Androgen Therapy Induces Muscle Protein Anabolism in Older Women

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Context: Normal healthy men and women undergo a gradual loss of skeletal muscle mass and strength with advancing age. While androgens are protein anabolic in older men, the metabolic effects in older women are poorly understood.

Objective and Design: The objective of this study was to determine whether oral administration of a synthetic derivative of testosterone [oxandrolone, Oxandrin (OX)] (7.5 mg orally twice daily for 14 d) to five older women (age, 65 ± 2 yr) would enhance skeletal muscle anabolic biomarkers including mixed muscle fractional synthetic rate (FSR), net phenylalanine balance, androgen receptor, and IGF-I protein expression at d 0, 5, and 14 of treatment. As a positive control, seven older men were examined after 14 d of OX (10 mg orally twice daily).

Aging can result in an involuntary decade-by-decade loss of skeletal muscle mass and function. It is likely due to a combination of key factors including inadequate nutrition (1, 2), hormonal alterations (3–6), reduced mitochondrial protein synthesis (7, 8), DNA alterations (8, 9), and physical inactivity (10, 11).

A progressive increase in adiposity and decrease in fat-free mass characterizes the aging process in both men and women. This underlying, subtle yet insidious imbalance between protein synthesis and breakdown with aging demands attention as our aging population grows. Interventional therapies such as androgen administration can positively influence protein metabolism and in recent years have been used by clinicians as a method to counter the negative effects of andropause and sarcopenia in aging males. Anabolic hormones such as oxandrolone [Oxandrin (OX)] and testosterone have been shown to increase mixed muscle protein synthesis in young (12) and older men (13) and to improve lean body mass and strength (13).

Little effort has been put forth to find anabolic agents that can be used effectively in older women to stem the effects of age-related muscle loss and strength. Although testosterone effectively stimulates muscle protein synthesis and enhances lean body mass and strength in men, the potential for virilization has limited its use in women. OX, a synthetic analog of testosterone, is an oral anabolic steroid currently used as an adjunctive therapy to promote weight gain in patients after surgery, chronic infections, and severe trauma (14). Additionally, OX is used by clinicians to treat children with growth disorders such as Turner’s syndrome and constitutional delay of growth and puberty (15, 16). Purported to induce fewer androgenic side effects, OX has caught the attention of clinicians for use in patients of both sexes. Numerous clinical studies have demonstrated the positive benefits of OX to various patient populations, including burns (14, 17, 18) and AIDS-wasting myopathy (19, 20). However, although androgens are protein anabolic to skeletal muscle in older men, we do not know whether androgens will induce skeletal muscle anabolism in older women. Therefore, we investigated the physiological, protein metabolic, and molecular effects of 14 d of OX administration on healthy older women, using older men as positive controls.

 Subjects and Methods

Healthy older women (n = 5) and men (n = 7) were recruited through the Sealy Center on Aging at the University of Texas Medical Branch (UTMB) (Table 1). All subjects provided written informed consent according to the guidelines established by the Institutional Review Board at UTMB. Eligibility was assessed by performing a medical screening including a physical examination, electrocardiogram, complete blood count, a metabolic panel, a hepatitis panel, an HIV test, and urinalysis. Exclusion criteria were heart or liver disease, hypo- or hypercoagulation disorders, vascular diseases, hypertension, diabetes, cancer, obesity, acute or chronic pulmonary diseases, infectious diseases, or an allergy to iodides. Subjects who had been taking anabolic steroids or corticosteroids in the past 6 months were also excluded, as were subjects engaged in regular aerobic or resistance exercise training. Subjects were asked to maintain their normal activities of daily living throughout the study and were fed a controlled meal by the General Clinical Research Center.

Results: Fourteen days of OX significantly increased skeletal muscle FSR in older women (d 0, 0.073 ± 0.006 vs. d 5, 0.092 ± 0.006 vs. d 14, 0.115 ± 0.007%/h) (P < 0.05, d 0 vs. d 14). Conversely, OX stimulated FSR in older men after only 5 d (d 0, 0.061 ± 0.003 vs. d 5, 0.101 ± 0.01 vs. d 14, 0.084 ± 0.01%/h) (P < 0.05, d 0 vs. d 5). Androgen receptor expression was significantly increased in older men by d 14, but had not increased in older women. No change was noted in IGF-I expression in either group. We conclude that the skeletal muscle of older women and men responds to androgen administration, although the time course of anabolism appears to be gender specific. (J Clin Endocrinol Metab 91: 3844–3849, 2006)
TABLE 1. Characteristics of subjects

<table>
<thead>
<tr>
<th></th>
<th>Older women (n = 5)</th>
<th>Older men (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>65 ± 2</td>
<td>72 ± 2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>166 ± 2</td>
<td>173 ± 3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>66 ± 1</td>
<td>80 ± 5</td>
</tr>
<tr>
<td>Day 14</td>
<td>67 ± 1</td>
<td>81 ± 5</td>
</tr>
</tbody>
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Values are mean ± SE.

