Effects of testosterone and exercise on muscle leanness in eugonadal men with AIDS wasting

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ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS) wasting syndrome (AWS) is a complication of human immunodeficiency virus (HIV) infection associated with loss of lean body mass (20), an independent predictor of mortality in this population (19). Reduction in lean body mass as assessed by quadriceps muscle cross-sectional area strongly predicts reductions in strength and functional capacity of leg muscles (13). Loss of lean body mass occurs in patients with AWS, despite adequate nutritional supplementation (18), and may result from increased catabolism and energy expenditure (11, 14) or inadequate protein synthesis (25).

Lean tissue density can be determined on the basis of X-ray attenuation value (4). Because fat has a strongly negative attenuation value on computed tomography (CT), a lower attenuation value is thought to reflect increased fat deposition within the muscle (5, 9, 21). Conversely, increased attenuation is consistent with increased leanness of the muscle. We previously reported significant effects of testosterone and training on muscle area and function in this group of eugonadal men with AWS (12). Although changes in muscle bulk may explain part of the functional loss observed in AWS, we are unaware of studies examining muscle composition as it relates to anabolic therapy in men with AWS. In this study we use single-slice CT to determine the effects of anabolic therapy on muscle leanness in this population.

SUBJECTS AND METHODS

Experimental Subjects

Fifty-four eugonadal (normal serum free testosterone >41.6 pmol/l) HIV-infected men with AWS [weight <90% of ideal body weight (IBW) or weight loss >10% of baseline weight] were recruited from the multidisciplinary HIV practice at the Massachusetts General Hospital and from newspaper advertisements. A screening visit assessed baseline weight, medication history, and testosterone level (23). Exclusionary criteria for participation in the study included diagnosis of new opportunistic infection within 6 wk of the study, unstable angina, aortic stenosis, uncontrolled hypertension, severe neuropathy, arthritis, or other contraindications to exercise. Additional exclusionary criteria included intractable diarrhea (>6 stools/day), current substance abuse, initiation of protease inhibitor use within 6 wk of...
study entry, abnormal prostate-specific antigen, symptomatic prostatism, history of prostate malignancy, bipolar disorder, Hb <9 g/dl, platelet count <50,000 cells/mm³, and serum creatinine >2.0 mg/dl (170 μmol/l). Patients receiving parenteral nutrition, megestrol acetate, glucocorticoids, androgen, estrogen, growth hormone, or other anabolic agent within 3 mo of the study were excluded. All patients provided written informed consent, and the study was approved by the Human Studies Committee of the Massachusetts General Hospital. Data on the effects of testosterone on body composition, muscle cross-sectional area, muscle function, and hormonal parameters from this group of patients were previously published (12).

Protocol

Participating patients were stratified for weight above or below 90% of IBW and were randomized to 1) testosterone (200 mg/wk im; Bio-Technology General, Iselin, NJ) or an identical placebo and 2) progressive resistance training or no training for 12 wk in a 2 × 2 factorial design. Randomization to testosterone was blinded to patient and investigator.

Patients were admitted to the General Clinical Research Center of the Massachusetts General Hospital for an inpatient baseline evaluation including body composition and muscle strength. Patients were instructed to self-administer intramuscular injections. Patients unable or unwilling to self-administer study medications (n = 43) received weekly injections by the nursing staff of the General Clinical Research Center. Patients randomized to placebo performed weekly injections of sesame oil with chlorobutanol as a preservative, which had the same viscosity and color as the active medication. Compliance was assessed by history, review of injection records, and empty vial counts. Patients completed 4-day food diaries before baseline and 12-wk visits.

Patients randomized to training underwent progressive strength training three times per week for 12 wk. A standardized dynamic progressive resistance training regimen, in which five Life Circuit machines (Life Fitness, Franklin Park, IL) were used, was supervised by a licensed physical therapist of Massachusetts General Hospital. Patients performed 1) leg extension, 2) leg curl, 3) leg press, 4) latissimus dorsi pull down, 5) arm curl, and 6) triceps extension using a predetermined weight. Baseline 1 repetition maximum (1 RM, defined as the maximal load that could be lifted throughout the joint range of motion once) was assessed by taking the best of three efforts. Each set consisted of six to eight repetitions. In weeks 1 and 2, patients performed two sets at 60% 1 RM. In weeks 3–6, patients performed two sets at 70% 1 RM. In weeks 7–9, patients performed two sets at 70% 1 RM and one set at 80% 1 RM. In weeks 10–12, patients performed three sets at 80% 1 RM.

All patients randomized to exercise performed 30 min of aerobic exercise on a stationary bicycle three times per week, including a 5-min warm-up period and a 5-min cool-down period. Target heart rate was 60–70% maximal predicted rate (220 – age in years). Attendance was monitored, and patients were asked to limit other exercise to normal daily activity during the study.

