

Testosterone Deficiency in Young Men: Marked Alterations in Whole Body Protein Kinetics, Strength, and Adiposity*

NELLY MAURAS, VALERIE HAYES, SUSAN WELCH, ANNIE RINI,
KEVIN HELGESON, MARYANNE DOKLER, JOHANNES D. VELDHUIS, AND
RANDALL J. URBAN

Nemours Children's Clinic, Jacksonville, Florida 32207; Divisions of Endocrinology (N.M., V.H., S.W., A.R.), Physical Therapy (K.H.), and Pediatric Surgery (M.D.), Department of Medicine (J.D.V.), University of Virginia Medical Center, Charlottesville, Virginia 22908; and the Department of Medicine (R.J.U.), University of Texas Medical Branch, Galveston, Texas 77555-1060

ABSTRACT

To investigate specific effects of androgens on whole body metabolism, we studied six healthy lean men (mean \pm SEM age, 23.2 ± 0.5 yr) before and after gonadal steroid suppression with a GnRH analog (Lupron), given twice, 3 weeks apart. Primed infusions of [13 C]leucine, indirect calorimetry, isokinetic dynamometry, growth factor measurements, and percutaneous muscle biopsies were performed at baseline (D1) and after 10 weeks of treatment (D2); each subject served as his own control. Testosterone concentrations were markedly suppressed after 10 weeks of treatment (D1, 535 ± 141 ng/dL; D2, 31 ± 9). Leucine's rate of appearance (index of proteolysis) was markedly suppressed after 10 weeks of hypogonadism (-13% ; $P = 0.01$) as well as the nonoxidative leucine disposal, an index of whole body protein synthesis (-13% ; $P = 0.01$) without any changes in plasma amino acid concentrations. All subjects studied after 10 weeks showed a decrease in fat-free mass, as measured by skinfold calipers and dual emission x-ray absorptiometry scans (D1, 56.5 ± 2.9 kg; D2, 54.4 ± 2.5 ; $P = 0.005$), and an increase in percent fat mass (D1, $19.2 \pm 2.5\%$; D2, $22.2 \pm 2.5\%$; $P = 0.001$). Rates of lipid oxidation decreased (-31% ; $P = 0.05$) after treatment, with parallel changes in resting energy expenditure (-9% ; $P = 0.05$). Mean and peak GH concentrations (measured every 10 min for 6 h) and GH production rates did not decrease after

testosterone deficiency, with an actual increase in basal secretion ($P < 0.02$). Plasma insulin-like growth factor I (IGF-I) concentrations did not change significantly after 10 weeks of treatment (D1, 227 ± 44 μ g/L; D2, 291 ± 60 ; $P = 0.08$). Isokinetic dynamometry of leg extensors at 60° and 180° /s was also decreased after 10 weeks of hypogonadism. Total ribonucleic acid (RNA) was isolated from muscle biopsy samples, and ribonuclease protection assays were performed using human complementary DNA clones for IGF-I, IGF-binding protein-4, myosin, and actin. Ten weeks after Lupron treatment, messenger RNA (mRNA) concentrations of IGF-I decreased significantly, whereas there was a trend toward higher IGF-binding protein-4 concentrations, with no change in myosin or actin mRNA concentrations.

In conclusion, testosterone deficiency in young men is associated with a marked decrease in measures of whole body protein anabolism, decreased strength, decreased fat oxidation, and increased adiposity. These effects of testosterone deficiency are independent of changes in peripheral GH production and IGF-I concentrations, even though im IGF-I mRNA concentrations decrease. These data suggest a direct effect of androgens on whole body lipid and protein metabolism. (*J Clin Endocrinol Metab* 83: 1886–1892, 1998)

A NUMBER of clinical studies using a variety of experimental designs have shown that androgenic hormones have potent protein anabolic effects in man. Testosterone administration increases mixed muscle protein synthesis in normal men (1), increases whole body protein synthesis in prepubertal boys (2), and increases skeletal muscle protein synthesis and strength in elderly (3) and hypogonadal men (4, 5). However, the mechanisms by which these changes occur remain incompletely understood, *i.e.* whether these changes are a result of androgens working directly through the androgen receptor or whether the anabolic effect

of testosterone is secondary to the enhancement of the GH/insulin-like growth factor I (IGF-I) axis or another system. The latter becomes particularly relevant in puberty, as exogenous and endogenous androgens markedly enhance GH release and hence IGF-I production (6, 7), because both GH and IGF-I are highly protein-anabolic in man (8, 9). In elderly men, both relative hyposomatotropism and androgen deficiency are increasingly better defined entities (4, 10); hence, the relative contributions of the androgen and GH/IGF-I systems to body composition changes are important when considering potential therapeutic interventions. We designed these studies to investigate whether short term testosterone deficiency 1) results in decreases in estimates of whole body protein synthesis, 2) results in decreases in lean body mass and increases adiposity, 3) affects measures of muscle strength, and 4) affects the above through the GH/IGF-I system. To accomplish this, a group of young, normal men were studied before and during 10 weeks of severe hypogonadism. Significant changes in body composition, particularly in protein and lipid metabolism, were observed.

