0021-972X/04/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 89(6):3033–3041

doi: 10.1210/jc.2003-031992

Recombinant Human Chorionic Gonadotropin But Not Dihydrotestosterone Alone Stimulates Osteoblastic Collagen Synthesis in Older Men with Partial Age-Related Androgen Deficiency

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Several randomized trials of androgen supplementation in older men have been undertaken. However, the relative contributions of testosterone (T) and estrogens on bone metabolism in aging men are controversial. Within the setting of two double-blind, placebo-controlled studies, we evaluated the effect of dihydrotestosterone (DHT) and recombinant human chorionic gonadotropin (rhCG) on bone turnover in healthy, community-dwelling older men with partial androgen deficiency (total T ≤ 15 nmol/liter). In the first study, 35 men (age 68.3 ± 6.8 yr; baseline T, 13.9 ± 3.3 nmol/liter) were randomized to receive either daily transdermal DHT (n = 17) or placebo for 3 months. In the second study, 40 men (age 67.4 ± 5.4 yr; baseline T, 11.4 ± 2.2 nmol/liter) were randomized to receive either rhCG sc (n = 20), two injections weekly, or placebo for 3 months. The following parameters were measured before, monthly during, and 1 month after treatment: serum T, estradiol (E2), and LH; markers of bone formation, serum amino-terminal propeptide of type I procollagen (S-PINP) and osteocalcin; markers of bone resorption, serum carboxyterminal cross-linked telopeptide of type I collagen and urinary deoxypyridinoline. Compared with placebo, treatment with DHT significantly increased serum DHT and suppressed LH and T levels, whereas E2 concentrations and markers of bone turnover did not change. In contrast, rhCG therapy significantly increased both T and E2, with the increases in E2 being supraphysiological. At the same time, rhCG significantly increased S-PINP concentrations with peak levels after 1 month (Δ40%; $P = 0.02$ compared with placebo). In contrast, serum osteocalcin and carboxyterminal cross-linked telopeptide of type I collagen and urinary deoxypyridinoline levels did not change. The change in S-PINP levels correlated with the change in E2 levels ($r = 0.59$; $P = 0.02$) but not with a change in T.

We conclude that in older men with partial age-related androgen deficiency, rhCG treatment stimulates osteoblastic collagen formation proportionally to increased E2 concentrations but does not alter markers of mature osteoblastic function or bone resorption. In contrast, treatment with a pure, nonaromatizable androgen (DHT) has no effect on bone turnover despite a 20-fold increase in serum levels. Bone resorption was not accelerated during unchanged (DHT) or increased (rhCG) E2 levels, suggesting that minimal E2 levels are needed to maintain stable resorption, although direct androgen receptor-mediated effects cannot be excluded. If androgen supplementation is required for aging men, aromatizable androgens with sufficient endogenous estrogenic activity may have the most beneficial effects on bone. (*J Clin Endocrinol Metab* 89: 3033–3041, 2004)

OSTEOPOROSIS IS A major cause of morbidity in men (1). The incidence of osteoporosis and osteoporosis-related fractures increase with age (2, 3), and among other factors, osteoporosis may be related to the age-associated gradual decline in sex hormones. Longitudinal population-based studies have consistently reported that serum testosterone (T) declines by 1–2% per year (4, 5). Several randomized placebo-controlled clinical trials of androgen supplementation in older men have therefore been undertaken to investigate the effect of T replacement therapy on muscle and physical performance in particular (6–13), but other androgen targets including bone have also been examined (7, 11, 14). Most of these studies show that T replacement in older men does not alter biochemical markers of bone turnover. In addition, T replacement did increase lumbar bone density only in those men with low pretreatment serum T concentrations (14). It remains unclear, however, whether the observed limited effect of T on bone turnover is due to a lack of estradiol (E2) or dihydrotestesterone (DHT), because both hormones are largely products of T metabolism. Specifically, the effects of DHT, a nonaromatizable androgen, or human chorionic gonadotropin (hCG), which is known to have profound effects on circulating E2 and T levels, have been little studied. These hormones may have differing effects on bone according to relative susceptibility to aromatization and/or 5α-reduction. T *per se* may act directly on osteoblasts via the androgen receptor or indirectly through the estrogen receptors via aromatization to E2. Approximately 60% of circulating E2 is derived from peripheral aromatization of circulating T, whereas approximately 20% is the product of peripheral conversion of estrone to E2, and 15–20% is directly

Abbreviations: CV, Coefficient of variation; DHT, dihydrotestosterone; E2, estradiol; rhCG, recombinant human chorionic gonadotropin; S-BAP, serum bone-specific alkaline phosphatase; S-ICTP, serum C-terminal telopeptide of type I collagen; S-OC, serum osteocalcin; S-PINP, serum amino-terminal propeptide of type I procollagen; T, testosterone; U-DPD, urinary free deoxypyridinoline.

secreted by the testes (15). Although gonadal hormones are essential for the accrual and maintenance of bone mass and both androgens and estrogens are important for skeletal homeostasis in men, the relative importance of each is little studied.