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study. At the end of each infusion study, all catheters were removed, and the subjects were fed a meal and discharged to home.

Analytical methods

Blood and muscle processing. Phenylalanine enrichments and concentrations in arterio-venous blood samples were determined using gas-chromatography mass-spectrometry (GC-MS) in electron capture mode (Hewlett-Packard, Palo Alto, CA) as previously described (12, 25).

Intracellular phenylalanine enrichments and concentrations from vastus lateralis muscle biopsy samples were analyzed using gas-chromatography mass-spectrometry (12, 25).

ICG concentration in infusate and serum samples was measured spectrophotometrically at λ = 805 nm.

Hormone assays. The serum concentration of total testosterone was measured with a commercial RIA kit (Diagnostic Products Corp., Los Angeles, CA). The analytical sensitivity and thus reportable range of serum testosterone for this kit was 4–1600 ng/dl. Intrassay coefficients of variation for this assay were less than 5%.

Western blot analysis. Protein was isolated from muscle biopsy samples by slicing frozen muscle into very small pieces using a clean razor blade and thawing the tissue in lysis buffer [150 mM NaCl, 10 mM Tris, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 5 mM EDTA] containing proteinase inhibitors (1 mM phenylmethylsulfonyl fluoride, 1 mM benzamide, 10 μg/ml aprotinin, 50 μg/ml leupeptin, 1 μg/ml pepstatin A) at a concentration of 3 ml of ice-cold lysis buffer per gram of tissue. The tissue was homogenized with a Dounce homogenizer (Kinematica AG, Littau, Switzerland) at 4 C and centrifuged at 15,000 × g for 20 min, the supernatant was removed and centrifuged again to result in total cell lysate. The androgen receptor (AR) antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) was incubated with 80 g of cell lysate run on standard SDS-polyacrylamide gel with a working solution concentration range of 1:15–20. The IGF-I antibody (Santa Cruz Biotechnology, Inc.) was incubated with 40 g of cell lysate run on standard SDS polyacrylamide gel with a working solution concentration range of 1:100–200. The actin antibody (Sigma, St. Louis, MO) was used with a working solution concentration range of 1:100–200. This antiactin antibody is a broad-based antibody that recognizes an epitope located on the N-terminal region of actin and demonstrates a broad reactivity among multiple actin isoforms in various species. The “housekeeping” antibody was used to correct the results for protein loading of the gel. Western analysis allows the direct measurement of protein expression in the muscle biopsy samples.

Calculations

Two well-established methodologies were used to measure phenylalanine kinetics in human skeletal muscle: phenylalanine net balance (NB) and direct amino acid incorporation via measurement of the mixed muscle FSR (26). A stable isotope of phenylalanine was used for this method because it is an essential amino acid and it is not oxidized in the muscle tissue. NB is therefore calculated as follows: NB = (CΝ – CNE)BF, where CΝ and CNE represent the phenylalanine concentrations in the femoral artery and vein, respectively, and BF represents leg blood flow, as determined by the ICG dye dilution method (22). Data are presented per 100 ml of leg volume.

FSR of mixed muscle proteins was determined by measuring the direct incorporation of L-[ring-2H3]phenylalanine into protein, using the precursor-product model: FSR = [(EP2 – EP1)/(Em x t)]×100, where EP1 and EP2 are the enrichments of bound L-[ring-2H3]phenylalanine in the first and second muscle biopsies, t is the time interval between biopsies, and Em is the mean L-[ring-2H3]phenylalanine enrichment in the muscle intracellular pool (27). The results are presented as percent per hour. All kinetic parameters presented in this paper were measured with subjects in the postabsorptive state.

Statistical analysis

Statistical analyses were performed with Dunnett’s simultaneous 95% confidence intervals, which yield 97.5% upper and lower limits on each of the primary parameters. Primary parameters include mixed muscle FSR and NB of phenylalanine. Significance levels were adjusted.
using Bonferroni’s inequalities to account for multiple comparisons made for both primary parameters (yielding simultaneous 95% joint lower confidence limits). Secondary parameters include AR and IGF-I expression. Statistical analysis was carried out using NCSS software (2004) (NCSS, Kaysville, UT). All parameters and descriptive data are presented as means ± SEM.

Results

Subjects

The mean anthropometric data are displayed in Table 1. There was no difference from baseline to d 14 of OX administration in both men and women for any of the measurements. However, when differences in height and weight between the men and women are taken into account, women on average were administered more OX (4.43 ± 0.05 vs. 3.99 ± 0.25 mg/kg/d) during the 14 d.

