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Growth Hormone (GH) Effects on Bone and Collagen Turnover in Healthy Adults and Its Potential as a Marker of GH Abuse in Sports: A Double Blind, Placebo-Controlled Study*

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ON BEHALF OF THE GH-2000 STUDY GROUP

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ABSTRACT

The effects of GH on bone remodeling in healthy adults have not been systematically investigated. An analysis of these effects might provide insights into GH physiology and might yield data useful for the detection of GH doping in sports. The aim of this study was to evaluate the effects of GH administration on biochemical markers of bone and collagen turnover in healthy volunteers. Ninety-nine healthy volunteers of both sexes were enrolled in a multicenter, randomized, double blind, placebo-controlled study and assigned to receive either placebo (40 subjects) or recombinant human GH (0.1 IU/kg·day in 29 subjects and 0.2 IU/kg·day in 30 subjects). The treatment duration was 28 days, followed by a 56-day wash-out period. The biochemical markers evaluated were the bone formation markers osteocalcin and C-terminal propeptide of type I procollagen, the resorption marker type I collagen telopeptide, and the soft tissue marker procollagen type III. All variables increased on days 21 and 28 in the two active treatment groups *vs.* levels in both the baseline ($P < 0.01$) and placebo ($P < 0.01$) groups. The increment was more pronounced in the 0.2 IU/kg·day group and remained significant on day 84 for procollagen type III (from 0.53 ± 0.13 to 0.61 ± 0.14 kU/L; $P < 0.02$) and osteocalcin (from 12.2 ± 2.9 to 14.6 ± 3.6 UG/L; $P < 0.02$), whereas levels of C-terminal propeptide of type I procollagen and type I collagen telopeptide declined after day 42 and were no longer significantly above baseline on day 84 (from 3.9 ± 1.2 to 5.1 ± 1.5 μg/L and from 174 ± 60 to 173 ± 53 μg/L, respectively). Gender-related differences were observed in the study; females were less responsive than males to GH administration with respect to procollagen type III and type I collagen telopeptide ($P < 0.001$).

In conclusion, exogenous GH administration affects the biochemical parameters of bone and collagen turnover in a dose- and gender-dependent manner. As GH-induced modifications of most markers, in particular procollagen type III and osteocalcin, persist after GH withdrawal, they may be suitable markers for detecting GH abuse. (*J Clin Endocrinol Metab* **85:** 1505–1512, 2000)

GH AFFECTS BONE remodeling, a process characterized by continuing bone resorption and formation. In childhood, GH stimulates bone formation in excess of bone resorption, and this promotes the accumulation of peak bone mass. GH seems to also be an important factor in the regulation of bone turnover in adult life (1). Indeed, disruption of physiological bone turnover is associated with such clinical conditions of altered GH activity as GH deficiency (GHD) and acromegaly. There is also evidence that GH and its local tissue effector, insulin-like growth factor I (IGF-I), are involved in the pathophysiology of osteoporosis (2).

Although prohibited in sport and potentially dangerous, GH use has progressively increased among athletes in an attempt to enhance their physical performance. GH abuse is a major problem not only in sports, but also among school children, according to a report of GH use in high school students in the U.S. (3). GH abuse is undesirable not only for the unfair competition, but also because the uncontrolled use of large doses of this hormone may increase the risk of serious side-effects. In particular, prolonged GH abuse may result in a syndrome similar to acromegaly, with the inherent risk of hypertension, diabetes, cardiomyopathy, and malignancy.

Currently, no approved method to detect GH doping is available. This is partly because exogenous GH is not easily distinguishable from endogenous GH and partly because GH is excreted in the urine in tiny and inconsistent amounts.

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In addition, GH has a short half-life (4, 5), a problem that cannot be circumvented by using the IGF-I assay because the increment in the plasma levels of this peptide is detectable for only 2 days after GH withdrawal (6). Thus, measurement of serum and urinary GH is not suitable for the detection of GH abuse. Given the present state of knowledge, an effective test would be one based on circulating, long lasting markers of GH administration. In this respect, bone and collagen markers represent the most promising ones to detect GH abuse because their changes seem to persist longer after GH withdrawal than those in GH and IGF-I (7). A potential limitation of this approach is the variation in the bone markers over time and in relation to the intensity of physical training.

The aim of this study was 2-fold: 1) to gain deeper insights into GH's effect on bone and collagen turnover in healthy subjects, and 2) to assess the usefulness of bone and collagen markers in the development of a GH doping test. For this purpose, osteocalcin, C-terminal propeptide of type I procollagen (PICP), C-terminal cross-linked telopeptide of collagen type I (ICTP), and procollagen type III N terminal extension peptide (PIIIP) were measured in a double blind, randomized, placebo-controlled study of healthy volunteers treated for 4 weeks with GH, at doses that simulate GH doping in sport.

Subjects and Methods

Subjects

This study was conducted in Denmark, Italy, Sweden, and the United Kingdom. Most of the subjects were recruited from the medical student population and army personnel. Subjects were eligible to participate if they were between 18–35 yr of age and had been training twice a week for at least 1 yr. Ninety-nine healthy volunteers (51 males and 48 females) entered the study. All women were using safe contraception, consisting of oral contraceptives in 7 and barrier methods in the remainder. The protocol was approved by the ethics committee and the National Health Authority of each country. All subjects gave written informed consent to their participation, and the study was conducted in accordance with the ethical principles of the Declaration of Helsinki and the guidelines of good clinical practice.