Randomized placebo-controlled studies examining the effect of either rhCG or DHT on bone turnover in elderly men have not previously been published, although such therapy has been assessed for physical and sexual performance efficacy (12, 13, 16). Due to differing effects on E2 in particular, contrasting the effects of these therapies on bone formation and resorption may clarify mechanistically the relative importance of androgenic or estrogenic effects. Within the setting of two contemporaneous double-blind, placebo-controlled studies, we therefore evaluated the effect of rhCG and DHT on bone turnover in healthy, community-dwelling older men with partial androgen deficiency using blood and urine samples stored *a priori* for future measurement of biochemical markers of bone turnover.

Subjects and Methods

Study design and population

Two separate but identically designed randomized, double-blind, placebo-controlled clinical trials, each of 3 months duration, were conducted in a single center. In the first study, DHT (70 mg daily) was administered as a transdermal gel (Andractim, Laboratoires Besins-Iscovesco, Paris, France) (12). The second trial used recombinant hCG (rhCG), 250 μg (5000 IU) twice weekly by self-administered sc injection (Ovidrel, Serono Australia, Sydney, Australia) (13).

In the DHT study, at randomization, 18 men were assigned to DHT and 19 to placebo. Two subjects discontinued the study after randomization, one man (on DHT) because he disliked the gel, and the other man (on placebo) due to self-perceived worsening of arthritis. In the rhCG study, 20 men were randomized to receive rhCG and 20 men to receive placebo, and there were no discontinuations.

Both studies were identically designed, were contemporaneously performed at the same institution, and recruited participants from the same population. Both studies included healthy ambulatory men over 60 yr of age with partial androgen deficiency, defined as a low serum total T (<15 nmol/liter) measured on two separate occasions. Men were excluded due to the presence of prostatic disease requiring medical or surgical treatment or significant chronic medical diseases likely to interfere with safe participation (including advanced chronic renal or liver disease, unstable chronic pulmonary or cardiovascular disease, uncontrolled or severe hypertension, hyperlipidemia, obstructive sleep apnea, polycythemia, or malignancy with poor prognosis). Patients were also excluded from the study if they were taking medications that were known to affect bone metabolism (*i.e.* glucocorticoids, calcitonin, anticonvulsants, vitamin D or analogs, androgens or other sex steroids, and antiandrogens). Study subjects received no payment for their participation. Both studies were approved by the Central Sydney Area Health Ethics Committee, according to the Declaration of Helsinki, and all participants provided written informed consent.

Laboratory measurements

Subjects were assessed and blood and urine specimens were obtained at baseline, monthly during the 3-month treatment period, and 1 month after cessation of treatment. Serum and urine samples were collected in the fasting state between 0830 and 1030 h, processed within 30 min, and stored at –20 C until analysis. Hormones were measured as described previously (12, 13, 17–19). Briefly, serum LH [coefficient of variation (CV), 5.0–7.4%], total T (CV, 7.8–12.7%), and SHBG (CV, 6.1–7.9%) were measured by commercial autoanalyzer immunoassays (LH assayed by Axsym, Abbot Laboratories, Abbott Park, IL; total T and SHBG assayed by Immulite, Diagnostics Products Corp., Los Angeles, CA). E2 was measured from unextracted serum samples using the Delfia assay (Perkin-Elmer Life Sciences, Rowville, Australia) (CV, 1.2–5.8%). DHT was measured by the permanganate method using a T antibody (C0457, Bioquest, North Ryde, Australia) (CV, 3.8–4.6%). Ether extracts of plasma samples were oxidized by exposure to 0.5% potassium permanganate for 30 min, which was terminated by ether extraction. Full procedural recovery was calculated for each sample using tritiated DHT.

Serum C-terminal telopeptide of type I collagen (S-ICTP; intra- and interassay CVs, <10%) and serum N-terminal propeptide of type I collagen (S-PINP; intra- and interassay CVs, <15% and <9%, respectively) were measured using competitive RIAs (Orion Diagnostica, Espoo, Finland) (20, 21). Serum bone-specific alkaline phosphatase (S-BAP; intra- and interassay CVs, <6.5%) was measured using an autoanalyzer immunoassay (Access Ostase, Beckman Coulter, Brea, CA) (22). Serum intact osteocalcin (S-OC; intra- and interassay CVs, <5% and <8%, respectively) and urinary free deoxypyridinoline (U-DPD; intra and interassay CVs: <15% and 20%, respectively) were measured using autoanalyzer immunoassays (Immulite) (23, 24). All samples from a single subject were run in duplicate back to back in one assay.

Statistical analysis

All data are expressed as means ± SD unless stated otherwise. Unpaired *t* test (two-sided), or Mann-Whitney *U* test for nonnormal distributed data, was used to identify demographic variables showing differences among groups. Differences in proportion were tested with Fisher's exact test. Response variables were calculated as the difference from baseline. DHT and rhCG effects on continuous response variables were estimated by the main effects of treatment (DHT or rhCG *vs.* placebo; baseline through to month 3) in a repeated-measures ANOVA model. Correlations were calculated using Pearson's linear regression coefficient. Data were analyzed by using SPSS version 10 (SPSS, Inc., Chicago, IL). A two-tailed P value less than 0.05 was considered significant.

