Skeletal muscle adaptations to testosterone and resistance training in men with COPD

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Divisions of 1Pulmonary and Critical Care Medicine and of 2Endocrinology, Diabetes, and Metabolism, The Burns and Allen Research Institute at Cedars-Sinai Medical Center, Los Angeles; 3David Geffen School of Medicine at UCLA, Los Angeles; 4Exercise Sciences Laboratory, El Camino College, Torrance; 5Division of Endocrinology, Metabolism, and Molecular Medicine, Drew University of Medicine and Science, Los Angeles; and 6Division of Respiratory and Critical Care Physiology and Medicine, Rehabilitation Clinical Trials Center, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, California

Submitted 5 February 2007; accepted in final form 20 July 2007

Lewis MI, Fournier M, Storer TW, Bhasin S, Porszasz J, Ren S-G, Da X, Casaburi R. Skeletal muscle adaptations to testosterone and resistance training in men with COPD. J Appl Physiol 103: 1299–1310, 2007. First published August 2, 2007; doi:10.1152/japplphysiol.00150.2007.—We recently reported increased leg lean mass and strength in men with chronic obstructive pulmonary disease (COPD) receiving 10 wk of testosterone (T) and leg resistance training (R) (Casaburi R, Bhasin S, Cosentino L, Porszasz J, Somfay A, Lewis M, Fournier M, Storer T. Am J Respir Crit Care Med 170: 870–878, 2004). The present study evaluates the role of muscle IGF and related factors as potential mechanisms for our findings, using quadriceps muscle biopsies from the same cohort. Patient groups were 1) weekly placebo (P) injections + no R; 2) P and R; 3) weekly injections of T + no R; and 4) T + R (TR). Muscle fibers were classified histochemically, and their cross-sectional areas (CSAs) and fiber density (number of fibers per unit area) were determined. Gene transcripts were determined by real-time PCR and protein expression by RIA. While no significant changes in fiber CSAs were noted across groups, increased trends were observed after 10 wk, and significant decrements in muscle fiber density were noted in all treated groups. A global increase in all myosin heavy chain (MyHC) mRNA isoforms was observed in TR patients. Muscle IGF-Iα and IGF-Iεc mRNAs were significantly increased with TR group. Muscle IGF-I protein was increased in all intervention groups (greatest in TR). While TR IGF-II mRNA was increased, protein levels were unaltered. IGF binding protein-4 mRNA was increased with TR. Myogenin mRNA was increased in both T groups, while MyoD and myostatin were unaltered. Muscle atrophy F-box mRNA tended to increase with TR. Our data suggest that the combined interventions produced an enhanced local anabolic milieu driven in large part by the muscle IGF system, despite potentially negative biochemical influences present in COPD patients.

anabolic steroids; emphysema; vastus lateralis; insulin-like growth factors; myogenic regulatory factors; myosin heavy chains; muscle fiber morphometry

In patients with chronic obstructive pulmonary disease (COPD), skeletal muscle dysfunction has recently been highlighted (2). This is important as it is associated with muscle wasting and weakness and contributes to exercise limitations in these patients (2, 4, 14, 20, 32). A number of potential mechanisms have been proposed (2). These include energy imbalance and nutritional depletion (5, 63), worsened disordered protein turnover with acute exacerbations (72), deconditioning and impaired energy metabolism (11, 13, 41, 50), corticosteroids (15), influences of inflammatory cytokines (16, 18), hypoxemia, and possible hormonal aberrations impacting on muscle protein turnover. It is thus of interest that reduced serum levels of testosterone and free testosterone have been reported in a substantial proportion of older male patients with COPD (38, 43).

In a recently published companion paper to the present study (12), we reported significantly increased leg lean mass and strength following weekly injection of testosterone and resistance training in men with COPD in whom serum testosterone levels were low.

The biochemical mechanisms responsible for improved skeletal muscle bulk and strength with anabolic steroids (i.e., androgens) are not well understood. We (46) and others (71) have reported increased muscle expression of insulin-like growth factor-I (IGF-I) following anabolic steroid administration together with alterations in muscle expression of several IGF binding proteins (BP), which would be expected to facilitate the local effect of IGF-I within the muscle. Thus we believe that the muscle IGF-I system is integral in mediating, at least in part, the growth-promoting influences of anabolic steroids. Furthermore, resistance training in both young and older men also results in increased expression of limb muscle IGF-I (54). However, in patients with COPD there are several factors that could potentially lead to dysfunction of the IGF pathways, thus offsetting the positive effects of anabolic steroids and/or resistance training. These include nutritional depletion, corticosteroids, tumor necrosis factor (TNF)-α, and other cytokines, all of which are known to depress IGF-I expression (22, 27, 69).

