to examine the effect of OX administration on mRNA concentrations of IGF-I and ARs.

A recent study in exercising rats indicates that the accretion of skeletal muscle may be dependent on an increased number of ARs (25). Inoue *et al.* (25) examined the physiological importance of the increase in ARs on exercise-induced muscle hypertrophy. They determined that the androgen pathway had a significant effect on exercise-induced muscle hypertrophy and found the hypertrophy to be associated

with an increased number of ARs in the exercised muscle (25). Moreover, a study conducted by Doumit *et al.* (26) found that pretreatment of porcine satellite cells with T for 24 h up-regulated AR, but did not alter the responsiveness of these cells to IGF-I or other growth factors. Similarly, we found an increased expression of AR mRNA with no change in im IGF-I mRNA concentrations after a short term administration of OX. These data along with our findings of increased mRNA concentrations of ARs with short term exposure to OX lend support to the contention that ARs may regulate, either directly or indirectly, the accumulation of DNA required for muscle growth.

More recent evidence lends support to the complementary roles of androgens, ARs, and IGF-I. Urban *et al.* (5) found increased mRNA concentrations of IGF-I in skeletal muscle of elderly men given 4 weeks of replacement doses of TE. Further, by inducing severe androgen deficiency in young men for 10 weeks, Mauras *et al.* (27) showed marked decreases in mRNA concentrations of IGF-I and suggested that within skeletal muscle tissue, androgens are necessary for local IGF-I production, independent of GH production and systemic IGF-I concentrations. In addition, new data from this study of androgen-deficient men indicate that ARs are significantly decreased in response to severe hypogonadism (28). Although there is no direct evidence that OX binds to the ARs, the findings of the present study and those reported by Hayes *et al.* (28) suggest that androgens may work directly through the androgen receptor to exert their effects on protein metabolism. Nevertheless, we do not know from the present study the physiological importance of the increased expression of mRNA for AR.

In summary, this study demonstrates that OX, administered once a day at a moderate dose (15 mg/day), promotes net muscle protein synthesis. Moreover, these data suggest that OX induced an increase in AR expression as a mechanism for the increase in net muscle protein synthesis.

## Acknowledgments

The investigators wish to thank the nurses and staff of the University of Texas Medical Branch General Clinical Research Center for their assistance with this project, Lakshmi McRae for nursing support, Zhi Ping Dong and Boro Starcevic for sample analysis, and BTG Pharmaceuticals Co., Inc., for providing the study drug.

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