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Responses of Markers of Bone and Collagen Turnover to Exercise, Growth Hormone (GH) Administration, and GH Withdrawal in Trained Adult Males*

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ABSTRACT

To examine the interactions between acute exercise and GH on markers of bone and collagen turnover and to assess the potential for detecting GH abuse in athletes using these markers, we studied 17 aerobically trained males (age, 26.9 ± 1.5 yr). Sequential studies of exercise, GH administration, and GH withdrawal were undertaken. A randomized, controlled study of rest *vs.* exercise showed that exercise did not change serum osteocalcin; other markers of formation increased transiently (each $P < 0.001$): bone-specific alkaline phosphatase (+16.1%), carboxyterminal propeptide of type I procollagen (+14.1%), and procollagen III N-terminal extension peptide (+5.0%). The carboxyterminal cross-linked telopeptide of type I collagen, a bone resorption marker, increased 9.7% ($P = 0.018$) in response to exercise. A randomized, double blind, placebo-controlled, parallel study of recombinant human GH treatment (0.15 IU/kg·day) for 1 week increased serum osteocalcin (net increase preexercise, +10.0%; $P = 0.017$), carboxyterminal propeptide of type I procollagen (+17.6%; $P = 0.002$), procollagen III N-terminal extension peptide (+48.4%; $P = 0.001$), and carboxyterminal cross-linked telopeptide of type I collagen (53.3%; $P = 0.009$). Disappearance half-times after cessation of recombinant human GH for pre- and postexercise markers ranged from 248–770 h. We conclude 1) endurance exercise transiently activates bone and collagen turnover; 2) brief GH administration results in similar but quantitatively greater augmentation; and 3) these data will assist in designing a GH detection strategy. (*J Clin Endocrinol Metab* **85:** 124–133, 2000)

NORMAL BONE development is influenced by hormonal, nutritional, physical, and genetic factors. Physical exercise is well known to have potent, but complex, effects (1), with resistance exercise and mechanical loading favoring increased bone mass. Endurance-type exercise is less well understood, but females with exercise-induced amenorrhea are clearly at risk of osteoporosis. Public health recommendations advise regular endurance-type or aerobic activity.

Acute exercise stimulates GH secretion (2). We have recently shown that acute endurance-type exercise also results in transient increments in all components of the insulin-like growth factor (IGF) ternary complex, IGF-I, IGF-binding protein-3 (IGFBP-3), and acid-labile subunit (ALS) (3a). GH and IGF-I stimulate bone turnover and result in long term augmentation of bone mass (3).

Recent evidence suggests that GH has been used by athletes in an attempt to enhance performance. Although adults deficient in GH production due to pituitary tumor or irradiation have been shown to increase lean body mass, exercise performance, and muscle strength and to decrease body fat after GH treatment (4, 5), no scientific documentation of enhanced performance in athletes currently exists. GH use is banned by the International Olympic Committee and other major sporting bodies, but there is currently no method to detect GH abuse by athletes. Abuse in the wider community is also suggested by the use of GH in schools in the U.S. (6). Development of a detection strategy for GH abuse is therefore essential. Short term abuse of GH causes few side-effects other than acute fluid retention, but long term use of GH can cause irreversible, disfiguring skeletal changes and produce cancer and other potentially fatal cardiac abnormalities, as seen in patients with acromegaly (7).

The prolonged effect of GH on markers of bone turnover (8) suggests that such markers may be useful to detect GH abuse. We have previously shown that detection of exogenous GH administration to nonelite athletes was not possible by measuring total serum GH, but serum IGF-I and binding proteins of the IGF ternary complex allowed distinction be-

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tween GH- and placebo-treated individuals in the majority of cases both before and after acute exercise (3a). There is also a recent report that non-22-kDa isoforms of GH will discriminate between endogenous serum GH and exogenously administered 22-kDa GH (9), but no data in the sporting context are currently available.

We therefore studied nonelite, athletic adult males, aiming to define the effects of 1) acute exercise, 2) GH administration (at rest and after acute endurance-type exercise), and 3) the washout or disappearance kinetics after cessation of GH administration (at rest and after endurance-type exercise) on markers of bone and collagen turnover.

Subjects and Methods

Subject selection

Seventeen subjects were selected using the following criteria: male gender, age 18–40 yr, high level of habitual aerobic activity defined as at least four 30-min sessions of continuous aerobic type exercise per week, high aerobic fitness defined as maximal oxygen uptake (VO_2max) more than 45 mL/kg·min, and no illnesses or medications known to impair exercise or to alter endocrine function.

Screening

A full physical examination was performed, and blood was taken for routine biochemistry, hematology, and serum testosterone, T_4, and T_3 measurements. Urine samples were tested to exclude glycosuria. Screening variables were normal in all subjects, except for borderline low serum testosterone in a subject with no clinical evidence of hypogonadism. Skinfold thicknesses were measured with a Harpenden caliper at standard sites (biceps, triceps, subscapular, abdominal, and suprailiac), and percent body fat was calculated (10). Maximal oxygen uptake (VO_2max) was measured by cycle ergometry and respiratory gas analysis (see screening exercise test).