The aim of this study was therefore to evaluate the potential role of the muscle IGF-I system and other key biochemical factors influenced by IGF-I (e.g., muscle regulatory factors) in promoting the increased leg lean muscle mass and strength following testosterone administration and resistance training in those COPD patients reported in our companion paper (12). Vastus lateralis muscle biopsies obtained from the same cohort of COPD patients were analyzed (12). We hypothesize that despite the potential negative regulatory factors described...
above, the autocrine/paracrine influence of the muscle IGF system does indeed contribute to the muscle adaptations reported in our recent study (12). Some of the results of this study have been previously reported in abstract form (26).

**METHODS**

**Subjects and Subjects Groups**

Forty patients from our original patient cohort of 47 subjects recently reported in a companion paper (12) consented to undergo muscle biopsies. These male patients with stable COPD were 55 to 80 yr old, had forced expiratory volume in 1 s (FEV1) of 60% predicted or less, and FEV1 to vital capacity ratio of 60% or less. Screening serum testosterone was 400 ng/dl or less (in the lower range for healthy elderly men). Patients with body weight <75% or >130% of ideal body weight were excluded.

The study subjects were randomized into four groups: 1) the P group had weekly placebo (P; sesame oil) injections intramuscularly; 2) the T group had weekly injections of 100 mg testosterone enanthate (T) in sesame oil intramuscularly; 3) the PR group had weekly P injections and resistance training (R); and 4) the TR group had weekly T injections and R.

The study duration was 10 wk. The resistance training sessions were conducted for 45 min, three times per week, and included three sets per session of each of the following: seated leg press, seated leg curl, seated leg extension, standing calf raise, and seated ankle dorsiflexion. Training intensity was set at 60% of the pretraining one-repetition maximum for the first 4 wk and increased as tolerated thereafter to 80% of the new one-repetition maximum obtained after 4 wk of training, for each exercise.

The companion paper and its online supplement have further demographic and methodological details (12). The study including muscle biopsies was approved by the Institutional Review Boards of the Burns and Allen Research Institute at Cedars-Sinai Medical Center and the Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, and written informed consent was obtained.

**Muscle Sample Preparation**

Percutaneous biopsies of the vastus lateralis were performed at the midportion of the right thigh (15 cm above the patella) under local anesthesia (1% lidocaine) using a needle biopsy technique with fibers perpendicular to cork surface), and then rapidly frozen with isopentane, which had been cooled to its melting point by liquid nitrogen. The fresh frozen muscle samples were stored at −80°C until analysis.

**Fiber-Type Proportions and Morphometry**

For the assessment of muscle fiber classification and morphometry, serial cross sections of the muscle sample were cut using a cryostat (Reichert-Jung, model 2800E, Nussloch, Germany) kept at −20°C. Serial muscle cross sections of 10-μm thickness were stained for myofibrillar adenosinetriphosphatase (mATPase) after alkaline (pH 9.6), and acid (pH 4.3 and 4.55) preincubations (10). One additional serial section was fixed in 2% paraformaldehyde at pH 7.4 for 2 min at room temperature and then preincubated at pH 9.6, in a modification of a previously described method (33). These various staining procedures allowed the classification of human muscle fibers into the main three fiber types, i.e., types I, IIa, and IIx. In previous studies, the histochemically determined fiber type was also verified immunohistochemically, with 95% or more correspondence between the mATPase-based classification and the major isoform of myosin heavy chain (MyHC) expressed in single muscle fibers (39). For each muscle sample, fiber-type proportions were determined from an analysis of 200–650 fibers within the entire cross section.

**Muscle Protein Analyses**

Protein extraction. Soluble protein was extracted from muscle samples in a 1:10 ratio of cold cell lysis buffer (Cell Signaling Technologies, Beverly, MA) according to manufacturer’s protocol. Homogenization was performed with a Polytron homogenizer (model PT-1200C, Kinematica, Newark, NJ), and homogenates were centrifuged at 14,000 rpm. The supernatant was aliquotted in microcentrifuge tubes. Protein concentration was determined using a commercial protein assay kit (Bio-Rad, Hercules, CA) based on the Bradford (8) method and measured with a spectrophotometer (SmartSpec 3000, Bio-Rad).

**Muscle mRNA Analyses**

Total RNA extraction. Total RNA was extracted from muscle samples with TRIzol reagent (Invitrogen, Carlsbad, CA) according to manufacturer’s protocol. Quality and concentrations of total RNA were determined with a spectrophotometer (SmartSpec 3000). Samples were stored at −80°C in RNase-free water until analysis. Two micrograms total RNA was reverse transcribed (RT) using oligo(dT) primers (Invitrogen) and Omniscript RT kit (Qiagen, Valencia, CA), and reactions yielded 20 μl of first-strand cDNA.
**Oligonucleotides.** The primers for the genes of interest and glyceroldehyde-3-phosphate dehydrogenase (GAPDH) were designed based on published human cDNA sequences using Primer3 on the WWW for general users and for biologist programmers (2004, Whitehead Institute for Biomedical Research, Cambridge, MA; see Ref. 61). Sequences of primers used for cDNA synthesis and real-time RT-PCR analysis, along with their accession numbers, are described in Table 1.

