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Research Article

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Calcium and Protein Kinetics in Prepubertal Boys

Positive Effects of Testosterone

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Abstract

We investigated the effects of 4–6-wk administration of testosterone on calcium and protein metabolism in six healthy prepubertal short boys (mean age \pm SE = 12.9 \pm 0.6 yr). At baseline, subjects received a 4-h infusion of L-[1-¹³C]leucine and L-[2-¹⁵N]glutamine, and were given ⁴²Ca intravenously, and ⁴⁴Ca PO. Testosterone enanthate (~ 3 mg/kg) was given I.M. 2 wk apart (two doses $n = 5$, three doses $n = 1$), and the study was repeated 4–5 d after the last injection.

After testosterone therapy, there were significant increases in serum testosterone and mean peak and total growth hormone concentrations. Net calcium absorption (V_a) and retention (V_{bal}) also increased (V_a 13.3 \pm 2.3 vs 21.5 \pm 2.3; mg \cdot kg⁻¹ \cdot d⁻¹, V_{bal} 8.0 \pm 2.1 vs 16.6 \pm 2.5, mg \cdot kg⁻¹ \cdot d⁻¹, $P < .05$ both), as well as Ca's net forward flow into bone and total exchangeable pool (16 and 20%, respectively). The rate of appearance of leucine (an indicator of proteolysis) increased by 17.6 \pm 5.9%, $P = 0.036$. Leucine oxidation decreased by 48.6 \pm 8.0%, $P = 0.004$; thus, nonoxidative leucine disappearance, which estimates protein synthesis, increased significantly by 34.4 \pm 7.7%, $P = 0.009$. Glutamine's rate of appearance also increased (+32%), mostly through enhanced glutamine de novo synthesis (+42%).

In conclusion, short term testosterone administration significantly increases calcium's retention and net forward flow into bone in prepubertal humans, as well as whole body estimates of protein and calcium anabolism. These effects may represent a pure androgen effect, an amplification of growth hormone's action or some combination of these factors. (*J. Clin. Invest.* 1994. 93:1014–1019.) Key words: testosterone • puberty • growth • leucine metabolism • calcium metabolism

Introduction

Many of the physical changes of puberty, such as the accelerated growth, the increase in body mass and muscle bulk, and the active mineralization of the bones that occur are mediated, at least in part, through the actions of sex steroids. Some of these effects may be directly related to changes in protein and calcium metabolism induced by the sex steroids or alternatively, they may be secondary to the cascade of events triggered

by the increase in the growth hormone (GH)¹ IGF-I production observed after sex steroid exposure (1, 2).

The critical role of estrogens in preventing bone loss in women after menopause has been firmly established (3, 4). Not surprisingly, female runners with secondary amenorrhea and hypoestrogenemia, as well as females with iatrogenic hypogonadism (e.g., luteinizing hormone-releasing hormone analogue-treated patients) have reduced lumbar spine bone mineral density associated, at least in part, to estrogen deficiency (5–7). More recently, data have accumulated, examining the role of both dietary intake of calcium and the adequacy of the sex steroid milieu in preservation of bone mineral density in children and hypogonadal males (8, 9). These studies strongly suggest that dietary supplementation with calcium alone is associated with a gain in bone mineral acquisition in prepubertal children (8) and that the timing of the onset of puberty is an important determinant of bone density later in life (9). During puberty there is active bone mineralization and rapidly changing calcium turnover pools that presumably impact bone accretion and perhaps growth. The administration of stable labeled tracers of calcium to adolescent children has recently shown that the total exchangeable pool (TEP) of calcium, which is that pool of calcium in metabolically active bone in equilibrium with plasma and extracellular fluid, is much greater than in younger children and adults (10). Also, testosterone administration to the cynomolgus monkey causes the rapidly changing calcium pool to increase significantly (11). Both of these studies also suggest that the alterations in calcium pools observed in puberty may be secondary to changes in the sex-steroidal milieu.