Study design

The study was double blind, randomized, and placebo controlled. The subjects were assigned to treatment with placebo or 0.1 or 0.2 IU/kg·day recombinant GH on the basis of a four randomized block design. These two treated groups will be referred to as low and high dose groups, respectively. GH (Genotropin, Pharmacia & Upjohn, Inc., Stockholm, Sweden; or Norditropin, Novo Nordisk, Gentofte, Denmark) was administered as daily sc self-injections at bedtime. Placebo preparations were reconstituted in a volume of solvent identical to that of GH preparations and were administered in a similar fashion. To minimize side-effects during the first week of treatment, the GH dose was half the target dose. In addition, the dose was reduced by 50% in the case of side-effects; the treatment was stopped if side-effects persisted. If a subject dropped out during the treatment phase, the wash-out phase started the following day.

Treatment protocol and follow-up studies

Before entering the study, a complete history and physical examination were performed in all subjects. Female subjects underwent a pregnancy test.

The study protocol consisted of 28 days of treatment followed by 56 days of wash-out. Study visits were scheduled every week during the treatment period and on days 30, 33, 42, and 84 during the wash-out period. Fasting blood glucose and systolic and diastolic blood pressures were measured at each visit. Resting blood samples were collected at baseline and on days 21 and 28 during treatment. After treatment, blood samples were collected on days 30, 33, 42, and 84. Blood sampling was performed at the same time of day in all subjects to minimize diurnal variations in bone marker concentrations. Twenty-nine subjects (15 males and 14 females) received the low GH dose, and 30 subjects (15 males and 15 females) received the high dose. Forty subjects (21 males and 19 females) received placebo (Table 1).

TABLE 1. Baseline characteristics of the subjects (n = 99)

	Placebo	GH	
		0.1 IU/kg·day	0.2 IU/kg·day
No. of subjects	40	29	30
Age (yr)	25.4 ± 4.5	25.6 ± 4.2	25.8 ± 3.3
Sex	21 M, 19 F	15 M, 14 F	15 M, 15 F
BMI	22.7 ± 3.2	23.1 ± 2.7	22.5 ± 2

BMI, Body mass index.

Analytical procedures

Serum samples were stored at −80 C until analysis. To reduce analytic variations, samples from each subject were analyzed in the same run. The laboratory staff was blinded to the treatment code, which was broken after the assay results were entered into the database.

Serum osteocalcin was assayed by a double antibody RIA (International CIS, Gif-sur Yvette, France) with intraassay coefficients of variation (CVs) of 8.2%, 5.6%, and 5.4% at serum concentrations of 3.6, 10.3, and 22.3 UG/L, respectively. Serum PICP concentrations were measured using a RIA (Orion Diagnostica, Espoo, Finland) with intraassay CVs of 6.2%, 11.3%, and 11.3% at serum concentrations of 112.2, 162.7, and 403.4 μg/L, respectively. Serum ICTP was measured using a RIA (Orion Diagnostica) with intraassay CVs of 5.6%, 7.2%, and 5.1% at serum concentrations of 5.5, 3.2, and 16.8 μg/L, respectively. The serum PIIIP concentration was determined by a RIA (International CIS, Gif-sur Yvette, France) with intraassay CVs of 5.7%, 9.1%, and 6.7% at serum concentrations of 0.95, 0.62, and 1.18 kU/L, respectively.

Statistical analysis

Differences among the treatment groups at each time point were assessed using ANOVA with Bonferroni multiple comparison test. Regression analysis was used to assess the relationship between the markers and age. To evaluate the effect of gender, subjects were stratified into males and females, and differences in the markers for each treatment group were assessed using one-way ANOVA.

To establish which combination of markers distinguished most reliably between the placebo and the active treatment groups, a linear discriminant analysis was performed using the SAS system (SAS Institute, Inc., Cary, NC). Initially, all four markers were included in the analysis, and then only those that showed the most significant change with GH treatment. A false positive rate was calculated for each combination to identify subjects who received placebo but were misclassified as having received active treatment. All data are presented as the mean ± SD.

Results

The baseline characteristics of the subjects enrolled in the study are shown in Table 1. Ten of the 29 subjects enrolled in the low dose group had side-effects during the study, and the dose was reduced in 2 of them. In the high dose group, 23 subjects had side-effects, and the dose was reduced in 7. In the placebo group, 8 subjects had side-effects. Among these, 1 subject reduced the dose and 1 subject stopped the treatment on day 14 because of headache and tachycardia. The most frequently reported side-effect was transient fluid