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Address all correspondence and requests for reprints to: Nelly Mauras, M.D., Nemours Children's Clinic, 807 Nira Street, Jacksonville, Florida 32207. E-mail: nmauras@nemours.org.

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Subjects and Methods

Subjects

These studies were approved by the Nemours Children's Clinic clinical research review committee and the Baptist Medical Center institutional review committee. Six healthy young males (mean \pm SEM age, 23.2 \pm 0.5 yr) participated in these studies after informed written consent was obtained. They were all within 5% of their ideal body weight (Metropolitan Life Insurance Tables).

Study design

For 3 days before admission to our Clinical Research Center, each subject consumed a weight maintenance diet consisting of approximately 34 Cal/kg and 1.7 g/kg protein/day. Subjects were instructed to keep the same pattern of weekly exercise during these studies.

The afternoon before the isotope tracer studies, subjects underwent body composition analysis using skinfold thickness measurements and dual emission x-ray absorptiometry (DEXA; Hologic 2000, Waltham, MA). Isokinetic dynamometry of the left knee extensors and flexors was performed using a Biodex Dynamometer (Biodex Corp., Shirley, NY). After a 10-min training session, followed by 30 min of rest, maximum torque production and work measures were recorded for isometric and isokinetic tests. Isometric tests, with the knee placed at 45° of flexion, were performed with five contractions for 5 s each, with 10 s of rest between contractions. Isokinetic tests were performed for knee extension and flexion at 60°/s for 5 repetitions and at 180°/s for 21 repetitions.

On the morning of the first study (D1), after an overnight 14-h fast, two iv heparin locks were placed, one in the antecubital vein for the infusion of isotopes and another in a contralateral hand vein kept heated for arterialized blood sampling (11). At 0800 h (time zero), a primed, dose constant infusion of L-[1-¹³C]leucine was started and was continued uninterrupted for the next 240 min (~4.5 μ mol/kg; 0.07 μ mol/kg·min). Multiple blood, breath, and urine samples were obtained at frequent intervals, as detailed below. Indirect calorimetry was performed three times during the study using a CPX-MAX calorimeter (Medical Graphics, St. Paul, MN).

Subjects were fed lunch at 300 min and were free to move around. A percutaneous muscle biopsy of the anterior quadriceps was performed under local anesthesia for the measurement of messenger ribonucleic acid (mRNA) gene expression of different proteins in muscle.

After the baseline study was completed, subjects began treatment approximately 1 week later with a long acting GnRH analog (GnRHa; Lupron, TAP Pharmaceuticals, Deerfield, IL), at a dose of 7.5 mg, im. Three weeks after the first injection, another injection was given, and the study was repeated identically 7 weeks later (D2).

Blood, urine, and breath samples

During the isotope infusions, blood was withdrawn at -5, 30, 90, 150, 180, 210, and 240 min in both studies for determination of the isotopic enrichments of α -ketoisocaproic acid (KIC). At 0, 120, and 240 min, blood samples were collected for the measurement of serum total and free testosterone, insulin, and glucose and plasma IGF-I and IGF-binding protein-3 (IGFBP-3) concentrations. Amino acid concentrations were also measured in the plasma samples. Serum samples were obtained at 10-min intervals for 6 h during these studies, from -60 min through 300 min for the assessment of GH concentration profiles. Expired air samples were obtained at -10, 0, 160, 180, 200, and 220 min on each study day to measure the expired labeled ¹³CO₂. A 4-h urine collection for measurement of urea nitrogen excretion was obtained during the 4-h isotope tracer infusions.

Isotopes

L-[1-¹³C]leucine (99% enriched; Cambridge Isotopes, Andover, MA) was determined to be sterile and pyrogen free and was prepared using 0.9% nonbacteriostatic saline.

