

# 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 [ $22 \pm 1$  ( $\pm$ 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  $53.5 \pm 3$  to  $68.3 \pm 5$  (mean  $\pm$  SE) nmol/min $\cdot$ 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, Ise-lin, 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

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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 [ $^{16,16,17-3}\text{H}$ ]T ( $d^3\text{T}$ ; 12  $\mu\text{g}/\text{mL}$ ; 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  $\times$  2-mm column (Keystone Scientific, Inc., Bellefonte, PA) and Hypersil BDS  $C_{18}$ , 20  $\times$  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  $d^3\text{T}$  (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  $d^3\text{T}$ , 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. Glycerinaldehyde 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'-AAATCAGCAGTCTTGGAAACC-3'; antisense, 5' CT-TCTGGGTCTTGGGCATGT 3'; oligonucleotide, 5'-CAAGCCCACAGGGTATGGCTCCAGCAGT-3'; AR: sense, 5'-GATGCTCTACTTCGC-CCCTGA-3'; antisense, 5'-CCCAGCAAATAGAATTCATGAC-3'; oligonucleotide, 5'-CTGGGTGTGGAAATAGATG-3'; and GAP: sense, 5'-GGTATCGTGAAGGACTCAT-3'; antisense, 5'-TCCACCACCTGT-TGCTGTA-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,

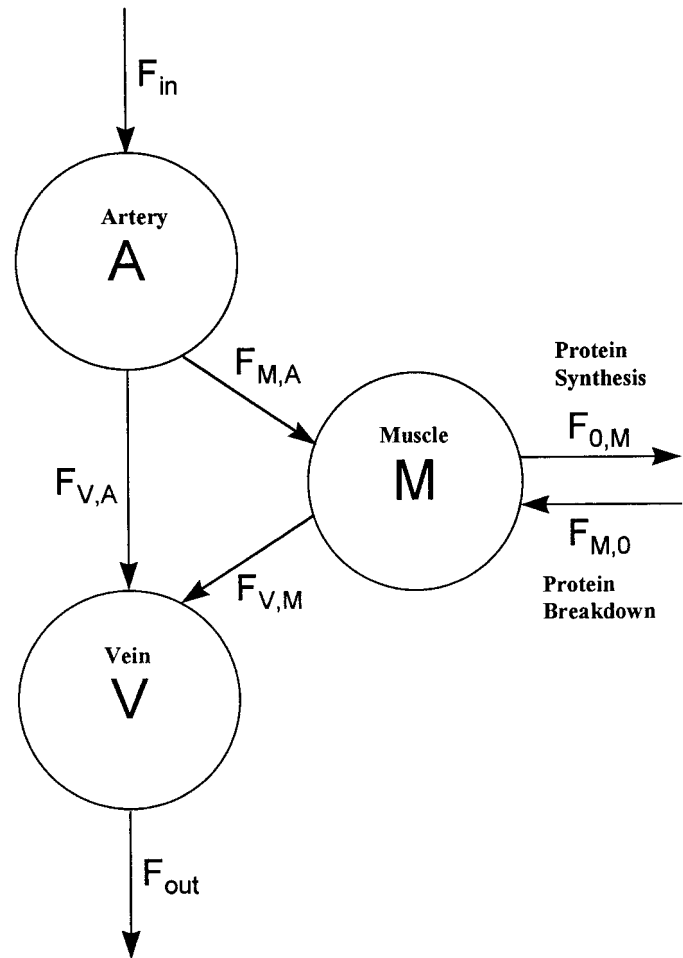


FIG. 2. Three-compartmental model of leg amino acid kinetics. Free amino acid pools in femoral artery (A), femoral vein (V), and muscle (M) are connected by arrows, indicating unidirectional amino acid flow between each compartment. Amino acids enter the leg via femoral artery ( $F_{in}$ ) and leave 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.