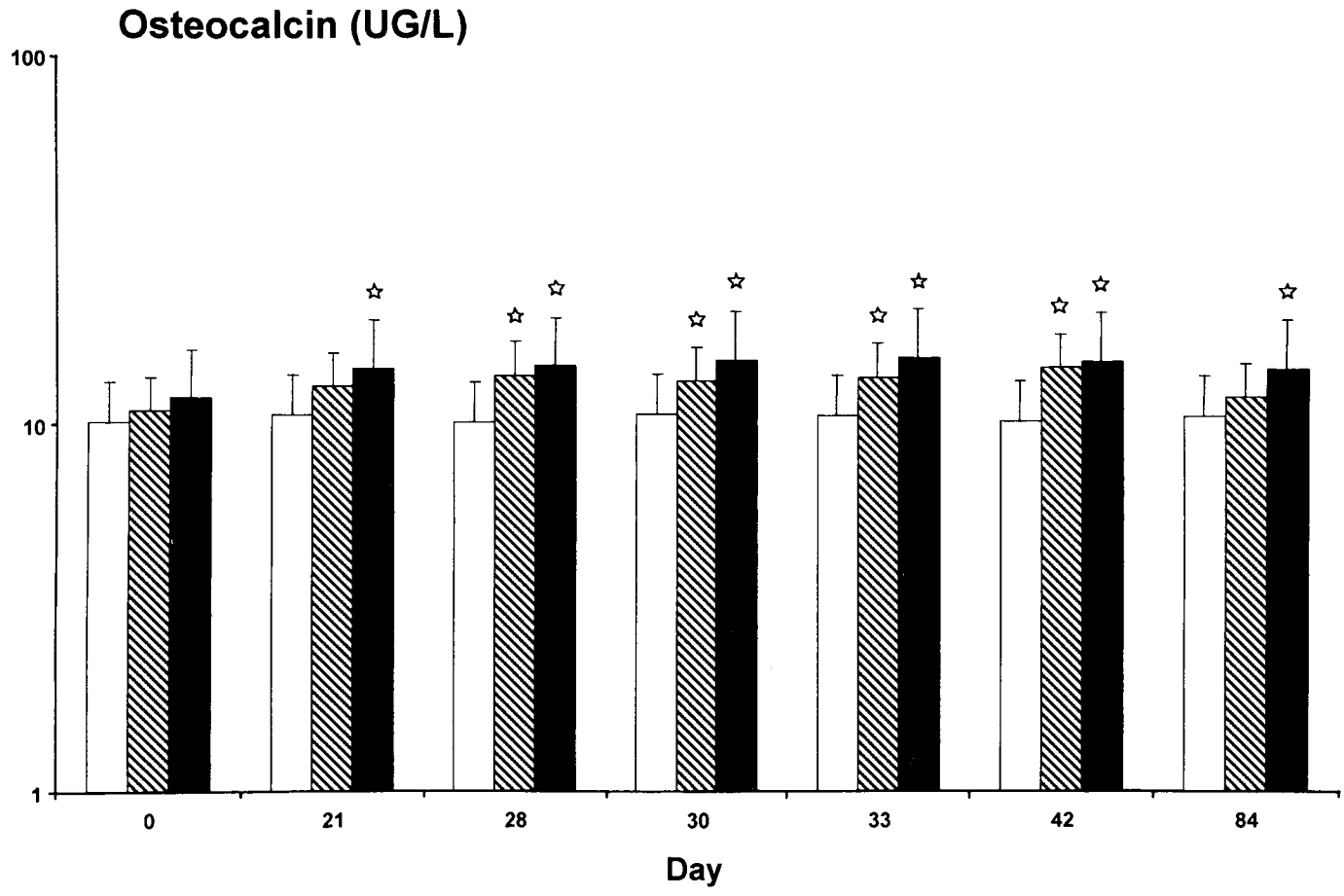

FIG. 1. Effect of GH administration (up to day 28) and withdrawal on serum osteocalcin concentrations in normal subjects. *Asterisks* indicate significant differences *vs.* placebo. □, Placebo; ▨, low dose group; ■, high dose group.

retention. Increased sweating and arthralgia were also reported.

Bone and soft tissue markers at baseline

There were no significant differences among the three treatment groups for any of the markers (Figs. 1–4). Regression analysis showed significant negative relationships between age and ICTP ($F = 31.1$; $P = 0.001$) and between age and PIIIP ($F = 13.1$; $P = 0.0005$). Males had significantly higher levels of osteocalcin ($P = 0.001$) and PICP ($P = 0.001$) than females.

Bone and soft tissue markers in the placebo group

None of the markers changed in the placebo group during treatment (Figs. 1–4). The coefficients of variation were 30% for osteocalcin, 29% for PICP, 21% for ICTP, and 24% for PIIIP. All women were eumenorrheic and entered the study at different phases of the menstrual cycle. No relationship was found for any of the markers with the day in the menstrual cycle.

Responses of bone and soft tissue markers to GH

During the treatment phase, all subjects taking GH showed an increase in the four markers measured. The magnitude and the time course of the response varied according to the individual markers and the GH dose.

Osteocalcin. Figure 1 shows the response of osteocalcin to GH administration. There was a significant difference between placebo and the high dose group from day 21 ($P < 0.005$) until day 84 ($P < 0.005$). The same was true for the low dose group, except for days 21 and 84. There were no significant differences between the two active treatment groups at any time point.

Basal osteocalcin levels were higher in males ($P < 0.0001$) than in females. In both the placebo and the two active treatment groups, the males had significantly higher values than the females at all time points ($P = 0.001$).

PICP (C-terminal propeptide of collagen type I). The response of PICP to GH is shown in Fig. 2. The treatment groups differed from placebo from day 21 ($P < 0.0001$) until day 33 ($P = 0.001$). There was no significant difference between the active treatment groups at any time point.

