

Testosterone Treatment in Adolescents with Delayed Puberty: Changes in Body Composition, Protein, Fat, and Glucose Metabolism*

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ABSTRACT

Previously, we demonstrated decreased protein breakdown and insulin resistance in pubertal adolescents compared with prepubertal children. Puberty-related increases in sex steroids and/or GH could be potentially responsible. In the present study, the effects of 4 months of testosterone enanthate (50 mg im every 2 weeks) on body composition, protein, fat, and glucose metabolism and insulin sensitivity were evaluated in adolescents with delayed puberty. Body composition was assessed by $H_2^{18}O$ -dilution principle. Protein breakdown, oxidation, and synthesis were measured during primed constant infusion of $[1-^{13}C]$ leucine. Whole-body lipolysis was measured during primed constant infusion of $[^2H_5]$ glycerol. Insulin action in suppressing proteolysis and lipolysis and stimulating glucose disposal was assessed during a stepwise hyperinsulinemic (10 and 40 $mU \cdot m^{-2} \cdot min$) euglycemic clamp. Fat and glucose oxidation rates were calculated from indirect calorimetry measurements.

After 4 months of testosterone treatment, height, weight, and fat free mass (FFM) increased and fat mass, percent body fat, plasma cholesterol, high- and low-density lipoproteins, and leptin levels decreased significantly. Whole-body proteolysis and protein oxidation were lower after testosterone treatment (proteolysis, 0.49 ± 0.03 vs.

0.54 ± 0.04 $g \cdot h \cdot kg$ FFM, $P = 0.032$; oxidation, 0.05 ± 0.01 vs. 0.09 ± 0.01 $g \cdot h \cdot kg$ FFM, $P = 0.015$). Protein synthesis was not different, and resting energy expenditure was not different. Total body lipolysis was not affected by testosterone treatment, however, fat oxidation was higher after testosterone (pre: 2.4 ± 0.7 vs. post: 3.5 ± 0.7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P = 0.031$). During the 40 $mU \cdot m^{-2} \cdot min$ hyperinsulinemia, insulin sensitivity of glucose metabolism was not affected with testosterone therapy (59.1 ± 8.8 vs. 57.1 ± 8.2 $\mu mol \cdot kg^{-1} \cdot min$ per $\mu U/mL$). However, metabolic clearance rate of insulin was higher posttestosterone (13.6 ± 1.1 vs. 16.7 ± 0.8 $mL \cdot kg^{-1} \cdot min$, $P = 0.004$).

In conclusion, after 4 months of low-dose testosterone treatment in adolescents with delayed puberty 1) FFM increases and fat mass and leptin levels decrease; 2) postabsorptive proteolysis and protein oxidation decrease; 3) fat oxidation increases; and 4) insulin sensitivity in glucose metabolism does not change, whereas insulin clearance increases. These longitudinal observations are in agreement with our previous cross-sectional studies of puberty and demonstrate sparing of protein breakdown of approximately 1.2 $g \cdot kg^{-1} \cdot day$ FFM, wasting of fat mass, but no change in insulin sensitivity after short periods of low-dose testosterone supplementation. (*J Clin Endocrinol Metab* 82: 3213–3220, 1997)

CROSS-SECTIONAL studies have demonstrated that during puberty, insulin action is diminished and is manifested in lower rates of insulin-stimulated glucose metabolism in pubertal compared with prepubertal or adult subjects (1–3). Moreover, during puberty there is a marked acceleration of growth with increasing body size, muscle mass, and changes in body composition (4). We recently demonstrated that whole-body proteolysis and protein oxidation were lower in pubertal adolescents compared with prepubertal children, whereas protein synthesis was comparable (5). The mechanism(s) and cause(s) responsible for the observed metabolic changes during puberty remain unclear.

There is a multitude of hormonal changes that occur during puberty, including increased sex steroids and increased GH/insulin-like growth factor-I (IGF-I) secretion (6). Whether or not pubertal changes in growth and mass accretion are the result of sex steroids vs. GH vs. a combination of

both remains unresolved. The important role of GH in pubertal growth spurt has been challenged by the demonstration that acceleration of height velocity into peak pubertal range by dihydrotestosterone was achieved without an increase in plasma GH (7). Also, the anabolic response to testosterone in the rat does not require the presence of the pituitary gland (8). Moreover, testosterone supplementation in several human models, including aging men (9), healthy young men (10), men with muscular dystrophy (11), hypogonadal men (12–14), and prepubertal boys (15) has been shown to have significant anabolic effect with increases in fat free mass (FFM). Regarding insulin resistance during puberty, both GH and testosterone are likely candidates. However, the transient nature of pubertal insulin resistance is out of tempo with the increasing sex steroids, which remain elevated in young adults while insulin resistance subsides.

The aim of the present investigation was to assess longitudinally the effects of low-dose testosterone supplementation in adolescents with delayed puberty on body composition, protein, fat, and glucose metabolism and insulin sensitivity.

Materials and Methods

Subjects

Seven healthy male subjects, age range 14.9–16.5 yr, with the diagnosis of constitutional delay in puberty, participated in this research. The

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studies were approved by the Human Rights Committee of Children's Hospital of Pittsburgh. Parents and participants gave written informed consent/assent after a thorough explanation of the proposed studies. All subjects were healthy as assessed by medical history, physical examination, and routine hematological and biochemical tests. Pubertal development was assessed by Tanner staging (4) and confirmed by measurement of plasma testosterone. All subjects were prepubertal in Tanner stage I (Table 1).

Experimental design

Each subject was studied twice, before and after 4 months of testosterone enanthate treatment of 50 mg im every 2 weeks. This dose was chosen for two reasons. First, to achieve mean serum testosterone levels commensurate with Tanner stage III puberty, coincident with peak height velocity in males (16, 17). Second, our clinical experience in patients with delayed puberty indicates that this dose regimen uniformly advances secondary sex characteristics and growth rate.