Androgenic hormones have an anabolic effect in immature castrated (12) and eugonadal animals (13), as well as in hypogonadal and growth hormone-deficient individuals (14, 15). Most of these anabolic effects are measured by ponderal changes and positive effects on nitrogen balance, and offer us no clue as to the specific mechanisms involved in these actions. Several studies have demonstrated the specific protein anabolic action of androgenic hormones. Athletes taking anabolic steroids have an increase in lean body mass (16); patients with myotonic dystrophy treated with testosterone had a significant increase in muscle protein synthesis, without any detectable change in whole body protein synthesis (17). Since these studies have been performed in the postpubertal male after years of exposure to their endogenous sex hormones, it is difficult to extend their physiological implications to the events at pu-

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1. Abbreviations used in this paper: α , true fractional calcium absorption; α KIC, α -ketoisocaproic acid; GH, growth hormone; IGFBP₃, insulin-like growth factor binding proteins 3; Ra, rate of appearance; TEP, total exchangeable pool; V_a , true dietary absorption of digested calcium; V_{bal} , net calcium retention; Vo^+ , net forward flow of calcium; Vo^- , bone resorption.

erty, when the body is exposed to androgenic hormones after more than a decade of very low exposure. Recent animal experiments have revealed a strong correlation between the size of the muscle free glutamine pool (the most abundant amino acid in the body) and the rate of protein synthesis *in vivo* (18, 19). Other studies suggest that glutamine *per se* may stimulate protein synthesis, and that the size of the available free glutamine pool in muscle may be an index of protein accretion (20–22). Whether such an association between the glutamine pools and whole body protein synthesis occurs during puberty remains to be determined.

The timing of onset of action and the mechanisms by which sex steroids mediate their effects on bone and protein metabolism are largely unexplored in prepubertal and adolescent children. Therefore, the present studies were designed to determine whether exogenous testosterone acutely affects bone and body protein metabolism in prepubertal boys and, if so, to determine whether such changes are the results of increases in bone accretion and protein synthesis and/or decreases in the rates of bone resorption and protein breakdown. In addition, we determined whether the changes in whole body protein anabolism occurred independent of or in conjunction with changes in the whole body pool and turnover of glutamine. To accomplish this, a group of six prepubertal boys received infusions of stable labeled tracers of leucine, glutamine, and calcium before and after a short course of testosterone therapy.

Methods

Subjects

Six healthy boys (mean age = 12.9 ± 0.6 yr) participated in this study after informed written consent was obtained from their parents and assent was obtained from the subjects. All were prepubertal on physical exam (Tanner stage I) and had baseline early morning plasma testosterone concentrations < 30 ng/dl, as well as normal blood chemistries, blood counts, and thyroid function. Subjects were recruited from our pediatric endocrine clinics and had varying degrees of normal variant short stature (mean height = 135.8 ± 4.7 cm) and/or delayed puberty.

Experimental design

Each subject was studied twice. 3 d before the first study (study 1), they consumed a weight maintenance diet of ~ 40 kcal \cdot kg⁻¹ \cdot d⁻¹ and 55% carbohydrates, 25% fat, and 20% protein. A regular calcium intake was encouraged. Meal plans were prepared by our nutritionist and consumed at home, and parents kept careful food records for determination of nutrient and calcium intake. Subjects were admitted to the Jacksonville Wolfson Children's Hospital Clinical Investigation Unit the afternoon before to the study. At 1800 h, a mixture of milk or fruit juice with a stable isotope of calcium, ⁴⁴Ca, (0.5 mg/kg) was consumed orally and a fractionated urine collection was begun and continued for the next 38 h for the measurement of calcium isotopic enrichments. The calcium mixture was prepared 8–12 h before ingestion for equilibration. The evening meal was consumed then, and subjects took nothing by mouth except for water subsequently until 12 noon the next day. At 0700 h the next morning (study 1), two intravenous catheters were placed, one in the antecubital vein for the infusion of substances, and the another in the dorsum of a hand vein for blood sampling, using the heated hand technique (23). At \sim 0800 h (time 0), a primed dose-constant infusion of L-[1-¹³C]leucine (~ 4.50 μ mol/kg; ~ 0.07 μ mol \cdot kg⁻¹ \cdot min⁻¹) was initiated and continued for the next 240 min. Simultaneously, an unprimed continuous infusion of L-[2-¹⁵N]glutamine (~ 0.23 μ mol \cdot kg⁻¹ \cdot min⁻¹) was initiated and continued for 4 h. At -10 min, a slow infusion of 0.6 mg/kg of ⁴²Ca was given over 10 min. Arterialized blood samples and breath samples were collected at frequent intervals as detailed below.