After stratification for gender, males had significantly higher baseline PICP values than females ($P = 0.0003$). In the placebo group, males had higher PICP values than females on days 21, 28, 30, and 33 ($P < 0.02$ to $P < 0.05$). In the high dose group, males had significantly higher PICP than females, which persisted until day 84 ($P < 0.05$ to $P < 0.005$),

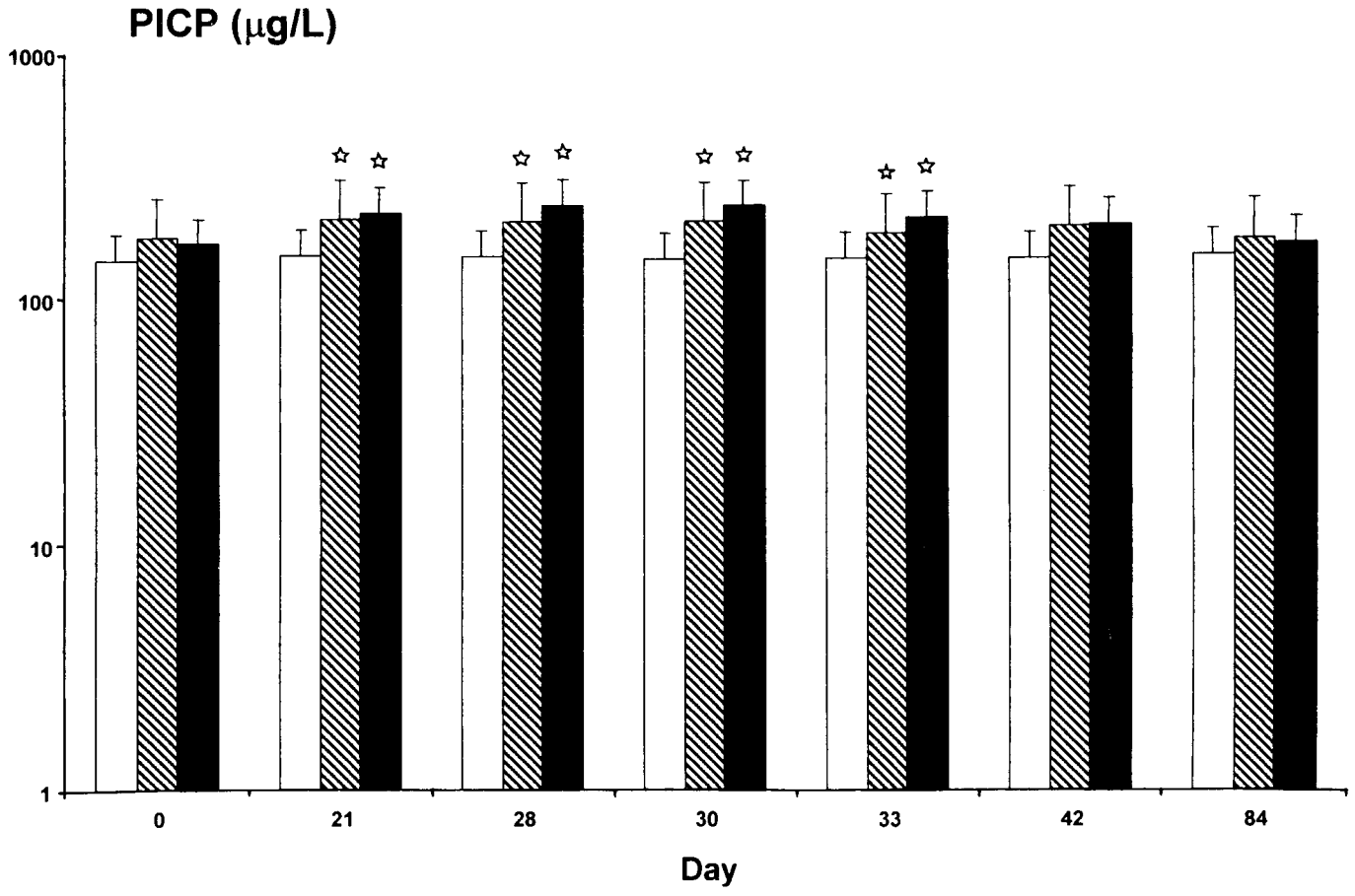

FIG. 2. Effect of GH administration (up to day 28) and withdrawal on serum PICP concentrations in normal subjects. *Asterisks* indicate significant differences *vs.* placebo. □, Placebo; ▨, low dose group; ■, high dose group.

except for day 30. In the low dose group, males had higher PICP on days 0, 21, 30, and 84.

ICTP. Figure 3 shows the response of ICTP to GH. There was a significant difference between placebo and both active treatment groups from days 21–42 ($P < 0.02$-$P < 0.0001$). The active treatment groups differed from each other from day 21 ($P < 0.02$) to day 33 ($P < 0.001$).

After stratification for gender, there were no significant differences between males and females in the placebo group at any time point. However, in the low dose group, males had a much greater response of ICTP than females at all visits ($P < 0.05$ to $P < 0.005$). In particular, in males, the ICTP concentration rose from the basal value of 5.5 ± 2.6 μg/L to the peak value of 10.8 ± 3.1 on day 28, whereas in females, ICTP rose from 3.9 ± 1.1 to 5.3 ± 1.5 μg/L. In the high dose group, males had a significantly greater response than females on days 21, 28, 33, and 42 ($P < 0.02$ to $P < 0.001$).