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:

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

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

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

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

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  $\pm$  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 ( $1.9 \pm 0.4$  ng/dL) and day 5 ( $2.2 \pm 0.3$  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 ( $0.48 \pm 0.06$  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 ( $449 \pm 35$  ng/dL) and day 3 ( $441 \pm 44$  ng/dL) of OX treatment. However, by day 5, total serum T concentrations were significantly reduced ( $282 \pm 45$  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 ( $98 \pm 10$  pg/mL;  $P < 0.001$ ) below day 0 ( $121 \pm 12$  pg/mL) and day 3 ( $126 \pm 9$  pg/mL) values. Hence, the total androgen concentration (T + OX) was reduced in parallel to the reduction in T (Fig. 3).

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

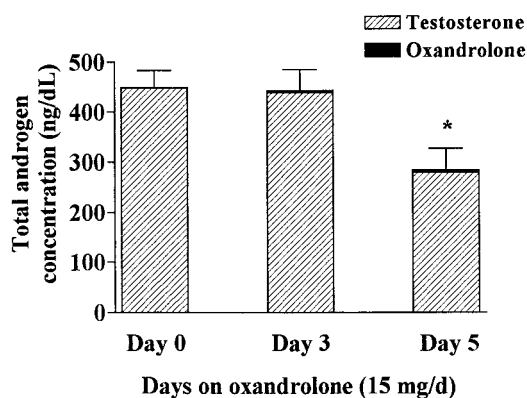
The model-derived parameters of leg muscle free amino acid kinetics of the five subjects in the control period and after 5 days of OX are shown in Table 2. OX treatment had no effect on amino acid delivery to ( $F_{in}$ ) or the release of labeled phenylalanine from the leg ( $F_{out}$ ). Moreover, the 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 <sup>a</sup>
260	0.0640 ± 0.0030	0.0722 ± 0.0016 <sup>a</sup>
280	0.0647 ± 0.0038	0.0672 ± 0.0025 <sup>a</sup>
300	0.0638 ± 0.0017	0.0715 ± 0.0022 <sup>a</sup>

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.

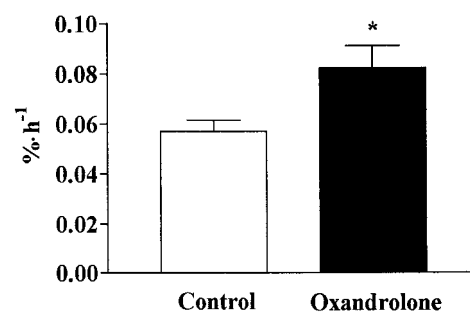
<sup>a</sup> *P* < 0.05.



**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).

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 ( $-30 \pm 6$ ) during the fasted control period to an approximate zero balance ( $-1 \pm 4$ ) during an overnight fast after 5 days of OX (Table 2; *P* < 0.05). Protein synthesis efficiency increased significantly from control to OX periods ( $24 \pm 0.03\%$  vs.  $39 \pm 0.07\%$ ; *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,  $2.3 \pm 0.6$ ; OX,  $3.1 \pm 0.5$ ). GAP concentration did not change. The data are presented as a ratio of the AR band density over the GAP band density.



**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 <sup>a</sup>
$F_{V,A}$ (functional shunting)	118 ± 35	102 ± 17
$F_{M,O}$ (protein breakdown)	83 ± 8	69 ± 8
$F_{O,M}$ (protein synthesis)	53 ± 3	68 ± 5 <sup>a</sup>
$Ra_M$ (intracellular appearance)	228 ± 16	193 ± 32
NB (net balance)	$-30 \pm 6$	$-1 \pm 4^a$

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

<sup>a</sup> Significantly different after 5 days of oxandrolone administration, *P* < 0.05.