Experimental Methods

Body composition analysis. Fasting weight and percent IBW were determined on the first day of each visit (1). Total body fat and lean mass were determined by dual-energy X-ray absorptiometry (DXA; QDR-4500 densitometer, Hologic, Waltham, MA) with a precision of 1.5% for lean body mass and 3% for whole body fat mass. Muscle cross-sectional area of the left leg was assessed by quantitative CT (General Electric RP high-speed helical CT scanner, Milwaukee, WI) (13). After symmetrical positioning of the legs, a coronal scout image was used to draw a line tangent to the femoral articular cortex at the hip, and a perpendicular line was constructed parallel to the long axis of the femur. A second line was constructed perpendicular to this line, extending from the femoral head to the most distal portion of the medial femoral condyle. This line was bisected to identify the middle of the femur. Total muscle anterior and posterior cross-sectional areas were determined using graphical analysis software provided by the manufacturer. The standard error of leg muscle area was ±1%. Mean muscle attenuation in Hounsfield units was determined in anterior and posterior left thigh muscle compartments and averaged for total mean attenuation with the use of a GE Advantage Windows workstation. The coefficient of variation for the measurement of midfemur thigh muscle attenuation in our laboratory is 2.4% (unpublished data).

Muscle strength testing. Upper and lower extremity muscle strength was assessed using the Quantitative Muscle Function Test as previously described (2, 12, 22). On the better of two repetitions, peak isometric force was measured for knee flexion and extension with maximal contraction held for 5 s.

Biochemical and immunologic assays. Serum total and free testosterone were measured by RIA kit (Diagnostics Products, Los Angeles, CA) with intra-assay coefficients of variation of 5–12% and 3.2–4.3%, respectively. CD4 lymphocyte counts were measured by flow cytometry (Becton-Dickinson Immunocytometry Systems, San Jose, CA). Viral load was determined using the AmpliCor HIV-1 Monitor Test (Roche Molecular Systems, Branchburg, NJ).

Statistical analysis. Patients were randomized simultaneously to 1) testosterone or placebo and 2) training or no training in a 2 × 2 factorial model to assess the independent effects of testosterone and training as specified in the data analytic plan (7, 8). Simple linear regression analysis was used to compare thigh muscle attenuation and relevant body composition parameters at baseline. Change in muscle attenuation from baseline was compared between treatment groups (testosterone vs. placebo and training vs. no training) using analysis of covariance, controlling for baseline values. To test for an interaction term between testosterone therapy and resistance training, we used analysis of covariance with an interaction term. A multivariate regression model was used to determine the independent effect of fat mass on thigh muscle attenuation, controlling for age, body mass index (BMI), total testosterone, viral load, whole body lean mass, and thigh muscle area. Descriptive statistics (change from baseline) are also reported for the individual treatment groups (placebo vs. exercise alone, testosterone alone, and combined-treatment group). In a secondary analysis, data from the individual groups are compared with the placebo/no-training group using Dunnett’s test.

RESULTS

Of the 101 patients screened, 69 were eligible to participate in the study. Of 54 patients randomized, 4 elected not to participate in the study before baseline evaluation and 7 dropped out before the end of study evaluation. No patients dropped out because of adverse events or side effects, and there were no significant differences between the number of dropouts in the different treatment groups. Among the 43 patients who
completed the study, 12 were in the placebo/no-training group, 10 were in the testosterone/no-training group, 10 were in the placebo/training group, and 11 were in the testosterone/training group. Compliance with testosterone/placebo injections was 98%. Compliance with resistance exercise training was 78% among patients randomized to exercise who completed the study. There were no deaths among patients in the protocol. Values are means ± SE unless otherwise indicated.

Baseline Clinical Characteristics

Table 1 presents the baseline characteristics of all patients in each treatment group. Patient groups did not differ with respect to age, CD4 count, viral load, total testosterone, free testosterone, weight, BMI, percent IBW, caloric intake, whole body fat content, whole body lean content, thigh muscle cross-sectional area, or initial thigh muscle attenuation on CT. Overall, the mean age of patients was 38.1 ± 0.8 yr. The mean duration since diagnosis of HIV infection was 5.9 ± 0.6 yr, and the mean time lapse since experiencing minimum weight was 2.6 ± 0.6 yr. BMI was 17.4–28.7 (mean 22.1 ± 0.4) kg/m². The mean percent IBW was 96.6 ± 1.6%. The percentage of body fat was 7–33% (mean 18.1 ± 0.8%). Patients had a baseline mean CD4 count of 369 ± 39 cells/mm³ and a viral load of 44,661 ± 15,037 copies. Baseline mean total and free testosterone values were 22.6 ± 0.9 nmol/l and 77.7 ± 3.5 pmol/l, respectively. Patients’ diets, which consisted of 2,896 ± 114 kcal with 78.8 ± 3.9 g protein, 99.8 ± 4.9 g fat, and 437 ± 18 g carbohydrate, did not significantly change over the 12-wk treatment period.