PIIIP. As shown in Fig. 4, PIIIP was significantly different in both the active treatment groups compared with the placebo group from day 21 ($P < 0.0001$) until day 42 in the low dose group and until day 84 in the high dose group. There was a significant difference between the two treatment groups from day 21 ($P = 0.001$) to day 42 ($P = 0.02$). The increment above baseline for this marker was the highest among the four markers studied and amounted to approximately 150% on days 28–30.

After stratification for gender, there were no significant differences between males and females in the placebo group at any of the time points of the study. However, in the low dose group, males had a greater response of PIIIP compared with females at all visits ($P < 0.001$). The peak increment in PIIIP concentration occurred on day 28 and was 1.5 ± 0.4 kU/L in males (basal, 0.57 ± 0.2) and 0.71 ± 0.2 in females (basal, 0.47 ± 0.1). In the high dose group, males had a significantly greater response than the females on days 21, 28, 33, and 42 ($P < 0.01$-$P < 0.001$).

Discriminant analysis

To establish which combination of markers distinguished most reliably between placebo and GH-treated groups, a discriminant analysis was performed. Initially, all four markers were included in the analysis, then various combinations of markers, and finally only one marker. Figure 5 shows the false positive rate at each time point for three combinations of markers. The best discrimination between the placebo and the GH-treated group occurred on day 28 for the discriminant function using one (PIIIP), two (ICTP and PIIIP), or four markers, as one, two, and one false positives occurred, respectively. If PICP was deleted from the analysis, the results

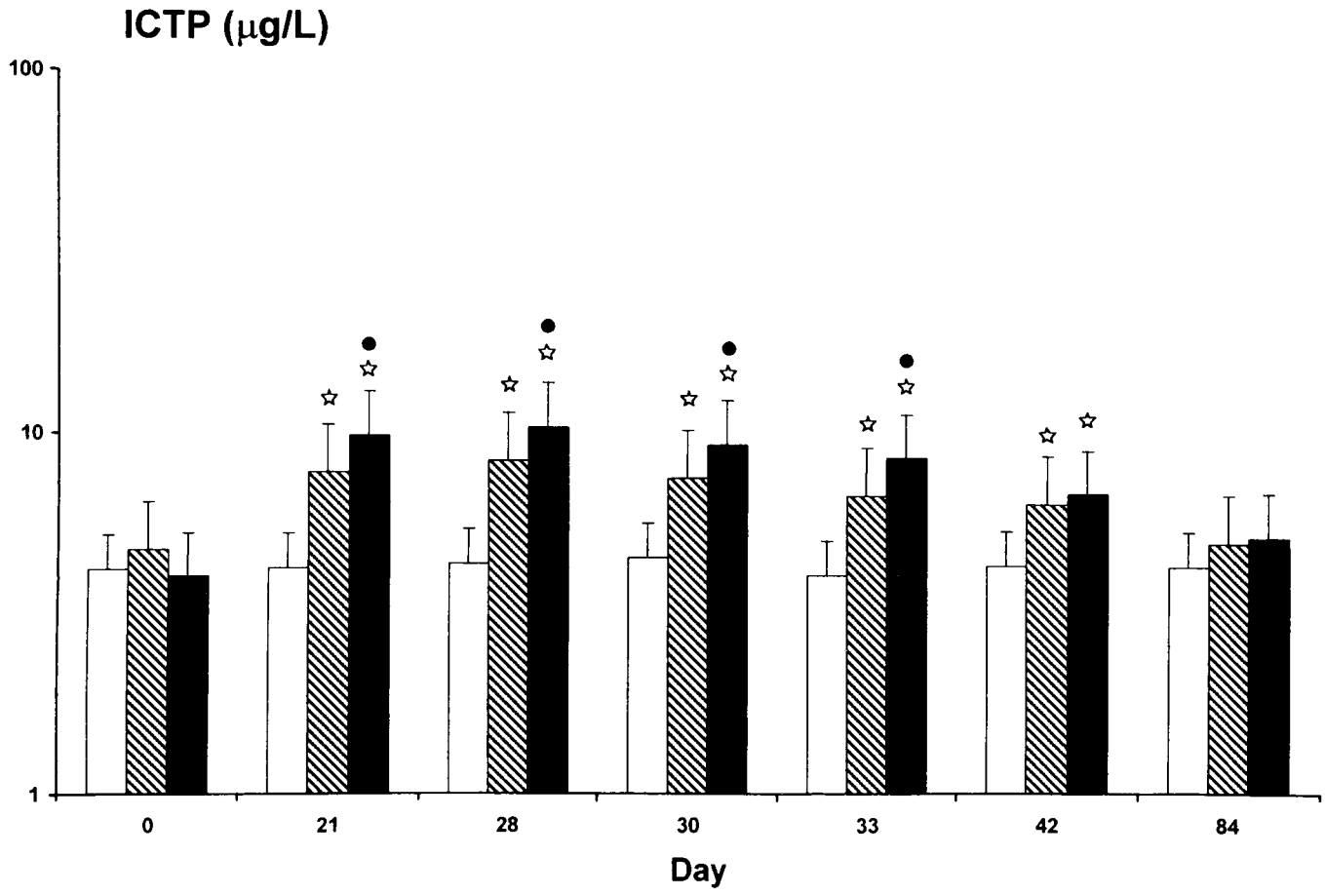

FIG. 3. Effect of GH administration (up to day 28) and withdrawal on serum ICTP concentrations in normal subjects. *Asterisks* and *circles* indicate significant differences *vs.* placebo and between low and high dose groups, respectively. □, Placebo; ▨, low dose group; ■, high dose group.

