Effects of Testosterone Replacement on Muscle Mass and Muscle Protein Synthesis in Hypogonadal Men—A Clinical Research Center Study*

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ABSTRACT

Testosterone replacement in hypogonadism has long been known to promote nitrogen retention and increase body density, but the mechanisms of nitrogen retention and body composition changes are poorly defined. We measured body composition and muscle protein synthesis in five hypogonadal men before and 6 months after initiating testosterone replacement. Body composition was examined using dual energy x-ray absorptiometry. Muscle mass was estimated both by excretion of creatinine on a meat-free diet and from appendicular mass measured using dual energy x-ray absorptiometry. Muscle protein synthesis was assessed by measuring the increment of \(^{13}C\)leucine in mixed muscle protein and myosin heavy chain during a continuous infusion of L-[\(^{13}C\)]leucine.

In all subjects there was an increase in fat-free mass (average, 15%; range, 10–22%; \(P = 0.02\)) and a decrease in fat mass (−11%; range, −0.4% to −22.0%; \(P = 0.03\)). Muscle mass also increased in everybody (mean, 20%; range, 11–32%; \(P = 0.04\)) such that 65% of the increase in fat-free mass could be attributed to accretion of muscle. The accumulation of muscle was associated with a 56% (\(P = 0.015\)) increase in the fractional synthesis rate of mixed skeletal muscle proteins and a trend toward a similar increase in the fractional synthesis rate of myosin heavy chain (46%; \(P = 0.098\)). We conclude that testosterone replacement in hypogonadal men enhanced skeletal muscle mass by stimulating the muscle protein synthesis rate. (J Clin Endocrinol Metab 81: 3469–3475, 1996)

The physiological role of testosterone as an anabolic hormone in men has been characterized by its ability to reversibly promote nitrogen retention (1) and increase body density (2) in eunuchoid patients. The composition of changes in body density induced by testosterone have been described in healthy men and consist of decreases in fat, particularly abdominal fat, mass accompanied by increased fat-free mass (FFM). The former is thought to be the consequence of decreased lipid uptake into the abdominal fat depot and decreased lipoprotein lipase activity combined with increased lipid release from the abdominal fat depot (3). Such changes are reported to be associated with decreased waist to hip ratio and improved insulin-induced glucose disposal (4). In otherwise healthy obese men, testosterone concentrations are lower than those in nonobese men, a phenomenon noted in the extreme in the superobese who may develop testosterone concentrations suggestive of hypogonadism (5, 6). The converse is expected, that hypogonadal men may accumulate fat and revert to normal with physiological testosterone replacement therapy. These men were testosterone deficient as the result of either primary hypogonadism (one patient with Klinefelter’s syndrome) or secondary hypogonadism (four patients; three postresection of nonfunctioning pituitary macroadenomas and one post resection of a craniopharyngioma) confirmed by total testosterone concentrations at screening of less than 6.9 nmol/L (200 ng/dL). Subjects were required to have taken stable doses of other replacement hormones for at least 1 yr and have no other active metabolic diseases (e.g. Cushing’s disease, acromegaly, etc.). Subjects were otherwise healthy as determined at a replacement in hypogonadism is whether FFM, particularly muscle mass, is increased by testosterone in physiological replacement in hypogonadal men as it is reported to be by supraphysiological concentrations in healthy young adults (7, 8) or by increasing testosterone levels in healthy older men (9). The effect of testosterone replacement on the FFM, particularly muscle mass, in hypogonadal men has yet to be defined. Thus, the effects of testosterone replacement therapy for hypogonadal men are usually described in terms of the augmentation of sexual function and secondary sex characteristics (10), rather than alteration of body composition, pending information regarding its influence on FFM and muscle.

We investigated the effect of testosterone at replacement doses on body composition, muscle mass, and skeletal muscle protein synthesis rates in postpubertal testosterone-deficient men. We hypothesized that testosterone replacement therapy would result in increased synthesis rates of skeletal muscle proteins with resultant increases in muscle mass and FFM.