## 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-

## ANDROGEN RECEPTOR

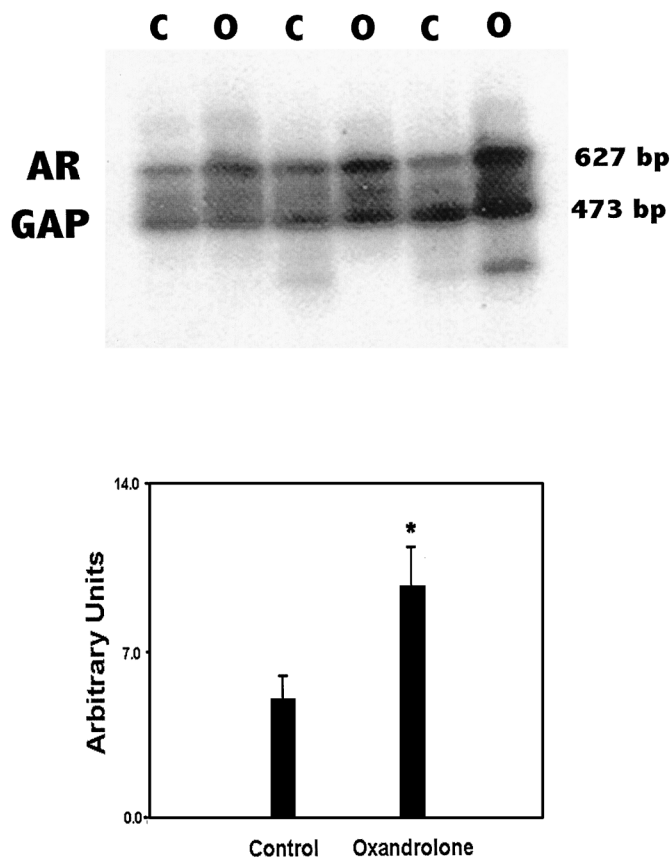


FIG. 5. AR mRNA concentrations. *Top*, Representative autoradiogram of a Southern blot hybridization of RT-PCR product from the total RNA of muscle biopsies from three subjects who were treated with OX (15 mg/day for 5 days). GAP was used as an internal control. C, Control; O, OX. *Bottom*, Graph of the mean  $\pm$  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 ( $2094 \pm 561$  ng/dL). Further, T was still in the upper physiological range ( $953 \pm 283$  ng/dL) by day 5 and was statistically different from preinjection T concentration ( $425 \pm 99$  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 ob-

served. 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.