**Real-time PCR.** Primer efficiency tests were performed to compare amplification efficiency between the target genes and the endogenous control gene (GAPDH). A PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, CA) with V1.76A software was used to set the reaction plate design to perform the real-time PCR. The experimental conditions were 95°C for 2 min, 95°C for 10 min, 95°C for 30 s, 60°C for 1 min, and set for 40 cycles. For each well, 65 ng of cDNA template was used with the master mix (Applied Biosystems) containing SYBR Green in a volume of 25 µl.

**Statistical Analysis**

The distribution of data was tested for normality. Statistical analysis was performed using ANOVA (SigmaStat v. 2.0, Jandel, Richmond, CA). If a significant interaction was found, post hoc analysis (Newman-Keuls test) was used to compare differences in independent groups. Intragroup analysis (baseline vs. after intervention) was performed using ANOVA (SigmaStat v. 2.0, Jandel, Richmond, CA). If a significant interaction was found, post hoc analysis (Newman-Keuls test) was used to compare differences in independent groups. Intragroup analysis (baseline vs. after intervention) was performed using a paired t-test. An α level of 0.05 was used to determine significance. Values are presented as means ± SE in Figs. 1–7 and means ± SD in Table 2.

**RESULTS**

**Demographic, Morphometric, and Physiological Data**

Demographics and pulmonary function tests are shown in Table 2. Of note, data for the current 40 patients studied were not significantly different from those reported for the original 47 COPD patients reported in Table 1 of the companion paper (12). Body composition changes and in particular leg muscle mass changes in the four groups are highlighted in Table 3 and Fig. 3 of the companion paper (12). In summary, both resistance-trained and testosterone groups significantly increased leg lean muscle mass. However, the leg lean muscle mass in the combined testosterone and resistance training group was significantly greater than in resistance training alone. Leg strength significantly improved following both interventions. However, the combination of testosterone and

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**Table 1. Primers used for cDNA synthesis and real-time RT-PCR analysis with sequence given in the 5’ to 3’ direction**

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<th>Primer Name</th>
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<th>Accession No.</th>
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<td>Antisense: GCC ATA GTG GAT CAG GGA GA</td>
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<tr>
<td>MyHC2A</td>
<td>Sense: GCA GAA GCT GGA GAA GAG GA</td>
<td>AF111784</td>
</tr>
<tr>
<td></td>
<td>Antisense: TGG TCT AGA GTG CCG GCC CAC AT</td>
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<tr>
<td>MyHC2X</td>
<td>Sense: GGA TAC CCA GCT CCA CCT AGA</td>
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<td>Antisense: GCC TGC TAG TCC CTC GAT CTC</td>
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<td>M25142</td>
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<td>Antisense: ATG CTG TGG TCC CAG CTT AG</td>
<td></td>
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<td>MyHcEmb</td>
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MyHC, myosin heavy chain; Emb and Neo refer to embryonic and neonatal isoform, respectively; IGF-I and IGF-II, insulin-like growth factor I and II; IGFBP, IGF binding proteins; MAFbx, muscle atrophy F-box; GAPDH, glyceroldehyde 3-phosphate dehydrogenase.
resistance training increased quadriceps strength significantly greater than testosterone alone.

**Vastus Lateralis Muscle Fiber Proportions and CSAs**

Muscle fiber-type proportions for the entire cohort of patients at baseline were type I, 41.9 ± 1.0%; type IIa, 29.0 ± 0.8%; and type IIx, 29.1 ± 0.9%. No significant changes in fiber proportions were noted following resistance training, testosterone administration, or the combination of both interventions (Fig. 1). The mean CSAs of muscle fibers at baseline in our cohort of patients were type I, 4.853 ± 254 μm²; type IIa, 4.729 ± 201 μm²; and type IIx, 3.602 ± 154 μm². The impact of the experimental interventions were analyzed in two ways: as the relative changes in muscle fiber CSA per fiber type across groups (Fig. 2, top), and the before vs. after changes in muscle fiber CSA per fiber type within specific groups. Despite significant increments in leg lean muscle mass in testosterone-treated patients in our companion paper (12), both sets of analyses yielded nonsignificant differences across all groups for changes in muscle fiber CSA, despite trends (e.g., a mean increase of 19.3% for the CSA of type IIx fibers in TR patients; Fig. 2, top). Despite this, significant decrements in muscle fiber density (i.e., fewer fibers per unit cross-sectional area) were observed after 10 wk in all three intervention groups (resistance training and/or testosterone) compared with no changes in the P patients (P < 0.05; Fig. 2, bottom) (see Muscle Morphometry: Critical Evaluation of Data for further analysis).