Body Composition

Baseline thigh muscle cross-sectional area correlated with weight ($r = 0.77, P < 0.0001$), BMI ($r = 0.73, P < 0.0001$), and lean body mass as assessed by DXA ($r = 0.82, P < 0.0001$). Baseline thigh muscle attenuation by CT inversely correlated with age ($r = -0.33, P = 0.02$), weight ($r = -0.30, P = 0.04$), BMI ($r = -0.32, P = 0.02$), and percent IBW ($r = -0.32, P = 0.02$). The strongest single correlation between thigh muscle attenuation and body composition parameters was between muscle attenuation and whole body fat mass ($r = -0.52, P = 0.0001$; Fig. 1). The relationship between muscle attenuation and whole body fat mass persisted in a standard least-squares multivariate model, controlling for age, BMI, total testosterone, viral load, lean body mass, and thigh muscle cross-sectional area (Table 2). Thigh muscle attenuation by CT did not correlate with baseline lean body mass by DXA ($r = -0.02, P = 0.90$).

**Anabolic Therapy**

Testosterone therapy and resistance training each significantly increased thigh muscle attenuation over the 3-mo treatment period (Fig. 2). No interaction was found between testosterone and training therapy. In the four-group comparison, the greatest relative effect

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**Table 1. Baseline characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n = 26)</th>
<th>Testosterone (n = 24)</th>
<th>P</th>
<th>No Training (n = 24)</th>
<th>Training (n = 26)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>38.7 ± 1.1</td>
<td>37.5 ± 1.2</td>
<td>0.47</td>
<td>37.3 ± 1.0</td>
<td>38.8 ± 1.3</td>
<td>0.33</td>
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<tr>
<td>CD4, cells/mm³</td>
<td>313 ± 47</td>
<td>430 ± 60</td>
<td>0.13</td>
<td>366 ± 59</td>
<td>372 ± 52</td>
<td>0.94</td>
</tr>
<tr>
<td>Viral load, copies</td>
<td>51,038 ± 20,499</td>
<td>37,752 ± 22,466</td>
<td>0.66</td>
<td>35,882 ± 21,112</td>
<td>52,764 ± 21,639</td>
<td>0.58</td>
</tr>
<tr>
<td>Testosterone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total, nmol/l</td>
<td>23.0 ± 1.4</td>
<td>22.3 ± 1.2</td>
<td>0.72</td>
<td>22.5 ± 1.6</td>
<td>22.7 ± 1.1</td>
<td>0.90</td>
</tr>
<tr>
<td>Free, pmol/l</td>
<td>75.6 ± 4.2</td>
<td>80.4 ± 5.5</td>
<td>0.47</td>
<td>74.2 ± 4.5</td>
<td>81.1 ± 4.9</td>
<td>0.32</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>69.5 ± 1.8</td>
<td>66.8 ± 1.7</td>
<td>0.28</td>
<td>68.2 ± 1.7</td>
<td>68.2 ± 1.9</td>
<td>0.99</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.3 ± 0.6</td>
<td>21.8 ± 0.4</td>
<td>0.50</td>
<td>22.1 ± 0.5</td>
<td>22.1 ± 0.5</td>
<td>0.93</td>
</tr>
<tr>
<td>Whole body DXA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat, kg</td>
<td>12.7 ± 0.9</td>
<td>11.8 ± 0.7</td>
<td>0.43</td>
<td>12.2 ± 0.8</td>
<td>12.3 ± 0.9</td>
<td>0.97</td>
</tr>
<tr>
<td>Lean, kg</td>
<td>53.0 ± 1.3</td>
<td>51.6 ± 1.4</td>
<td>0.46</td>
<td>52.3 ± 1.3</td>
<td>52.4 ± 1.4</td>
<td>0.93</td>
</tr>
<tr>
<td>Thigh muscle area, mm²</td>
<td>13,464 ± 404</td>
<td>13,438 ± 443</td>
<td>0.96</td>
<td>13,085 ± 417</td>
<td>13,790 ± 416</td>
<td>0.24</td>
</tr>
<tr>
<td>Thigh muscle attenuation, HU</td>
<td>49.5 ± 0.7</td>
<td>49.1 ± 0.9</td>
<td>0.71</td>
<td>49.4 ± 0.8</td>
<td>49.2 ± 0.8</td>
<td>0.81</td>
</tr>
</tbody>
</table>