Subjects and Methods

Subjects

We studied five men before and after 6 months of testosterone replacement therapy. These men were testosterone deficient as the result of either primary hypogonadism (one patient with Klinefelter's syndrome) or secondary hypogonadism (four patients; three postresection of nonfunctioning pituitary macroadenomas and one post resection of a craniopharyngioma) confirmed by total testosterone concentrations at screening of less than 6.9 nmol/L (200 ng/dL). Subjects were required to have taken stable doses of other replacement hormones for at least 1 yr and have no other active metabolic diseases (e.g. Cushing's disease, acromegaly, etc.). Subjects were otherwise healthy as determined at
screening visit by medical history, physical examination, and routine clinical laboratory testing of blood chemistry (SMA-20) and complete blood count, with the exception of gastroesophageal reflux disease in two subjects (one receiving ranitidine therapy and one receiving cimetidine therapy), essential hypertension in another subject (clonidine and chlorthalidone therapy), and a remote history of depression in one subject (Pamelor and Klonepin therapy). Medications and dosages remained unchanged throughout participation in the study. Subjects had either not received testosterone therapy after the diagnosis of testosterone deficiency or had not received such therapy for at least 2 yr before entry into the study.

Protocol
Subjects gave written informed consent for participation in the protocol and procedures as approved by the University of Vermont committee on human research for the medical sciences. Following the screening visit, subjects were given a 3-day weight-maintaining creatine-free (i.e. no meat or nuts) diet with 1.5 g/kg/day protein to assure adequate nutrition in each volunteer. Energy intake was determined by estimates derived from 3-day diet diaries and Harris-Benedict calculation of basal energy needs. The diet was consumed on the first day at home with the assistance of a very specific menu and a food scale lent to measure amounts accurately. The diet was consumed on the following 2 days at the Clinical Research Center as prepared by the Clinical Research Center dietitian. Subjects completed a 3-day physical activity diary as previously described (11) and were asked to include specific notation for any formal resistance exercise or strength training. Subjects were then admitted to the University of Vermont Clinical Research Center to undertake a 48-h urine collection for creatinine excretion while continuing a creatine-free, weight-maintaining diet.

Body composition analysis
Body composition pre- and posttreatment with testosterone was evaluated by dual energy x-ray absorptiometry (Lunar, Madison, WI), as recently reviewed (12). Total body muscle mass was estimated by 2 methods: 48-h creatinine excretion (13) and measurement of appendicular FFM using dual energy x-ray absorptiometry (14).

After the baseline study, subjects were given biweekly injections of testosterone cypionate that were gradually increased over 1 month to a dose of 3 mg/kg every 2 weeks. The dose was adjusted biweekly with the aim of achieving a nadir (i.e. immediately before injection) total testosterone concentration of 10.4-13.9 nmol/L (300-400 ng/dL) and was administered for 6 months (Fig. 1). The goal concentrations were approximately achieved in all subjects at all times (<15.0 nmol/L) with the exception of one subject who had a value above this range at the 4 month visit, resulting in a decrease in testosterone dosage from 300 to 250 mg biweekly between the 4 and 6 month visit. Other medications remained unchanged for the duration of study participation. Subjects were not instructed to maintain any prescribed level of physical activity other than to maintain their usual activity and to avoid any habitual resistance exercise that had not been undertaken before study entry. They were questioned about habitual physical activity and were asked to complete bimonthly the same activity diary completed at baseline. Subjects were questioned by the investigator at each bimonthly visit to identify any habitual changes in activity not reflected in the diaries. Specific activity prescriptions were not made because of the low likelihood of compliance during a long (6-month) study in free-living subjects. After completing 6 months of testosterone replacement therapy, subjects repeated all baseline procedures. Posttreatment procedures were performed 1 week after the final testosterone cypionate injection.

Infusion and muscle biopsy studies
Subjects received nothing by mouth except water and replacement hormones for 8 h before infusion studies. A venous catheter was inserted at 2200 h on the night before the study, and the line was maintained by normal saline infusion until saline was switched over to the stable isotope (nonradioactive) leucine at 0200 h without disturbing the sleep. A primed continuous infusion of [1-13C]leucine (7.35 µmol/kg FFM bolus, 9.38 µmol/kg FFM/h infusion) after a bolus of NaH13CO3 (2.9 µmol/kg FFM) to prime the bicarbonate pool for measurement of leucine oxidation rate. Plasma samples were taken at baseline and every 30 min from 4–8 h of infusion for measurement of [1-13C]-ketoisocaproate (KIC) isotopic enrichment from an “arterialized” heated dorsal hand vein as previously described (15). Expired air samples were taken for measurement of 13CO2 every 30 min from 4–8 h. Samples for other hormones, amino acid, lipid, lipoprotein, and metabolic substrate concentrations were also taken hourly from 4–8 h. Muscle biopsies were taken percutaneously from the vastus lateralis muscle after 4 h (0600 h) and 8 h (1000 h) of isotope infusion (i.e. first biopsy taken after 12 h of fasting) and immediately frozen in liquid nitrogen for later analysis.