**Biochemical Analyses of the Vastus Lateralis**

**Muscle MyHC mRNA.** Analyses of muscle MyHC mRNA abundances across and within groups for fold changes (i.e., values at the end of the intervention vs. the baseline) are depicted in Fig. 3. We included the analysis of developmental MyHC isoforms [i.e., embryonic (Emb) and neonatal (Neon)] and MyHC Slow α/β (usually expressed in the myocardium), as these may be reexpressed with remodeling, injury, and repair. Values are expressed as fold change from baseline (horizontal line); thus a value of onefold indicates no change following intervention. Across the groups, significant increments in mRNA abundances were observed in the TR patients for MyHC Slow/α (P < 0.05), MyHC2α (P > 0.05), MyHC2X (P < 0.05), MyHC E (P < 0.001), and MyHC Neo (P < 0.01) compared with P patients. Further, the abundance of MyHC E mRNA in TR patients was greater than that observed in the PR group (P < 0.05). For T patients, significant increments were observed for MyHC Slow/β (P = 0.05) compared with P, and for MyHC E compared with P (P < 0.001) and PR patients (P < 0.01). There was a trend for increased MyHC Neo in T patients (P = 0.06) compared with P. In PR patients, increased abundance of MyHC 2A was observed compared with P (P < 0.05).

**IGF system.** MUSCLE IGF-I mRNA. Analysis across groups revealed a significant relative increment in IGF-IIa mRNA (similar to circulating and liver form of IGF-I) abundance in the TR patients compared with P (P < 0.01), PR (P < 0.01), and T (P < 0.05) groups following the 10-wk intervention (Fig. 4). Of interest, there was a significant positive correlation between the change in muscle mass in TR patients, as reported in our companion paper (12), and the increased abundance of IGF-IIa mRNA (r = 0.62; P = 0.05). There was also a tendency for the increase in abundance of IGF-IIa mRNA to be greater in the T patients compared with the P group (P = 0.09). The change in abundance of the spliced variant of IGF-I, IGF-Ieb (spliced form mostly found in the liver, but present at low levels in muscle), was similar across the groups after 10 wk (Fig. 4). The abundance of the muscle stretch-sensitive IGF-I spliced variant, IGF-Iec (also called mechanogrowth factor, which is induced by stretch, exercise, and injury in muscle), was significantly increased in both T and TR groups compared with P (P < 0.001) and PR patients (P < 0.001 and P < 0.01, respectively; Fig. 4).

**MUSCLE IGF-I PROTEIN.** Following the 10-wk experimental period, analysis of relative change in IGF-I protein expression across the groups revealed significantly increases for TR patients compared with P (P < 0.01), PR (P < 0.05), and T (P < 0.05) groups (Fig. 5, left). Within each group, significantly increased IGF-I expression (change before vs. after) was observed for PR (20%; P < 0.05), T (30%; P < 0.05), and TR (90%; P < 0.001) (Fig. 5, left).

**MUSCLE IGF-II mRNA.** Across groups, abundance of IGF-II mRNA was significantly greater for TR patients compared with P (P < 0.01), PR (P < 0.05), and T (P < 0.01) patients following 10 wk (Fig. 4, bottom).

**MUSCLE IGF-II PROTEIN.** In contrast, no significant differences in IGF-II muscle protein expression were observed across groups or as a result of the 10-wk intervention in any group (Fig. 5, right).

**MUSCLE IGFBP mRNAs.** Over the 10-wk experimental period, no differences in the changes in IGFBP-3,-5, and -6 mRNA abundances were observed between the groups (Fig. 6). In contrast, the relative increase in mRNA abundance for IGFBP-4 in the TR group was significantly greater than in all other groups [P (P < 0.05), PR (P < 0.01), and T (P < 0.05)] (Fig. 6). There was a trend for a small decrease in the abun-

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Table 2. Demographics and pulmonary function results at study entry

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<th>Age, yr</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>FEV1, liters</th>
<th>FEV1, %predicted</th>
<th>TLC, liters</th>
<th>DLCO, ml·min⁻¹·mmHg⁻¹</th>
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<tr>
<td>Placebo + No Training (n = 11)</td>
<td>Placebo + Resistance Training (n = 8)</td>
<td>Testosterone + No Training (n = 11)</td>
<td>Testosterone + Resistance Training (n = 10)</td>
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<tr>
<td>68.6 ± 8.6</td>
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<td>176.8 ± 5.6</td>
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<td>179.8 ± 8.4</td>
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<td>82.2 ± 14.4</td>
<td>85.6 ± 15.2</td>
<td>81.0 ± 22.2</td>
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<td>1.26 ± 0.44</td>
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Values are mean ± SD. FEV1, forced expiratory volume in 1 s; VC, vital capacity; TLC, total lung capacity; DLCO, diffusing capacity of the lung for CO.
dance of IGFBP-4 mRNA in PR compared with P patients ($P = 0.07$).

Muscle myostatin, MyoD, and myogenin mRNAs. Across the groups, no significant changes were observed for either myostatin or MyoD mRNA muscle abundances [i.e., the relative abundance of IGFBP-4 mRNA in PR compared with P patients ($P = 0.07$)].

Muscle atrophy F-box. Despite a mean 1.6-fold increase in muscle atrophy F-box (MAFbx) mRNA abundance in TR patients compared with the other groups, this trend did not achieve statistical significance ($P = 0.06$ to 0.1; Fig. 7).