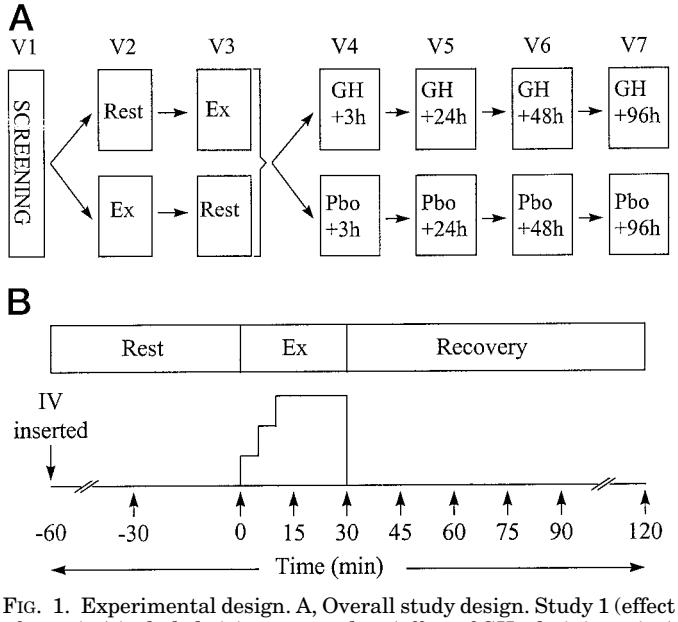

FIG. 1. Experimental design. A, Overall study design. Study 1 (effect of exercise) included visits 2–3; study 2 (effect of GH administration) included the pretreatment exercise study (visit 2 or 3) and visit 4; and study 3 (effect of GH withdrawal) included visits 4–7. B, Protocol for visits 2–7; for rest day (visits 2 or 3 only), chair-sitting was substituted for exercise from 0–30 min.

Overall study design (Fig. 1a)

Subjects attended for seven visits. After screening (visit 1), three consecutive studies were performed to assess the affects of acute exercise, GH administration, and GH withdrawal on markers of bone and collagen turnover. An identical protocol was used on each postscreening visit (Fig. 1b), with the exception of a resting visit in study 1 in which exercise was omitted, and chair sitting was substituted to control for the effect of posture. The effect of acute exercise (study 1) was assessed with a repeat measures design where subjects were randomized (arbitrary assignment) to a rest day or an exercise day, and the alternative condition was performed within 48 h (visits 2 and 3). The effect of GH treatment (study 2) was assessed with a parallel, double blind, placebo-controlled study. A computer-generated code was used for randomization. Subjects self-administered recombinant human GH (rhGH; Genotropin, Pharmacia & Upjohn, Inc., Stockholm, Sweden) or identical placebo at a dose of 0.15 IU/kg·day by sc abdominal injection for 7 days, the first 6 days at 2000 h and the final dose 3 h before admission for testing. Response to the exercise day protocol was assessed both before and after treatment (pretreatment exercise visit and visit 4). This parallel design was continued after treatment was withdrawn (study 3), providing data on disappearance kinetics of bone markers 3 h after the last dose (visit 4), then 24, 48, and 96 h later (visits 5, 6, and 7, respectively), both at rest and in response to acute exercise.

Individual study protocol (Fig. 1b)

Each subject was studied after a 3-h fast in the late afternoon or evening at an identical time. Body weight was recorded in a hospital gown. At −60 min a vein in the nondominant cubital fossa was cannulated using local anaesthetic. Subjects were rested in bed in a semirecumbent position, and baseline blood samples were taken at −30 min. A second set of baseline samples was taken 30 min later (0 min), after which subjects exercised for 30 min on a cycle ergometer (exercise day), or sat on a chair to simulate the upright posture of exercise for 30 min (rest day). Blood samples were taken during exercise/chair-sitting at 15 and 30 min. Subjects returned to bed where blood samples were taken in a semirecumbent position at 45, 60, 75, 90, and 120 min. Subjects drank 250 mL water immediately after exercise and again at 60 min to compensate for water lost in sweating. Chair-sitting subjects were given 50 mL water at these two times to ensure a uniform protocol. Serum free T_4 (fT_4), free T_3 (fT_3), and testosterone were determined at baseline at each study, and serum GH, IGF-I, osteocalcin, bone-specific alkaline phosphatase (BS-ALP), carboxyterminal propeptide of type I procollagen (PICP), procollagen III N-terminal extension peptide (PIIIP), and carboxyterminal cross-linked telopeptide of type I collagen (ICTP) were determined at all time points.

Exercise testing

Exercise testing was performed using an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Grunningen, Holland) and Medical Graphics CPX-D Cardiopulmonary Exercise Testing System (Medical Graphics, Birmingham, UK). Expired gas was sampled continuously at the mouth. The concentration of dried gas was measured with analyzers accurate to ~1%: zirconia oxide O_2 analyzer (response time, <80 ms) and infrared CO_2 analyzer (response time, <130 ms). Gas volume was measured with a bidirectional differential pressure preVent pneumotach (accuracy, ~3%). A 12-lead electrocardiogram was monitored during and after exercise.

Screening exercise test. For screening purposes VO_2max was assessed. Subjects cycled to exhaustion with a starting workload of 1.5 watts/kg BW, using a smooth ramp of 25 watts/min, at a cycling cadence of 80 rpm with feet strapped to the pedals. Workload at VO_2max was used to calculate the submaximal protocol for the main studies. Where the oxygen uptake reached a plateau and the workload continued to rise, the workload at which the plateau first occurred was regarded as workload at VO_2max.

Submaximal exercise protocol. All subsequent submaximal exercise tests used an identical protocol, consisting of three consecutive stages: stage 1 was 5 min at 1 watt/kg, stage 2 was 5 min at 2 watts/kg, and stage 3 was 20 min at 65% of the workload achieved at VO_2max (corresponding to approximately 80% VO_2max).

The protocol was approved by the ethics committee of Guys and St Thomas's Hospital (London, UK). Subjects gave informed, written consent.

Assays

All samples from one patient were assayed in two batches: batch 1, visits 2, 3, and 4; and batch 2, visits 5, 6, and 7. Laboratory staff were blinded to the treatment code, which was broken after results were entered into a database. Analytes responding to GH treatment at visit 4 were considered to be potential doping markers and assayed for all visits; BS-ALP did not respond and therefore was not analyzed beyond visit 4.