At 240 min, the amino acid tracer infusions were stopped and the subjects ate lunch. At 1600 h, the last blood sample was obtained, and the subjects were discharged with instructions, to complete the urine collection through 0800 h the next morning. For 5 d after discharge, two urine samples were collected daily (in the morning and evening) and kept refrigerated for the measurement of calcium isotopic enrichments.

After the baseline study was completed, five of the six boys received an intramuscular injection of 100 mg of testosterone enanthate, and one subject (subject 3) received 75 mg. 2 wk from the last injection, the same testosterone enanthate dose was repeated, except that subject 3 received 50 mg. The subjects were studied a second time in an identical fashion 4–5 d after the last injection (study 2). Because of a viral illness, subject 2 could not be studied after the second testosterone injection, hence a third injection was given 2 wk after the second one, and the patient was studied 4–5 d after the last injection. The average dose of testosterone was 3 mg/kg. The mode of administration of testosterone enanthate chosen has been shown to produce nearly constant levels of testosterone 4–5 d after injection (24). The doses chosen stem from our clinical experience in youngsters with delayed puberty, in whom similar doses of testosterone promote masculinization and short-term linear growth.

Blood and breath samples

On each study day, arterialized venous blood samples were obtained at min -20 , -15 , -5 , 0 , 10 , 15 , 20 , 30 , 40 , 60 , 80 , 100 , 120 , 140 , 160 , 180 , 200 , and 240 for plasma enrichments of [1-¹³C]leucine, [2-¹⁵N]glutamine, ⁴⁴Ca, and ⁴²Ca. An additional sample was obtained at 480 min for calcium enrichments. Serum GH concentrations were measured at 20-min intervals throughout the infusion study. Plasma IGF-I, IGF binding protein 3 (IGFBP₃), and serum testosterone concentrations were also measured at periodic intervals. Expired air samples were collected to calculate the rate of expiratory loss of ¹³CO₂ at min -20 , -15 , -5 , 120 , 160 , 220 , and 240 .

Assays

All analyses of leucine and glutamine kinetics were performed at the Nemours Children's Clinic Mass Spectroscopy Core Laboratory. The analysis of the calcium kinetics was performed either at the Section on Metabolic Analysis and Mass Spectrometry, National Institute of Child Health and Human Development (Bethesda, MD) or at the United States Department of Agriculture/Agricultural Research Service Children's Nutrition Research Center (Houston, TX). The plasma enrichments of L-[1-¹³C]leucine and L-[2-¹⁵N]glutamine were determined by gas chromatography–mass spectrometry (5970 GC-MS; Hewlett Packard, Palo Alto, CA), as previously described (25, 26). ¹³CO₂ enrichments were determined using an automated dual-inlet isotope mass ratio mass spectrometry (27, 28).

Total carbon dioxide production (\dot{V} CO₂) was determined at three different times during the infusions using a calorimeter (PX-MAX; Medical Graphics Corp., St. Paul, MN). Plasma IGF-I and IGFBP₃ concentrations, as well as serum testosterone were determined by radioimmunoassay (Endocrine Sciences Labs, Calabassas Hill, CA). Growth hormone was measured in duplicate using the immunoradiometric assay of the Nichols Institute (San Juan Capistrano, CA).

Total calcium was measured on all urine samples by flame atomic absorption mass spectrophotometry. Calcium isotope ratios were determined by thermal ionization mass spectrometry using either a Thermoquad or a thermal ionization mass spectrometer (model 261; Finnigan MAT, Bremen, Germany) as previously described (29–32).

Isotopes

99% enriched L-[1-¹³C]leucine (Merck, Sharpe and Dohme, St. Louis, MO) and 99% enriched L-[2-¹⁵N]glutamine (Tracer Technologies, Somerville, MA) were determined to be sterile and pyrogen free (limulus lysate assay) and diluted with 0.9% bacteriostatic saline. Sterile solutions of ⁴²Ca and ⁴⁴Ca were prepared by the National Institutes of Health pharmacy and also tested for pyrogenicity and sterility before

their use. Calcium isotopes were obtained from Oak Ridge National Laboratory (Oak Ridge, TN) as calcium carbonate, dissolved in a hydrochloric acid solution and infused as a calcium chloride salt.