Assays

Plasma enrichments of [1-¹³C]KIC were determined at the Nemours Children's Clinic Core Endocrine/Metabolic Laboratory by gas chro-

matography-mass spectrometry as previously described (12). ¹³CO₂ enrichments were determined using a dual inlet isotope ratio mass spectrometer (13, 14). The intraassay coefficient of variation (CV) for the isotopic enrichments of [1-¹³C]KIC was 1.1%, and that for ¹³CO₂ was 0.22%. Plasma amino acid concentrations were measured by an ion exchange method using a Beckman 6300 Amino Acid Analyzer (Beckman Instruments, Fullerton, CA) with an intraassay CV of 2.5%. Total serum testosterone was measured by RIA using kits from Diagnostic Products Corp. (Los Angeles, CA), and free testosterone was measured by radioimmunoassay using a kit from Diagnostic Systems Laboratories (Houston, TX) at the Mayo Clinic General Clinical Research Center (GCRC) Core Laboratory (Rochester, MN), with intraassay CVs of 13.6% and 11%, respectively. IGF-I and IGFBP-3 concentrations were measured by radioimmunoassays with intraassay CVs of 6% and 5%, respectively. Insulin was measured by a chemiluminescence assay with a CV of 5%. GH was measured by a highly sensitive chemiluminescence assay at the University of Virginia GCRC Core Laboratory with an intraassay CV of 4.6%. Glucose was measured by a glucose oxidase method using a Beckman Glucose Analyzer (Beckman Instruments). Urinary nitrogen excretion was measured using a Kodak Ektachem urease method (Rochester, NY). Substrate oxidation and energy expenditure rates were measured by indirect calorimetry using a mouthpiece with a CPX-MAX Calorimeter (Medical Graphics Corp.).

Ribonuclease protection assays

Percutaneous muscle biopsy samples were placed in liquid nitrogen and kept frozen at -70 C until assayed. Total RNA was isolated from the samples using RNAzol B (Tel-Test, Friendswood, TX). The ribonuclease protection assay was performed as described previously (3, 15), using human complementary DNA (cDNA) clones for IGF-I, IGFBP-4, actin, and myosin. The IGF-I and IGFBP-4 clones have been described previously (3). The myosin cDNA clone contains the myosin light chains MLC₁ and MLC₃ from fast skeletal muscle fibers (16, 17). These were cloned from human fetal tissue and are the products of alternative splicing of one gene (17). They are the major isoforms of adult skeletal muscle (17). The α -actin cDNA clone is a full-length clone described by Gunning *et al.* (18). The amount of total RNA used for each assay is as follows: IGF-I, 15 μ g; IGFBP-4, 10 μ g; and actin and myosin, 2 μ g. The RNA-protected bands were detected with a 425E Phosphor Imager (Molecular Dynamics, Sunnyvale, CA), and band intensities measured with the ImageQuant analysis program. All bands were corrected for loading differences by simultaneously measuring band densities of the housekeeping gene, human glyceraldehyde 3-phosphate dehydrogenase (G3PD; Ambion, Austin, TX).

Calculations

Leucine kinetics. Isotope dilution methods using the essential amino acid leucine were used in these experiments. This model assumes that at steady state in the post absorptive state the rate of appearance (Ra) of the tracer equals its disappearance (Rd), and hence, Rd can be partitioned into oxidative and nonoxidative losses or nonoxidative leucine disposal (NOLD). The latter serves as an index of whole body protein synthesis. Plasma enrichments of [1-¹³C]KIC were used as the index of intracellular enrichment of leucine in the reciprocal pool model (19, 20). All estimates were made at near steady state, between 160-240 min of infusion. The Ra of leucine, leucine oxidation rates, and NOLD were calculated as previously described (20).

Substrate oxidation rates. These combustion equations calculate the oxidation of substrates (sugars, lipids, and proteins) from the rates of O₂ and CO₂ exchanged and total nitrogen excretion in the urine as previously described (21).

Deconvolution. The GH concentration series was analyzed using deconvolution mathematical modeling, and the amplitude, the frequency of GH bursts, as well as the GH production rates were measured as previously described (22).

Body composition. DEXA scan data were used to estimate body composition changes. Fat-free mass (FFM) represents the sum of nonfat mass plus bone mineral content, as calculated using the tissue composition reference bar of Hologic.

Statistics. Each subject served as his own control; hence, paired Student's *t* tests were used to compare differences after treatment. Significance was established at $P < 0.05$.

Results

Circulating androgens and body composition

Table 1 shows the changes in total and free testosterone concentrations in these subjects during the 10 weeks of the experiments. Both were markedly suppressed, similar to levels found in prepuberty or very early puberty. There was a marked correlation between the body composition measurements made by skinfold thickness vs DEXA (FFM: $r^2 = 0.9$; $P = 1.0 \times 10^{-9}$; fat mass: $r^2 = 0.83$; $P = 4.5 \times 10^{-8}$); hence, only data generated by DEXA are reported. There were no significant changes in total body weight or BMI during these experiments; however, there was a significant decrease in FFM ($P = 0.005$) and a concomitant increase in percent fat mass during hypogonadism ($P = 0.001$).