All participants were admitted to the General Clinical Research Center at Children's Hospital of Pittsburgh on the previous afternoon for testing the following morning. Experiments were performed after 10–12 h of overnight fasting. The posttreatment study was performed the week after the last injection of testosterone. Subjects were prescribed a weight-maintaining diet containing 55% carbohydrate, 30% fat, and 15% protein for a week before and during the hospital stay. For each study, two intravenous catheters were inserted, one in a forearm vein for administration of test infusions and the second on the dorsum of the contralateral heated hand for sampling of arterialized venous blood. Pre- and posttestosterone evaluations were identical.

Leucine turnover studies

Leucine turnover studies were performed by the prime constant infusion of [$1\text{-}^{13}\text{C}$]leucine at baseline and during a hyperinsulinemic-euglycemic clamp as described by us previously (5). In the basal post-absorptive state a primed ($5\ \mu\text{mol}/\text{kg}$) constant-rate infusion ($6\ \mu\text{mol}\cdot\text{kg}\cdot\text{h}$) of [$1\text{-}^{13}\text{C}$]leucine (99 atom percent excess ^{13}C) was started 3 h before the clamp (Tracer Technologies, Somerville, MA). The pyrogen-free isotope was dissolved in 0.9% sodium chloride and sterilized by passing through a Millipore filter (Bedford, MA) $0.22\ \mu\text{m}$ in size. The bicarbonate pool was primed by a $1.5\text{-}\mu\text{mol}/\text{kg}$ bolus of $\text{NaH}^{13}\text{CO}_3$ at the beginning of each study. Before the start of the isotope infusion and 60 min after, arterialized blood samples were obtained every 30 min for determination of [$1\text{-}^{13}\text{C}$]ketoisocaproate (KIC). Breath samples were collected at -180 , -30 , 0 , and $+5$ min using a Hans Rudolph one-way nonbreathing valve connected to a 2-liter anesthesia bag. An aliquot of each breath sample was trapped in an evacuated glass tube for the subsequent analysis of ^{13}C enrichment of expired CO_2 (5).

Total-body lipolysis

Lipolysis was measured at baseline after overnight fasting and during a hyperinsulinemic-euglycemic clamp by the use of a primed ($1.2\ \mu\text{mol}/\text{kg}$) constant rate ($0.08\ \mu\text{mol}/\text{kg}\cdot\text{min}^{-1}$) infusion of [$^2\text{H}_5$]glycerol (Isotec, Miamisburg, OH), which was started 3 h before the clamp (3).

TABLE 1. Body composition and hormonal profile before and after testosterone treatment

	Pretreatment	Posttreatment	P
Weight (kg)	47.9 \pm 4.6	52.7 \pm 4.2	.0003
Height (cm)	149.3 \pm 2.5	154.1 \pm 2.5	.0002
FFM (kg)	37.5 \pm 1.9	45.5 \pm 2.2	.002
Fat mass (kg)	10.5 \pm 2.9	7.2 \pm 2.9	.011
% Body fat	19.9 \pm 3.9	12.1 \pm 4.2	.008
Leptin (ng/mL)	20.5 \pm 7.4	9.6 \pm 3.6	.045
Testosterone (ng/dL)	23 \pm 4	422 \pm 45	.0001
IGF-I (ng/mL)	210 \pm 28	505 \pm 40	.00004
Mean nocturnal GH (ng/mL) ^a	2.5 \pm 0.5	6.0 \pm 0.8	.0004

^a Mean nocturnal GH is expressed as mean concentration of 19 blood samples drawn every 20 min from 0000–0600 h.

Insulin-stimulated glucose metabolism

After the baseline isotopic infusion period, *in vivo* glucose metabolism and insulin action was evaluated by the hyperinsulinemic-euglycemic clamp (3). Intravenous crystalline insulin (Humulin; Lilly, Indianapolis, IN) was infused at a constant rate of $10\ \text{mU}$ and $40\ \text{mU}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$, each for 2 h. The lower insulin infusion rate was chosen to study suppression of proteolysis and lipolysis. The higher insulin infusion rate was chosen to produce high physiological insulin concentrations to study insulin-stimulated glucose disposal (R_d), oxidation, and nonoxidative R_d . Plasma glucose was clamped at $100\ \text{mg}/\text{dL}$ with a variable rate infusion of 20% dextrose. The rate of glucose infusion was adjusted based on arterialized plasma glucose measurement every 5 min. Blood was sampled every 10–15 min for determination of plasma insulin, leucine, glycerol, free fatty acids (FFA), and plasma isotopic enrichment of KIC and glycerol.

Indirect calorimetry

Continuous indirect calorimetry by a ventilated hood system (Deltatrac Metabolic monitor, SensorMedics, Anaheim, CA) was used to measure CO_2 production, O_2 consumption, and respiratory quotient (18). Measurements were made for 30 min at baseline before insulin infusion and at the end of each 2-h study period.

Body composition was assessed from the determination of total body water using H_2^{18}O -dilution principle reported by us previously (19). Overnight blood was sampled every 20 min from 0000 h until 0600 h for measurement of GH levels. Fasting blood was obtained for determination of plasma leptin, testosterone, and IGF-I concentrations.