Serum osteocalcin was measured by RIA (OSTK-PR *in vitro* test kit, CIS Biointernational, Oris Industries, Gif-Sur-Yvette Cedex, France); the intraassay coefficients of variation (cv) were 3.7% and 3% at 3.8 and 24.7 ng/mL, and the interassay cv were 6.6% and 5.5% at 3.8 and 24.7 ng/mL. Serum B-S ALP was measured by immunoradiometric assay (IRMA; Tandem-R Ostase, Hybritech Europe, Liege, Belgium); intraassay cv were 6.7%, 4.2%, and 3.7% at 13.2, 26.7, and 48.6 μg/L, and interassay cv were 8.1%, 7.2%, and 7.0% at 11.7, 40.6, and 77.4 μg/L. Serum PICP was measured by RIA (Orion Diagnostica, Espoo, Finland); the intraassay cv were 2.1% and 3.2% at 103 and 415 μg/L, and the interassay cv were 4.1% and 4.0% at 105 and 435 μg/L. Serum PIIIP was measured by a two-stage sandwich RIA (Cis Biointernational; as above); total assay cv (intra- plus interassay) were 9.1%, 5.7%, and 6.8% at 0.62, 0.95, and 1.18 μg/L. Serum ICTP was measured by RIA (Orion Diagnostica); the intraassay cv were 6.2% and 4.4% at 3.8 and 11.2 μg/L, and the interassay cv were 7.9% and 6.5% at 3.3 and 10.5 μg/L. Serum total IGF-I was measured by RIA after acid-ethanol extraction as previously reported (3a). Serum fT_4 and fT_3 were measured in a Bayer Corp. (Oberlin, OH) ACS:180 analyzer with a chemiluminescent end point. The fT_4 intraassay cv were 4.9% and 6.0% at 12.0 and 20.0 pmol/L, and the interassay cv were 10.0% and 9.6% at 10.5 and 21.5 pmol/L. The fT_3 intraassay cv were 3.8% and 2.1% at 2.9 and 5.2 pmol/L, and the interassay cv were 6.2% and 5.6% at 2.9 and 5.2 pmol/L. Serum testosterone was measured by a Bayer Corp. ACS:180 analyzer with a chemiluminescent end point. The intraassay cv was 3.8% at 9.1 and 24.9 nmol/L, and the interassay cv were 3.8% and 7.6% at 9.1 and 24.9. Hematocrit, collected at the start and the end of exercise only, was measured immediately in duplicate by microcentrifugation.

Statistics

Differences in subject characteristics at baseline between GH and placebo groups were assessed with Student's *t* test. Effects of exercise were assessed by split plot, repeat measures ANOVA using a general linear model (SPSS 7.5 for Windows<SPSS, Inc., Chicago, IL), with within-subject factors being condition (rest *vs.* exercise) and time point, and the between-subject factor being study order. Effects of GH treatment were assessed similarly, with within-subject factors being visit (pre- *vs.* posttreatment) and time point, and between-subject factors being treatment and study order if it was significant in the prior analyses. To illustrate relative responses to treatment and withdrawal, data for individual analytes were converted into SD scores, using the means and SDs of resting, pretreatment values as reference data. Descriptions of disappearance half-times involved exponential curve fitting to the group mean data in the GH-treated group alone for declining values (visits 5–7 for all except osteocalcin, where values declined from visit 4). Simple linear regression analysis was used to assess relationships between variables. Results are reported as the mean ± SEM.

Results

Subjects

We studied 17 males; 1 subject (placebo group) withdrew before treatment due to a training injury. Eight subjects were randomized to each treatment group. There were no statistically significant differences in physical or performance characteristics between those randomized to GH or placebo treatments, although the placebo group tended to be heavier, but not fatter, than the GH group (see Table 1). Compliance, as assessed by vial count, approached 100% in both groups. There were few side effects reported by those who had received rhGH treatment: 1 individual noted facial puffiness and flushing, and another felt heaviness in his thighs. Two reported a subjective sensation that the standard exercise protocol felt more difficult after rhGH treatment. Symptoms disappeared within 24 h of cessation. Before intervention, several subjects had basal values above the reference ranges [see Table 2; osteocalcin (n = 1), 19.0 μg/L; PICP (n = 3), up to 301.5 μg/L; ICTP (n = 2), up to 5.66 μg/L] and below the reference range [PIIIP (n = 1), 0.25 μg/L].

Effect of acute exercise (see Table 2 and Fig. 2)

Serum osteocalcin increased by 5.8% from 0–15 minutes postexercise. This change was not significant compared to that on the rest day ($P = 0.08$). All other markers increased significantly in response to exercise compared to rest ($P < 0.001$, condition × time point): serum BS-ALP transiently, +7.4% (+16.1% in response to acute exercise compared to the rest day, +8.7%); PICP transiently, +9.2% (+14.1% exercise *vs.* +4.9% rest); PIIIP transiently, +10.2% (+5.0% exercise *vs.* −5.2% rest); and ICTP, a persistent increase of +7.0% (+9.7% exercise *vs.* +2.7% rest).

Hematocrit values before and after upright posture on the rest day were 36.8 ± 0.5% (range, 33.5–40.4%) and 37.7 ± 0.6% (range, 32.5–41.6%), respectively, and values before and after acute exercise were 39.2 ± 0.7% (range, 35.8–46.3%) and 40.9 ± 0.6% (range, 36.3–47%), respectively. There was no significant effect of exercise on hematocrit ($P = 0.3$). Although individual hematocrit values fell below Queensland Health Pathology Services range of 35–51%, repeated mea-

TABLE 1. Subject characteristics

	All subjects	GH group	Placebo group	*P* value
Age	26.9 ± 1.5	28.3 ± 2.8	25.5 ± 1.5	0.4
Ht (cm)	176.9 ± 1.1	175.4 ± 1.0	178.2 ± 2.0	0.23
Wt (kg)	73.9 ± 2.2	69.5 ± 2.8	76.7 ± 2.8	0.09
BMI (kg/m^2)	23.6 ± 0.6	22.6 ± 0.7	24.2 ± 0.9	0.16
Fat (%)	17.3 ± 1.1	16.0 ± 1.8	18.5 ± 1.6	0.32
Waist/hip ratio	0.84 ± 0.02	0.83 ± 0.02	0.85 ± 0.02	0.5
VO_2max (L/min)	4.09 ± 0.09	3.93 ± 0.11	4.26 ± 0.15	0.1
VO_2max (mL/min·kg)	56. ± 1.2	57 ± 1.6	56 ± 1.5	0.58

All subjects (n = 17) underwent both resting and exercise studies. Subjects were subsequently randomized to either GH (n = 8) or placebo (n = 8) treatment groups. There were no statistically significant differences between treatment groups ($P < 0.05$), assessed with the Student's *t* test. BMI, Body mass index. Data represent the mean ± SEM.

