Metabolism
Clinical and Experimental

VOL 44, NO 4 APRIL 1995

Effect of Testosterone on Bone Density and Bone Metabolism in Adolescent Male Hypogonadism

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To assess the influence of gonadal steroid testosterone (T) on bone mineral status in males during puberty, we observed the response of cortical bone density and serum biochemical parameters of bone metabolism to T treatment in 12 adolescent patients with hypogonadotropic hypogonadism (11 with both gonadotropin and growth hormone deficiency and one with isolated gonadotropin deficiency). The 12 patients aged 15 to 21 years (Tanner stage I to II) were divided into two groups: group 1 (n = 6) given T treatment for 2 consecutive years, and group 2 (n = 6) without T treatment for the first year and then with T treatment for the second year. Cortical bone density measured in the radius was less than the age-matched mean value for normal subjects in all 12 patients (groups 1 and 2) at the start of the study. Bone density in group 1 increased significantly during the 2-year T treatment period, but did not increase in group 2 during the first year without T treatment, although an increase was observed during the subsequent year with T treatment. Among circulating biochemical factors such as osteocalcin, parathyroid hormone (PTH), 25-hydroxyvitamin D (25-OHD), and 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D], only osteocalcin showed an increase in response to T treatment in both groups. Levels of insulin-like growth factor-I (IGF-I) remained consistently low and did not change in any patients except one with isolated gonadotropin deficiency. These data indicate that the impaired mineralization due to T deficiency during puberty may be reversed to some extent by extrinsic T replacement even after puberty, and that T exerts effects on bone tissue directly even in the absence of systemic growth hormone and IGF-I.

BONE FORMATION and mineralization are governed by many factors such as genetics, nutrition, various hormones, and physical activity. Abnormalities in these factors are thought to be responsible for decreased bone formation or mineralization.[1,2] Puberty is known to influence bone growth; during this period, accumulation of skeletal mass occurs through linear growth and an increase in bone density.[3,4] Both postmenopausal women and young women with delayed menarche or delayed pubertal development have been demonstrated to have associated osteoporosis. Therefore, gonadal function is considered important in both young and postmenopausal women for maintenance of normal bone mineral content.[5]

On the other hand, adult men with hypogonadism are known to have associated osteoporosis.[6] Premature osteoporosis has also been reported in men with hypogonadism associated with hyperprolactinemia,[7] Klinefelter's syndrome,[8] idiopathic hypogonadotropic hypogonadism,[9] and delayed puberty.[10,11] In males, deficiency of gonadal steroid hormone (testosterone [T]) during adolescence may be an important risk factor for future development of osteoporosis. Therefore, the effects of T on bone have been studied,[12-14] although there are few data on the effects of long-term androgen replacement on bone mineral density and bone metabolism.

To elucidate these aspects, we assessed the effects of gonadal steroid T replacement on bone mineral density and bone metabolism in patients with adolescent male hypogonadism for 2 years.

SUBJECTS AND METHODS

Patients

Twelve males aged 15 to 21 years (median age, 18) with delayed puberty (external genitalia Tanner stage I to II) were selected. One patient (no. 1) had idiopathic isolated gonadotropin deficiency, and the other 11 had proven growth hormone deficiency associated with gonadotropin deficiency. They had documented growth hormone deficiency, with peak blood levels of growth hormone less than 7.0 ng/mL (<322 pmol/L) after insulin-induced hypoglyce-

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Submitted January 13, 1992; accepted July 1, 1994.
Presented in part at the 26th Annual Meeting of the Japanese Society for Pediatric Endocrinology, Kanazawa, Japan, October 2, 1992.
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0026-0495/95/4404-0001$03.00/0

mia and arginine infusion. All patients had been found to have low plasma baseline T levels of less than 78 ng/dL (2.8 nmol/L) and low serum gonadotropin concentrations in response to intravenous bolus infusion of luteinizing hormone–releasing hormone. Human growth hormone therapy had been started at least by age 11 years in all patients. Two patients (no. 7 and 8) were receiving human growth hormone therapy at the time of examination (0.03 IU/kg/wk); their bone ages were 11 years and 12 years, 6 months, respectively (by the atlas of Greulich and Pyles). The remaining patients had discontinued growth hormone therapy 1 to 2 years previously.