Calculations

Calcium kinetics. The mathematical methods used to calculate true fractional calcium absorption (α) from dual tracers have been described previously (32). The true fractional absorption of calcium is calculated as the ratio of the accumulated oral vs intravenous tracer in urine 24 h after administration:

$$\alpha = \frac{\int_0^t {}^{44}\text{Ca in urine}}{\int_0^t {}^{42}\text{Ca in urine}}$$

V_a (true dietary calcium absorption) is then calculated as

$$V_a = V_i(\text{total calcium intake}) \cdot \alpha.$$

V_{ndo} (endogenous fecal calcium excretion) was estimated from published data (30). V_{bal} (net calcium retention) is then calculated as

$$V_{bal} = V_a - (V_u + V_{ndo}) = V_o^+ - V_o^-,$$

where V_u is the total urinary calcium excretion rate, V_o^+ is the rate of bone accretion, and V_o^- is the rate of bone resorption. The TEP of calcium and the net forward flow of calcium into deep bone (V_o^+) may be calculated from the rate of disappearance of the intravenous tracer in serum over time, using a three-term fit of exponentials as previously described (10). TEP represents the calcium in metabolically active bone that is in equilibrium with the plasma compartment and the extracellular fluid. Finally, V_t (bone turnover) can be calculated from the expression $V_t = V_o^+ + V_u + V_{ndo}$, and V_o^- can be estimated by difference, as shown previously (10).

Leucine kinetics. The plasma enrichment of α -ketoisocaproic acid (α KIC) was used as an index of the intracellular enrichment of leucine (reciprocal pool model). All estimates of whole body leucine metabolism were made at near substrate and isotopic steady state, between 180–240 min. The rate of appearance (Ra) of isotope, the rate of leucine oxidation, and the nonoxidative leucine disappearance were calculated as previously described (33, 34). Because of analytical problems with the α KIC assay in patient 2, the enrichments of plasma α KIC were calculated as 70% of the plasma enrichment of leucine (33, 34).

Glutamine kinetics. Similar equations were used for the calculations of glutamine kinetics (35). However, since glutamine is a nonessential amino acid, its Ra has two components: release from protein breakdown and glutamine de novo synthesis. Release from protein breakdown was estimated from the leucine Ra, assuming contents of 8.0 g of leucine and 13.9 g of glutamine plus glutamate for every 100 g of protein (36–37).

The rise of [$2\text{-}^{15}\text{N}$]glutamine enrichment to plateau was fitted into an exponential curve using nonlinear regression (35):

$$E_p = E_{inf}(1 - e^{-kt}),$$

where E_p is the enrichment in plasma as a function of the sampling time t (min). The fitted parameters were the enrichment at plateau E_{inf} (mol% excess) and the rate constant for amino acid turnover k (min^{-1}). The size of the tracer-miscible glutamine pool was then calculated as $\frac{\text{Ra}_{\text{GLN}}}{k}$ for each individual infusion study.

Statistics

All results are expressed as mean \pm SE. For normally distributed data, one-tailed paired Student's t test was used to calculate differences between the means in different groups. Otherwise, Wilcoxon signed ranks test was used to calculate the differences between groups. Significance was established at $P < .05$.

Results

Table I summarizes the mean changes in body weight and plasma testosterone, IGF-1, IGFBP₃, and GH concentrations during the sampling times at baseline (study 1) and after testosterone therapy (study 2). Each IGF-1 and IGFBP₃ concentration represents the average of three different measurements during a 4-h period; the GH concentrations represent both the mean and peak GH values obtained during every 20 min sampling for 4 h.

There were significant increases in body weight, as well as the expected increase in serum testosterone and IGF-1 concentrations after testosterone administration. Only modest yet significant increases in IGFBP₃ concentrations were observed after androgenic exposure.