Results

At baseline, groups in both studies as defined by treatment assignment were well matched for age, hormone levels, and biochemical markers of bone turnover. Men in the placebo group of the DHT study were heavier ($P = 0.05$), had a higher body mass index ($P = 0.02$), and had lower T concentrations ($P = 0.045$) (Table 1).

Treatment with DHT

Plasma DHT concentrations increased during treatment (between-group effect, $P < 0.001$), resulting in a marked decrease in plasma T ($P < 0.001$) and LH ($P < 0.001$). All hormonal changes had returned to baseline at 1 month after cessation of treatment. Serum E2 levels remained unchanged during DHT treatment ($P = 0.33$; Table 2). Treatment with DHT did not induce any significant changes in markers of bone formation (S-PINP, $P = 0.87$; S-OC, $P = 0.54$; S-BAP, $P = 0.69$) or of bone resorption (S-ICTP, $P = 0.11$; U-DPD, $P = 0.36$) (Fig. 1).

Treatment with rhCG

Treatment with rhCG increased serum T and E2 and suppressed LH levels (between-group effect, $P < 0.001$). In all subjects except two, T concentrations remained well within the young eugonadal reference range (11.0–35.0 nmol/liter) throughout the whole study period. In these two men, T was elevated only on one occasion, 1 month after commencing rhCG therapy. In contrast, serum E2 levels were above the reference range for young adult men (80–180 pmol/liter) in all but one man treated with rhCG. All hormonal changes

TABLE 1. Baseline characteristics by study and treatment assignment

Characteristics	DHT study		rhCG study	
	Placebo (n = 18)	DHT (n = 17)	Placebo (n = 20)	rhCG (n = 20)
Age (yr)	68.0 ± 6.0	71.7 ± 7.0	66.3 ± 4.8	68.7 ± 5.8
Body weight (kg)	85.8 ± 12.4[a]	78.3 ± 9.7	82.0 ± 15.8	82.6 ± 12.5
Height (cm)	172 ± 6	173 ± 6	174 ± 6	176 ± 7
Body mass index (kg/m^2)	28.9 ± 3.9[b]	26.0 ± 2.7	27.2 ± 5.3	26.7 ± 3.0
Smokers (%)	9 (50)	8 (47)	9 (47)	10 (53)
Testosterone (nmol/liter)	12.8 ± 3.1[b]	15.0 ± 3.1	11.8 ± 2.2	11.1 ± 2.2
DHT (nmol/liter)	1.4 ± 0.3	1.4 ± 0.4	NM	NM
Estradiol (pmol/liter)	165 ± 49	169 ± 39	125 ± 36	127 ± 22
SHBG (nmol/liter)	33.9 ± 9.1	37.9 ± 8.2	29.4 ± 12.9	35.4 ± 12.1
LH (IU/liter)	5.1 ± 3.3	4.5 ± 2.0	5.7 ± 4.5	5.3 ± 4.5
Bone alkaline phosphatase (U/liter)	28.6 ± 11.7	23.1 ± 6.7	NM	NM
S-PINP (μg/liter)	43.2 ± 16.5	33.7 ± 7.4	34.3 ± 8.7	40.6 ± 13.9
Osteocalcin (μg/liter)	1.0 ± 1.0	0.8 ± 0.3	0.7 ± 0.5	0.8 ± 0.9
S-ICTP (μg/liter)	3.8 ± 0.6	3.6 ± 0.4	3.8 ± 0.7	4.2 ± 1.0
U-DPD/creatinine (nM/mM)	6.6 ± 2.0	5.6 ± 0.8	5.8 ± 1.2	6.2 ± 1.1

Values are expressed as mean ± SD or frequency (%). NM, Not measured.
P values are given for differences between the placebo and treatment groups: [a] $P = 0.05$ *vs.* DHT group; [b] $P < 0.05$ *vs.* DHT group.

TABLE 2. Hormone values at baseline, during the 3-month treatment period, and 1 month after cessation of treatment for both studies

	Trial group	Baseline	1 month	2 months	3 months	4 months	Between-group effect
T (nmol/liter)	DHT	15.0 ± 3.1	6.6 ± 2.9	6.5 ± 2.5	5.6 ± 2.6	14.4 ± 4.4	$P < 0.001$
	Placebo	12.8 ± 3.1	12.2 ± 2.4	11.9 ± 1.9	12.6 ± 2.9	12.6 ± 2.7	
	rhCG	11.1 ± 2.2	25.4 ± 9.7	25.1 ± 6.8	21.2 ± 6.1	14.5 ± 4.6	$P < 0.001$
	Placebo	11.8 ± 2.2	13.5 ± 3.6	14.5 ± 4.4	14.1 ± 2.9	14.5 ± 3.5	
DHT (nmol/liter)	DHT	1.4 ± 0.4	16.3 ± 9.6	16.6 ± 13.8	22.3 ± 15.1	3.0 ± 4.6	$P < 0.001$
	Placebo	1.4 ± 0.3	1.4 ± 0.3	1.4 ± 0.4	1.4 ± 0.4	1.3 ± 0.4	
	rhCG	NM	NM	NM	NM	NM	
	Placebo	NM	NM	NM	NM	NM	
E2 (pmol/liter)	DHT	169 ± 39	179 ± 99	162 ± 47	141 ± 40	181 ± 74	$P = 0.33$
	Placebo	165 ± 49	182 ± 50	168 ± 62	170 ± 55	177 ± 61	
	rhCG	127 ± 22	309 ± 97	327 ± 118	307 ± 108	136 ± 39	$P < 0.001$
	Placebo	125 ± 36	128 ± 29	125 ± 30	132 ± 27	125 ± 30	