Values are means ± SE. BMI, body mass index; DXA, dual-energy X-ray absorptiometry; HU, Hounsfield units.
was observed in the combined-therapy group (Fig. 3). At the end of the 3-mo treatment period, the testosterone treatment group \((n = 21)\) had mean trough total and free testosterone levels of \(37.0 \pm 2.2 \text{ nmol/l}\) and \(147.7 \pm 9.0 \text{ pmol/l}\), respectively. Of the 21 patients randomized to testosterone, 10 demonstrated end-of-study trough testosterone values above the normal range for total and free testosterone. In the group randomized to placebo \((n = 22)\), mean total and free testosterone levels were \(24.5 \pm 1.5 \text{ nmol/l}\) and \(78.0 \pm 4.2 \text{ pmol/l}\), respectively.

## DISCUSSION

AWS is associated with a loss of lean body and muscle mass. Single-slice CT at the midthigh is easily performed and provides a more accurate assessment of muscle cross-sectional area and composition than anthropometry (4). CT uniquely provides information on adipose and lean tissue volumes (3, 21) as well as tissue density information with normal muscle attenuation values in the range of water density (6). CT radiation is attenuated differently in certain tissues, and the measured attenuation values represent the average density of the pixels in the image area analyzed (16). Adipose and muscle tissues have markedly different attenuation values, with positive values for muscle and negative values for adipose tissue. Although CT provides a noninvasive assessment of gross morphological changes, this technique does not provide direct evidence of muscle changes at the cellular level. However, replacement of normal muscle with fat, as observed in certain neuromuscular diseases, has been shown to result in a lowering of muscle tissue attenuation on CT (15). In addition, thigh muscle attenuation by single-slice CT has previously been reported to vary inversely with BMI in obese subjects and patients with diabetes (17). Our findings demonstrate that thigh muscle attenuation by single-slice CT in eugonadal patients with AWS is highly inversely predictive of whole body fat mass. In support of this finding, baseline thigh muscle attenuation values also negatively correlate with weight, BMI, and percent IBW. Our data suggest that average muscle attenuation on single-slice CT may be considered an alternate marker of body composition reflecting muscle specific leanness or degree of adiposity. This regional response to anabolic therapies cannot be determined with measures of whole body composition.

We previously reported that therapy with testosterone and progressive resistance training independently increase thigh muscle cross-sectional area and lean body mass in this group of eugonadal patients with AIDS wasting.
AWS (12). In this analysis, we have shown that testosterone and training therapies independently increase muscle attenuation, suggesting that these anabolic strategies increase the leanness of muscle in addition to muscle cross-sectional area. Exercise therapy may have additional benefits on cardiovascular health, including high-density lipoprotein, not generally attributed to therapy with testosterone. Although the effects of both therapies on muscle leanness appeared additive, this study was not powered to detect differences between the combined-therapy and monotherapy groups. Additionally, testosterone dosing was supraphysiological. Therefore, the effect of physiological testosterone replacement therapy on muscle attenuation in hypogonadal men with AWS remains unknown.

Analysis of regional specific changes in body composition as assessed by attenuation value on CT may have several important applications. For example, in a different population of HIV-infected patients with the lipodystrophy syndrome, changes in intramuscular fat content (as assessed by attenuation value) may correlate with changes in insulin sensitivity, as has previously been shown in normal and obese subjects (10, 24). Investigation of region-specific body composition at baseline and longitudinally in response to a therapy may provide insight into the pathogenesis of the disease being investigated as well as the mechanism of action of the therapy. Needle muscle biopsies were not obtained as part of this investigation but should be considered in future studies. Direct analysis of muscle tissue could confirm changes in muscle composition as assessed by noninvasive imaging techniques.

To our knowledge, this is the first study to investigate the site-specific effects of testosterone and exercise on muscle composition in patients with AWS. Our data suggest that anabolic strategies for AWS alter muscle cross-sectional area and composition. Use of CT scan to assess these dual parameters is feasible and provides important information not typically obtained using other methods of body composition assessment such as DXA and anthropometry. Furthermore, single-slice CT of the thigh provides information that correlates with overall body composition of the patient. Further studies are necessary to investigate this relationship between muscle composition and function and determine the independent effects of anabolic strategies on these parameters in HIV and other wasting syndromes. Nevertheless, our data suggest that testosterone and progressive resistance training alter muscle composition in addition to increasing muscle area in men with AWS.

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Informed consent was obtained from the patients, and guidelines for human experimentation of the US Department of Health and Human Services and the Massachusetts General Hospital Subcommittee on Human Studies were followed in the conduct of this research.

REFERENCES