Protein kinetics and plasma amino acids

There was a remarkable decrease in the Ra of leucine, a measure of whole body proteolysis, after hypogonadism, which was accompanied by a parallel decrease in the measure of whole body protein synthesis (Fig. 1). These differences were comparable whether the data were expressed as total kilograms or kilograms of FFM. There were no significant changes in plasma amino acid concentrations after treatment (Table 2).

Substrate oxidation and energy expenditure rates

Table 3 summarizes results of the changes in the rates of carbohydrate, protein, and lipid oxidation as measured by indirect calorimetry. There were no significant changes in carbohydrate and protein oxidation rates, but there was a clear trend toward lower lipid oxidation rates after 10 weeks of hypogonadism (-31% ; $P = 0.05$); hence, the resting energy expenditure was also lower after treatment (-9% ; $P = 0.05$).

Muscle strength

Isokinetic dynamometry of the knee extensors showed lower strength at 60° and $180^\circ/s$ after 10 weeks of hypogonadism [at $60^\circ/s$: D1, 186 ± 15 Newton meters; D2, 175 ± 13 ; $P = 0.01$; at $180^\circ/s$: D1, 146 ± 11 ; D2, 135 ± 8 ; $P = 0.07$; (by one-tailed test, $P = 0.035$); Fig. 2]. The isometric testing results were: D1, 166 ± 13 Newton meters; D2, 158 ± 13 ($P = 0.21$); the isokinetic testing results of the flexors were: D1,

104 ± 13 ; D2, 95 ± 8 ($P = 0.17$) at 60° ; and D1, 85 ± 8 ; D2, 76 ± 3 ($P = 0.13$) at 180° .

GH, IGF-I, IGFBP-3, insulin, and glucose concentrations

Deconvolution analysis of the GH concentration series performed by frequent blood sampling (every 10 min) for 6 h revealed no decreases in the mean or peak GH concentrations in these young men during the hypogonadal state or in the GH production rates (Table 4). Actually, basal GH secretion was increased after induction of hypogonadism. Comparably, the circulating IGF-I concentrations did not change significantly despite 10 weeks of hypogonadism, with an actual trend toward higher concentrations on D2 ($P = 0.08$). IGFBP-3 concentrations increased during treatment ($P < 0.05$). Insulin concentrations showed no significant increase during treatment, whereas circulating glucose concentrations remained normal (Table 5).

Muscle mRNA concentrations of specific proteins

IGF-I mRNA concentrations were significantly decreased after 10 weeks of hypogonadism (D1, 5.4 ± 0.4 ; D2, 3.7 ± 0.4 ; $P = 0.04$; Fig. 3). Concentrations of the inhibitory IGFBP-4 tended to be increased, but did not reach significance (D1, 1.1 ± 0.2 ; D2, 1.7 ± 0.4 ; $P = 0.2$). Actin and myosin mRNA concentrations did not change with hypogonadism (actin: D1, 1.7 ± 0.3 ; D2, 2.0 ± 0.5 ; $P = 0.5$; myosin: D1, 1.4 ± 0.5 ; D2, 1.1 ± 0.3 ; $P = 0.5$).

Discussion

Short term (10-week) severe androgen deficiency in young men was associated with marked changes in whole body metabolism, including decreased rates of whole body protein turnover and protein synthesis, decreased FFM, and increased adiposity. This was also associated with decreased fat oxidation, decreased resting energy expenditure, and decreased muscle strength, clearly supporting the pivotal role of androgens in the maintenance of normal body composition in man.

These changes in body composition are congruent with the well established observations of increased adiposity and decreased lean body mass of both hypogonadal and elderly men reported previously (3–5). Bhasin *et al.* showed significant anabolic changes in body composition and strength in hypogonadal men treated for 10 weeks with testosterone, but no changes in the whole body protein turnover/synthesis rates (5). These differences can be readily accounted for by the acuteness and severity of the androgen deficiency observed in the present studies, as testosterone concentrations decreased from the mid-500 ng/dL range to the prepubertal to early pubertal range (~ 30 ng/dL). The changes reported here, however, are opposite; they mirror those observed by us in prepubertal boys treated with testosterone (2) and confirm our previous findings that testosterone increases both the rates of whole body proteolysis (as measured by the leucine Ra) and protein synthesis (NOLD) with a net anabolic effect. These changes in the rates of whole body kinetics were not due to depletion of the plasma amino acid pool, as the concentrations of all amino acids after 10 weeks of hypogo-