Analytical methods
Amino acids. Amino acid concentrations were determined in plasma by fluorescence detection after precolumn derivatization with o-phthalaldehyde and reverse phase C18, high performance liquid chromatography (HPLC) as previously described (Hewlett-Packard 1090 series 2, 1046 fluorescence detector, Palo Alto, CA) (16).

Substrates and hormones. Concentrations of β-hydroxybutyrate, glycerol, pyruvate, and FFA were determined as previously described (17). Free testosterone concentrations were measured by initially evaluating the total concentration with RIA following extraction chromatography and subsequently determining the free fraction using equilibrium dialysis (Nichols Institute, San Juan Capistrano, CA). Glucagon, insulin, insulin-like growth factor I (IGF-I), and cortisol concentrations were analyzed by RIA. Insulin and cortisol were measured in serum. Glucagon and IGF-I were measured in ethylenediaminetetraacetate plasma. Total and free IGF-I were analyzed using a immunoradiometric assay kit from Diagnostic Systems Laboratory (Webster, TX). These procedures employ immunoradiometric principles first described by Miles et al. (18), including a simple extraction step in which IGF-I is separated from its binding proteins in serum (19). Triglyceride, cholesterol, and high density lipoprotein C (HDLc) were measured using a Roche Reagent kit (Roche Diagnostic Systems, Branchburg, NJ), and low density lipoprotein C (LDLc) was calculated from total cholesterol, HDL and, very low density lipoprotein fractions.

Isotope enrichments. Plasma: Enrichment of [13C]KIC was determined by gas chromatography-mass spectrometry with selected ion monitoring and electron impact ionization of a t-butyldimethylsilyl derivative as previously described (20), except that the sample was incubated with o-phenylenediamine to prevent enolization of KIC by creation of a quinoxalinol ring. KIC enrichment was monitored at m/z 260: 259.

Expired air: The enrichment of 13CO2 in expired air was measured by isotope ratio mass spectrometry (IRMS; VG Isogas series II, UK, 12, 14).
The total CO₂ production rate was measured using indirect calorimetry with an infrared CO₂ analyzer (Deltatrac, Sensormedics, Anaheim, CA).

**Mixed skeletal muscle protein** [1-¹³C]Leucine incorporation into skeletal muscle protein was measured as previously described (21, 22), except that leucine was isolated by a HPLC technique (23). Briefly, approximately 50-mg muscle biopsy specimens were homogenized at the temperature of liquid nitrogen and placed in ice-cold buffer, and protein was precipitated using trichloroacetic acid. The protein precipitate was hydrolyzed in 6 N HCl at 110 °C for 24 h to constituent amino acids. The hydrolysate was poured over a 100- to 200-mesh cation exchange resin (50W-8X, Bio-Rad Laboratories, Richmond, CA) for partial purification of amino acids and eluted using 3 mol/L NH₄OH. Leucine was isolated without prior derivatization using a reverse phase C₁₈ column with HPLC as previously described (21). Isotopic enrichment of the leucine carboxyl carbon was measured by IRMS of CO₂ after liberation of CO₂ from leucine by ninhydrin reaction (22). Myosin heavy chain purification was conducted as previously described (24), using a preparative gel electrophoresis technique. The purity of isolated myosin heavy chain was confirmed by analyzing an aliquot on 4-20% SDS-polyacrylamide minigradient gels and silver staining of protein. Approximately 150 mg skeletal muscle were homogenized in ice-cold SDS pyrophosphate buffer and centrifuged. The supernatant containing protein was subjected to preparative continuous gel electrophoresis. Isolated myosin heavy chain was hydrolyzed in 6 N HCl, and the leucine was separated as described for mixed muscle proteins. The enrichment of labeled leucine in myosin heavy chain protein was determined by IRMS using the ninhydrin method (23).