P values are related to differences between treatment and placebo groups. Values are expressed as mean ± SD. NM, Not measured. Reference ranges: T, 8.7–33 nmol/liter; DHT, 0.8–3.3 nmol/liter; E2, 57–206 pmol/liter.

had returned to baseline by 1 month after cessation of treatment (Table 2).

Treatment with rhCG resulted in a significant increase in S-PINP (between-group effect, $P = 0.02$), which peaked at 1 month (increase by 40%), was sustained throughout the treatment period, and completely reversed 1 month after treatment was stopped. In contrast, no significant treatment effects were seen for S-OC ($P = 0.32$), S-ICTP ($P = 0.86$), and U-DPD levels ($P = 0.10$) (Fig. 2).

Correlation analysis

At baseline, no significant correlations were found between hormone values and markers of bone formation and resorption. Furthermore, baseline T, E2, or DHT levels did not correlate with the change in bone turnover markers (as assessed as difference between baseline levels and peak levels after 1 month). During rhCG treatment, however, the change in S-PINP levels correlated positively with the change in serum E2 levels ($r = 0.59$; $P = 0.02$), whereas no significant correlations were seen between S-PINP and the change in T levels (Fig. 3). The change in U-DPD was correlated with the change in E2 levels ($r = 0.65$; $P = 0.002$) (Fig. 4) only, whereas changes in S-OC and S-ICTP were not correlated with the changes in E2 or T. In the total group of DHT-treated subjects (n = 20), the change in serum DHT was correlated with the change in S-OC ($r = 0.49$; $P = 0.04$). After removal of one patient with extremely low values for both ΔDHT and ΔS-OC, the association was weaker, but the trend was still visible ($r = 0.44$; $P = 0.09$). No associations were observed with S-PINP, S-ICTP, and U-DPD or between any marker and the change in serum E2.

Discussion

The randomized placebo-controlled study examined the effect of rhCG on bone turnover and showed that 250 μg (5000 IU) rhCG administered sc twice weekly increases S-PINP but has no effect on S-OC levels. Because S-PINP is generated from newly synthesized collagen in a stoichiometric fashion, it is considered a measure of newly formed type I collagen and therefore a marker of early osteoblastic function (25). In contrast, although the exact function of S-OC is unknown, its strong affinity for hydroxyapatite crystals of mineralized bone suggests that it is a marker of mature osteoblasts and hence a later marker of osteoblast differentiation (26).

The effect of rhCG on S-PINP was maximal at 1 month, sustained for the entire 3-month treatment period, and was completely reversed 1 month after the end of therapy. In-