DISCUSSION

While both resistance training and testosterone treatment alone exerted positive biochemical and morphometric influences, their combination, as in our previous companion paper (12), had the greatest impact. This includes improved morphometric parameters, increased mRNA abundances of all major MyHC isoforms, and enhanced expression of muscle IGF-I and other components of the muscle IGF system, together with increased myogenin expression, a myogenic regulatory factor (MRF) influenced by IGF-I.

Muscle Morphometry: Critical Evaluation of Data

In the present study, the significant decrease in the number of fibers per unit area of muscle (fiber density) in all interventions changes in abundance before vs. after the experimental period (Fig. 7). In contrast, the fold change in myogenin mRNA abundance was significantly greater in T ($P < 0.01$) and TR ($P < 0.001$) compared with P patients, and there was only a trend for an increase in PR patients ($P = 0.06$; Fig. 7).

Fig. 1. Muscle fiber proportions in placebo (P), placebo + resistance training (PR), testosterone (T), and testosterone + resistance training (TR) patients before and after the 10-wk intervention. No differences were observed between groups at baseline, and no changes as a result of the interventions were detected. Values are means ± SE.

Fig. 2. Change in muscle fiber cross-sectional areas (CSAs; top) and muscle fiber density (bottom) after 10 wk in the 4 subject groups. Top: CSAs of all fiber types were unchanged after 10 wk in P subjects. In other groups, changes in individual fiber CSA (types I, Ila, IIX) did not achieve statistical significance. Nevertheless, incremental trends in CSAs were observed. For example, PR increased by 10.0–11.7% and T increased by 6.8–7.1%. In TR subjects, type Ila CSAs increased by 7.2%, while a 19.3% increase was noted in type IIX fibers; in type III fibers, a 7.6% reduction in CSA was observed. Bottom: in P patients, no changes were observed in fiber density after 10 wk. Significant decreases in fiber density (i.e., fewer fibers per unit area) were observed for intervention groups (PR, 12.8%; T, 12.7%; and TR, 11.9%). *Significantly different from P. Values are means ± SE for top and bottom.
tion groups suggests an increase in muscle mass per unit area. This is consistent with the increment in leg lean muscle mass in all three intervention groups reported using dual-energy X-ray absorptiometry (DEXA), in our companion paper (12). Similarly, we reported an increase in cumulative muscle fiber area and reduction in relative interstitial space per unit area of diaphragm muscle in hamsters given nandrolone (45). We suggested that the enhanced contractile mass per unit area of the diaphragm in part explained the increased force-generating capacity of the diaphragm in nandrolone-treated animals (45). Similarly, an increased contractile mass per unit area of the vastus lateralis could contribute to the increased leg strength observed in our patients receiving testosterone and/or resistance training (12).

However, closer inspection of our data suggests that further mechanistic considerations are necessary, as the reduction in muscle fiber density (similar across all interventions groups) cannot explain the significantly greater increase in leg press strength reported in the patients receiving the combination of testosterone and resistance training (12). Of interest also are the wide variances in fiber CSAs and different directions of changes for different fiber types in the TR patients (see Fig. 2). Indeed, the finding that the mean CSA of type I fibers tended to decrease compared with the 19.3% mean increase in the CSA of type IIX fibers is intriguing. We explored, in individual TR patients, the variances in fiber size for each type following the 10-wk intervention. We found significantly greater variances in fiber size for each type following the intervention (e.g., 2- to 3-fold increment) in each TR patient compared with baseline values (Fournier, preliminary data). We postulate that these interesting findings may be the result of two possible mechanisms.

The first mechanism is that muscle fiber hypertrophy induced by the interventions may stress the metabolic and diffusion reserves as well as the critical myonuclear domain size of very large fibers, resulting in splitting. This has been well described in rats and cats following weight lifting exercise (31, 37) and in stretch-enlarged avian muscle (3). Morphologically, the longitudinal split muscle fibers were observed as a “pinching off” of a segment from the parent fiber (37). In patients with neuromuscular disorders, longitudinal splitting of mainly type I fibers was reported in muscle biopsies exhibiting significant fiber hypertrophy (64). We believe that muscle fiber splitting is a positive adaptive response to maintain optimal homeostasis in muscle tissue. A second possible mechanism is that of de novo muscle fiber formation. With this in mind, an increase in muscle satellite cell number and enhanced expression of androgen receptors on satellite cells have been reported with testosterone treatment in male subjects and human satellite cell cultures, respectively (65, 66). Further, increased satellite cell activation and presence of small fibers were demonstrated in forearm muscles of cats following weight-lifting exercises due to presumed de novo fiber formation (28). In preliminary studies (Fournier, unpublished observations), we have observed significant increments in the number of myonuclei per muscle fiber with TR. Thus either or both mechanisms could contribute to enhanced variances in fiber size in response to testosterone and/or resistance training. In addition, other influences may contribute to variances in fiber size observed in this study. These include regional variations in fiber size within the muscle depending on site and depth, age, preexisting comorbidities and levels of exercise, etc. (19, 21, 47, 48, 56, 59).