Serum testosterone concentrations

Total serum testosterone concentrations remained unchanged from d 0 (20.8 ± 5.9 ng/dl) to d 5 (23.1 ± 4.3 ng/dl) and d 14 (23.86 ± 4.5 ng/dl) in the women (Fig. 2A). However, the men demonstrated a significant decrease in total serum testosterone concentration from d 0 to d 5 and 14 (111.5–23.86 ng/dl) to d 5 and 14 (111.5 ± 10.4 and 76.7 ± 12 ng/dl, respectively; P < 0.05). Thus, OX shifted both groups from a more protein catabolic state to a more protein anabolic condition.

Phenylalanine kinetics

FIG. 2. Total testosterone concentration in older women (A) and older men (B). Total serum testosterone concentrations in older women remained unchanged from d 0 to d 5 and 14. *, Total serum testosterone decreased significantly from d 0 to d 5 and 14 in the older men, P < 0.05.

Serum testosterone concentrations

Total serum testosterone concentrations remained unchanged from d 0 (20.8 ± 5.9 ng/dl) to d 5 (23.1 ± 4.3 ng/dl) and d 14 (23.86 ± 4.5 ng/dl) in the women (Fig. 2A). However, the men demonstrated a significant decrease in total serum testosterone concentration from d 0 to d 5 and 14 (111.5± 36 ng/dl) to d 5 and 14 (111.5 ± 10.4 and 76.7 ± 12 ng/dl, respectively; P < 0.05) (Fig. 2B).

Leg phenylalanine kinetics

Figure 3 displays the NB of phenylalanine measured in the postabsorptive state at d 0, 5, and 14 of OX for both the men and women. Phenylalanine NB improved in both groups from d 0 to d 14. OX significantly increased NB in the positive control group (men) from −30.5 ± 5.3 at d 0 to −14.3 ± 1.5 nmol-min⁻¹·100 ml leg⁻¹ (P < 0.05). Likewise, OX significantly shifted NB in a positive direction in the women from −16 ± 0.88 at d 0 to −6.5 ± 3.2 nmol-min⁻¹·100 ml leg⁻¹ (P < 0.05). Thus, OX shifted both groups from a more protein catabolic state to a more protein anabolic condition.

FIG. 3. Net balance of phenylalanine in a group of healthy older men and women at d 0 (white bars), d 5 (gray bars), and d 14 (hatched) of OX. *, Significant difference from d 0 to d 14 in the older men and women, P < 0.05.

FSR of mixed muscle

FIG. 4. The FSR of mixed muscle in a group of healthy older men and women at d 0 (white bars), d 5 (gray bars), and d 14 (hatched) of OX. *, Significant difference from d 0 to d 5 in the older men and from d 0 to d 14 in the older women, P < 0.05.

FSR of mixed muscle

Figure 4 shows the postabsorptive skeletal muscle FSR data at d 0, 5, and 14 of OX administration. OX administration resulted in a progressive increase in mixed muscle FSR in the women throughout the 14 d (0.073 ± 0.006, 0.092 ± 0.006, and 0.115 ± 0.007%/h), with d 14 FSR significantly different from d 0 (P < 0.05). FSR in the positive control men showed a significant increase by d 5 (0.061 ± 0.003%/h, d 0, and 0.101 ± 0.012%/h, d 5; P < 0.05); however, although still elevated,
d 14 FSR in the men was no longer statistically different from basal (0.084 ± 0.01%/h).

**AR and IGF-I protein expression**

Figure 5 displays the densitometry data for AR measured at d 0, 5, and 14 for both groups. The data are presented as a ratio of the AR band density over the actin band density, respectively. Actin concentration did not change. AR protein expression was significantly increased in the older men by d 14, with no change noted in the women at either d 5 or d 14 (Fig. 5; *P* < 0.05). IGF-I protein expression remained unchanged after OX administration in both the older women and men.

**Discussion**

We examined the response of muscle protein kinetics to OX administration in healthy older women, using an older male cohort as a positive control. We chose OX as our administered androgen in women due to its purported fewer androgenic side effects. OX is currently used by clinicians to treat children with growth disorders such as Turner’s syndrome and constitutional growth and puberty (15, 16). Our previous experience with OX in young men has shown it to be anabolic to skeletal muscle (12), although not as potent as testosterone enanthisate (28).

We have previously demonstrated that the skeletal muscle of older (13) and younger (12) men is protein anabolic to long- and short-term androgen administration, respectively. Here, we show that 14 d of a moderate dose of OX is sufficient to stimulate muscle protein synthesis in healthy older women and men, despite being postabsorptive during the pre-post testing conditions. However, the time course of muscle protein anabolism between older women and men appears to be different because FSR was not significantly elevated in the women until d 14. Conversely, FSR in the positive control men was significantly elevated on d 5 but had declined somewhat by d 14. A decline of this proportion in FSR in the men is not unexpected because OX significantly decreased endogenous testosterone production by d 5 in the older men.