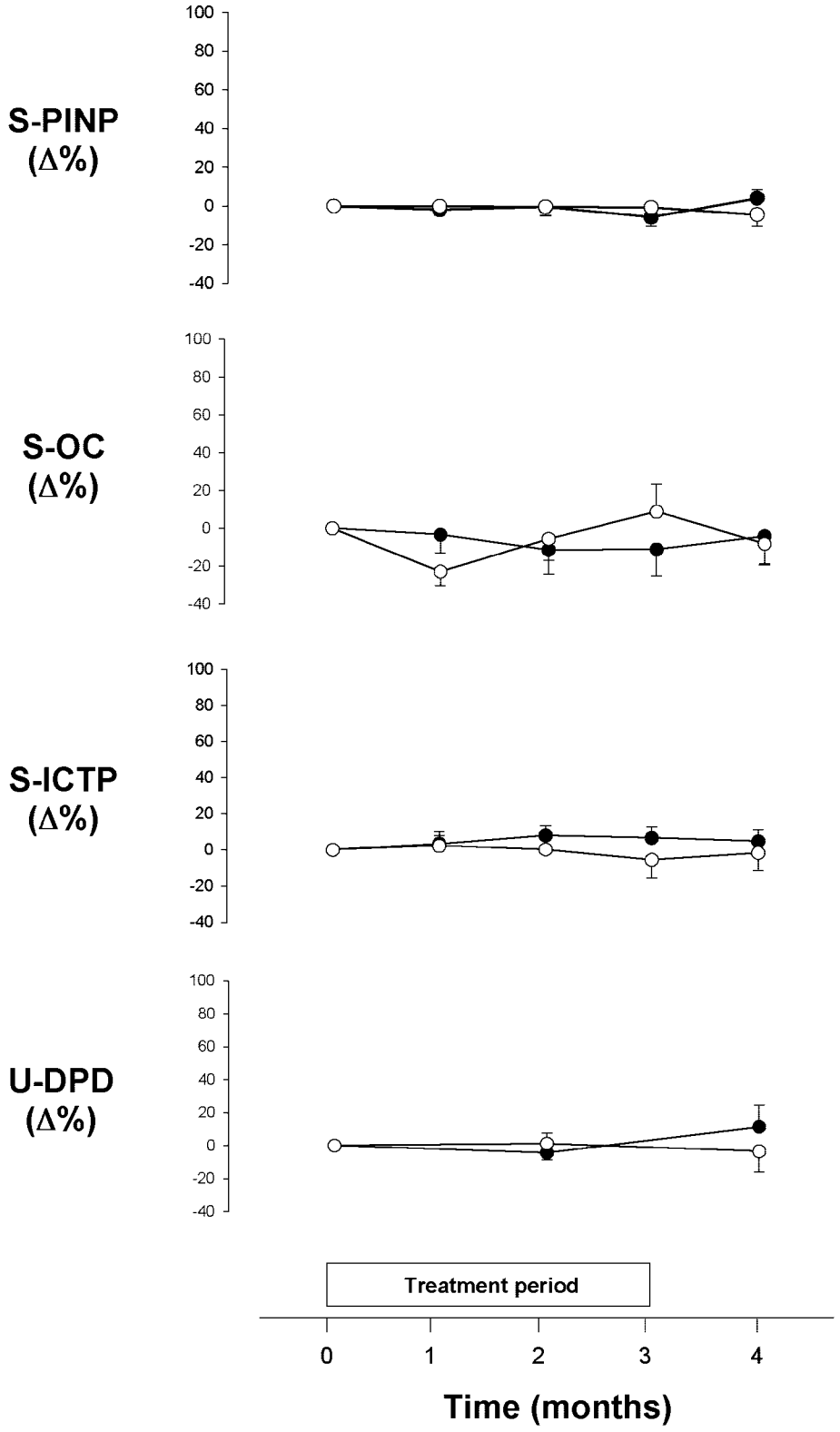

FIG. 1. Treatment with DHT or placebo. Mean percent changes (±SEM) in bone turnover markers among 35 men before, during, and after daily application of 70 mg DHT for 3 months. No significant differences (repeated-measures general linear model) between the treatment (●) and placebo (○) groups were observed during the 3-month treatment period.

terestingly, a significant correlation was seen between the change in S-PINP and the change in serum E2 levels, whereas no such relationship was noted for serum T. Furthermore, treatment with DHT induced no change in bone formation (S-PINP, S-BAP, or S-OC) despite a 20-fold increase in serum DHT levels, but in the presence of unaltered E2 levels. Other randomized placebo-controlled studies have consistently shown no effect of T therapy on biochemical markers of bone