**Muscle Fiber Proportions and MyHC mRNA Abundances**

In the present study, histochemical procedures for ATPase were employed to classify muscle fibers into the major types.
It is now well described that hybrid fibers coexpressing different MyHCs are present, although generally accounting for small percentages of the total proportions in aggregate. This type of analysis cannot be performed from an ATPase-based approach. Changes in MyHC mRNAs most attributed to T include MyHC_{Slow/β} and MyHC_{Emb} isoforms, while those most attributed to resistance training include the fast isoforms (MyHC_{2A} and MyHC_{2X}). The predominant changes in the abundances of MyHC mRNA isoforms, however, occurred in the TR patients. Of interest, there appears to be a global increase in expression of the various major isoforms (slow/β, 2A, 2X) in these patients. It is unclear whether protein expression after the 10-wk intervention would track the mRNA abundances. If this were the case, a global increase in MyHCs might in part explain the preserved fiber-type proportions in the TR patients. Testosterone treatment was associated with an increase in abundance of developmental MyHCs. Of interest, immunohistochemically, MyHC_{Emb} appeared to be expressed only in very small fibers, while MyHC_{Neo} was expressed in larger fibers of varying sizes and types (Fournier, unpublished observations). We postulate that the expression of MyHC_{Emb} in very small fibers reflects the presence of new fiber formation as a component of the remodeling process as described above.

### Muscle Biochemistry

Some provocative biochemical data were evident following the various treatments. Although gene expression studies were conducted at only one time point after 10 wk of treatment, it should be emphasized, however, that treatments were continued throughout the study, and so the data obtained with the final biopsy reflect ongoing stimuli over 10 wk and should not be judged as one might the impact of an initial stimulus studied 10 wk later. We interpret mRNA changes therefore to reflect both recent and ongoing influences of the interventions. Nevertheless, early changes in signaling pathways may have been missed. This is, however, an unavoidable limitation in human studies, which constrains the number of invasively derived samples as in our study.

In the ensuing discussion, we will compare and contrast our data with those in the literature and, where possible, distinguish anabolic steroid effects from those of resistance training. Studies evaluating acute responses over hours to several days were not included as these responses are not relevant to the long-term interventions we employed.

**IGF-Ia mRNA, IGF-I protein, and anabolic steroids.** In recent years, there has been increasing appreciation that the hypertrophic response of muscle fibers to anabolic steroids is due at least in part to the anabolic effects of IGF-I acting locally in an autocrine/paracrine fashion. We previously reported this in the diaphragm of rats receiving nandrolone (46). Further, altered expression of muscle IGFBPs was noted and interpreted as facilitating local muscle IGF-I action. It should be noted that most assays of IGF-I mRNA in muscle previously reported in the literature likely reflect the IGF-Ia isoform. In elderly men given testosterone, similar increments in IGF-I mRNA abundance were reported in the vastus lateralis muscle (71). More recently, the increment in muscle IGF-I mRNA abundance in elderly men was reported to be greater with the combination of testosterone and growth hormone (GH) than GH alone (9). Furthermore, testosterone administration pro-
duced sustained increments in IGF-I muscle protein expression over a 6-mo period while the expression of androgen receptors in muscle increased for only the first 4 wk (23). It should be emphasized, however, that healthy elderly men represent a distinct separate patient population from elderly men with COPD in whom skeletal muscle dysfunction has been highlighted (2). Nevertheless, we postulated that testosterone would still have the potential to significantly impact on the local IGF system (perhaps in an attenuated fashion) despite the potential negative regulatory factors present in our patients with COPD. The significant increase in the abundance of IGF-IEa in the TR patients is thus in line with this hypothesis. Furthermore, the change in the abundance of IGF-IEa mRNA in the TR patients is thus in line with this hypothesis. Sixteen weeks of resistance training in the young and older men revealed a twofold increase in vastus lateralis muscle IGF-IEa mRNA for both groups compared with baseline (54). Similar results were reported in other studies study following 12 wk of resistance training in young males (42). Of interest, in the present study, similar abundances of IGF-IEa mRNA in resistance-trained and other groups after 10 wk may reflect the insensitivity of measurements performed at limited time frames, in which a transient rise might have been missed. Furthermore, in elderly patients with COPD, the stimulus may have been insufficient to alter the expression of this spliced variant at any time point.

IGF-IEa mRNA, IGF-I protein, and resistance training. There is a paucity of data on local muscle IGF-I responses to resistance training and no data in the COPD population. Sixteen weeks of resistance training in the young and older men revealed a twofold increase in vastus lateralis muscle IGF-IEa mRNA for both groups compared with baseline (54). Similar results were reported in other studies study following 12 wk of resistance training in healthy elderly males (34) and after 8 wk of strength training in young men (42). Increased immunohistochemical staining for IGF-I was reported in vastus lateralis biopsies from elderly subjects after 10 wk of strength training (24). Thus resistance training might be expected to promote a local anabolic milieu. It is therefore not unexpected that in our study the combination of testosterone and resistance training had enhanced effects. Furthermore, both resistance training itself and IGF-I may enhance expression of key MRFs (25, 52, 57).