Several important factors may account for the anabolic differences between OX and testosterone in women and men and may provide additional clarity to our findings. In particular, testosterone enanthesate is administered in a lipid base that is readily stored in adipose tissue and released slowly, providing a sustained duration of action. Testosterone levels can rise to supraphysiological levels within 24 h, with levels gradually declining to hypogonadal levels (29). In contrast, we showed previously that OX reached peak levels in young men by 10 h but dropped significantly by 18 h (12). Despite the rapidity of the metabolism of OX and the significant fall in serum androgen levels in comparison to that previously seen with testosterone, OX was significantly anabolic to the skeletal muscle of young men (12).

OX stimulated muscle protein anabolism in the older women and men by increasing muscle protein synthesis, as muscle protein breakdown did not change. These findings are consistent with a previous study with young men (12) in that the early and/or short-term response of androgens on skeletal muscle occurs via an increase in muscle protein synthesis. Also similar to findings from our previous 5-d study of OX in young men, outward amino acid transport trended downward from d 0 to d 5 in the older men (data not shown). This finding, combined with a significant increase in muscle protein synthesis at d 5 and no change in intracellular appearance of amino acids (data not shown), provides evidence of increased intracellular reutilization of amino acids in the older men. In the older women, the model-derived muscle protein breakdown data are trending downward by d 14, suggesting that a portion of the early and/or short-term effect of OX in the women may also be affecting proteolysis.

In the present study, we were able to show a significant increase in muscle protein synthesis in women during the fasted state after only 14 d of OX administration. Our previous study using OX in younger men indicated that the potent protein synthetic response of OX was sufficient to ameliorate the net amino acid efflux and protein catabolism associated with an overnight fast (12). We know that during the fasted state muscle protein breakdown is normally much higher than muscle protein synthesis (21, 30). In the present study, our NB data show that muscle protein breakdown is higher than muscle protein synthesis in both older groups in the postabsorptive state. Although OX was able to overcome the catabolic effect of fasting in the women at d 14, the significant reduction in endogenous testosterone in combination with the fasting condition in the men resulted in a nonstatistically significant elevation in FSR at d 14 in the men.

Unfortunately, the invasive nature of these studies, in combination with the older age of our study population, often limits the number of subjects that meet the rigorous inclusion criteria. Although the small subject numbers in this study are not ideal, we previously showed OX capable of inducing statistically significant increases in the FSR of mus-
Significance in human studies of protein metabolism with six men (12). Moreover, we commonly use and report statistical differences from those previously studied in men. Overall, these data suggest that the skeletal muscle of women was anabolically responsive to an androgen. Finally, it is important to note the rationale behind the differences in doses administered between the older men and women. As a reminder, the women received 7.5 mg twice a day and the men received 10 mg twice a day (i.e., 20 mg/d). The rationale for this dosing regimen was 2-fold. First, the dosing for the men was selected because it is the maximum approved dose for men, whereas the dosing for the women was chosen to be 7.5 mg twice a day, which when normalized for body weight of the smaller women resulted in a comparable per kilogram of body weight dosage to the men. Because the women were on average 14 kg lighter than the men, once normalized per kilogram body weight, the doses were very similar between the women (4.43 mg/kg) and men (3.99 mg/kg), respectively.

Although the intracellular mechanism stimulating muscle protein anabolism requires further clarification, it is clear that OX improves net protein balance of skeletal muscle. This effect is pronounced in the fasted state because net protein balance becomes less negative in both groups. Thus, OX administration when combined with the anabolic stimulus of a meal may ameliorate the loss of skeletal muscle nitrogen in older women by preventing the loss of intracellular amino acids. This retention may lead to lean muscle mass accretion over time.

The ability to reverse the inevitable losses in lean body mass using oral anabolic agents has considerable clinical implications. Given that most trauma and burn patients are acutely hypercatabolic and most cancer and AIDS patients are chronically catabolic, efficient reutilization of intracellular amino acids is necessary for continued maintenance of the metabolic state (12, 13, 32). Further research is needed in these groups to better understand the intracellular mechanisms stimulating muscle protein anabolism and the time frame needed for protein accretion to occur.

In summary, the present study demonstrates that the administration of OX in moderate doses will stimulate muscle protein anabolism in women. Moreover, these data suggest that the mechanism in which this is accomplished differs from those previously studied in men. Overall, these data suggest that the skeletal muscle of women is anabolically responsive to an androgen and further suggests that women may achieve similar benefits of longer-term androgen supplementation as demonstrated in their older male counterparts.

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**References**


**Androgens and Older Women and Men**

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