TABLE 1. Changes in circulating androgens and body composition (by DEXA) at baseline (D1) and after 10 weeks of hypogonadism (D2)

	D1	D2	P value
Testosterone (ng/dL)	535 ± 141	31.0 ± 9.0	0.02
Free testosterone (pg/mL)	23.1 ± 3.1	2.1 ± 0.5	0.001
Wt (kg)	72.7 ± 3.6	71.6 ± 3.0	0.30
BMI (kg/m^2)	23.8 ± 0.8	23.4 ± 0.8	0.30
FFM (kg)	56.5 ± 2.9	54.4 ± 2.5	0.005
% FFM	77.9 ± 2.4	76.2 ± 2.5	0.018
FM (kg)	15.8 ± 1.9	16.9 ± 2.0	0.05
% Fat mass	19.2 ± 2.5	22.2 ± 2.5	0.001
Bone mineral content (g)	2.52 ± 0.09	2.64 ± 0.17	NS

WHOLE BODY PROTEIN KINETICS IN YOUNG MEN DURING HYPOGONADISM

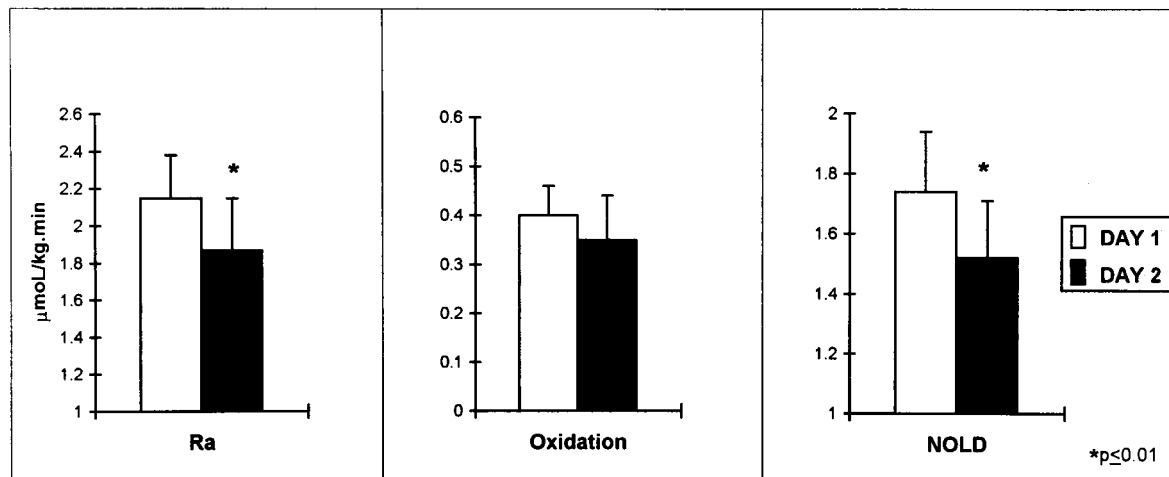


FIG. 1. Changes in rates of whole body proteolysis (leucine Ra: D1, 2.15 ± 0.23 ; D2, 1.87 ± 0.28), oxidation (D1, 0.40 ± 0.06 ; D2, 0.35 ± 0.09), and whole body protein synthesis (NOLD; D1, 1.74 ± 0.20 ; D2, 1.52 ± 0.19), before (D1) and after (D2) 10 weeks of hypogonadism. The trends are the same if data are expressed as micromoles per kg FFM/min.

TABLE 2. Mean (\pm SEM) individual plasma amino acid concentrations before (D1) and after (D2) 10 weeks of hypogonadism