### Calculations

Equation I is as follows:

\[ FSR = \frac{E_{\text{inf}} - E_{\text{EIC}}}{E_{\text{KIC}}} \times 100 \]  \hspace{1cm} (1)

Myosin and the mixed muscle protein fractional synthesis rate (FSR), representing the portion of the myosin heavy chain mass or mixed skeletal muscle protein mass within the body that is produced in a given period of time (expressed as percentage per h), was calculated as previously described (14), where \( E_{\text{inf}} \) represents leucine enrichment in muscle proteins (myosin and mixed muscle) at time \( t \) (4 h) and time \( t + 8 \) (8 h) through the infusion of 1-[¹³C]leucine. \( E_{\text{EIC}} \) represents the steady state isotopic enrichment of plasma ¹³C/KIC between 4–8 h. The ¹³C/KIC enrichment is used as a measure of intracellular leucine enrichment. ¹³C/KIC enrichment is close to the isotope enrichment of leucyl-transfer ribonucleic acid in skeletal muscle (25), which is the obligatory precursor of protein synthesis.

Endogenous whole body leucine flux (Q) representing protein degradation rate was calculated as previously described (26):

\[ Q = \frac{E_{i}}{F_{\text{KIC}}} - 1 \]  \hspace{1cm} (2)

where \( i \) is the isotope infusion rate, \( E_{i} \) is the isotope enrichment of infusionate, and \( E_{\text{KIC}} \) is the ¹³C/KIC enrichment at the plateau. The whole body leucine oxidation rate (C) is calculated using KIC enrichment (E_KIC) at the plateau as the precursor pool as previously described (17, 21, 22). Nonoxidative whole body leucine disposal, taken to represent the protein synthesis rate, is calculated as the difference between leucine flux and leucine oxidation, because besides irreversible loss as oxidation, the only other fate of leucine is its incorporation into protein.

Whole body muscle mass estimated from 48-h creatinine excretion, collected during a creatine-free diet, was calculated as previously described (13). Muscle mass was separately estimated from appendicular FFM measured using dual energy x-ray absorptiometry, assuming that appendicular muscle mass represents 75% of whole body muscle mass (14).

The total body muscle protein synthesis rate, representing the mass of muscle protein produced in a given period of time (expressed as grams per h), was estimated (22) by extrapolating fractional synthesis rate data from vastus lateral is muscle to total body muscle protein by multiplying the FSR by the calculated muscle protein mass. We assumed that the protein content in muscle is 17%, as estimated in the present samples and similar to that discussed previously (21, 22). For comparison with the muscle protein synthesis rate, the whole body protein synthesis rate was estimated by dividing the nonoxidative whole body leucine disposal rate (expressed as micromoles per h) by 0.08 on the assumption that leucine accounts for approximately 8% of body protein.

### Statistical analysis

Paired data were analyzed by paired t test. Data in which sampling was performed over multiple time points in a steady state were analyzed by calculating the mean of the values over the steady state and comparing treatment phases within subjects by paired t test. Steady state was confirmed by subjecting data from the sampling period to a linear model ANOVA, analyzing the effect of time of sampling. Values are presented as the mean ± sem unless otherwise stated.

### Results

The following paragraphs describe measurements made at baseline and after testosterone replacement therapy.

#### Body composition

Body weight increased in all subjects from a mean of 89.19 ± 8.51 to 95.41 ± 8.69 kg (P = 0.04) with testosterone replacement. FFM increased in all subjects from a mean of 58.15 ± 3.05 kg pretestosterone to 66.86 ± 2.87 kg after 6 months of replacement therapy (mean increase, 15%; range, 10–22%; P = 0.02; Fig. 2). This change was accompanied by a decrease in fat mass from a mean of 31.04 ± 6.75 kg pretestosterone to 27.65 ± 6.75 kg with 6 months of testosterone replacement (mean decrease, 11%; range, 4–22%; P = 0.03; Fig. 2). The increase in FFM was in part related to an increase in muscle mass. Calculated using the creatinine excretion rate as described above, the mean increase in muscle mass was 20% (range, 11–32%; P = 0.04; Fig. 3), from 27.94 ± 5.40 to 33.25 ± 6.03 kg. Using an alternative calculation based on dual energy x-ray absorptiometry described above, an extrapolation from the appendicular FFM, the mean increase in muscle mass was 13% (range, 7–18%; P < 0.01; Fig. 3) from 36.68 ± 1.88 to 41.27 ± 1.92 kg.