Our results thus demonstrate a distinct impact of both resistance training and testosterone on IGF-1 muscle expression with a positive interactive influence of the combined interventions. IGF-IEb. IGF-IEb is a spliced variant of IGF-I, which has greater abundance in the liver than in extrahepatic tissues (67). While expressed in skeletal muscle, its precise function is unknown. There are no reports of the effect of anabolic steroids on muscle IGF-IEb mRNA abundance. However, increased abundance of IGF-IEb mRNA was reported in the vastus lateralis of healthy elderly men following 5 wk of resistance training (34). Of note, after 12 wk, levels had decreased back to baseline values (34). In healthy young men, 8 wk of resistance training failed to increase vastus lateralis levels of IGF-IEb mRNA (42). We thus suggest that in the present study, similar abundances of IGF-IEb mRNA in resistance-trained and other groups after 10 wk may reflect the insensitivity of measurements performed at limited time frames, in which a transient rise might have been missed. Furthermore, in elderly patients with COPD, the stimulus may have been insufficient to alter the expression of this spliced variant at any time point.

IGF-IEc. The response of another spliced variant of IGF-I, IGF-IEc (also called mechanogrowth factor; 29), that is sensitive to muscle load and stretch was also examined. There are data in humans to suggest that increased mechanical activity shifts splicing of the IGF-I gene toward IGF-IEc (34, 35). Indeed, enhanced expression of the splice variant has been reported in elderly male subjects following 5 and 12 wk of resistance training (34). By contrast, no significant increase in IGF-IEc mRNA levels was observed after 8 wk of strength training in young males (42). Of interest, in the present study, IGF-IEc abundances were significantly increased only for the testosterone-treated groups. Thus anabolic steroids appear to be a potent stimulus for the expression of this spliced variant. No response with resistance training may reflect an elderly COPD population.

IGF-II. There are no reports of the effect of anabolic steroids on muscle IGF-II expression. In elderly men subjected to resistance training, vastus lateralis biopsies at 14 and 24 wk
failed to show enhanced IGF-II mRNA expression (68). IGF-II has an important role in myoblast differentiation, prevention of apoptotic cell death, and repair following injury (67). As postulated above, extreme hypertrophy of muscle fibers, which outstrip their nuclear domain size and diffusion reserves, could...
lead to de novo fiber synthesis and/or fiber splitting. Furthermore, resistance training could result in muscle fiber injury (58). In the present study, the increased abundance of IGF-II and myogenin (a MRF) in the TR patients might thus reflect muscle reparative processes, remodeling, and stimuli for new fiber formation (25, 49). The increased abundance of IGF-II mRNA in TR patients was not reflected by concomitant changes in protein expression. This likely reflects posttranscriptional influences.

IGFBPs. Enhanced expression of IGFBP-4 was observed in TR patients. This was unexpected, as IGFBP-4 has been shown to inhibit IGF action in both in vitro and in vivo models, by preventing binding of IGFs to their receptors (74). These results are opposite to those reported by us in rats (46) and in elderly men (71), following the administration of anabolic steroids. The specific roles of IGFBPs in vivo and in different tissues are still not fully understood and are the subject of intense research (62). Of interest, IGFBP-4 knockout mice are smaller than their wild-type littermates, suggesting that IGFBP-4 may be needed to support myogenesis and possibly IGF-II action (55). We thus speculate that our IGFBP-4 data in the TR patients reflect myogenesis and remodeling.

MRFs. The MRFs were studied because 1) IGF-I can induce the expression of myogenin (1, 53, 70); and 2) the MRFs, like IGF-I and IGF-II, play an integral role in muscle injury, repair, and regeneration (51). There are no studies reporting the effect of anabolic steroid administration on limb muscle MRF expression. Myogenin, MyoD, and MRF4 mRNA abundances, however, have been reported to be significantly elevated following acute resistance exercise in male subjects (57) and following 16 wk of resistance training in young and older adults with lower expression in the elderly (40). In the latter study, protein levels were not significantly increased (40). In contrast, muscle myogenin protein increased following 10 wk of resistance training in young subjects (36).

In the present study, the increased expression of myogenin mRNA in T and TR patients and a trend for increase in the PR group supports further enhancement of an anabolic stimulus within the muscle by both interventions. Our findings may be explained by the influences of increased IGF-I expression. Furthermore, both IGF-I and myogenin have been reported to be upregulated in skeletal muscle with injury and regeneration (51) and would clearly be important factors in the repair processes and the differentiation of newly formed myoblasts, as postulated above (25). MyoD is an important factor in the transcriptional control of myogenesis. Its expression is not temporally linked to myogenin, and thus nonparallel changes in their expression are not surprising, especially at a single 10-wk time point. Furthermore, protein expression of the regulatory factors was not determined. We thus cannot exclude important contributions from MyoD.