Calcium kinetics. Table II summarizes the changes in calcium kinetics after the administration of testosterone in the subjects studied. The fractional absorption of calcium (α), V_a , and V_{bal} increased significantly after testosterone therapy. V_o^+ and the TEP of calcium were also increased by testosterone administration by 16 and 20%, respectively; however, the number of subjects was too small to achieve statistical significance. The value of V_o^- was essentially unchanged after testosterone therapy. However, the relative contribution of bone resorption to bone turnover (parameter E) was lower after testosterone therapy. Fig. 1 represents the mean changes in V_a , V_{bal} , and V_o^+ before and after testosterone therapy.

Leucine kinetics. Data are available in five subjects. The sixth patient had intermittent intravenous pump failure during study 2, so his data are not included in this analysis. The rate of [$1\text{-}^{13}\text{C}$]leucine infusions was identical for both study days, $0.07 \pm 0.003 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. At steady state, the plasma enrichment of [$1\text{-}^{13}\text{C}$] α KIC was 3.24 ± 0.39 and 2.86 ± 0.19 mol% for studies 1 and 2, respectively. The rate of $^{13}\text{CO}_2$ expired was much lower on the second study date as compared to the first one, 0.005 ± 0.001 vs $0.013 \pm 0.001 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, whereas the $\dot{V}\text{CO}_2$ was similar on both study days, 185 ± 11 vs $171 \pm 16 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for study days 1 and 2, respectively. In each patient studied, there was a significant increase in the Ra of leucine (1.80 ± 0.08 vs $2.11 \pm 0.08 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $P = 0.36$) and a concomitant decrease in the rate of leucine oxidation (0.26 ± 0.03 vs $0.13 \pm 0.02 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $P = 0.004$) with a net increase in the nonoxidative rate of leucine disappearance (1.52 ± 0.09 vs $2.03 \pm 0.09 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$,

Table I. Mean \pm SE of Weights and Serum Testosterone, IGF-I, IGFBP₃, and GH Concentrations in Subjects at Baseline (Study 1) and after Testosterone Exposure (Study 2)

	Study 1	Study 2	P
Weight (kg)	35.2 \pm 5.2	37.3 \pm 5.5	<0.001
Testosterone (ng/dl)	14.2 \pm 4.2	831 \pm 102	<0.001
IGF-I (ng/dl)	156 \pm 25	261 \pm 37	0.005
IGFBP ₃ (mg/l)	2.1 \pm 0.2	2.5 \pm 0.3	0.017
Mean GH (ng/ml)	2.2 \pm 0.6	6.6 \pm 1.7	0.030
Peak GH (ng/ml)	10.3 \pm 3.1	22.3 \pm 5.8	<0.050

The average dose of testosterone enanthate was ~ 3 mg/kg given twice in five subjects and three times in one subject. The doses were given 2 wk apart and subjects studied 4–5 d from the last injection.

Table II. Summary of Calcium Kinetics (n = 6)

	Study 1	Study 2
V_i (mg/d)	1280±107	1470±100*
α	0.35±0.07	0.52±0.04*
V_a (mg/kg per d)	13.3±2.3	21.5±2.3*
V_u (mg/kg per d)	3.9±0.5	3.6±0.4
V_{bal} (mg/kg per d)	8.0±2.1	16.6±2.5*
V_o^- (mg/kg per d)	61.1±6.4	63.9±6.1
V_o^+ (mg/kg per d)	72.4±8.7	83.8±6.3
V_t (mg/kg per d)	74.4±6.0	85.5±6.7
TEP (mg/kg)	255.7±30.3	305.6±38.8
$E = V_o^-/V_t$	0.81±0.04	0.75±0.03

* $P \leq 0.05$. V_i , dietary calcium intake; V_u , total urinary calcium excretion; V_t , bone turnover rate; E , contribution to bone resorption to bone turnover.

$P = 0.009$), an indicator of protein synthesis, after relatively short-term exposure to testosterone (Fig. 2).