#### Synthesis rate of mixed skeletal muscle protein and myosin heavy chain

The fractional synthesis rate of mixed skeletal muscle proteins increased during testosterone treatment (P = 0.015). The mean increase was 56% from 0.042 ± 0.004%/h before testosterone replacement to 0.065 ± 0.008%/h after 6 months of treatment. Parallel trends were noted for changes in the fractional synthesis rate of skeletal muscle myosin heavy chain, in that all but one subject experienced increased fractional myosin synthesis. The mean increase was 46% (0.033 ± 0.001% to 0.047 ± 0.007%/h), but due to slightly greater variability of the myosin synthesis measurement, this was not statistically different from baseline rates (P = 0.098). In all cases, the myosin fractional synthesis rate was lower than that for mixed skeletal muscle proteins (baseline, 0.033 ± 0.0006 vs. 0.042 ± 0.004%/h (P = 0.05); after testosterone therapy, 0.047 ± 0.007 vs. 0.65 ± 0.008%/h (P = 0.03)).

Estimates of total muscle protein synthesis rates were calculated from the fractional muscle protein synthesis rate and muscle mass. Using calculated muscle masses based on either creatinine excretion or appendicular FFM, increases in skeletal muscle protein synthesis rates were noted in all subjects. The synthesis rate based on creatinine excretion...
Fig. 2. Changes in body composition during testosterone replacement in hypogonadal men. Measurements performed on individual patients are presented before testosterone replacement (pre-T) and after 6 months of replacement (post-T). A and B are FFM and fat mass, respectively, determined by dual energy x-ray absorptiometry. *P < 0.05; †P < 0.01 (vs. pre-T).

Increased 87% (from 2.14 ± 0.55 to 4.00 ± 1.05 g/h; P = 0.03; Fig. 3) during testosterone replacement therapy. The total muscle protein synthesis rate, estimated using appendicular FFM, increased 71% (from 2.69 ± 0.36 to 4.61 ± 0.68 g/h; P = 0.01; Fig. 3). The percentage of calculated whole body protein synthesis rate attributable to muscle increased from 21 ± 5% to 31 ± 7% during testosterone replacement (creatinine excretion used to estimate muscle mass; P = 0.04; Fig. 4).

Whole body leucine kinetics and amino acids (Table 1)

Endogenous leucine flux increased 21% with testosterone (P = 0.04), but when examined per unit FFM, remained unchanged from baseline with testosterone replacement. No changes were noted in leucine oxidation rate whether examined as a total measurement or per unit FFM. Nonoxidative leucine disposal (taken to reflect whole body protein synthesis) increased 28% (P = 0.04) with testosterone replacement, but was unchanged when expressed per unit FFM.

Plasma concentrations of amino acids were largely unchanged during testosterone replacement (14 amino acids measured; data not given), with the exception of threonine, which increased by 19%. Branched chain amino acid concentrations tended to decrease slightly during testosterone replacement (leucine, −6%; valine, −3%; isoleucine, −2%), as did the transamination product of leucine, KIC (−4%; Table 1). The decrease in leucine concentration even when leucine flux tends to increase and leucine oxidation tends to decrease supports an increased protein synthesis.

Substrate and lipid concentrations (Table 2)

No changes were noted in the concentrations of other metabolic substrates during testosterone replacement. The unaffected substrates include nonesterified fatty acids, glycerol, and β-hydroxybutyrate. Likewise, no significant changes were noted in lipid concentrations. Minor decreases in HDL-C were not statistically significant (P = 0.07).

Hormone concentrations (Table 2)

Concentrations of hormones other than testosterone during the posttreatment studies were not different from baseline. Other hormone concentrations measured include those of insulin, glucagon, and IGF-I.

Physical activity

Habitual energy expenditure estimated from bimonthly 3-day activity diaries did not change during testosterone replacement. Similarly, there was no increase in the number of reported episodes of resistance exercise performed by the subjects during treatment. The one exception was a single subject who reported moving furniture during 1 day after 4 months of treatment. The same subject reported lifting dumbbells (10 lb) for 1 week only during the fourth month of testosterone treatment, but abandoned formal resistance exercise of his own accord for the remaining 2 months of the study. Mean physical activity (estimated energy expended expressed as kilocalories per kg/15 min) at baseline was 0.46 ± 0.05. After 2 months of testosterone replacement, mean physical activity was 0.50 ± 0.08; after 4 months, it was 0.48 ± 0.07; and after 6 months of therapy, it was 0.48 ± 0.07.