on day 28 were identical to those based on the four markers. However, on days 30 and 33, the false positive rates were slightly better when all markers were included compared to those without PICP (2.7% and 7.6% *vs.* 5.5% and 12.8%, respectively. The four markers discriminant function on day 28 was $F_1 = 0.789 + 2.628PIIIP + 0.2PICP + 0.99ICTP - 1.327osteocalcin$. Note that the marker value should be the natural logarithm of the original scale. On day 0 (basal condition), the value of the function for all cases (n = 99) was -1.755 ± 0.793. For day 28, the sensitivities of one-, two-, and four-marker tests were 0.84, 0.87, and 0.84, respectively. The corresponding specificity measures were 0.95, 0.90, and 0.97, respectively.

Discussion

The results of this study show that 1) bone and collagen turnover is markedly increased by GH administration in healthy volunteers; and 2) GH-induced changes in the bone and collagen markers persist long after GH withdrawal and, therefore, may provide a reasonable basis on which to devise a robust test for GH doping.

Factors influencing bone and soft tissue markers at baseline

At baseline, the factors found to influence the bone and collagen markers were age and gender. Age had a significant negative relationship only with the soft tissue marker, PIIIP, and the bone resorption marker, ICTP. These results concur with those of a study of elite athletes (Healey, M. L. *et al.*, manuscript in preparation). In the case of gender, the males had significantly higher levels of the bone formation markers PICP and osteocalcin. This finding is new and suggests a gender-related difference in bone turnover.

Variations in bone and soft tissue markers in the placebo group

Our data from the placebo group indicate that in normal subjects the bone and soft tissue markers are very stable over time. This is an important finding, because bone markers have been reported to undergo diurnal variation (8), a factor that would not be desirable for a reliable marker of GH usage.

The menstrual cycle did not have any effect on the markers. However, in a study based on 10 women, bone marker values varied according to the phase of the menstrual cycle (9). The discrepancy between this finding and our data may be explained by the fact that the menstrual cycle is a short event in terms of the bone-remodeling process. Moreover, the regularity of the menstrual cycles and oral contraceptive pill usage may have more impact on bone turnover.