TABLE 2. Responses of markers of bone and collagen turnover to acute exercise

Analyte	(reference range)	−30 min	0 min	Peak	120 min
Osteocalcin (0.9–18.0 μg/L)	Rest	11.7 ± 0.8 (8.3; 17.8)	11.9 ± 0.8 (8.2; 18.4)	11.9 ± 0.8 (8.3; 18.4)	12.2 ± 0.8 (8.6; 18.7)
	Ex	11.7 ± 0.7 (8.4; 18.1)	11.9 ± 0.8 (8.1; 17.5)	12.6 ± 0.8 (8.6; 19.1)	12.5 ± 0.9 (8.1; 19.5)
BS-ALP (3.7–21.2 μg/L)	Rest	9.8 ± 0.7 (5.6; 15.1)	10.4 ± 0.7 (6.3; 15.4)	11.1 ± 9.0 (6.8; 16.2)	10.2 ± 0.7 (5.8; 15.0)
	Ex	10.3 ± 0.8 (6.3; 15.4)	11.2 ± 0.9 (6.7; 17.1)	13.0 ± 1.0 (7.9; 19.2)	10.5 ± 0.8 (6.5; 15.8)
PICP (38–202 μg/L)	Rest	177.3 ± 16.1 (100.2; 283.9)	178.8 ± 15.8 (101.4; 287.2)	185.2 ± 18.5 (106.0; 303.4)	180.4 ± 17.1 (106.8; 301.9)
	Ex	160.0 ± 12.8 (108.7; 233.0)	172.75 ± 14.0 (114.7; 251.3)	198.6 ± 16.2 (127.7; 293.4)	149.5 ± 12.7 (93.7; 229.4)
PIIIP (0.3–0.8 U/mL)	Rest	0.427 ± 0.021 (0.295; 0.557)	0.424 ± 0.021 (0.306; 0.550)	0.403 ± 0.021 (0.227; 0.505)	0.418 ± 0.022 (0.286; 0.552)
	Ex	0.445 ± 0.028 (0.285; 0.626)	0.460 ± 0.024 (0.325; 0.618)	0.488 ± 0.026 (0.326; 0.626)	0.442 ± 0.027 (0.284; 0.602)
ICTP (0.8–5.0 μg/L)	Rest	3.50 ± 0.23 (2.42; 5.32)	3.63 ± 0.24 (2.43; 5.46)	3.64 ± 0.23 (2.62; 5.20)	3.86 ± 0.25 (2.76; 6.00)
	Ex	3.75 ± 0.23 (2.62; 5.27)	3.87 ± 0.23 (2.66; 5.23)	4.28 ± 0.25 (3.01; 5.79)	4.48 ± 0.29 (2.88; 6.37)

Subjects (n = 17) underwent rest and exercise (Ex) studies in randomized order. Time was measured relative to the start of exercise, and peak values occurred at the end of exercise, except for osteocalcin, which occurred 15 min later. Data represent the mean ± SEM (5th percentile; 95th percentile).

FIG. 2. The effect of acute endurance-type exercise on markers of bone and soft tissue collagen turnover. Subjects (n = 17) underwent random sequence exercise study (semirecumbency from −30 until 0 min; exercise from 0–30 min, with 10 min two-stage warm-up and 20 min at 65% of the workload at VO_2max; semirecumbency from 30–120 min; *solid symbols and continuous line*) or rest study (upright posture during equivalent exercise period indicated by *box*; *open symbols and dotted line*).

sures on other visits were within this range, and no individual had an abnormal hemoglobin value. As previously reported, serum total GH increased from 5.2 ± 2.2 mU/L before exercise to a peak of 72.8 ± 10.9 mU/L at the end of exercise ($P = 0.0001$), and serum total IGF-I increased from 25.2 ± 0.2 nmol/L before exercise to 30.3 ± 0.2 nmol/L at the end of exercise ($P = 0.0001$) (3a).

At baseline on the exercise day there were significant negative associations between age and markers of formation (osteocalcin: $r^2 = 0.30$; $P = 0.028$; PIIIP: $r^2 = 0.49$; $P = 0.003$) and resorption (ICTP: $r^2 = 0.49$; $P = 0.003$). There were no associations between markers and stature (height, weight), fitness (VO_2 max and VO_2 max per kg), body composition (body mass index and percent body fat by skinfold thickness), hormonal status (fT_4, fT_3, and testosterone), or peak GH response to exercise. Basal IGF-I was weakly associated only with ICTP ($r^2 = 0.25$; $P = 0.047$). There were associations between markers at baseline (osteocalcin *vs.* PICP: $r^2 = 0.45$; $P = 0.004$; osteocalcin *vs.* ICTP: $r^2 = 0.30$; $P = 0.029$; PIIIP *vs.* ICTP: $r^2 = 0.69$; $P < 0.0001$). BS-ALP was not associated with any other marker. There were no associations between changes in markers in response to exercise and either age, stature, fitness, body composition, hormonal status, or peak GH response to exercise. Increments in ICTP in response to exercise were associated with increments in PIIIP ($r^2 = 0.49$; $P = 0.002$) and PICP ($r^2 = 0.27$; $P = 0.038$).