Amino acid ($\mu\text{mol/L}$)	D1	D2	P values
Alanine	248 \pm 47	246 \pm 29	0.88
α -Amino-adipic	0 \pm 0	0 \pm 0	0.36
α -Amino-N-butyric	30 \pm 5	25 \pm 4	0.17
Arginine	72 \pm 12	70 \pm 9	0.59
Asparagine	32 \pm 5	29 \pm 2	0.44
Aspartic acid	7 \pm 1	6 \pm 0	0.35
Citrulline	29 \pm 5	28 \pm 1	0.73
Cystathionine	6 \pm 1	2 \pm 1	0.03
Cystine	15 \pm 5	27 \pm 7	0.07
Glutamic acid	62 \pm 11	58 \pm 7	0.37
Glutamine	477 \pm 75	492 \pm 26	0.57
Glycine	167 \pm 28	187 \pm 14	0.13
Histidine	78 \pm 13	75 \pm 6	0.57
Homocystine	0 \pm 0	0 \pm 0	
Hydroxyproline	12 \pm 2	13 \pm 1	0.31
Isoleucine	69 \pm 11	60 \pm 6	0.13
Leucine	158 \pm 25	145 \pm 8	0.29
Lysine	191 \pm 30	192 \pm 15	0.97
Methionine	32 \pm 6	27 \pm 2	0.12
1-Methyl histidine	1 \pm 1	0 \pm 0	0.18
3-Methyl histidine	1 \pm 1	1 \pm 1	0.49
Ornithine	52 \pm 9	49 \pm 4	0.39
Phenylalanine	56 \pm 9	56 \pm 3	0.97
Phosphoethanolamine	0 \pm 0	0 \pm 0	
Proline	151 \pm 25	136 \pm 10	0.06
Sarcosine	0 \pm 0	0 \pm 0	
Serine	94 \pm 16	97 \pm 7	0.57
Taurine	37 \pm 6	41 \pm 4	0.13
Threonine	116 \pm 20	126 \pm 13	0.16
Tyrosine	61 \pm 11	65 \pm 5	0.35
Valine	235 \pm 36	230 \pm 15	0.75

nadism were comparable to baseline levels. As the availability of amino acids from endogenous sources declined, despite the maintenance of oxidative rates, there was a net decrease in protein synthesis and, overall, less anabolism after treatment. These changes in whole body kinetics were

TABLE 3. Substrate oxidation and resting energy expenditure rates in six young men before (D1) and after 10 weeks of hypogonadism (D2)

	D1	D2	P value
Carbohydrate oxidation	4.1 \pm 0.8	7.3 \pm 2.1	0.30
Protein oxidation	6.7 \pm 0.5	5.8 \pm 0.6	0.30
Lipid oxidation	18.5 \pm 1.6	12.8 \pm 1.6	0.05
Resting energy expenditure	30.1 \pm 2.0	27.4 \pm 1.6	0.05

All units are kilocalories per kg FFM/day (FFM by DEXA).

accompanied by marked reductions in quadriceps muscle strength, as measured by isokinetic dynamometry of the knee extensors at 60° and 180°. The isometric test did not show any detectable changes during these studies; the latter we believe to be due to the inability of these subjects to give a consistent maximum effort during the test.

The induction of hypogonadism in these young men was not accompanied by any decrease in systemic GH or IGF-I production in any of the subjects studied, as measured by deconvolution analysis of the pulsatile GH concentration series using a highly sensitive assay. Neither mean and peak GH and IGF-I concentrations nor GH production rates were decreased by the severe decrease in testosterone concentrations. The basal GH secretory rate increased after 10 weeks of hypogonadism, suggestive of changes in GH-binding protein levels or GH distribution volume. We do not believe that the lack of change in GH production (with an actual increase in basal secretion) was affected by the lack of nighttime sampling. First, the differences in day/night pulsatility are particularly pronounced in puberty, and our subjects are young adult, postpubertal males. Second, the studies were performed during a 14- to 18-h fast, which typically enhances GH production and overall pulsatility (23). Third, the studies are paired, making comparisons by far more robust. The severe metabolic changes observed here appear to be directly related to androgen deficiency and not due to GH/IGF-I

FIG. 2. Muscle strength, as measured by extension peak torque (Newton meters), of leg extensors (quadriceps) before (D1) and after 10 weeks of hypogonadism (D2). This was measured at 60°/s (best of five repetitions).