Glutamine kinetics. Glutamine Ra increased after testosterone administration, from 6.97 ± 0.54 to $9.22 \pm 0.59 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $P < 0.05$. This change of ~ 32% was accounted for by increases in both glutamine release from protein breakdown (2.78 ± 0.12 vs $3.26 \pm 0.12 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), and glutamine de novo synthesis (4.30 ± 1.31 vs $6.10 \pm 1.40 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). Since no priming dose was used during the labeled glutamine infusion, the increase in plasma [2- ^{15}N]-glutamine from each infusion study could be fitted to a curve using nonlinear regression. Fig. 3 shows a plot of the composite time course for the six patients before and after testosterone treatment. While the rate constant parameter k did not vary (0.029 ± 0.003 vs $0.030 \pm 0.004 \text{ min}^{-1}$ after testosterone) on the two study days, there was an increase in the size of tracer-miscible glutamine pool (251 ± 23 vs $330 \pm 43 \mu\text{mol}/\text{kg}$ after testosterone therapy); however, this increase failed to reach statistical significance.

Discussion

Both androgens and estrogens have been used extensively in the treatment of youngsters with exaggerated delay of entry into puberty. In boys, aromatizable (testosterone) and nonaro-

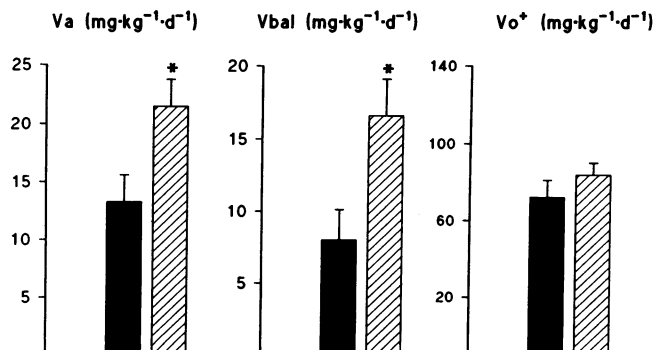


Figure 1. Changes in V_a , V_{bal} , and V_o^+ in prepubertal children treated with testosterone ($n = 6$). ■, Study 1; ▨, study 2; * $P < 0.05$.

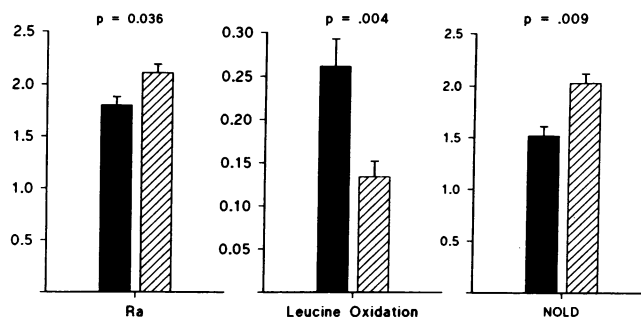


Figure 2. Mean±SE of the rates of appearance, leucine oxidation, and nonoxidative leucine disappearance (NOLD) in five prepubertal boys before (■) and after (▨) treatment with testosterone enanthate for 4–6 wk. Note the difference in the ordinate for the middle panel (leucine oxidation). Units = $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$.

matizable (oxandrolone) androgenic hormones seem to have a positive effect on linear growth and, if continued, on virilization (38–39). The specific bodily changes mediating such an effect have not been studied in detail, specifically the changes in protein accretion and the accrual of calcium into bone.

In these studies on prepubertal boys, we have shown positive changes in all parameters of bone accretion and calcium retention after short-term testosterone therapy. The size of the pool of metabolically active calcium (TEP) was also increased by the administration of androgenic steroids, and the relative contribution of bone resorption to bone turnover was decreased after this short term exposure to testosterone, all of which is compatible with increased growth. Bone mineral density has been shown to be significantly decreased in young adult males who experienced a delayed entry into puberty (constitutional delay of puberty) as compared with men who entered puberty normally (9). Similarly, bone density was increased in hypogonadal young men given testosterone substitution therapy (40). Since calcium is a major component of trabecular bone, our findings in the studies reported here strongly suggest that a pivotal role of both sex steroidal hormones and the tim-