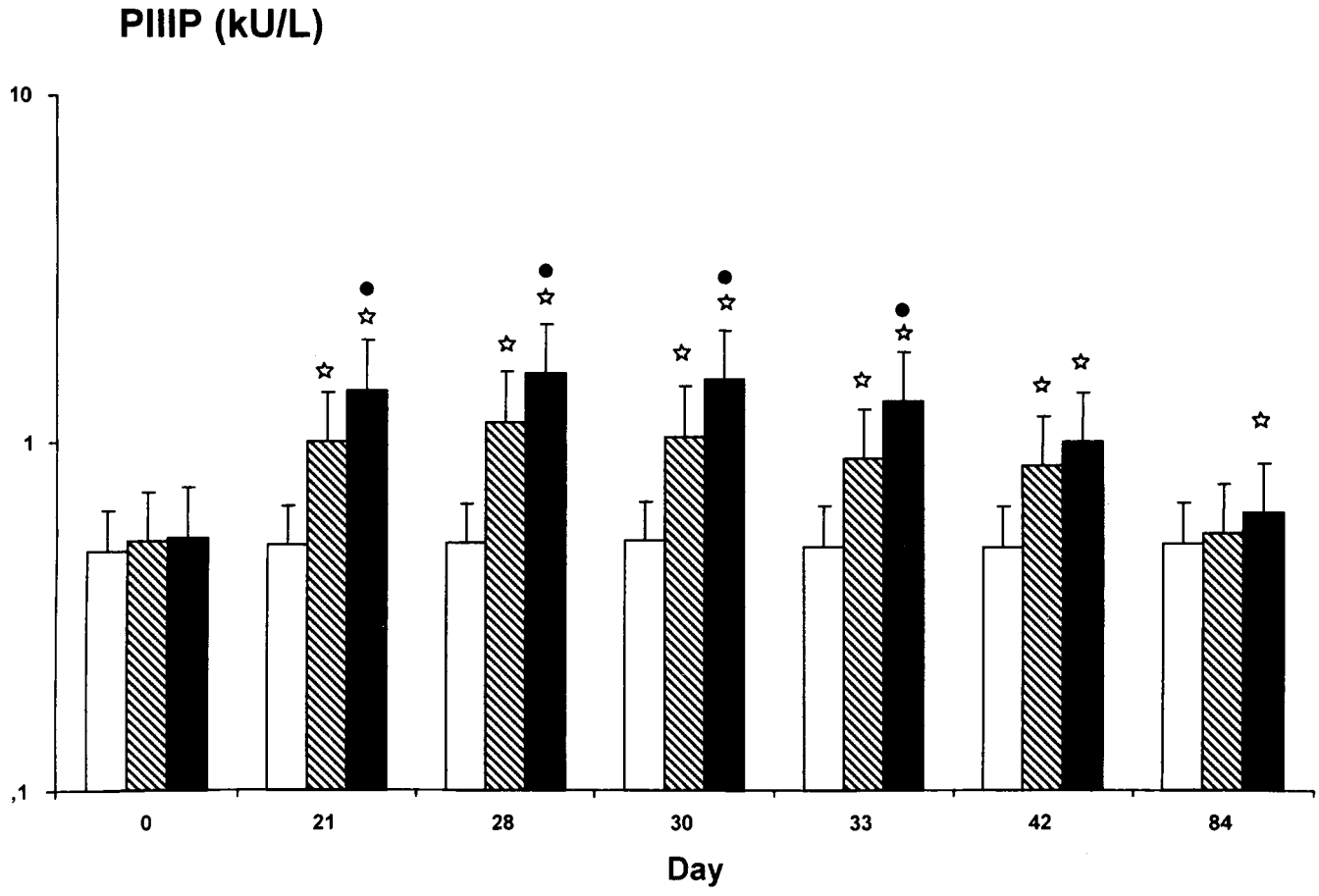

FIG. 4. Effect of GH administration (up to day 28) and subsequent withdrawal on serum PIIIP concentrations in normal subjects. *Asterisks* and *circles* indicate significant differences *vs.* placebo and between low and high dose groups, respectively. □, Placebo; ▨, low dose group; ■, high dose group.

Responses of bone and soft tissue markers to GH

Time course. Our data demonstrate that GH is able to augment bone turnover in healthy adults. All of the markers significantly increased in response to GH during the 28-day treatment phase. The most remarkable increases were shown by ICTP and PIIIP, whereas osteocalcin and PIIIP remained significantly elevated until day 84. The fact that both bone markers of resorption (ICTP) and formation (osteocalcin and PICP) were increased indicates a general acceleration of bone turnover. However, the stimulation of bone formation was more sustained, as supported by the persistent elevation of osteocalcin, whereas the resorption marker ICTP remained elevated for a shorter period, *i.e.* up to day 42.

The effects of GH administration on bone turnover in healthy adults have been poorly investigated, and there is no study on collagen turnover. Our data concur with those of a previous study (7) in which 20 men were given GH (0.1 IU/kg·day) for 1 week. Urinary hydroxyproline/creatinine and calcium/creatinine ratios were increased during treatment and remained elevated for 4 and 2 weeks, respectively. Serum osteocalcin increased during treatment and remained high for 6 months. In line with our results, the resorption markers declined within a few weeks, whereas there was a prolonged effect on stimulation of bone formation markers. However, that study was based on a small number of men alone, and the treatment phase was much shorter than in our study. In addition, there was no placebo group to monitor any spontaneous variation in the markers, and only one dose of GH was used. Finally, only bone turnover markers were assessed.

There are extensive data demonstrating that GH deficiency is associated with a disruption of bone turnover and that long term GH replacement therapy reverses these abnormalities (10). The response to GH therapy is typically biphasic (11), with an initial increase in bone resorption, followed by an increase in bone formation. The transition point when bone formation is stimulated more than bone resorption occurs after about 6 months, as reflected by the change in BMD. This biphasic response of bone turnover to GH therapy is similar to that observed in our study, although the transition point seems to occur sooner (between days 42–84) in our normal volunteers than in GHD patients. This is suggested by the prolonged elevation of the bone-specific marker osteocalcin and the early decline of ICTP.

Effect of GH dose. The higher dose of GH had a greater effect on PIIIP and on the bone resorption marker ICTP. In contrast, the formation markers (osteocalcin and PICP) did not differ significantly between the two dosage groups. Our data sug-