Response to treatment (see Table 3 and Figs. 3 and 4)

Serum osteocalcin increased significantly in response to GH treatment (net basal values, +10.0%; $P = 0.015$, visit × treatment), but the response to exercise was not augmented ($P > 0.05$, visit × treatment × time point). BS-ALP did not change with either placebo or GH treatment, and the acute response to exercise was maintained. PICP increased significantly in response to GH treatment (net basal values, +17.6%; $P = 0.05$, visit × treatment), and the response to acute exercise was exaggerated (pre-GH, +15.3%; post-GH, +20.3%; $P = 0.009$, visit × treatment × time point). PIIIP increased significantly in response to GH treatment (net basal values, +48.4%; $P = 0.006$, visit × treatment), but the response to exercise was not altered ($P = 0.11$). ICTP increased significantly in response to GH treatment (net basal values, +53.3%; $P = 0.009$, visit × treatment), and the response to acute exercise was exaggerated (pre-GH, +8.4%; post-GH, +11.3%; $P = 0.027$, visit × treatment × time point).

As previously described, hematocrit did not change in response to GH treatment (3a). Baseline serum GH increased in response to GH treatment (pre-GH, 1.6 ± 0.7; post-GH, 89.2 ± 11.6 nmol/L), and the GH response to exercise was attenuated (pre-GH: preexercise, 4.3 ± 2.2; end of exercise, 49.7 ± 17.5; post-GH: preexercise, 81.2 ± 11.6; end of exercise, 98.2 ± 12.6). Serum IGF- I at baseline increased with GH treatment (pre-GH, 24.5 ± 3.2; post-GH, 69.6 ± 8.1; $P = 0.0001$). Age, stature, fitness, body composition, hormonal status, and peak GH response to exercise were not associated with either baseline or posttreatment increments in bone and collagen turnover markers.

Response to withdrawal (see Table 4 and Fig. 5)

Responses to treatment and withdrawal of GH in preexercise absolute values and relative responses in SD scores are shown in Table 4 and Fig. 5, respectively. All markers began to decline immediately after cessation of GH or with a delay of 24 h. Disappearance half-times calculated from individual curves for both pre- and postexercise data were as follows: osteocalcin, 770 and 693 h; PICP, 433 and 408 h; PIIIP, 693 and 770 h; and ICTP, 248 and 289 h, respectively.

Serum fT_4 fell after GH treatment ($P = 0.032$, treatment × visit), and serum fT_3 rose ($P = 0.003$, treatment × visit; Table 5). Serum total testosterone was not affected by rhGH administration ($P = 0.14$; Table 5). As previously reported, after cessation of rhGH administration, the serum GH response to exercise was inhibited at visits 5 and 7. After cessation of rhGH administration, the preexercise serum total IGF-I concentration in the GH group remained elevated 24 h later (69.6 ± 8.1 and 70.3 ± 8.8 nmol/L at visits 4 and 5, respectively), then declined in an exponential fashion, approaching basal values by approximately 96 h after cessation of rhGH.

Discussion

The main findings from this study are that in young athletic males 1) acute endurance-type exercise stimulates markers of bone and soft tissue formation and bone resorption; 2) GH administration results in a much larger increase in these markers; 3) such changes persist in some cases for at least

TABLE 3. Responses of markers of bone and collagen turnover to GH administration

Analyte		Pretreatment		Posttreatment	
		0 min	Peak	0 min	Peak
Osteocalcin (μg/L)	Pbo	12.0 ± 0.9 (9.9; 16.1)	12.5 ± 1.0 (9.7; 15.2)	12.1 ± 0.9 (9.6; 16.1)	13.1 ± 1.2 (9.8; 17.5)
	GH	12.4 ± 1.1 (9.0; 17.3)	13.3 ± 1.4 (8.8; 18.8)	15.0 ± 1.2 (10.9; 19.5)	16.2 ± 1.1 (11.6; 20.2)
BS-ALP (μg/L)	Pbo	10.6 ± 1.0 (7.6; 14.4)	12.2 ± 1.4 (8.6; 17.4)	10.0 ± 0.8 (7.5; 12.8)	12.5 ± 1.3 (8.7; 18.3)
	GH	11.7 ± 1.6 (5.5; 17.2)	13.5 ± 1.7 (7.6; 19.8)	11.0 ± 1.6 (5.8; 17.0)	13.5 ± 2.0 (7.2; 20.7)
PICP (μg/L)	Pbo	193.1 ± 23.4 (117.6; 288.7)	224.2 ± 27.6 (126.5; 328.8)	211.7 ± 29.13 (114.5; 316.7)	238.2 ± 31.1 (136.3; 349.8)
	GH	156.1 ± 17.1 (116.3; 228.7)	176.9 ± 18.6 (137.6; 257.9)	203.6 ± 16.6 (174.6; 279.9)	244.9 ± 20.9 (197.3; 336.5)
PIIIP (U/mL)	Pbo	0.440 ± 0.022 (0.350; 0.505)	0.464 ± 0.033 (0.339; 0.593)	0.411 ± 0.021 (0.318; 0.473)	0.458 ± 0.020 (0.374; 0.523)
	GH	0.474 ± 0.046 (0.309; 0.642)	0.497 ± 0.044 (0.318; 0.638)	0.668 ± 0.070 (0.402; 0.906)	0.769 ± 0.094 (0.435; 1.131)
ICTP (μg/L)	Pbo	3.60 ± 0.23 (3.04; 4.62)	4.03 ± 0.29 (3.36; 5.41)	3.42 ± 0.27 (2.56; 4.59)	3.99 ± 0.36 (3.11; 5.60)
	GH	4.13 ± 0.43 (2.61; 5.65)	4.47 ± 0.44 (2.86; 5.87)	6.21 ± 0.66 (3.85; 8.63)	6.91 ± 0.73 (4.10; 9.14)

Subjects were randomly assigned to receive placebo (Pbo) or GH (n = 8 each) at a dose of 0.15 IU/kg·day for 7 days. Time measured relative to the start of exercise, and peak values occurred at the end of exercise, except for osteocalcin, which occurred 15 min later. Data represent the mean ± SEM (5th percentile; 95th percentile).