Discussion

The present study demonstrates that testosterone replacement therapy for 6 months in hypogonadal men produced clinically significant increases in FFM and decreases in fat mass. The increased FFM is in large part related to an increase in the mass of skeletal muscle. An increase in the rate of leucine incorporation into skeletal muscle in the current study indicates that the mechanism underlying the increase in muscle mass is due to an increase in the synthesis of muscle.
proteins. Although a decline in muscle protein breakdown is not excluded as a cause, leucine flux reflecting whole body protein breakdown did not decrease. Furthermore, a trend toward an increased synthesis rate of myosin heavy chain, a novel measurement of the present study, implies that included in the muscle proteins displaying increased synthesis are those responsible for the contractile function of muscle. This latter trend may be of importance given the sexually dimorphic responses of myosin synthesis in different muscles of many animal models of testosterone action and the lack of basic information regarding the actions of androgens and androgen receptors in human muscle (8, 27, 28). The concordance of findings suggesting an increase in muscle mass in the present study, including similar changes in muscle mass estimates by dual energy x-ray absorptiometry, creatinine excretion, and muscle protein amino acid isotope incorporation helps to minimize the importance of the limitations of any one of the measurement techniques. This is meaningful given the potential for testosterone administration to promote retention of body fluid that may falsely elevate estimates of lean mass measured by dual energy x-ray absorptiometry alone.

The testosterone replacement regimen employed in the present study produced testosterone concentrations nearly identical, at both the first week peak and the second week nadir, to those previously reported to represent physiological testosterone replacement in hypogonadal males (29). These testosterone concentrations contrast with those of other studies reporting an effect of testosterone to increase muscle protein synthesis, in that these latter studies involved...
administration of testosterone to normal eugonadal males in doses that produced supraphysiological concentrations 1 week after injection that were 41–51% higher than those in the current study in hypogonadal men and clearly above the normal male range (10). The fact that the present study achieved physiological testosterone concentrations demonstrates the importance of testosterone for maintenance of muscle protein synthesis in the normal male range. Likewise, the fact that subjects in the current study did not increase habitual physical activity or formal resistance training indicates that the effect of testosterone to augment muscle protein synthesis is unrelated to the encouragement of physical exercise.

Subjects in the present study were adults between the ages of 33–57 yr. It is not clear that the responses seen in the present subjects can be extrapolated across all ages to the very young and the very old. Although muscle accretion was not specifically examined, Mauras and co-workers (30) showed that prepubertal boys experience anabolism in response to increases in testosterone concentration from the prepubertal to the adult male range characterized by calcium accumulation and increased whole body protein synthesis. The increase in whole body protein synthesis in the boys was more dramatic than that in the adults participating in the present study and was associated with parallel differences in the responses of other anabolic factors such as IGF-I, factors probably diminished in our adult subjects, four of whom lacked pituitary function. It is possible that more complete hormone replacement therapy in the hypopituitary subjects, including GH as well as testosterone, may have augmented the increase in muscle mass noted over the 6 months of therapy in our subjects. GH administration to GH-deficient adults is known to increase muscle size (31), probably related to the effect of GH to specifically stimulate muscle protein synthesis (32). However, the increased muscle mass and muscle protein synthesis of pituitary hormone-deficient and nondeficient subjects alike in the present study suggests a role of testosterone to increase muscle mass that is distinct from that of GH.

The comparison between the boys and adult men suggests that the effect of testosterone to augment protein synthesis may be amplified in the young. It is not clear whether an amplified response of whole body protein synthesis to testosterone in the young is attributable to increases in muscle protein synthesis or that of other tissues. Our subjects experienced an increase in muscle protein synthesis relative to whole body protein synthesis, such that the contribution of the muscle protein synthesis rate to that of the whole body rate increased from 21% to 31%, the latter approximating the contribution in normal adult males (22). By contrast, testosterone supplementation in older patients designed to achieve testosterone concentrations in the normal middle-age adult male range, as reported by Tenover (mean age, 67 yr), produces increases in FFM that are 5-fold less than those re-