The endocrine status of the patients' (no. 1 to 12) thyroid function was normal (thyroid hormone replacement had not been performed), and basal levels of cortisol and prolactin in serum were within normal limits, as were serum levels of calcium, phosphorus, and creatinine. The skeletal ages of the patients (except for no. 7 and 8) were 14 to 18 years. No patients had received prior androgen replacement therapy.

Study Design

The entire period of the study was 24 months. The study consisted of an initial randomized, open-study phase over a period of 12 months, with two parallel groups of six patients treated with T (group 1) and six untreated patients who served as controls (group 2). After the initial 12-month open phase, all patients in both groups were treated with T for another 12 months.

T (T enanthate 125 mg intramuscular) was administered every 2 months. Bone density (distal radius) in each patient was measured at the start of the study and at 12 and 24 months (except for patient no. 1, in whom bone density was measured at the start and at 3, 12, and 24 months). As biochemical parameters of bone metabolism, serum levels of osteocalcin, parathyroid hormone (PTH), vitamin D metabolites 25-hydroxyvitamin D (25-OHD) and 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D], and insulin-like growth factor-I (IGF-I) were assayed at the start of the study and at 12 and 24 months.

All patients gave informed consent for participation in the study, which was approved by the ethics committee of our institution.

Bone Density Determination

Bone density (cortical bone) was determined by ^{125}I single-photon absorptiometry; quadruplicate readings were obtained from the junction of the proximal two thirds and distal one third of the nondominant radius. Bone density was measured as grams per centimeter squared (bone mineral content in grams per centimeter divided by bone width in centimeters). The coefficient of variation for repeated measurements over a period of 1 year was 1.5% to 2.0% for measurement of a phantom. The age-matched controls were 117 normal males aged 12 to 30 years who had no systemic diseases, including renal disease and growth disorders.

Biochemical Parameter Assays

Blood samples were taken between 10 AM and noon (at least 1 week after cessation of human growth hormone injection in patients receiving therapy), and serum was stored at −20°C until assayed.

Osteocalcin was determined by radioimmunoassay. The antiserum used was raised in rabbits immunized with purified intact calf osteocalcin. Standards and samples were analyzed in duplicate. The sensitivity of the assay was 0.44 ng/mL (0.07 nmol/L), and intraassay and interassay coefficients of variation were less than 5% and less than 10%, respectively. The mean serum osteocalcin concentration in 68 normal males aged 17 to 23 years was 15.8 ± 2.5 ng/mL (2.5 ± 0.4 nmol/L, mean ± SD).

PTH was determined by radioimmunoassay using an antibody directed against the midregion of the molecule (amino acids 43 to 68). The mean serum PTH level in 68 normal males aged 17 to 23 years was 250 ± 65 pg/mL (25 ± 6.5 pmol/L).

Vitamin D metabolites 25-OHD and 1,25-$(OH)_2$D were assayed by competitive protein binding assay and radioreceptor assay, respectively. Details of the methods have been described previously.[15] The concentration of serum 25-OHD in 24 normal males aged 17 to 20 years was 17.4 ± 3.1 ng/mL (43.5 ± 7.8 nmol/L), and serum 1,25-$(OH)_2$D was 41.6 ± 8.1 pg/mL (90.7 ± 17.7 pmol/L).

IGF-I level was measured by radioimmunoassay, after extraction by acid ethanol to separate IGF-I from IGF-binding proteins; details of the method have been described elsewhere.[16] The mean serum level in 17 normal controls aged 17 to 23 years was 280 ± 85 ng/mL; at this age, IGF-I levels less than 40 ng/mL corresponded roughly to plasma IGF-I levels less than 0.14 U/mL as determined by the direct IGF-I assay using a Nichols kit (San Juan Capistrano, CA).

Statistical Analysis

Comparison of all parameters between groups 1 and 2 was made by Student's nonpaired *t* test. Comparisons of all baseline and follow-up parameters within each group were made by Student's paired *t* test.

RESULTS

Bone Density

Radial bone density at the first determination was significantly less than (−3.6 to −0.9 SD) that of age- and sex-matched controls in both group 1 ($P < .0001$) and group 2 ($P < .0001$).

At 12 months, bone density was increased in all six patients in group 1 ($P < .05$). In contrast, bone density in group 2 did not show a significant increase; in three of six patients it had not changed or had decreased. Statistical analysis for group comparison during the initial period of 12 months showed that the increase in bone density in group 1 was highly significant ($P < .01$) as compared with group 2.