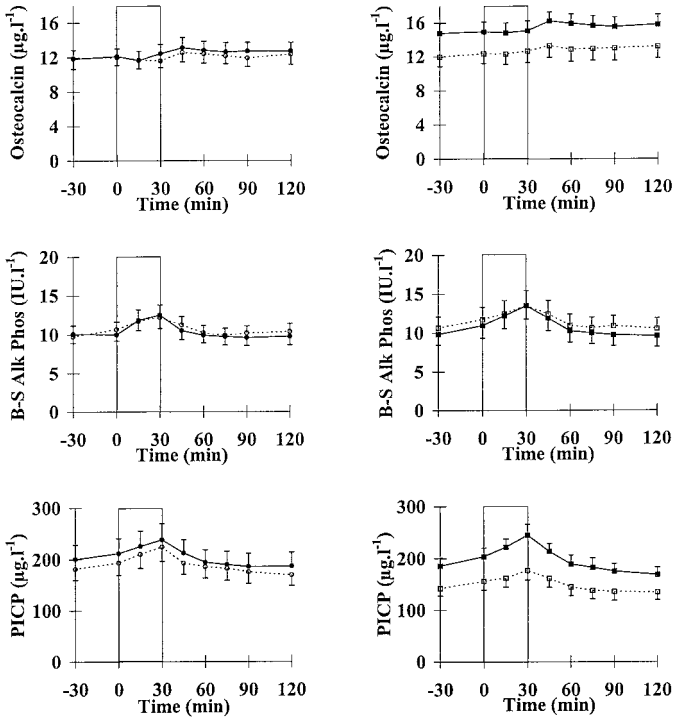

FIG. 3. The effect of rhGH treatment on markers of bone formation. Subjects underwent identical exercise tests as those described in Fig. 1 before (*open symbols*) or after (*closed symbols*) randomization to treatment with placebo (*circles, left panel*) or rhGH (0.15 IU/kg·day; *squares, right panel*) for 7 days.

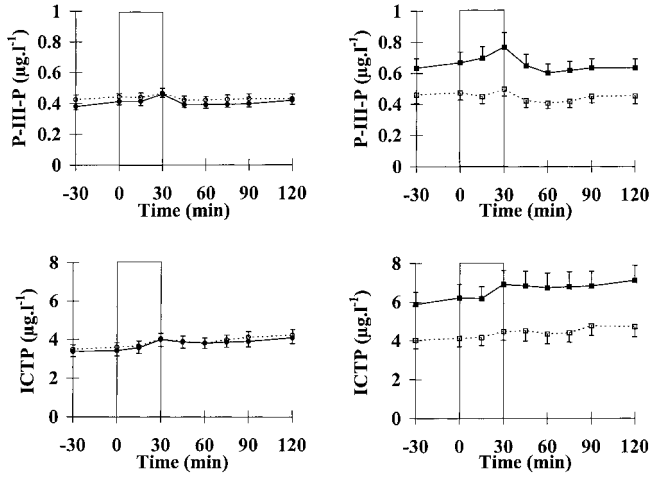

FIG. 4. The effect of rhGH treatment on markers of soft tissue collagen formation and bone resorption. Subjects underwent identical exercise tests as those described in Fig. 1 before (*open symbols*) or after (*closed symbols*) randomization to treatment with placebo (*circles, left panel*) or rhGH (0.15 IU/kg·day; *squares, right panel*) for 7 days.

96 h; and 4) markers that appear promising for the purpose of detecting GH abuse include PIIIP and ICTP, as they exhibit small changes in response to acute exercise and large enduring changes in response to treatment with little variation in placebo-treated individuals over time.

Response to acute exercise

The markers of bone formation reported in this study reflect different stages of osteoblastic cell function (11, 12). For example, osteocalcin and BS-ALP are generated during bone mineralization (13, 14). Earlier phases of the bone-remodeling process involve the deposition of collagen scaffolding, which generates PICP (15). In contrast to these bone-specific markers, PIIIP does not appear to be present in bone (except during callus formation after fracture), reflecting extraosseous collagen formation, particularly in normal ligament and tendinous structures and in pathological states of collagen deposition, such as hepatic fibrosis (16–18). ICTP is thought to be a marker of bone resorption (11).

The net changes in markers of bone and collagen turnover in response to acute exercise exceeded those attributable to changes due to hemoconcentration. The formulas of van Beaumont *et al.* (19, 20) predict mean changes in concentration of analytes due to hemoconcentrations of 7% and 4% due to exercise and upright posture, respectively, across the 30-min exercise/sitting period. Our finding of no statistically significant change in serum osteocalcin with acute exercise is consistent with either 1) no change in production, possibly due to its relationship to a more delayed phase in the bone-remodeling cycle; or 2) a real increase in production that was missed due to diffusion of this small molecule (5.8 kDa) from the vascular space (21). The small, late rise in osteocalcin during recovery seen in our study is consistent with a delayed elevation documented in athletes 60 min after exercise (22). BS-ALP increased by 15.3% at the end of exercise, but appeared to be the most posturally dependent marker, increasing by 11.4% on the rest day in response to upright posture. Transient, parallel increases in other collagen formation markers (PICP, +14.1%; PIIIP, +5.0%; during exercise *vs.* −5.2% across upright posture) were also noted. ICTP, a marker of bone resorption, increased by 9.7% by the end of exercise and remained consistently elevated thereafter. Correlation analysis revealed concordance between markers in response to exercise. We concluded 1) the exercise-induced changes in serum markers of bone and collagen turnover cannot be explained by hemoconcentration; 2) bone and soft tissue formation and bone resorption were augmented during the brief, endurance-type exercise protocol employed; and 3) there was a coupling of formation and resorption in response to this stimulus.