Analytical methods

Plasma glucose was measured at the bedside by the glucose oxidase method using a YSI glucose analyzer (Yellow Springs Instrument, Yellow Springs, OH). For the other biochemical determinations, pre- and posttreatment samples were analyzed simultaneously in the same assay. Blood samples were immediately processed after collection. Serum and plasma were separated by centrifugation at $4\ \text{C}$ and frozen at $-80\ \text{C}$ until further measurement. Plasma insulin was analyzed by RIA (20), testosterone by double-antibody RIA (ICN Biomedical, Costa Mesa, CA), GH by double-antibody RIA (Diagnostic Products Corp., Los Angeles, CA). IGF-I was measured by RIA after acid-ethanol extraction (Nichols Institute Diagnostics, San Juan Capistrano, CA). Cholesterol, high-density lipoprotein (HDL), and triglyceride measurements were performed using U.S. Centers for Disease Control protocols as reported before (21). low-density lipoprotein (LDL) cholesterol was computed using cholesterol, HDL cholesterol, and triglyceride levels. Serum leptin was measured by double-antibody RIA using ^{125}I -labeled human leptin as tracer and rabbit antihuman leptin antibodies as previously described (22) (Linco, St. Louis, MO). Urinary nitrogen was measured by the Kjeldahl method (23). Amino acid analysis was performed by reverse-phase high-performance liquid chromatography (Millipore, Waters Chromatography Division, Milford, MA) using a phenyl isothiocyanate derivative (5). The ^{13}C enrichment of the expired CO_2 was measured after separation of the CO_2 by cryogenic distillation (5). The isotopic enrichment of plasma KIC was measured with electron impact ionization and selective ion monitoring of the bis (trimethylsilyl)trifluoroacetamide derivative as described previously (24). Plasma KIC enrichment was measured on Hewlett-Packard model 5890 series II gas chromatograph-5971 mass spectrometer (Hewlett-Packard, Palo Alto, CA) with a selective ion monitoring software package. The ions monitored were mass to charge ratio (m/z) 232 for (M) and 233 for ($M + 1$). Deuterium enrichment of glycerol in the plasma was determined with a slight modification of a previously described method (25). Plasma samples were deproteinized with methanol. The supernatant was dried in a vacuum centrifuge. Pentafluoropropyl derivatives of glycerol were prepared by adding pentafluoropropionic anhydride and ethyl acetate to the dried samples. Derivatized samples were analyzed for ^2H enrichment by gas chromatography-mass spectrometry. Selected ion monitoring software was used to monitor charge-to-mass ratio (m/z) 367 for (M) and 372 for ($M + 5$), representing unlabeled and $^2\text{H}_5$ -labeled glycerol, respectively. Standard curves of known enrichments of glycerol and KIC were performed with each assay.

Calculations

Proteolysis. Leucine kinetics were calculated with the reciprocal pool model (26) during the following periods: -30-0 min, the basal post-absorptive period; 90-120 min of the 10 mU·m⁻²·min insulin step; and 210-240 min of the 40 mU·m⁻²·min insulin step of the two-step sequential clamp. During each of these periods four consecutive samples, 10 min apart, demonstrated the presence of a steady state plateau in plasma isotopic enrichment of KIC, with coefficients of variation of <5%. At baseline, whole-body leucine turnover and oxidation were calculated using [¹³C]KIC mole percent excess according to steady state tracer kinetic equations (5). Nonoxidative leucine disposal was the difference between whole-body leucine flux and leucine oxidation and represented a parameter of protein synthesis. Leucine turnover was extrapolated to whole-body protein turnover with the assumption that 1 g of protein contains 590 μmol leucine (27). During the hyperinsulinemic clamp, leucine oxidation could not be calculated from ¹³C-enrichment of expired CO₂ because of the contribution of naturally occurring ¹³C in the exogenously infused glucose. Therefore, only proteolysis was assessed during hyperinsulinemia.

Total-body lipolysis. The rate of appearance (R_a) of endogenous glycerol in plasma was calculated during the last 30 min of the postabsorptive (basal) period and of each hyperinsulinemic step according to steady state tracer dilution equations (3, 28). A steady state plateau of glycerol isotopic enrichment was achieved in the subjects before the start of the clamp experiment and during the last 30 min of each hyperinsulinemic step. Glycerol R_a was expressed in micromoles per minute per kilogram body weight (μmol·kg⁻¹·min). Plasma FFA R_a was calculated by multiplying glycerol R_a by three, because when a triglyceride molecule is hydrolyzed, one glycerol molecule and three fatty-acid molecules are produced.

Insulin-stimulated glucose R_d. This was calculated during the last 30 min of the 40 mU·m⁻²·min⁻¹ hyperinsulinemic-euglycemic clamp step. Under steady state conditions of euglycemia, the rate of exogenous glucose infusion is equal to the rate of insulin-stimulated glucose disposal. Insulin at this dose level inhibits hepatic glucose production in prepubertal and pubertal subjects (29). Glucose R_d was expressed in micromoles per minute per kilogram body weight (μmol·kg⁻¹·min).

Basal and insulin-stimulated carbohydrate oxidation rates and lipid oxidation were calculated according to the formulas of Frayn (30) from the indirect calorimetric data by averaging the data for 30 min before the beginning of the insulin infusion and for the last 30 min during insulin infusion. Nonoxidate glucose R_d was estimated by subtracting the rate of glucose oxidation from the total-body insulin-stimulated R_d during the last 30 min of the clamp.

Insulin sensitivity was calculated by dividing insulin-stimulated glucose R_d by steady state plasma insulin concentration during the 40 mU·m⁻²·min hyperinsulinemic step as described previously (31). Metabolic clearance rate of insulin was calculated by dividing insulin infusion rate by the δ increase in circulating insulin concentrations during the 40 mU·m⁻²·min hyperinsulinemic steady state as described by DeFronzo *et al.* (31).

Statistical analysis. Data are presented as mean ± SE. Paired *t* test was used to compare pre- and posttreatment data. To evaluate univariate relationships, least-squares regression analysis was applied. Multiple regression analysis was used to assess multivariate relationships. The goodness of fit of the model was measured by R-square (R²), the coefficient of determination, which is the square of multiple correlation coefficient between the dependent and independent variables (32). Statistical significance is implied by *P* < 0.05.

Results

Body composition

After 4 months of testosterone treatment, both height and weight increased significantly. FFM increased in all subjects. The mean increase in FFM was 7.6 ± 1.5 kg in absolute terms or 20.7 ± 4.2%. This change was accompanied by a decrease in total body fat, percent body fat, and plasma leptin levels

(Table 1). Serum testosterone, mean nocturnal GH, and IGF-I levels increased significantly (Table 1). Plasma leptin levels correlated positively with fat mass (*r* = 0.86, *P* < 0.001) and percent body fat (*r* = 0.79, *P* < 0.001) and inversely with mean nocturnal GH (*r* = -0.71, *P* = 0.002), but not testosterone levels.