At 24 months, bone density had increased further in group 1 in comparison to the value at 12 months ($P < .05$). Bone density of patients in group 2, who had been treated with T during the final 12 months, showed a significant increase in comparison to the pretreatment value at 12 months ($P < .01$) (Fig 1).

Biochemical Parameters

Mean levels of circulating osteocalcin, PTH, 25-OHD, and 1,25-$(OH)_2$D in groups 1 and 2 at initial assay showed no significant differences in comparison to levels in normal subjects. Serum IGF-I levels were markedly low, except in one patient (no. 1) with isolated gonadotropin deficiency who had no growth hormone deficiency. Serum IGF-I levels were low even in patients (no. 7 and 8) who received growth hormone therapy during the study. For group comparison of parameters (osteocalcin, PTH, 25-OHD, 1,25-$(OH)_2$D, and IGF-I), there were no significant differences between groups 1 and 2.

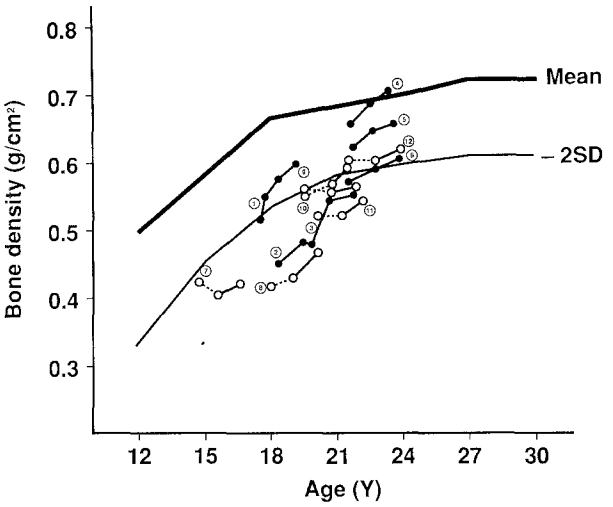

Fig 1. Changes in bone density in patients with male hypogonadism. (●) group 1 with T treatment; (○- - -○) group 2 without T treatment; (○—○) group 2 with T treatment.

At 12 months after T treatment, the serum osteocalcin level in group 1 was increased significantly in comparison to the pretreatment level ($P < .05$) and remained elevated above the pretreatment level at 24 months. The level of osteocalcin did not increase in group 2 during the initial 12 months without T treatment but increased significantly during the subsequent 12 months of T treatment, as compared with the pretreatment level ($P < .05$). PTH, 25-OHD, and 1,25-$(OH)_2$D showed no significant change in both groups. IGF-I level did not change except in one patient (no. 1) with isolated gonadotropin deficiency (Table 1).

DISCUSSION

Skeletal mass in adulthood is the result of both the amount of bone gained during growth and its subsequent rate of loss.[1,4,17] A marked increase in cortical bone density and trabecular bone density during puberty and adolescence has been reported.[1,3,4] Therefore, it seems that factors affecting bone mineralization during growth are important determinants of future skeletal resistance to mechanical forces.

Growth hormone, sex steroids, and IGF-I are important regulators of bone growth during puberty.[1-4,18,19] In normal puberty, the marked accretion of mineralized bone is supported by biochemical findings indicating an increase in the concentration of osteocalcin (a marker of bone formation) in serum; in boys, the level of osteocalcin has been demonstrated to increase in parallel with increased plasma T concentration.[20] Lower bone density in association with reduced serum osteocalcin concentration and the reverse change in response to human growth hormone treatment in patients with growth hormone deficiency have been well documented.[21] Shore et al[22] demonstrated that the reduction of bone density was more pronounced during puberty in some patients with growth hormone deficiency even after treatment with human growth hormone. This observation