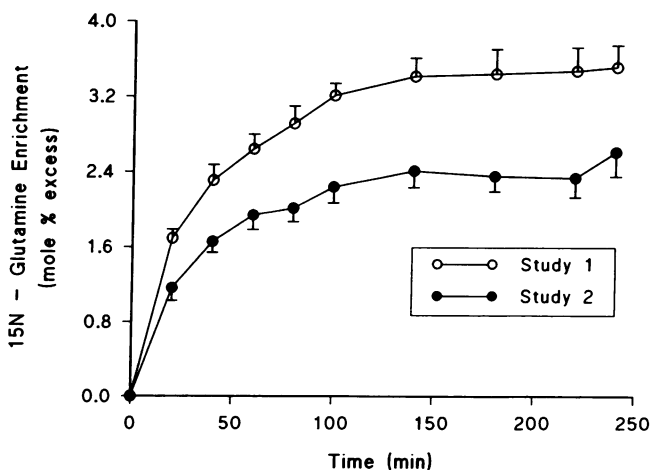


Figure 3. Composite time course of plasma glutamine amino ^{15}N enrichments during unprimed infusion of L-[2- ^{15}N]glutamine. Enrichments were averaged at each time point for the six patients studied. ○, Baseline infusion (study 1); ●, posttestosterone infusion (study 2).

ing of exposure to these hormones in the adequate development of the density of the axial skeleton in man.

The anabolic effects of sex-steroidal hormones on protein and calcium accretion may depend not only on the kind of hormone used, but also on the length of exposure and the sex of the individual as well. Using infusions of stable isotopic tracers of leucine, we have also shown that even short-term exposure to androgenic hormones has a specific yet significant effect on estimates of whole body protein synthesis and proteolysis with an over-all improvement in leucine and presumably protein balance. As shown previously (1), mean GH concentrations and plasma IGF-I concentrations increased significantly with exogenous testosterone therapy. However, GH alone appears to mediate its effects on protein kinetics by selectively increasing estimates of protein synthesis with no apparent effect on estimates of proteolysis (33), whereas exogenous testosterone significantly affects estimates of both proteolysis and protein synthesis in the youngsters studied here.

Our results on whole body protein kinetics differ from that of Griggs and colleagues (17), who found no change in whole body protein anabolism after androgen administration despite increases in muscle protein synthesis in a group of adults with myotonic dystrophy. The differential effect of exogenous testosterone on whole body protein may be caused by the hypoandrogenic state of the boys studied here, contrary to the adults reported previously, who presumably had adequate virilization secondary to the endogenous rise of testosterone. It is conceivable that the amplifying effect of testosterone (and GH) on whole body protein metabolism is exerted most dramatically during the narrow window of the pubertal years in which the body is first exposed to androgens after more than a decade of nonexposure.

We observed a significant increase in glutamine production in the present study, which can indeed be accounted for by an enhanced rate of glutamine synthesis. This may, in turn, be caused by stimulation of the enzyme glutamine-synthetase by testosterone, or alternatively, to increased availability of precursor substrates. We have shown previously that branched-chain amino acids are a major source of glutamine nitrogen (41): increased release of amino acids from greater rates of proteolysis may thus have promoted glutamine synthesis. We observed a similar pattern (~ 40% rise in glutamine synthesis, with an ~ 15% rise in proteolysis) in healthy adults receiving high physiological doses of cortisol (42). Both sex steroids and glucocorticoids increased the rate of appearance of leucine. However, while the glucocorticosteroid administration results in no change in estimates of protein synthesis and directs the excess leucine to oxidative loss, sex steroids significantly reduce the oxidative losses of this essential amino acid while concomitantly increasing estimates of protein synthesis.

We conclude that short-term testosterone therapy in the prepubertal human (a) increases bone calcium accretion by increasing calcium absorption, calcium retention, and net forward flow of calcium into bone while having no effect on bone resorption; (b) in addition, there is a specific and significant increase in estimates of leucine and presumably whole body protein balance after short-term androgen exposure by increasing the rate of leucine entering protein more than that released from protein, suggesting significant redistribution of body amino acid into different protein pools; and (c) we observed comparable increases in glutamine turnover pools and the amount of glutamine released from muscle and glutamine's de

novo synthesis, but could not relate the changes in leucine metabolism to those of the glutamine exchangeable pool. The latter suggests that the changes in whole body protein metabolism induced by testosterone are not mediated predominantly by changes in the glutamine pool.

We postulate a specific amplifying effect of androgenic hormones on protein and calcium metabolism in prepubertal children potentiating growth. Such an effect on protein may result from either a direct impact of androgens or at least partially mediated through the endogenous increase of circulating GH. These specific acute effects of low doses of androgens may be specific for the time of puberty.

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