Plasma lipid concentrations, insulin, and substrate levels

After 4 months of testosterone treatment, fasting plasma cholesterol, HDL, and LDL were lower, whereas triglycerides and very low-density lipoproteins were not different (Table 2). Testosterone treatment did not result in any change in basal circulating glucose, insulin, FFA, glycerol and leucine levels.

Protein metabolism

Basal period (Fig. 1). After 4 months of testosterone treatment, whole-body protein turnover (protein breakdown) and protein oxidation were lower (breakdown: 0.49 ± 0.03 *vs.* 0.54 ± 0.04 g·h·kg FFM, *P* = 0.032; oxidation: 0.05 ± 0.01 *vs.* 0.09 ± 0.01 g·h·kg FFM, *P* = 0.015). However, protein synthesis was not different (0.44 ± 0.03 *vs.* 0.45 ± 0.04 g·h·kg FFM). After testosterone therapy, the fraction of protein turnover that was oxidized decreased (before: 16.3 ± 3.0 *vs.* after: 10.2 ± 2.2%, *P* = 0.025) in favor of synthesis (before: 83.7 ± 3.0 *vs.* after: 89.8 ± 2.2%, *P* = 0.025). Resting energy expenditure per FFM did not change with testosterone treatment (37.5 ± 1.5 *vs.* 36.8 ± 1.6 kcal·24 h·kg FFM). Resting energy expenditure correlated positively with protein synthesis (*r* = 0.54, *P* = 0.02).

Hyperinsulinemic period (Fig. 2). During the 10 mU·m⁻²·min step of the hyperinsulinemic-euglycemic clamp, proteolysis was similar before and after testosterone treatment (0.48 ± 0.04 *vs.* 0.45 ± 0.03 g·h·kg FFM). However, absolute suppression in protein breakdown as well as percent suppression from baseline were lower after 4 months of testosterone (absolute: 0.06 ± 0.01 *vs.* 0.03 ± 0.01 gm·hr·kg FFM, *P* = 0.04; percent suppression: 11.3 ± 1.8 *vs.* 6.2 ± 2.2%, *P* = 0.059) (Fig. 2).

During the 40 mU·m⁻²·min insulin clamp, there was no difference in protein breakdown before (0.40 ± 0.03) and after testosterone (0.39 ± 0.03 g·h·kg FFM). Absolute suppression in protein breakdown as well as percent suppression from baseline were lower after 4 months of testosterone

TABLE 2. Fasting plasma lipid concentrations, insulin, and substrate levels before and after testosterone treatment

	Pretreatment	Posttreatment	<i>P</i>
Cholesterol (mg/dL)	167 ± 7	143 ± 7	0.007
Triglycerides (mg/dL)	145 ± 32	132 ± 31	NS
LDL (mg/dL)	108 ± 8	93 ± 7	0.06
HDL (mg/dL)	41 ± 5	33 ± 4	0.003
VLDL (mg/dL)	18 ± 2	18 ± 3	NS
Insulin (μU/mL)	14.3 ± 2.0	15.5 ± 2.3	NS
Glucose (mg/dL)	97 ± 2	99 ± 4	NS
FFA (mM)	0.35 ± 0.06	0.41 ± 0.08	NS
Glycerol (μM)	29 ± 3	29 ± 3	NS
Leucine (μM)	151 ± 17	157 ± 20	NS

NS, Not significant.

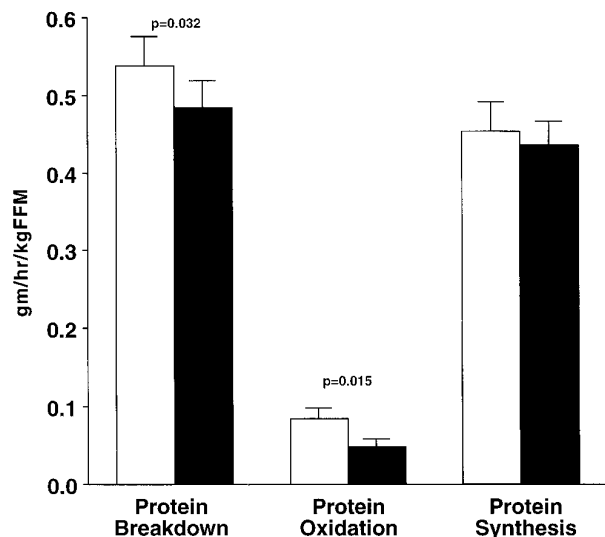


FIG. 1. Whole-body protein breakdown, oxidation, and synthesis before (open bars) and after (filled bars) 4 months of testosterone.

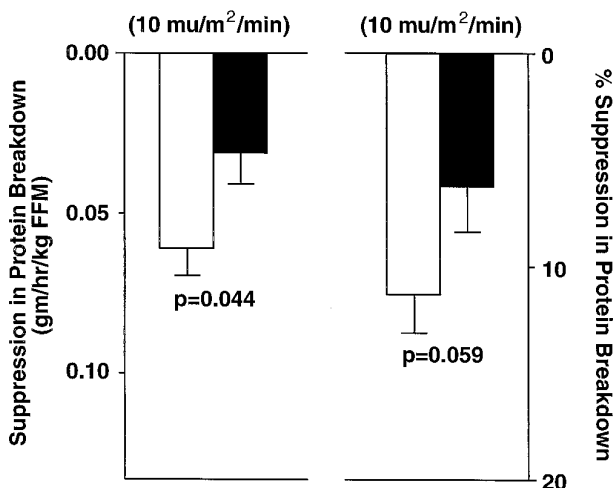


FIG. 2. Absolute (left) and percent suppression (right) in proteolysis during 10 mU·m⁻²·min hyperinsulinemic clamp before (open bars) and after (filled bars) testosterone.