Unlike our study, most other studies examining the effects of endurance-type exercise on markers of bone turnover do not have appropriate rest controls and/or do not take into account the effects of hemoconcentration. Brahm *et al.* initially suggested that changes in serum bone markers with acute exercise could be mostly explained by hemoconcentration (21). They later demonstrated, however, that the exercising limb acutely produced markers of bone turnover (23), supporting our conclusions. Unfortunately, this excellent but invasive study did not include nonexercising controls. Other uncontrolled studies examining low intensity endurance-type activity or brief high intensity or resistance exercise showed no acute change (24–27), increased markers of bone formation and resorption (22, 28), or transiently decreased markers (24). Previous studies using short duration, high intensity exercise showed no rise in PICP or ICTP in response to exercise (24, 25, 28), suggesting that the du-

TABLE 4. The effects of GH administration and GH withdrawal on markers of bone and collagen turnover

		Ex	V4	V5	V6	V7
Osteocalcin (μg/L)	Pbo	11.9 ± 1.0	11.8 ± 1.2	9.9 ± 0.6	10.1 ± 1.1	10.2 ± 0.7
	GH	12.0 ± 1.1	14.8 ± 1.2 (1/8)	14.0 ± 1.0 (4/8)	13.8 ± 0.7 (4/8)	13.5 ± 0.9 (3/7)
PICP (μg/L)	Pbo	181.6 ± 21.5	200.6 ± 27.8	184.5 ± 22.1	190.8 ± 25.2	167.0 ± 16.9
	GH	142.2 ± 14.5	186.0 ± 15.0 (0/8)	212.7 ± 17.6 (2/8)	200.2 ± 14.2 (1/8)	188.6 ± 9.7 (2/8)
PIIIP (U/mL)	Pbo	0.425 ± 0.030	0.380 ± 0.020	0.460 ± 0.026	0.463 ± 0.032	0.449 ± 0.030
	GH	0.461 ± 0.053	0.634 ± 0.061 (6/8)	0.760 ± 0.051 (7/8)	0.750 ± 0.060 (7/8)	0.709 ± 0.069 (5/7)
ICTP (μg/L)	Pbo	3.47 ± 0.24	3.37 ± 0.27	3.09 ± 0.21	3.09 ± 0.27	3.09 ± 0.23
	GH	4.01 ± 0.42	5.87 ± 0.62 (6/8)	6.55 ± 0.57 (7/8)	5.81 ± 0.47 (7/8)	5.31 ± 0.51 (6/7)

Values are at −30 min relative to exercise. Ex represents visit 2 or 3 (exercise day), and V4, 5, 6, and 7 (represent visits at 3, 27, 51, and 99 h, respectively, after cessation of GH or placebo (Pbo) treatment. Numbers in *parentheses* represent the proportion of subjects in the GH group outside the range in the placebo group at each time point.

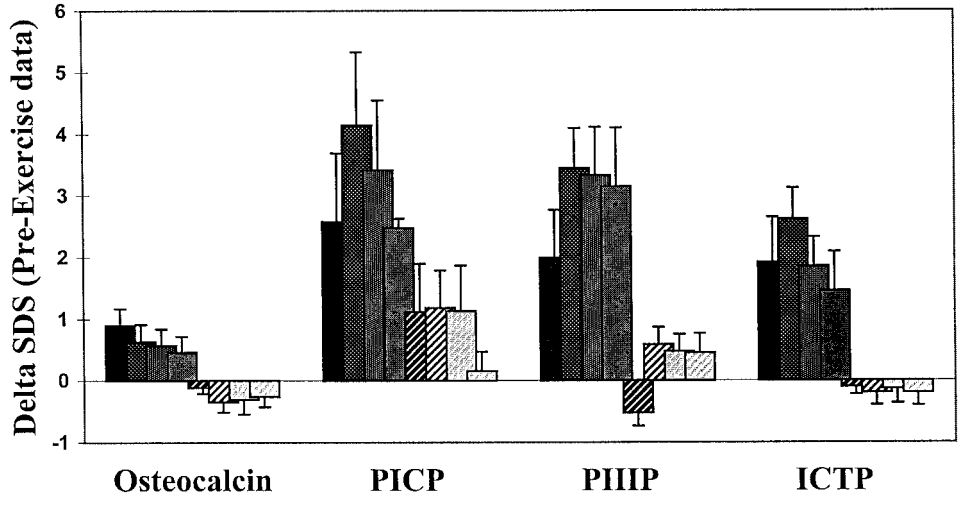

FIG. 5. The relative responses of markers of bone and soft tissue collagen turnover to GH treatment. Data have been transformed into SD scores (SDS) using the mean and SD from the pretreatment, preexercise data for the entire study group. Results report the differences in SD scores (mean ± SEM) from pretreatment visit to visits 4, 5, 6, and 7 (3, 27, 51, and 99 h after the last dose of rhGH, respectively) in the GH group (*black, dark gray, midgray*, and *light gray*, respectively) and placebo group (*black, dark gray*, *midgray*, and *light gray oblique stripes*, respectively).

ration of exercise is an important element in the response of bone markers to acute exercise. In addition, uncontrolled studies of the delayed effects of exercise show either reductions in bone formation after marathon running (29), or increases in bone formation and resorption (21, 26) in the 24–48 h after activity.

The concurrent increases in GH, GH-binding protein, IGF-I, IGFBP-3, and ALS (3a) are unlikely to cause the increase in bone and soft tissue markers in response to acute exercise, as exogenous GH administration to normal adults takes several days to increase markers of bone turnover (8). Concurrent exercise-stimulated, regulatory hormones (PTH, cortisol, and testosterone) (30, 31) or metabolic acidosis (which inhibits osteoblastic and stimulates osteoclastic activity) (32) are also unlikely stimuli. In contrast, physical strain on bones due to load bearing results in stimulation of bone formation and inhibition of bone resorption (1), and resistance training results in quantitatively greater bone marker responses than endurance-type training (33). We therefore hypothesize that markers may also be washed out of tissues by mechanical stress inducing microdamage and leakage or by alterations in blood flow that affect tissue-bound mobile pools, a theory supported by increases in PIIIP in long distance male runners after a 24-h competitive run (34), but not after a 24-h cross country ski race (35).