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### References

1. Brodsky IG, Balagopal P, Nair KS. 1996 Effects of testosterone replacement on muscle mass and muscle protein synthesis in hypogonadal men—a clinical research center study. *J Clin Endocrinol Metab.* 81:3469–3475.
2. Katznelson L, Kinkelstein JS, Schoenfeld DA, Rosenthal DI, Anderson EJ, Klibanski A. 1996 Increase in bone density and lean body mass during testosterone administration in men with acquired hypogonadism. *J Clin Endocrinol Metab.* 81:4358–4365.
3. Bhasin S, Storer TW, Berman N, et al. 1997 Testosterone replacement increases fat-free mass and muscle size in hypogonadal men. *J Clin Endocrinol Metab.* 82:407–413.
4. Tenover JS. 1992 Effects of testosterone supplementation in the aging male. *J Clin Endocrinol Metab.* 75:1092–1098.
5. Urban RJ, Bodenbuc C, Gillison C, et al. 1995 Testosterone administration to elderly men increases skeletal muscle strength and protein synthesis. *Am J Physiol.* 269:E820–E826.
6. Grinspoon S, Corcoran C, Lee K, et al. 1996 Loss of lean body mass and muscle mass correlates with androgen levels in hypogonadal men with acquired immunodeficiency syndrome wasting. *J Clin Endocrinol Metab.* 81:4051–4058.
7. Ferrando AA, Tipton KD, Doyle D, Phillips SM, Cortiella J, Wolfe RR. 1998 Testosterone injection stimulates net protein synthesis but not tissue amino acid transport. *Am J Physiol.* 275:E864–E871.
8. Griggs RC, Kingston W, Jozefowicz RF, Herr BE, Forbes G, Halliday D. 1989 Effect of testosterone on muscle mass and muscle protein synthesis. *J Appl Physiol.* 66:498–503.
9. Zhang X-J, Chinkes DL, Sakurai Y, Wolfe RR. 1996 An isotopic method for measurement of muscle protein fractional breakdown rate *in vivo*. *Am J Physiol.* 270:E759–E767.
10. Berger JR, Pall L, Hall CD, Simpson DM, Berry PS, Dudley R. 1996 Oxandrolone in AIDS-wasting myopathy. *AIDS.* 10:1657–1662.
11. Demling RH, DeSanti L. 1997 Oxandrolone, and anabolic steroid, significantly increases the rate of weight gain in the recovery phase after major burns. *J Trauma.* 43:47–51.
12. Wilson DM, McCauley E, Brown DR, Dudley R. 1995 Oxandrolone therapy in constitutionally delayed growth and puberty. Bio-Technology General Corporation Cooperative Study Group. *Pediatrics.* 96:1095–1000.
13. Rosenfeld RG, Attie KM, Frane J, et al. 1998 Growth hormone therapy of Turner's syndrome: beneficial effect on adult height. *J Pediatr.* 132:319–324.
14. Fenichel G, Pestronk A, Florence J, Robison V, Hemelt V. 1997 A beneficial effect of oxandrolone in the treatment of Duchenne muscular dystrophy: a pilot study. *Neurology.* 48:1225–1226.
15. Biolo G, Chinkes D, Zhang X, Wolfe RR. 1992 A new model to determine *in vivo* the relationship between amino acid transmembrane transport and protein kinetics in muscle. *J Parenter Enteral Nutr.* 16:305–315.
16. Biolo G, Fleming RYD, Maggi SP, Wolfe RR. 1995 Transmembrane transport and intracellular kinetics of amino acids in human skeletal muscle. *Am J Physiol.* 268:E75–E84.
17. Wolfe RR. 1992 Radioactive and stable isotope tracers in biomedicine: principles and practices in kinetic analysis. New York: Wiley-Liss.
18. Bergstrom J, Furst L, Noree L-O, Vinnars E. 1974 Intracellular free amino acid concentration in human muscle tissue. *J Appl Physiol.* 36:693–697.
19. Calder AG, Anderson SE, Grant I, McNurlan MA, Garlick PJ. 1992 The determination of low  $d_5$ -phenylalanine enrichment (0.002–0.09 atom percent excess), after conversion to phenylethylamine, in relation to protein turnover studies by gas chromatography/electron ionization mass spectrometry. *Rapid Commun Mass Spectr.* 6:421–424.
20. Phillips SM, Tipton KD, Aarsland A, Wolf SE, Wolfe RR. 1997 Mixed muscle protein synthesis and breakdown after resistance exercise in humans. *Am J Physiol.* 273:E99–E107.
21. Sokol RZ, Palacios A, Campfield LA, Saul C, Swerdloff RS. 1982 Comparison of the kinetics of injectible testosterone in eugonadal and hypogonadal men. *Fertil Steril.* 37:425–430.
22. Biolo G, Fleming D, Wolfe RR. 1998 Physiologic hyperinsulinemia stimulates protein synthesis and enhances transport of selected amino acids in human skeletal muscle. *J Clin Invest.* 95:811–819.
23. Kimura N, Mizokami A, Oonuma T, Sasano H, Nagura H. 1993 Immunocytochemical localization of androgen receptor with polyclonal antibody in paraffin-embedded human tissues. *J Histochem Cytochem.* 41:671–678.
24. Chang C, Kokontis J, Liao S. 1988 Structural analysis of complementary DNA and amino acid sequences of human and rat androgen receptors. *Proc Natl Acad Sci USA.* 85:7211–7215.
25. Inoue K, Yamasaki S, Fushiki T, Okada Y, Sugimoto E. 1994 Androgen receptor antagonist suppresses exercise-induced hypertrophy of skeletal muscle. *Eur J Appl Physiol.* 69:88–91.
26. Doumit ME, Cook DR, Merkel RA. 1996 Testosterone up-regulates androgen receptors and decreases differentiation of porcine myogenic satellite cells *in vitro*. *Endocrinology.* 137:1385–1394.
27. Mauras N, Hayes V, Welch S, et al. 1998 Testosterone deficiency in young men: marked alterations in whole body protein kinetics, strength, and adiposity. *J Clin Endocrinol Metab.* 83:1886–1892.
28. Hayes V, Jiang J, Urban RJ, Mauras N. IGF-I and androgenic steroids: synergistic effects in man [Abstract]. *Proc of the 81st Annual Meet of The Endocrine Soc.* 1999. p. 571.