(absolute: 0.14 ± 0.03 vs. 0.10 ± 0.03 g·h·kg FFM, $P = 0.002$; percent suppression: 25.2 ± 3.3 vs. $19.2 \pm 4.0\%$, $P = 0.003$). However, clamp steady state insulin levels were also lower (Fig. 3). After correcting for the lower insulin concentrations, differences in pre- and posttestosterone suppression of proteolysis during high-rate insulinemia disappeared.

Fat metabolism

Basal period. Pretestosterone glycerol R_a (lipolysis) (5.0 ± 0.6 $\mu\text{mol}\cdot\text{kg}\cdot\text{min}$) and FFA R_a (15.0 ± 1.9 $\mu\text{mol}\cdot\text{kg}\cdot\text{min}$) were similar to posttestosterone values (5.1 ± 1.2 $\mu\text{mol}\cdot\text{kg}\cdot\text{min}$ and 15.3 ± 3.6 $\mu\text{mol}\cdot\text{kg}\cdot\text{min}$, respectively). However, fat oxidation was significantly higher posttestosterone therapy (pre-: 2.4 ± 0.7 vs. post-: 3.5 ± 0.7 $\mu\text{mol}\cdot\text{kg}\cdot\text{min}$, $P = 0.031$).

Hyperinsulinemic period. During the low-rate insulin infusion (10 mU·m⁻²·min), FFA (0.14 ± 0.02 vs. 0.16 ± 0.03 mM) and glycerol (17 ± 2 vs. 17 ± 2 μM) were not different. Posttes-

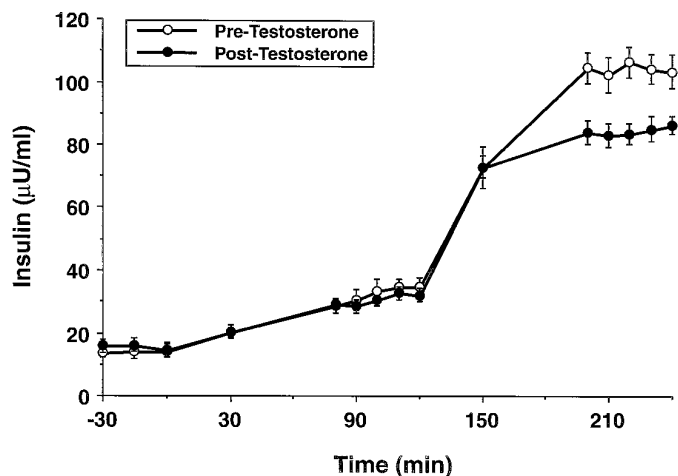


FIG. 3. Plasma insulin concentrations at baseline (-30-0 min) and during low-rate (90-120 min) and high-rate (210-240 min) hyperinsulinemic clamp before (○) and after (●) testosterone treatment.

tosterone therapy, glycerol R_a was lower (pre-: 1.9 ± 0.3 vs. 1.5 ± 0.2 $\mu\text{mol}\cdot\text{kg}\cdot\text{min}$, $P = 0.026$), whereas fat oxidation was higher (2.0 ± 0.3 vs. 3.0 ± 0.6 $\mu\text{mol}\cdot\text{kg}\cdot\text{min}$, $P = 0.037$). However, both absolute and percent suppression in lipolysis as well as in fat oxidation, were not significantly different before and after therapy. (Absolute suppression in lipolysis pre- vs. post-: 3.1 ± 0.4 vs. 3.5 ± 1.0 $\mu\text{mol}\cdot\text{kg}\cdot\text{min}$, percent suppression: 61 ± 2 vs. $67 \pm 2\%$; absolute suppression in fat oxidation: 0.9 ± 0.5 vs. 0.7 ± 0.1 , percent suppression: 36 ± 7 vs. $21 \pm 6\%$).

During the 40 mU·m⁻²·min insulin clamp step, pre- and posttestosterone FFA (0.08 ± 0.2 vs. 0.07 ± 0.02 mM) and glycerol (14 ± 1 vs. 14 ± 2 μM) levels were not different. Glycerol R_a was lower after testosterone (0.9 ± 0.1 vs. 0.7 ± 0.1 $\mu\text{mol}\cdot\text{kg}\cdot\text{min}$, $P = 0.01$), however, absolute and percent suppression in glycerol R_a were not significantly different before and after testosterone (absolute suppression: 4.1 ± 0.5 vs. 4.4 ± 0.2 $\mu\text{mol}\cdot\text{kg}\cdot\text{min}$; percent suppression: 81 ± 2 vs. $85 \pm 1\%$). Pre- and posttestosterone fat oxidation, absolute suppression, and percent suppression in fat oxidation were not significantly different (pre- vs. post- fat oxidation: 0.8 ± 0.4 vs. 1.5 ± 0.5 $\mu\text{mol}\cdot\text{kg}\cdot\text{min}$; absolute suppression: 2.3 ± 0.6 vs. 2.2 ± 0.5 $\mu\text{mol}\cdot\text{kg}\cdot\text{min}$ percent suppression: 88 ± 10 vs. $63 \pm 15\%$).