Myostatin. Myostatin, a skeletal muscle growth inhibitor, is a member of the transforming growth factor-β superfamily. Enhanced expression is associated with muscle wasting. In the present study, muscle myostatin mRNA abundance was not significantly affected in any of the intervention groups, although modest trends for reduction were seen in all three. A significant reduction in myostatin gene expression has been reported in human subjects following strength training in one study (60) but not in another (42). We cannot exclude, however, decreased myostatin protein expression after our 10-wk intervention.

MAFbx. MAFbx, also known as atrogin-1, is a muscle-specific E3 ligase and an important mediator of the ubiquitin-proteasome system, the major pathway through which muscle protein degradation occurs (7, 30). An increase in both MAFbx and MuRF1 mRNA was recently reported following 8 wk of resistance training (44). While the apparent increase in MAFbx in the TR patients appears at face value counterintuitive (as this is the group that experienced the greatest increased in muscle mass), other feasible postulates for this direction of change can be made. In keeping with our postulate of muscle remodeling including muscle fiber splitting, injury, and repair, upregulation of MAFbx may well be necessary to clear myofibrillar debris and optimize the local milieu for myogenesis. Indeed, myofibrillar disassembly is a necessary step before activation of the ubiquitin-proteasome system (17).

Limitations of Present Study

While this study provides an abundance of new and intriguing data that should prompt multiple future lines of investigation, several limitations should be highlighted. While we considered adding two control populations, i.e., normal healthy age-matched subjects and age-matched COPD patients but with normal serum testosterone levels, this was not undertaken for several reasons. First, it is well established that distinct differences in both limb and respiratory muscles exist in COPD patients compared with controls. Thus the response to testosterone and to resistance training is likely very different. Furthermore, in the present study, each COPD patient acted as his own control, providing more relevant data for this patient population. Logistical issues as well as excessive costs and time commitment also precluded adding two extra groups to the existing four groups studied. Indeed, the present study, funded by two major grants, took several years to complete. In addition, it was unclear whether this would provide cost-effective additional clarifications regarding restoration of muscle function and/or biochemical markers by the interventions.

While comparisons from the literature were cited regarding the impact of anabolic steroids or resistance training on limb muscle, in our discussion, the relevance of these comparisons remain very limited. These studies were mainly performed in normal subjects and thus exhibit no disease specificity. In addition, studies are often in young individuals in whom adaptations differ from those in the elderly (e.g., 54).

Morphometric and biochemical analyses on human muscle tissue are limited by great variances between subjects. Thus the total number of subjects per group that were studied was relatively low and likely accounted for several trends that were observed in this study.

Both mRNA and protein analyses were performed. The amount of tissue required for protein analysis (e.g., immunoblot, RIA) limited the number of assays that could be performed. While the use of mRNA analysis may be limited by the fact that changes in abundance may well have occurred earlier than after 10 wk, some intriguing data were nevertheless found and are of interest (i.e., transient changes in muscle gene expression may have been missed). This was limited by the fact that we did not consider a greater number of biopsies justified, as this intervention was invasive and provided no
direct benefits to the patients. We thus elected to limit our analysis to those adaptations present at the conclusion of the study.

**Summary**

In summary, the increased leg muscle mass and strength in male COPD patients with low serum testosterone levels, who received testosterone, resistance training, or the combination (12), was associated with increased cumulative fiber CSA per unit area of muscle, as suggested by decreased fiber density. This should result in increased contractile muscle protein per unit area. Furthermore, the combined interventions produced a distinctly enhanced local anabolic milieu driven in large part by the muscle IGF system, despite potentially negative biochemical influences present in patients with COPD. While our data provide strong inferences for the morphometric and biochemical mechanisms underlying improved lean leg structure and function, as reported in our companion paper (12), they do not provide conclusive cause-and-effect relationships. Last, our data also provide strong inferences that fiber remodeling and/or new fiber formation was induced, as adaptations to the combined interventions challenge fiber diffusion reserve and/or critical myonuclear domain size associated with rapidly induced changes in fiber morphometry.

**ACKNOWLEDGMENTS**

BioTechnology General (Iselin, NJ) generously donated testosterone enanthate for this study.

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**GRANTS**

This research was supported by funds from Tobacco-Related Disease Research Program of the University of California Grants 7RT-0114 and 6RT-036, National Heart, Lung, and Blood Institute Grant HL-071227, and the Research Program of the University of California Grants 7RT-0114 and GRANTS and function, as reported in our companion paper (12), they do not provide conclusive cause-and-effect relationships. Last, our data also provide strong inferences that fiber remodeling and/or new fiber formation was induced, as adaptations to the combined interventions challenge fiber diffusion reserve and/or critical myonuclear domain size associated with rapidly induced changes in fiber morphometry.

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