TABLE 2. Substrate, lipid, and hormone concentrations in plasma

<table>
<thead>
<tr>
<th></th>
<th>Pretestosterone therapy</th>
<th>Posttestosterone therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol</td>
<td>0.10 ± 0.01</td>
<td>0.08 ± 0.02</td>
</tr>
<tr>
<td>Nonesterified fatty acids</td>
<td>0.48 ± 0.06</td>
<td>0.52 ± 0.05</td>
</tr>
<tr>
<td>β-Hydroxybutyrate</td>
<td>482 ± 69</td>
<td>624 ± 146</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>178 ± 26</td>
<td>179 ± 31</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>165 ± 60</td>
<td>191 ± 54</td>
</tr>
<tr>
<td>HDL</td>
<td>27 ± 1</td>
<td>22 ± 2</td>
</tr>
<tr>
<td>LDL</td>
<td>114 ± 19</td>
<td>119 ± 22</td>
</tr>
<tr>
<td>Free testosterone</td>
<td>41.6 ± 27.7</td>
<td>1081.7 ± 97.1</td>
</tr>
<tr>
<td>Total testosterone</td>
<td>3.7 ± 1.9</td>
<td>44.3 ± 4.0</td>
</tr>
<tr>
<td>Insulin</td>
<td>66 ± 12</td>
<td>84 ± 18</td>
</tr>
<tr>
<td>Glucagon</td>
<td>113 ± 7</td>
<td>124 ± 9</td>
</tr>
<tr>
<td>Total IGF-I</td>
<td>85 ± 16</td>
<td>114 ± 12</td>
</tr>
<tr>
<td>Free IGF-I</td>
<td>1.7 ± 0.3</td>
<td>2.3 ± 0.4</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM. Glycerol and nonesterified fatty acids are expressed as millimoles per L; β-Hydroxybutyrate is expressed as micromoles per L. Lipid concentrations are expressed as milligrams per dl. Free testosterone values are presented as picomoles per L (conversion factor to pg/mL = 28.84). Total testosterone values are presented as nanomoles per L (conversion factor to ng/dL = 28.84). Insulin is a serum concentration expressed as picomoles per L (conversion factor to μU/mL = 0.1867). Glucagon is expressed as nanograms per L. IGF-I is expressed as nanograms per mL. Therapeutic values were drawn 1 week after previous testosterone injection after 6 months of therapy. The nadir testosterone (total) value at the end of 6 months of therapy was 15.3 ± 4.1 nmol/L. Values pre- and posttherapy are not significantly different, with the exception of testosterone and free testosterone, which increased by virtue of the study design.

Values are the mean ± SEM. 

* P < 0.05.

**P < 0.01.
ported in the present study, a finding that may have stemmed from a shorter duration of treatment (3 months) or from a diminished response to testosterone of lean tissues in older men (33).

The effect of testosterone to increase muscle mass in the hypogonadal patients of the present study may represent a favorable clinical effect of testosterone that should be examined in the context of other known metabolic effects of the hormone. Testosterone is known to decrease the HDL-C concentration, a phenomenon believed to contribute to the decreased longevity of males. (34) This concern is tempered by studies finding no effect of testosterone to decrease HDL-C when concentrations are physiological rather than pharmacological (35). Concentrations of HDL-C in the present study did not decrease significantly, but a trend toward decreased levels, given the small sample size, does not allow one to rule out a possible effect of testosterone replacement to adversely affect lipids in hypogonadal men. Analogous to this, hyperinsulinemia, suggesting the development of insulin resistance, has been noted in males taking supraphysiologic doses of androgen, but improved insulin sensitivity has been reported with testosterone supplementation in the physiological range (4). Our subjects did not experience changes in the insulin concentration. However, a nonsignificant trend toward increased insulin concentration during testosterone replacement, 1 week after testosterone cypionate injection, suggests that those who replace testosterone clinically in hypogonadal men may need to be watchful for periods of transient insulin resistance during therapy.

We conclude that testosterone replacement to the normal male range is important for the maintenance of muscle protein synthesis rate and muscle mass in adult hypogonadal men. Muscle protein synthesis promoted by testosterone may include that of both contractile proteins such as myosin heavy chain as well as noncontractile proteins. The effects of testosterone do not appear to be mediated by an influence of testosterone on physical activity. Testosterone replacement can be prescribed for hypogonadal men as a therapy to promote anabolism of skeletal muscle and possibly other lean tissues as well as to decrease fat mass and enhance sexual function and secondary sex characteristics.

Acknowledgments

We gratefully acknowledge the expert technical assistance of G. C. Ford, D. B. Eberstein, G. Bress, V. Houck, and P. Q. Baumann. We appreciate the valuable assistance of the nursing staff of the University of Vermont General Clinical Research Center in providing clinical care for our volunteers and in expertly procuring biological samples.

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