Glucose metabolism

Fasting glucose oxidation was not different pre- (14.4 ± 1.7 $\mu\text{mol}\cdot\text{kg}\cdot\text{min}$) and posttestosterone (14.7 ± 1.6 $\mu\text{mol}\cdot\text{kg}\cdot\text{min}$). The steady state insulin concentrations during the 10 mU·m⁻²·min hyperinsulinemia were similar pre- and posttestosterone (Fig. 3). The steady state insulin concentrations during the 40 mU·m⁻²·min hyperinsulinemia were significantly lower after 4 months of testosterone (Fig. 3). Metabolic clearance rate of insulin after testosterone was significantly higher (Table 3). During the high-rate insulin infusion clamp, posttestosterone insulin-stimulated glucose disposal was lower (60.5 ± 7.6 vs. 47.2 ± 6.1 $\mu\text{mol}\cdot\text{kg}\cdot\text{min}$, $P = 0.03$) and nonoxidative glucose disposal was lower (36.2 ± 5.8 vs. 24.4 ± 3.8 $\mu\text{mol}\cdot\text{kg}\cdot\text{min}$, $P = 0.031$), but glucose oxidation

TABLE 3. Metabolic clearance rate of insulin before and after testosterone

Clearance rate	Pretreatment	Posttreatment	<i>P</i>
mL/min	627 ± 23	869 ± 59	0.002
mL·min·m ²	449 ± 19	582 ± 25	0.002
mL·min·kg	13.6 ± 1.1	16.7 ± 0.8	0.004
mL·min·kg FFM	16.8 ± 0.7	19.1 ± 1.0	0.043

was not different (24.1 ± 2.3 vs. 23.1 ± 2.5 $\mu\text{mol}\cdot\text{kg}\cdot\text{min}$) (Fig. 4). When glucose disposal was corrected per unit of insulin, insulin sensitivity before and after testosterone therapy was unchanged (59.1 ± 8.8 vs. 57.1 ± 8.2 $\mu\text{mol}\cdot\text{kg}\cdot\text{min}$ per $\mu\text{U}\cdot\text{ml}$) (Fig. 4). Results were similar when data were expressed per kilogram FFM.

Correlations

Postabsorptive protein oxidation correlated with mean nocturnal GH ($r = -0.77$, $P = 0.001$) (Fig. 5) and with plasma testosterone concentrations ($r = -0.60$, $P = 0.012$). However, in a multiple regression analysis with protein oxidation as the dependent variable and mean nocturnal GH and testosterone as the independent variables, GH was the only significant predictor of protein oxidation ($P = 0.02$). The R-square for the reduced model (only including GH) was 0.59 ($P = 0.001$), and the R-square for the full model (GH + testosterone) was 0.60 ($P = 0.006$). Otherwise, there were no relationships between protein turnover parameters and either GH, testosterone, or IGF-I.

Mean nocturnal GH levels correlated positively with basal fat oxidation ($r = 0.66$, $P = 0.005$) (Fig. 5) and inversely with fat mass ($r = -0.67$, $P = 0.004$) and percent body fat ($r = -0.73$, $P = 0.001$) but not with rates of lipolysis. Testosterone levels did not correlate with fat oxidation or lipolysis.

Neither GH nor testosterone correlated with insulin-stimulated glucose disposal or insulin sensitivity. Metabolic clearance rate of insulin correlated with testosterone ($r = 0.72$, $P = 0.002$) and mean nocturnal GH ($r = 0.69$, $P = 0.003$). However, in a multiple regression analysis, testosterone was the only predictor explaining 52% of the variance in insulin clearance.

Discussion

The present study of adolescents with delayed puberty demonstrates that 4 months of low-dose testosterone therapy, which achieves plasma testosterone levels commensurate with Tanner III puberty, results in the following: 1) approximately an 8 kg or 21% increase in FFM; 2) a decrease in total fat mass, percent body fat, and leptin concentration; 3) on average a 10% decrease in whole-body proteolysis, a 25% decrease in protein oxidation, and a 6% rechanneling of protein breakdown from irreversible loss (oxidation) to protein synthesis; 4) reductions in plasma cholesterol and HDL and LDL levels; 5) an increase in fat oxidation; 6) an increased metabolic clearance rate of insulin; and 7) no change in insulin sensitivity of glucose metabolism.

Studies in the literature of normal (10, 11, 33, 34), hypogonadal (12, 35), aging (9), and ill men (11, 36) uniformly agree that testosterone treatment results in increased lean body mass. However, the controversy continues as to the mech-

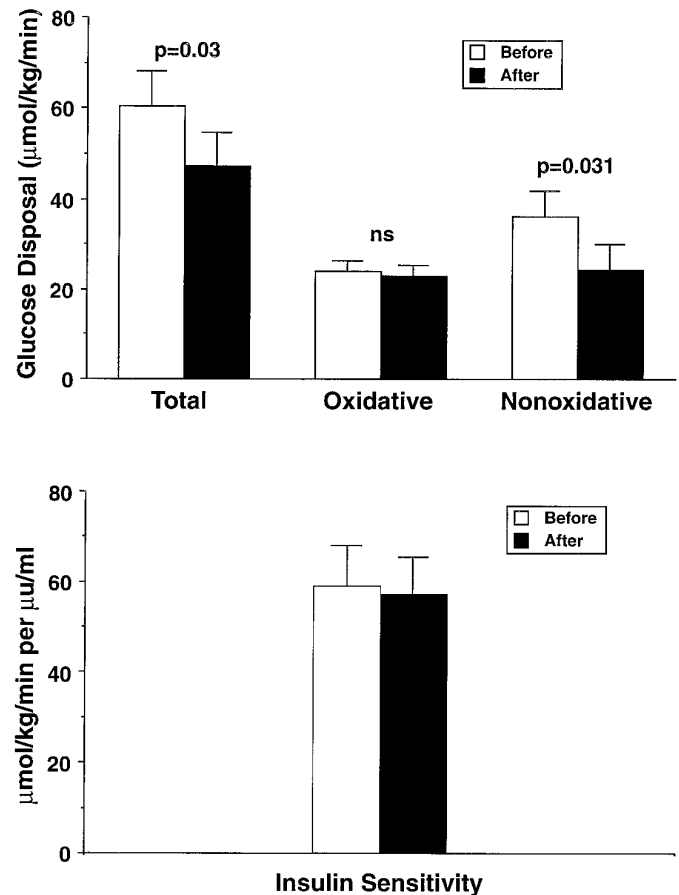


FIG. 4. Insulin stimulated total, oxidative, and nonoxidative glucose disposal during 40 $\text{mU}\cdot\text{m}^2\cdot\text{min}$ hyperinsulinemic clamp (upper) and insulin sensitivity (lower) before (open bars) and after (filled bars) testosterone therapy.