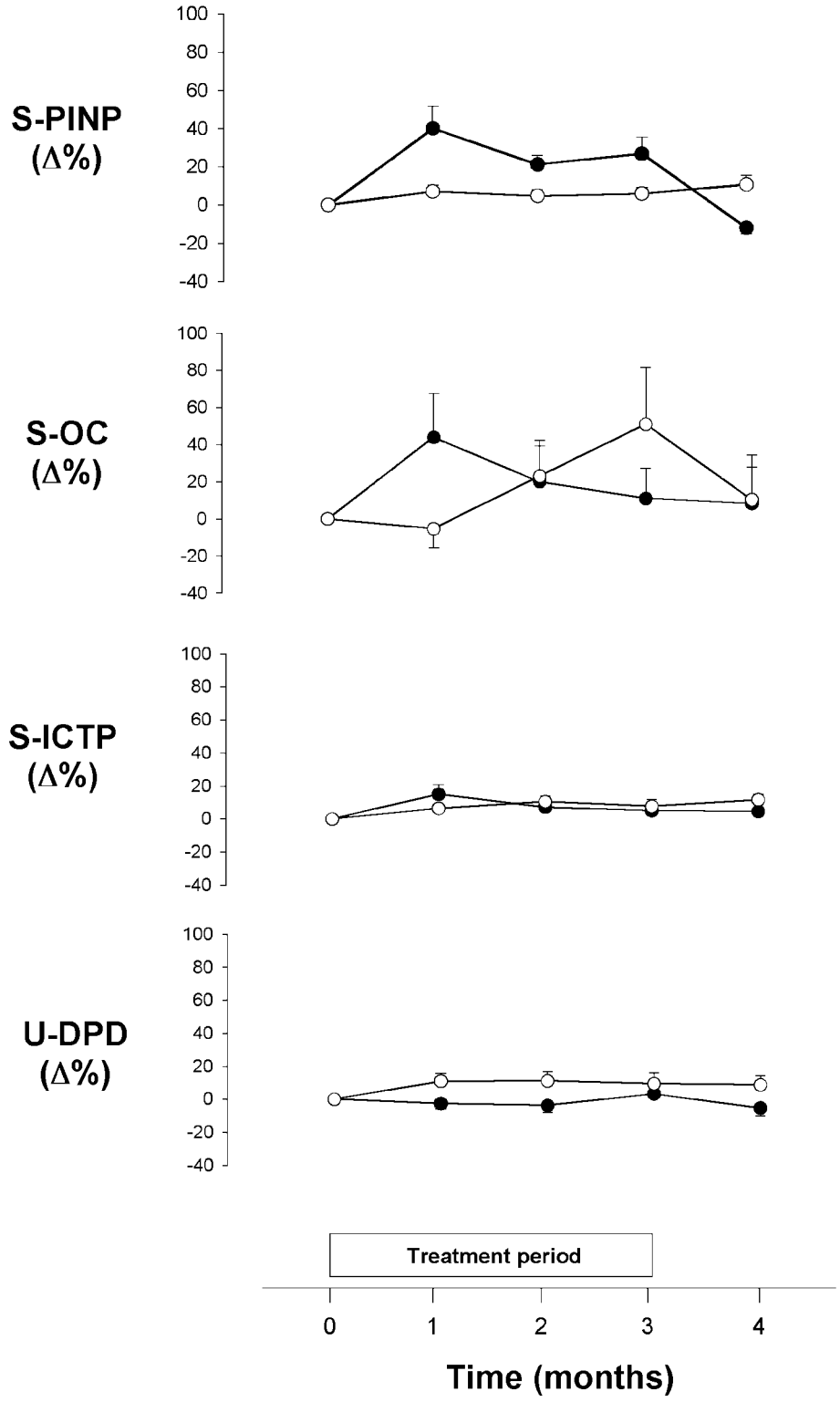

FIG. 2. Treatment with rhCG or placebo. Mean percent changes (±SEM) in bone turnover markers among 40 men before, during, and after twice-weekly sc injections of 250 μg (5000 IU) rhCG for 3 months. Note the significant increase in S-PINP levels in the treatment (●) compared with the placebo group (○) ($P = 0.002$, repeated-measures general linear model). In contrast, no consistent changes were seen in serum levels of OC and ICTP, or U-DPD.

formation in older men (7, 11, 14). Taken together, these data suggest that serum E2, rather than DHT or T, may be responsible for the early and sustained surge in osteoblastic collagen synthesis in these older, partially androgen-deficient men.

These results also seem to confirm that an anabolic effect of E2 on bone turnover can occur within 1 month (27, 28) but differs from and extends these findings in several respects. Based on a study by Falahati-Nini *et al.* (27), acute sex hormone withdrawal in elderly men resulted in a decrease in