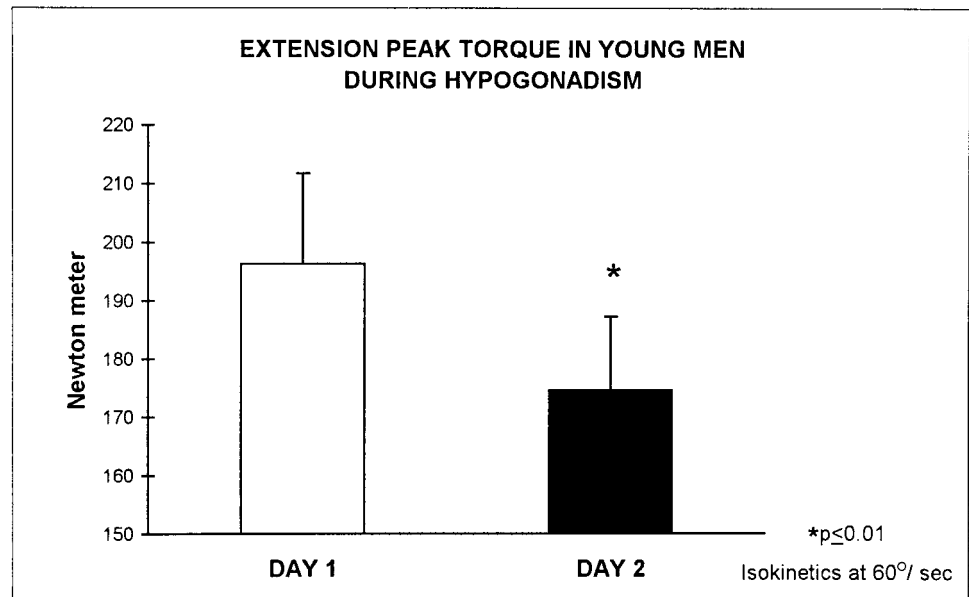


TABLE 4. Deconvolution of GH concentration profiles

	Basal (D1)	Hypogonadal (D2)	P value
Mean conc. ($\mu\text{g/L}$)	0.65 ± 0.19	0.97 ± 0.25	0.16
Peak conc. ($\mu\text{g/L}$)	3.49 ± 1.04	4.21 ± 1.03	0.54
Basal secretion ($\mu\text{g/L} \cdot \text{min}$)	0.0022 ± 0.0006	0.0048 ± 0.0012	0.02
Half-duration (min)	24 ± 4	20 ± 4	0.46
Half-life (min)	16 ± 1	15 ± 2	0.77
Bursts/6 h	5.3 ± 0.8	4.7 ± 0.8	0.62
Interburst interval (min)	73 ± 12	74 ± 21	0.96
Mass ($\mu\text{g/L}$)	2.8 ± 1.0	3.1 ± 0.9	0.73
Production rate ($\mu\text{g/L} \cdot 6 \text{ h}$)	12.7 ± 4.1	14.9 ± 4.9	0.72
Integrated ($\mu\text{g/L} \cdot \text{min}$)	232 ± 67	329 ± 92	0.18

TABLE 5. Hormones, growth factors, and glucose in six young men before (D1) and after 10 weeks of hypogonadism (D2)

	D1	D2	P value
IGF-I ($\mu\text{g/L}$)	227 ± 44	291 ± 60	0.08
IGFBP-3 (mg/L)	3.34 ± 0.29	3.81 ± 0.24	<0.05
Insulin (pmol/L)	29 ± 8	35 ± 7	0.14
Glucose (mmol/L)	4.9 ± 0.2	4.9 ± 0.1	NS

deficiency. Additionally, the IGFBP-3 concentrations were mildly, but significantly, increased during treatment with GnRHa. As IGFBP3 is a GH-dependent protein, it is possible that the bioactivity of GH may be increased and/or the small basal GH secretion rates may have influenced binding protein levels on D2, resulting in an increase in IGFBP-3. As IGFBP-3 may potentiate the effects of IGF-I *in vivo* (24), the rise in IGFBP-3 may represent a compensatory phenomena to maintain a basic anabolic rate in severe androgen deficiency.

There were, however, significant decreases in im mRNA concentrations for IGF-I and a trend toward increased IGFBP-4 gene expression, the main inhibitory binding protein for IGF-I in muscle (25). The gene expression for actin and myosin in muscle was not altered by the systemic decrease in testosterone concentrations. These observations are congruent with the observation made in elderly men treated with testosterone (3) and suggest that, within skeletal muscle

tissue, androgens are necessary for local IGF-I production, independent of GH production and systemic IGF-I concentrations. IGF-I and its type I receptor are ubiquitously expressed in skeletal muscle and appear to be important in both the proliferation and differentiation of skeletal myocytes (26). Even though the gene expression of actin and myosin, the main contractile proteins of skeletal muscle, were not altered during severe hypogonadism, testosterone deficiency was associated with a marked decrease in measures of muscle strength, indicating that other mechanisms besides changes in muscle protein expression are affected by this severe degree of androgen deficiency. Data reported to date do not show an effect of the GH/IGF-I system to enhance muscle strength (27), whereas testosterone administration to normal men has been shown to increase FFM and muscle size in normal men (28). In addition, the decrease in systemic, noncontractile protein synthesis and increased adiposity may also play a role in the decrease in strength observed here.