anisms responsible for lean body mass accretion. Following testosterone treatment, whole-body protein breakdown has been shown to increase (15) or not change (12, 33), protein oxidation has been found to decrease (15, 33) or not change (12), and protein synthesis found to increase (15) or not change (12, 33). These conflicting results most likely stem from the variability in experimental design, dose of testosterone administered (50–250 mg/week), duration of treatment (1–12 months), age of study subjects (12–70 yr old), and mode of expressing turnover data (micromoles per hour vs. micromoles per kilogram per hour vs. micromoles per kilogram FFM per hour). For example, in one study proteolysis increased with testosterone when expressed in micromoles per hour, but was unchanged when expressed per FFM (12). The only other pediatric investigation demonstrated an increase in proteolysis after testosterone. However, the data were expressed per kilogram of body weight and not per metabolically active FFM (15). Because of significant changes in body composition with testosterone, we elected to express the protein turnover data per metabolically active FFM. Our results demonstrate that after testosterone treatment both protein breakdown and protein oxidation decrease. Even though absolute protein synthesis does not change, the fractional synthetic rate increases from 84–90%. These results are

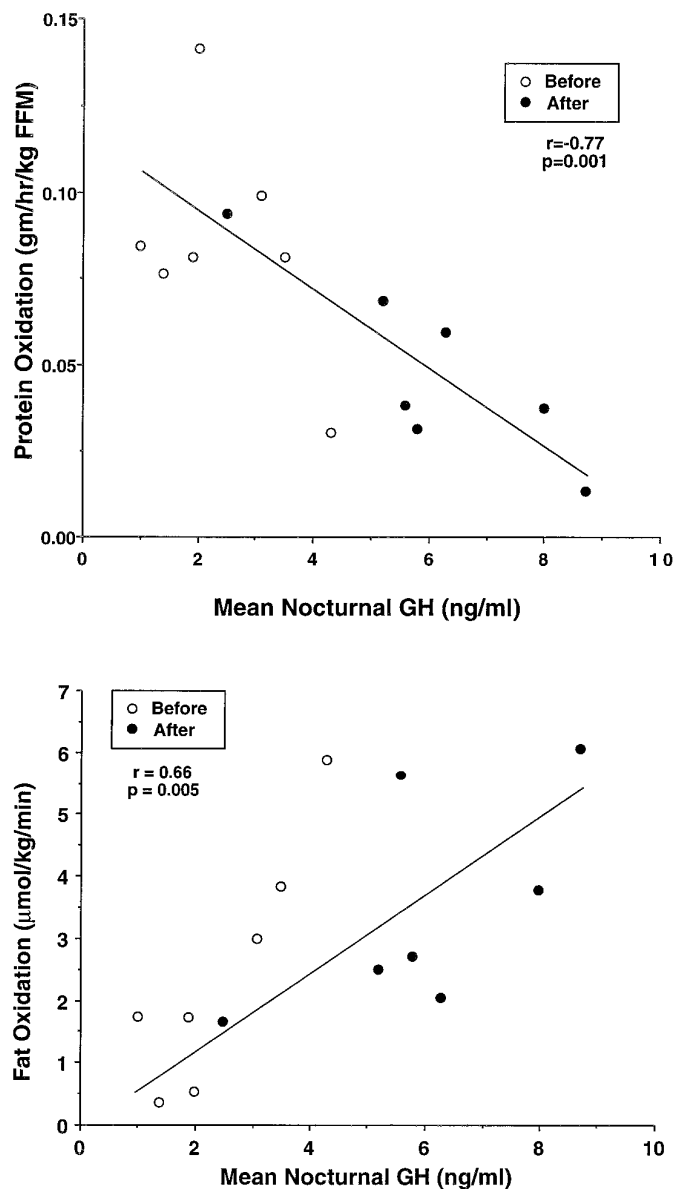


FIG. 5. Correlation between mean nocturnal GH and basal protein oxidation (*upper*) and mean nocturnal GH and fat oxidation (*lower*).

consistent with muscle biopsy studies, which have shown that the fractional synthetic rate of skeletal muscle proteins increases after testosterone replacement in hypogonadal and elderly men (12, 37). The present findings are in agreement with our previous cross-sectional studies in healthy children in whom whole-body proteolysis and protein oxidation were 12% and 24% lower, respectively, in pubertal adolescents compared with prepubertal children (5).

Growth and mass accretion involve net deposition of protein. Therefore, the rate of synthesis must be greater than breakdown. Waterlow (27) proposed that the most energy efficient and/or economical way of achieving this would be by a reduction in the rate of breakdown. Our cross-sectional studies of puberty and the present longitudinal studies support this concept by demonstrating lower rates of proteolysis and protein oxidation during puberty and/or after testos-

terone supplementation. Thus, it appears that the approximate 21% gain in FFM occurred in an energy efficient manner by a reduction in protein breakdown. The observed decrease in protein breakdown after testosterone therapy would translate to sparing of protein on average of 1.2 g·day·kg FFM. This figure is almost identical to the 1 g·day·kg FFM derived from our previous cross-sectional studies of puberty (5). Moreover, the approximate 5.8 kg of protein spared during the 4 months of treatment is very close to the mean 8 kg gain in FFM.

Whether or not the observed changes in protein turnover are secondary to testosterone per se or mediated through its effect on GH and IGF-I cannot be concluded with certainty in the present study. The role of testosterone in modulating the somatotrophic axis and enhancing GH secretion during puberty and in adulthood is well established (7, 38, 39). However, investigations evaluating whole-body protein turnover before and after testosterone have not aimed at specifically assessing effects of testosterone independent of GH changes. In the present study the multiple regression analysis would suggest that the major predictor of protein oxidation is mean nocturnal GH, explaining 59% of the variability, whereas testosterone has no added contribution. However, several observations in the literature would suggest that testosterone has a distinct role from that of GH to increase FFM. In a study of adult men who lacked pituitary GH function, testosterone replacement resulted in increased fractional synthetic rate of muscle protein and accretion of muscle mass despite no change in IGF-I levels (12). In another study of adult men, leucine oxidation decreased after testosterone despite no change in GH (33). In boys with constitutional delay in growth and adolescence, replacement with dihydrotestosterone resulted in acceleration of height velocity into the peak pubertal range without an increase in plasma GH (7). In Wistar rats, despite hypophysectomy a significant anabolic response was observed after testosterone (8). In contrast, both GH and IGF-I have been shown repeatedly to modulate protein metabolism with important anabolic actions (40). It is our hypothesis that during puberty both testosterone and GH/IGF-I have distinct and independent effects on protein turnover. Future studies identical to the present study but using dihydrotestosterone would be of value in testing the metabolic actions of sex steroids independent of GH.