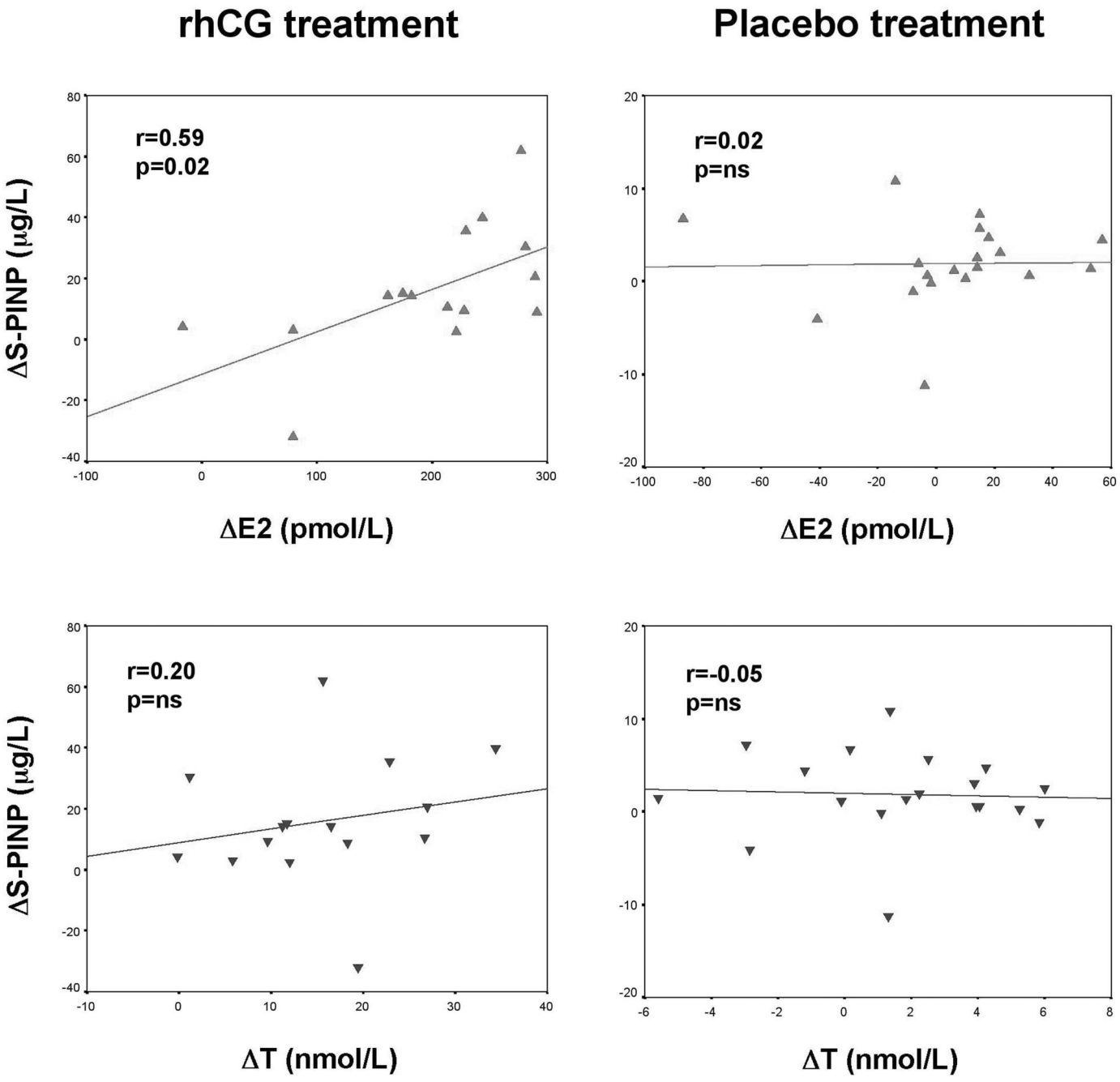

FIG. 3. Relationship between the change in serum E2 or T levels and the change in serum PINP levels in rhCG-treated men (n = 20). Changes in bone markers were calculated as the difference between baseline and peak levels after 1 month of treatment and correlated with changes in hormones during the same time period.

S-PINP and S-OC levels, which could be prevented by selective E2 replacement. In contrast, no such effect on bone formation had been observed in a study of sex steroid withdrawal for a period of 12 wk (29). This observation may, however, have been confounded by the overall increase in bone turnover that had occurred by the time the measurements were made. In addition, our study shows that therapy resulting in supraphysiological E2 levels has an effect specifically on early osteoblastic function, whereas later osteoblastic differentiation cannot be further induced by these higher E2 concentrations. Subcutaneous E2 implants in postmenopausal women (28) that result in comparable elevations in serum E2 also increases mean S-PINP (30%) and S-OC (10%) comparably. Other prospective nonrandomized studies of long-term supraphysiological sc E2 therapy in women have also shown an anabolic effect on bone (30, 31). Studies in female rats also confirm that high-dose E2 has anabolic skeletal effects indicated by increased bone formation and bone mass (32–34), although *in vitro* reports of the effects of estrogen on DNA synthesis, proliferation, and bone matrix

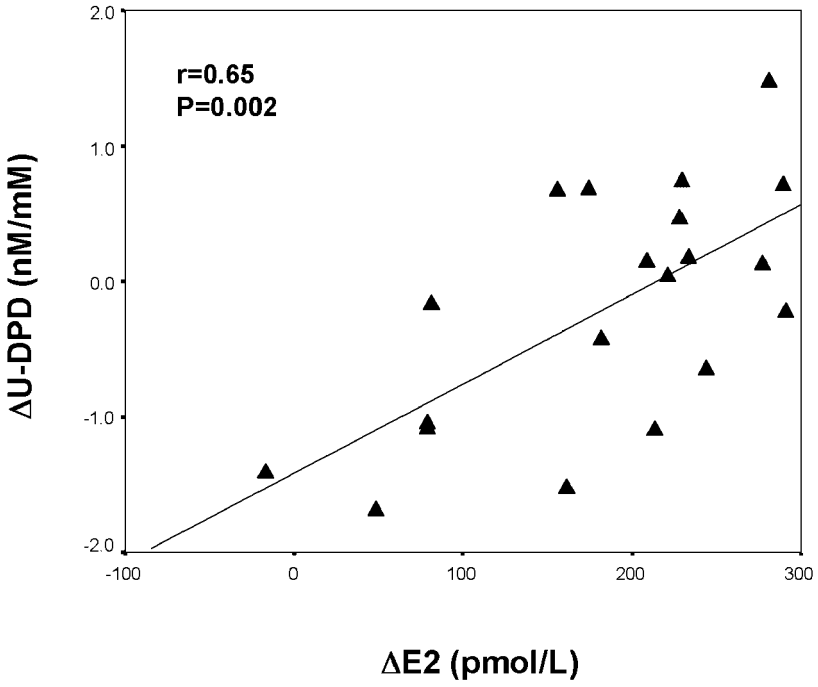

FIG. 4. Relationship between the change in serum E2 levels and the change in U-DPD levels in rhCG-treated men (n = 20).

protein production of type I collagen, alkaline phosphatase, and osteocalcin are conflicting (35–38).