Young men treated with a GnRHa were less efficient in their oxidation of fat, with a consequent decrease in the resting energy expenditure, which probably explains the increase in adiposity and the decrease in lean body mass observed during these experiments. The mechanisms for these findings are not completely understood; however, several considerations apply. First, concentrations of GH, a signifi-

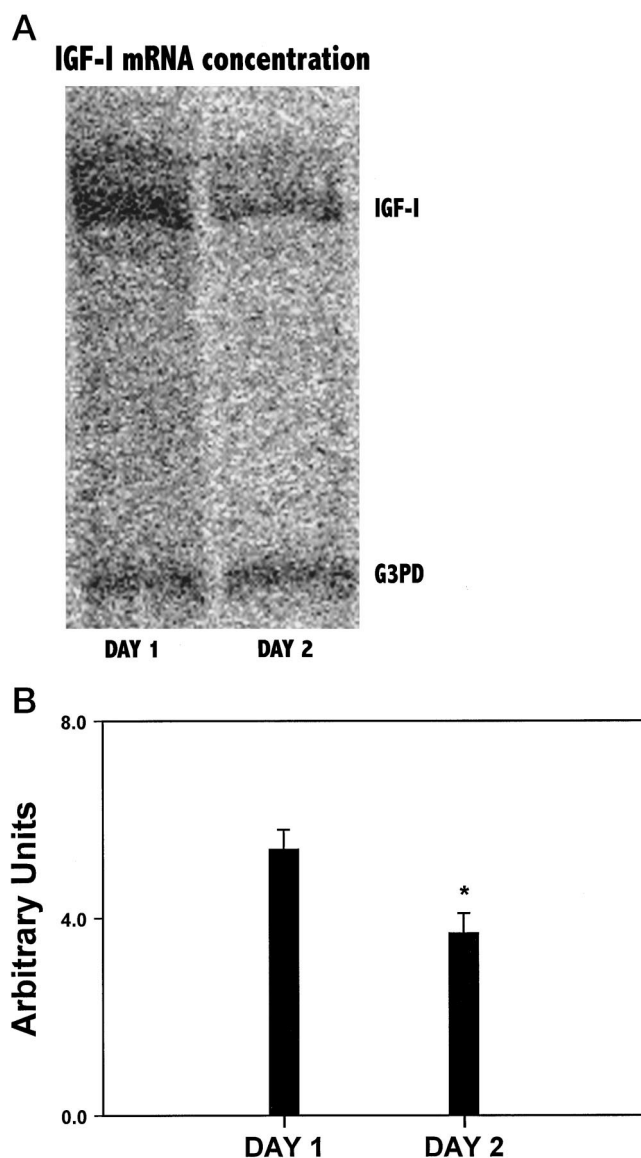


FIG. 3. The top panel shows representative IGF-I bands from a ribonuclease protection assay as detected on a phosphorimager screen. The lower bands are the G3PD bands used to normalize the data. The lower panel shows the mean \pm SE of the densities (normalized to G3PD) at baseline (day 1) and after Lupron treatment (day 2) for the six men. The asterisk indicates statistical significance ($P \leq 0.05$).

cant lipolytic hormone, did not decrease during these experiments. Insulin concentrations, on the other hand, did not increase significantly after hypogonadism. Plasma catecholamines were not measured in this paradigm; however, the studies were paired identically in each subject; hence, it is unlikely that the level of stress, and hence catecholamine production, would have been altered. Androgenic hormones, on the other hand, have been shown to stimulate lipolysis in a variety of species and experimental designs. Testosterone treatment of rat adipose precursor cells causes an increase in the number of β -adrenergic receptors as well as externalization of those receptors, and increases the forskolin-induced (cAMP-mediated) lipolysis (29, 30). In addition, testosterone increases triacylglycerol lipase activity (31).

When given to hypophysectomized rats, testosterone does not affect lipolysis, but when given in conjunction with GH, it normalizes lipolysis to a greater extent than GH alone, demonstrating that GH and testosterone have additive effects on lipolysis (32). Even though lipolysis was not directly measured in the present experiments, the observed decreases in lipid oxidation rates and significant increases in overall adiposity, despite the presence of normal GH production, are strongly suggestive that testosterone *per se* has significant effects on the regulation of fat metabolism.

In summary, severe androgen deficiency in young men was associated with decreased lean body mass and increased adiposity, decreased lipid oxidation and energy expenditure rates, decreased rates of whole body protein synthesis, and decreased leg muscle strength. These findings were not associated with changes in circulating amino acid concentrations. These changes were associated with decreased gene expression for IGF-I in muscle, but no peripheral decreases in GH and IGF-I production. We conclude that androgens can directly affect systemic protein synthesis, independent of the effect of peripheral GH and IGF-I. The latter may be important when an anabolic effect is the desired effect in the treatment of both elderly and young men.

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