Endurance-trained athletes and habitual exercisers have higher bone mineral density, especially in load-bearing sites, than age-matched nonexercising subjects (1), but only minor differences in markers of bone turnover. Cross-sectional studies show normal osteocalcin and BS-ALP in endurance-trained athletes and lower PICP and ICTP compared to age- and gender-matched controls (36, 37). Interventional studies have shown that serum osteocalcin and other markers of formation increase in response to exercise training of periods ranging from 5 weeks to 18 months (38–41), but that levels may transiently decline during the first 4 weeks and return to pretraining levels by 8 weeks of training (33, 42). We therefore conclude that 1) repeated bouts of acute exercise result in repetitive remodeling phases and exposure of bone to augmented levels of circulating growth factors (GH, IGF-I, and possibly other GH-stimulated, bone anabolic agents, such as IGFBP-5) (43); 2) stimulation of bone turnover by these endocrine factors would modify whole body bone mineral density in a direction dependant upon the balance of formation and resorption; 3) the long term effects of GH and IGF-I on bone favor formation (3, 43); and 4) autocrine, paracrine, and especially mechanical forces have additional local effects. Our data also suggest that age may be a negative determinant of the bone marker response to exercise.

Response to GH treatment

To our knowledge, this is the first study to assess the effects of GH treatment on bone markers in athletes. We demonstrated large increases in serum concentrations of markers of bone and collagen formation and bone resorption.

TABLE 5. Free T_4, free T_3, and total testosterone responses to exercise, GH administration, and GH withdrawal

Analyte		Rest	Exercise	V4	V5	V6	V7
Free T_4 (pmol/L)	Pbo	13.7 ± 1.1	14.0 ± 1.0	13.6 ± 1.1	13.6 ± 1.2	13.1 ± 1.1	12.7 ± 0.8
	GH	13.5 ± 0.3	14.4 ± 0.4	13.5 ± 0.5	12.0 ± 0.4	11.5 ± 0.4	12.4 ± 0.5
Free T_3 (pmol/L)	Pbo	4.3 ± 0.2	4.6 ± 0.2	4.5 ± 0.2	4.3 ± 0.2	4.4 ± 0.2	4.5 ± 0.1
	GH	4.5 ± 0.1	4.6 ± 0.1	5.5 ± 0.2	5.2 ± 0.2	4.7 ± 0.3	4.7 ± 0.2
Total testosterone (nmol/L)	Pbo	18.35 ± 2.2	17.3 ± 2.2	19.4 ± 2.3	18.5 ± 2.3	15.7 ± 1.4	17.7 ± 1.3
	GH	18.7 ± 2.5	20.8 ± 2.6	19.6 ± 2.3	18.8 ± 2.6	16.1 ± 2.9	15.4 ± 2.2

Values were collected at −30 min (basal/resting) at visits 2 and 3 (rest and exercise) and at visits 4, 5, 6, and 7 (at 3, 27, 51, and 99 h, respectively) after cessation of GH or placebo treatment.

These findings are in keeping with a large literature showing similar responses in GH-deficient adults and in normal adults (8, 44–51). Similarly, chronic elevation of serum GH, as in acromegaly, is also associated with increased bone turnover (52, 53) and increased IGF expression in cortical bone (54).

Concordance between increases in markers of bone formation and resorption after GH administration is consistent with the linkage of these processes. In adults with GH deficiency, GH administration results in a biphasic response, where bone resorption predominates in the first several months, followed by a dominant effect on formation (3). In our short term study, we have not been able to identify any physical, hormonal, or training-related factors that modify the bone and soft tissue marker responses to GH. Differences in the time course of responses may explain some of the differences between the markers. For example, BS-ALP in our study did not respond to GH treatment for 1 week, but others have shown either an initial fall or an elevation that is only seen after several months of treatment (8). We omitted further consideration of this marker in the light of the failure to respond to GH; further studies examining the delayed response may yet establish BS-ALP as a marker of GH abuse in sport.

We demonstrated an augmented response to acute exercise after GH administration for PICP and ICTP. We speculate that this exaggerated response represents an extension of the effects responsible for the acute exercise response. Bone is clearly rendered metabolically more active by GH, and hence, exercise-induced mechanical or blood flow-related events (see above) may release greater amounts of bone turnover markers.

GH treatment withdrawal

Elevations in markers of bone and collagen turnover after GH administration persisted for at least 96 h after cessation of treatment. The disappearance half-times are descriptive and limited by the relatively short observation period. Nevertheless, these data suggest that markers of bone and soft tissue turnover might detect abuse of GH for periods considerably longer than those obtained by assessing components of the GH/IGF axis (3a). Others have shown increased markers of bone and collagen turnover persisting for up to several weeks after cessation of GH administration (8, 55). Data from the placebo-treated control group are vital in assessing the utility of an agent as a potential marker of GH abuse, analogous to day to day variability in training athletes and postcompetition situations. For example, the reduction in osteocalcin in the placebo control group between visits 4–7 is consistent with decreased concentrations seen in men 24 h after a 28-km or marathon race (29, 56).

Development of a test for GH abuse in sports

The use of markers of GH action as a basis for a doping detection strategy demands clear distinction between an individual result and appropriate reference data. Several of our subjects had preinterventional values outside the reference ranges, which were constructed from nonathletic populations. We believe that this reflected the effects of training and highlights the need for condition-specific reference ranges. Reference data in elite athletes for markers of bone and soft tissue turnover markers in either blood or urine do not currently exist, but are being collected as part of the GH-2000 project, a large collaborative study group concerned with development of such a test. Consideration is being given to factors that are likely to influence this normative data, such as age, gender, racial background, and sporting discipline. A number of other factors will need to be assessed, including diurnal and seasonal variation in markers (57, 58), effects of heavy training or competition (29, 56), injury [especially for PIIIP (18), undiagnosed acromegaly, and other illnesses (59–63)], and other medications. Our data suggest that PIIIP and ICTP may be potential markers of GH abuse due to 1) small changes in response to acute exercise, 2) much larger increments in response to even short term GH administration, 3) day to day stability within subjects (individual data not shown), 4) separation of GH- and placebo-treated individuals in up to 87.5% of cases, and 5) persistence of elevated concentrations for up to 96 h after cessation of GH administration both before and after acute exercise.

Finally, a test using the combined strategy of markers of GH action in both the IGF/IGFBP axis and bone markers may improve the sensitivity of either approach alone.

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