Importantly, our data are not inconsistent with androgens having direct effects on osteoblastic cells, especially in androgen-deficient men. Androgens can directly increase production of type I collagen (39, 40), and DHT has previously been shown to stimulate osteoblast proliferation in several *in vitro* models (41). In men rendered androgen deficient pharmacologically (27), selective E2 replacement was able to prevent the decrease in S-PINP and S-OC, whereas selective T replacement (achieved by simultaneously blocking aromatization to E2) prevented the decrease in S-OC but not in S-PINP. Neither the DHT study nor the rhCG study showed an effect of sex steroids on S-OC levels. However, there tended to be an association between the change in DHT and the change in S-OC levels, suggesting that androgens might, at least in part, exert a direct effect on bone mineralization. However, because intact S-OC was measured in our study, we cannot completely exclude the possibility that unavoidable variability in sample collection and handling instability may have contributed to a type II error in detecting an effect on S-OC. Nevertheless, these data show convincingly for the first time in men that therapy resulting in supraphysiological E2 concentrations can increase bone formation even in the presence of adequate androgen exposure. Presumably, the degree of androgen deficiency in these relatively healthy older men was not sufficient to show anabolic effects mediated via the androgen receptor.

Neither rhCG nor DHT treatment altered biochemical markers of bone resorption (U-DPD or S-ICTP). Although E2 is known to inhibit osteoclast proliferation and differentiation, population-based epidemiological and interventional studies suggest that in the male skeleton, no further antiresorptive effect occurs above a threshold E2 concentration of 96–129 pmol/liter (27, 42–44). Similarly, in a small number of men with almost entirely absent estrogen action, arising from mutations of estrogen receptor α (45) or aromatase enzyme (46, 47) the lack of bone mineralization can be reversed with E2 replacement (47, 48). Because baseline mean E2 levels were 126 $\pm$ 30 pmol/liter in the rhCG study and 167 $\pm$ 43 pmol/liter in the DHT study, and therapy did not lower these values below the presumed threshold, no effect on bone resorption occurred. However, the change in serum E2 levels positively correlated with the change in U-DPD levels, indicating that men with the greatest increase in serum E2 after rhCG also had the most pronounced rise in U-DPD levels. In this regard it is noteworthy that circulating E2 levels remained unchanged, whereas serum T levels significantly decreased during treatment with nonaromatizable DHT. We hypothesize that even though serum T levels decreased to approximately 40% of baseline, the remaining concentrations still provided enough substrate to maintain serum E2 levels in a eugonadal range. Furthermore, the adrenals may substantially contribute to serum E2 levels, as small amounts of E2 are generated by peripheral conversion of adrenal estrone.

Clearly, the net effect of androgenic therapy on bone mineral density will vary depending on the specific drug used, the resultant effect on serum androgen and estrogen concentrations, and the net effect on bone formation and bone resorption. Aromatization is clearly an important consideration (49), particularly in the presence of increased bone turnover. However, controlled studies, particularly in older men, show that the net effect of T supplementation on bone mass is likely to be modest (7, 11, 14, 50), although a single uncontrolled study reported greater effects (50). Furthermore, the magnitude of effect on bone turnover may be determined by the degree of androgen deficiency. In the study by Snyder *et al.* (14), a significant effect of T treatment on bone mineral density was observed only in older men

with pretreatment T concentrations less than 6.9 nmol/liter after 3 yr of supplementation. In our studies, few such men had such low baseline T concentrations, which may partly explain why we did not observe a correlation between pretreatment hormone levels and the changes in bone turnover markers. However, as our study was only of short duration, a long-term effect of rhCG or DHT on bone turnover and, ultimately on bone mass, cannot be ruled out.

Together with our findings, treatment of partial androgen-deficient elderly men with aromatizable or nonaromatizable T, even in supraphysiological doses, is likely to have only modest effects on bone turnover. In particular, the increase in collagen synthesis with rhCG therapy may not be sustained for longer periods. As our study was limited to a short treatment over 3 months, however, we cannot estimate for how long this anabolic effect on osteoblasts would be sustained. Importantly, neither DHT nor rhCG worsened net bone turnover, suggesting that longer-term use of either of these compounds for prostate protection or physical performance efficacy should not adversely affect bone. Nevertheless, longer studies are required to determine the net beneficial or adverse effect of sex hormones on bone mass and fracture rates in older men.

We conclude that in healthy men with partial age-related androgen deficiency, short-term treatment with rhCG stimulates osteoblastic collagen formation but has no effect on bone resorption or later mature osteoblastic function, whereas DHT in pharmacological doses has no effect on bone turnover. These data suggest that in the presence of adequate androgen exposure, supraphysiological E2 exposure can further increase early bone formation. In contrast, further increase in E2 exposure above a low threshold does not reduce bone resorption in men. Further studies are required to determine the long-term effect of DHT and rhCG on bone turnover.

Acknowledgments

Received November 17, 2003. Accepted February 26, 2004.

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C.M. is the recipient of a medical research fellowship from the Swiss National Science Foundation (No. 81BS-67544), and P.Y.L. was supported by fellowships from the National Health and Medical Research Council of Australia (ID 262025) and the Royal Australasian College of Physicians.

This work was presented in part at the 25th Annual Meeting of the American Society for Bone and Mineral Research, Minneapolis, Minnesota, 2003.

C.M. and P.Y.L. have contributed equally to this work.

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