Testosterone has been shown to decrease adipose tissue mass by several mechanisms (41–44). In the rat model, testosterone treatment resulted in increased responsiveness to catecholamine induced-lipolysis through an up-regulation of β -adrenergic receptor density and a postreceptor effect (41, 42). In humans, information about testosterone and adipose tissue have evolved from observations of steroid hormone involvement in the metabolic regulation of regional fat distribution (45). Young men with high testosterone secretion have low visceral fat, whereas aging men with low testosterone have abdominal obesity. Testosterone treatment of the latter was followed by a reduction in visceral fat mass, assessed by computerized tomography (46). This diminution of fat mass was associated with an increase in the lipolytic responsiveness of isolated adipocytes to norepinephrine, and a dramatic decrease in lipoprotein lipase activity (43). More-

over, testosterone supplementation inhibited *in vivo* triglyceride uptake and *in vitro* lipoprotein lipase activity, the main metabolic pathway regulating adipocyte triglyceride uptake (47). Our study demonstrated increased fat oxidation after testosterone supplementation. Furthermore, fat oxidation correlated inversely with body fat mass ($r = -0.68, P = 0.01$). Because rates of lipolysis were not affected by testosterone, the increased fat oxidation would translate to decreased fat reesterification. Thus, decreased adipose tissue triglyceride synthesis would be the most likely mechanism responsible for the reduction in fat mass in these adolescents treated with testosterone. The rates of total body lipolysis in this group of children with delayed adolescence are almost twice as high compared with our findings in normal children (3). In abdominally obese men with lower testosterone levels triglyceride turnover was higher in abdominal *vs.* femoral adipose tissue (47). Based on such findings, it is possible that the higher lipid turnover rates in these adolescents with low testosterone compared with normal adolescents is secondary to higher lipid turnover rates in abdominal adipose tissue. We, however, did not assess regional adiposity in these patients. There are no other investigations in the literature similar to ours for comparative purposes.

Whether or not the observed changes in lipolysis and fat oxidation before and after testosterone therapy are caused by a testosterone effect alone or in combination with GH remains unresolved at the moment. The regression analysis in the present study would suggest that testosterone mediated increases in GH modulate fat oxidation. This is not only in agreement with the well-defined metabolic actions of GH (48), but with observations of additive effects of GH and testosterone on lipolysis (49). Testosterone treatment alone had no effect on lipolysis in adipocytes isolated from hypophysectomized rats, whereas testosterone and GH in combination restored the lipolytic response to isoproterenol (49). Additional studies in humans are needed to specifically investigate the role of testosterone *vs.* GH alone or in combination on rates of fat oxidation and reesterification during puberty.

Even though this investigation was not designed to evaluate the role of leptin in reproduction (50), the observations in this study make such a role in humans questionable. In agreement with previous reports, leptin reflected fat mass before and after testosterone. Moreover, leptin levels did not increase rather decreased after testosterone commensurate with the decrease in fat mass.

Androgens are physiological regulators of plasma lipids, particularly HDL cholesterol (51). Both endogenous and exogenous androgens have a suppressive effect on HDL in males, with little effect on other plasma lipoproteins. Limited data in the pediatric age group suggest that the decrease in HDL level in boys at puberty is related to an increase in testosterone concentration (52). In boys with delayed adolescence, two doses of 100–200 mg testosterone enanthate 1 month apart was associated with reductions in HDL levels (52). Our findings are in agreement, showing lower HDL levels after low dose testosterone, as well as lower total cholesterol. Similar to adult studies, there was no change in plasma triglyceride levels (33, 53).

For a long time, androgens were considered to decrease

glucose tolerance and induce insulin resistance (54). However, lately these effects have been questioned based on observations of 1) negative association between plasma testosterone and insulin in healthy adult men (55); 2) insulin resistance in castrated rats, which is reversed with testosterone replacement (56); 3) improved insulin sensitivity in relatively hypogonadal obese men after testosterone treatment (46); and 4) no change in insulin sensitivity following testosterone administration to healthy men (57). Our present findings are in agreement with the latter study. Four months of low-dose testosterone treatment of adolescents with delayed puberty did not impair insulin sensitivity. Interestingly however, insulin clearance posttestosterone increased significantly. In hypogonadal men, plasma insulin concentrations were lower after testosterone supplementation, suggestive of increased insulin clearance, but this was not measured (58). The nature or the mechanism(s) responsible for the observed increase in metabolic clearance rate of insulin in the present study is unclear. It could be hypothesized that changes in body composition with remarkable increases in linear growth and FFM are responsible. In support of such a theory is previous observations of lower insulin clearance in obese subjects that increases with weight loss (59).

In summary, our present investigation demonstrates that in adolescents with delayed puberty, 4 months of low-dose testosterone therapy that achieves serum testosterone levels commensurate with Tanner stage III puberty results in an average sparing of protein breakdown of 1.2 g·day⁻¹·kg FFM. This, in conjunction with increased fat oxidation, results in significant gains in FFM with loss of fat mass and lowering of leptin levels. This low-dose testosterone therapy is not associated with impairment of *in vivo* insulin action, but is associated with enhanced insulin clearance. Additional studies are needed to differentiate the metabolic actions of sex steroids separate from GH during puberty and the cause of increased insulin clearance.

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