Table 1. Changes in Biochemical Parameters in Patients With Male Hypogonadism

	Osteocalcin (ng/mL)			PTH (pg/mL)			25-OHD (ng/mL)			1,25-$(OH)_2$D (pg/mL)			IGF-I (ng/mL)		
Case No.	Start	12 Months	24 Months	Start	12 Months	24 Months	Start	12 Months	24 Months	Start	12 Months	24 Months	Start	12 Months	24 Months
Group 1 with T treatment (0-24 months)															
1	13	18	17	110	160	150	14	18	17	47	45	44	78	90	94
2	17	19	18	180	180	180	17	26	23	38	45	49	39	40	37
3	13	18	19	300	320	310	19	15	15	45	40	41	37	35	35
4	6	10	9	280	210	260	15	17	16	39	43	34	32	33	34
5	23	27	26	240	220	220	17	14	19	35	37	33	22	18	19
6	21	24	24	250	260	240	15	14	15	46	44	39	21	20	20
Mean ± SD	16 ± 6	19 ± 5	19 ± 5	226 ± 64	225 ± 53	227 ± 52	16 ± 2	17 ± 4	18 ± 3	42 ± 5	43 ± 3	40 ± 6	38 ± 19	39 ± 24	40 ± 25
		* NS *			NS NS NS			NS NS NS			NS NS NS			NS NS NS	
Group 2 without (0-12 months) and with (12-24 months) T treatment															
7	17	14	19	120	100	110	18	19	27	36	31	33	55	60	57
8	14	15	20	370	380	340	15	10	11	40	40	39	61	67	60
9	21	13	25	320	320	340	19	21	19	53	46	41	27	19	25
10	23	23	27	150	200	210	12	19	15	38	39	35	34	31	34
11	9	12	15	230	300	240	13	14	17	42	39	31	24	23	27
12	18	16	19	320	420	390	12	17	16	51	40	49	23	21	25
Mean ± SD	17 ± 5	16 ± 4	21 ± 4	252 ± 93	287 ± 108	272 ± 95	15 ± 3	17 ± 4	18 ± 5	43 ± 6	39 ± 4	38 ± 6	37 ± 15	37 ± 19	38 ± 15
		NS * *			NS NS NS			NS NS NS			NS NS NS			NS NS NS	
Normal range	11-22			120-380			10-24			24-58			120-560		

*Statistically significant difference between values, $P < .05$.

†Expressed as 95% confidence ranges.

may indicate that insufficient gonadal sex steroid secretion during puberty results in failure of bone mineralization in patients who have associated growth hormone and gonadotropin deficiency (hypopituitarism associated with hypogonadism).

In the present study, it appeared that cortical bone density was decreased in adolescent male patients with hypogonadism in comparison to normal controls. Gonadal sex steroid T deficiency seemed to be responsible for this impaired bone mineralization. Patients with growth hormone deficiency had received growth hormone therapy; therefore, growth hormone deficiency might have been less responsible for the impaired bone mineralization than T deficiency in these patients. Furthermore, the fact that bone density increased in response to T treatment and that these changes were associated with increases in serum osteocalcin levels may imply a normal process of bone mineralization like that occurring during puberty. Therefore, T deficiency during the pubertal period may be critical for bone mineralization, although this status may be reversed to some extent after puberty by T treatment.

With regard to the effect of T on bone tissue, the hormone may promote bone mineralization in several ways, including activation of the PTH–vitamin D axis, enhancement of growth hormone secretion followed by enhanced IGF-I action on bone, and probably a direct effect on bone.[1-3,12-14,17-19] In the present study, normal concentrations of PTH and vitamin D metabolites 25-OHD and $1,25\text{-}(OH)_2D$ before and during T replacement seemed to indicate that the PTH–vitamin D axis in patients with hypogonadism was not deranged, even though a decrease in serum $1,25\text{-}(OH)_2D$ concentration due to a reduction of renal 1α-hydroxylase activity mediated by T deficiency has been demonstrated to be the pathogenetic mechanism of osteoporosis in male hypogonadism.[23]

Furthermore, the dominant effect of T on bone tissue may be direct rather than indirect, since osteocalcin increased in the absence of changes in levels of calcium-regulating hormones and IGF-I binding protein–free IGF-I. In fact, 11 of the 12 patients had growth hormone deficiency; thus, the possibility of osteocalcin production by bone cells through the action of increased circulating IGF-I[18,24] (caused by enhancement of pituitary growth hormone secretion through the action of T) seemed unlikely, although T could have effects on local IGF-I levels in bone tissue that are not reflected by the serum concentration. An experimental study using hypophysectomized animals has proved that T exerts direct effects on bone tissue without growth hormone effects,[25] and it has been demonstrated that human osteoblasts have receptors for T.[26]

In summary, deficient T secretion during puberty may impair bone mineralization, but the altered mineral status is likely to be reversed to some extent by treatment with T from adolescence onward. T may exert effects on bone tissue even in the absence of systemic growth hormone and IGF-I.

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