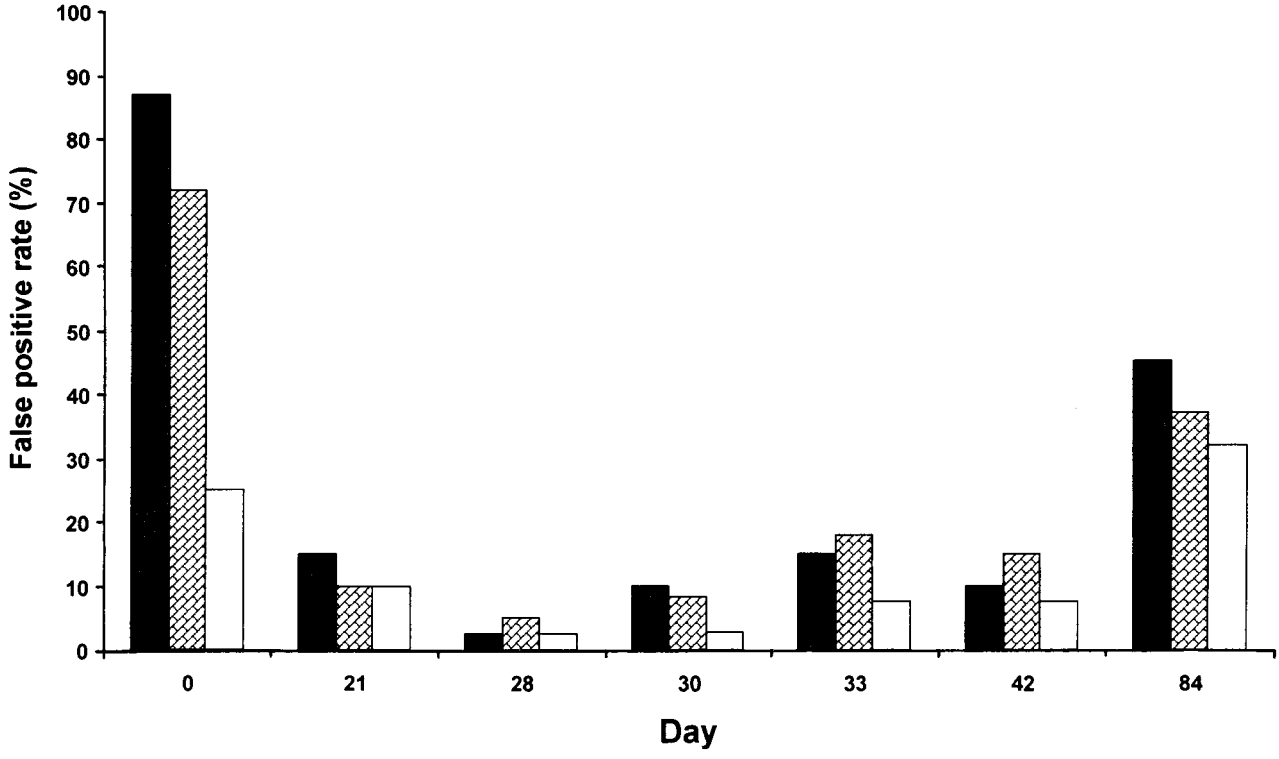

FIG. 5. Percentage of subjects erroneously diagnosed as having taken GH by discriminant analysis based on a single marker (PIIIP; ■), two markers (ICTP and PIIIP □; ▨), and four markers.

gest that the higher GH dose accentuates the response pattern of bone and soft tissue turnover to GH rather than changing its nature. This finding concurs with other reports. In a study of GHD patients, GH administration resulted in a dose-dependent increase in the markers of bone and collagen turnover (12). In addition, in a study using 2 yr of GH replacement therapy induced a similar dose-dependent response (13).

Influence of gender. At baseline, males had higher levels of formation markers than females. This difference is probably due to the fact that men usually reach peak bone mass somewhat later than women. In addition, it clearly appears that women are more resistant than men to the effect of GH on bone and soft tissue turnover. The men showed a significantly greater response in the measured markers, most impressively in the low dose group for PIIIP and ICTP. The increment in these markers was 3-fold greater in males compared with females.

Our observation of a gender-related difference in the response to GH is in agreement with a previous report that GHD men are more responsive to GH therapy than women (14). It has been reported that oral estrogen blocks hepatic IGF-I production and consequently suppresses connective tissue and bone metabolism (15). However, in our study only 7 of a total of 48 women were taking the oral contraceptive pill, so it is unlikely that this factor could account for the resistance of women to GH.

Data implications in doping strategy

The present study indicates that bone and soft tissue turnover responds quite rapidly to GH administration and that the relative markers are potentially useful in the detection of GH doping. Discriminant analysis showed that these markers are very reliable in distinguishing between placebo- and GH-treated individuals. It must be stressed, however, that the present study was performed on amateurs, whose commitment to sport and degree of training may be considerably different from those of elite athletes. In addition, our study involved only Caucasian subjects, and thus, the conclusions cannot be extended to athletes of different races. For these reasons, the present data highlight the potential usefulness of the bone markers in a doping test, but further experimental work is necessary for validation and implementation purposes.

A recent report points to the possibility of distinguishing between recombinant and natural human GH by simultaneous RIA of the two GH isoforms (20 and 22 kDa) (16). This may be a new powerful tool to reveal GH doping by a direct approach. However, due to the very short half-life of GH (4, 5), the diagnostic window of the method is unlikely to extend beyond 24 h after the last GH injection. In contrast, the present data suggest that some of the markers monitored may be very useful in detecting a much earlier episode of GH doping. Indeed, the increase in osteocalcin and PIIIP in our study persisted for 8 weeks after GH withdrawal.

Clinical implications

The demonstration that GH activates bone turnover with a prolonged phase of bone formation in healthy adults may be relevant to such clinical conditions as osteoporosis, acromegaly, and GH deficiency. Osteoporosis is frequent in aging, during which the activity of the GH/IGF-I axis declines

progressively. Yet, there is no clear evidence that GH therapy is beneficial in osteoporosis. In addition, the current approaches to osteoporosis rely essentially on agents that reduce bone resorption. Our demonstration that GH administration affects bone formation markers encourages long term trials designed to explore the possibility that GH replacement therapy in elderly people with low IGF-I levels may prevent osteoporotic fractures.

The diagnosis of acromegaly is usually underestimated and delayed. In addition, it is not clearly established how to monitor the efficacy of therapy. In a previous study, PIIIP levels were elevated in acromegalic patients, and they declined after surgical or medical treatment (17). In our study, PIIIP clearly discriminated between the placebo-treated and the GH-treated groups. This raises the question of whether PIIIP may be a useful adjunct to IGF-I in the early diagnosis of acromegaly and for assessment of the treatment efficacy of this disease.

The appropriate dose of GH to use in GHD patients is still a matter of debate. Our study points to PIIIP as a potential marker of GH biological activity in GHD patients, which makes PIIIP a candidate marker with which to monitor the adequacy of replacement therapy. In addition, GHD men are more responsive to GH replacement than women (14). Hence, the current opinion that GHD women must be treated with higher GH doses than men. Our study demonstrates that the difference between sexes remains when high doses of GH are used, suggesting that there is a gender-related difference in the bone responsiveness that cannot be easily reversed by increasing the GH dose.

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