

# Responses of the Growth Hormone (GH) and Insulin-Like Growth Factor Axis to Exercise, GH Administration, and GH Withdrawal in Trained Adult Males: A Potential Test for GH Abuse in Sport\*

JENNIFER D. WALLACE, ROSS C. CUNEO, ROBERT BAXTER, HANS ØRSKOV, NICOLA KEAY, CLAIRE PENTECOST, ROLF DALL, THORD ROSÉN, JENS OTTO JØRGENSEN, ANTONIO CITTADINI, SALVATORE LONGOBARDI, LUIGI SACCA, JENS SANDAHL CHRISTIANSEN, BENGT-ÅKE BENGTSSON, AND PETER H. SÖNKSEN

*Metabolic Research Unit, Department of Medicine, University of Queensland, Princess Alexandra Hospital (J.D.W., R.C.C.), Brisbane 4102, Australia; Kolling Institute of Medical Research, Royal North Shore Hospital, University of Sydney (R.B.), Sydney 2065, Australia; the Department of Endocrinology, Aarhus Community Hospital (H.Ø., R.D., J.O.J., J.S.C.), Aarhus, Denmark; the Department of Endocrinology, St. Thomas's Hospital (N.K., C.P., P.H.S.), London, United Kingdom SE1 7EH; the Research Center for Endocrinology and Metabolism, Sahlgrenska Hospital (T.R., B.-Å.B.), S-41345 Göteborg, Sweden; and the Department of Endocrinology, Frederico II Hospital (A.C., L.S.), 80131 Napoli, Italy*

## ABSTRACT

GH abuse by elite athletes is currently undetectable. To define suitable markers of GH doping, we assessed the effects of acute exercise, GH administration, and GH withdrawal on the GH/insulin-like growth factor (IGF) axis in athletic adult males. Acute endurance-type exercise increased serum GH, GH-binding protein (GHBP), total IGF-I, IGF-binding protein (IGFBP)-3, and acid-labile subunit (ALS), each peaking at the end of exercise. IGFBP-1 increased after exercise was completed. Free IGF-I did not change with exercise. Recombinant human GH treatment (0.15 IU/kg-day) for 1 week increased serum total IGF-I, IGFBP-3, and ALS, exaggerating the responses to exercise. IGFBP-2 and IGFBP-1 were trivially suppressed. After GH with-

drawal, the GH response to identical exercise was suppressed. Total IGF-I, IGFBP-3, and ALS returned to baseline over 3–4 days. In summary, 1) acute exercise transiently increased all components of the IGF-I ternary complex, possibly due to mobilization of preformed intact complexes; 2) GH pretreatment augmented the exercise-induced changes in ternary complexes; 3) postexercise IGFBP-1 increments may protect against delayed onset hypoglycemia; 4) serum total IGF-I, IGFBP-3, and ALS may be suitable markers of GH abuse; and 5) differences in disappearance times altered the sensitivity of each marker for detecting GH abuse. (*J Clin Endocrinol Metab* 84: 3591–3601, 1999)

GH IS A POLYPEPTIDE hormone suspected of being used by elite athletes to enhance sporting performance. Discovery of recombinant human GH (rhGH) in the possessions of Chinese swimmers bound for the 1998 World Swimming Championships and similar problems at the Tour de France cycling event in 1998 strongly suggest the abuse of GH at an elite level. This problem may affect the broader community, as shown by a report of GH use in highschool students in the U.S. (1).

The rationale for the use of exogenous GH to enhance

athletic performance is multifactorial. GH administration during childhood may augment adult height. GH's anabolic and lipolytic actions (2) are demonstrated in studies in adults with GH deficiency, where modest doses of rhGH have been shown to increase lean body mass, skeletal muscle mass, proximal muscle force, and maximal and submaximal aerobic performance and to reduce fat mass (3). Other potentially beneficial effects of GH administration in adults with GH deficiency include increased cardiac output during exercise, increased sweating rates and improved thermal homeostasis, lipolysis to provide fuel for endurance sports, and possibly enhanced ligamentous strength and wound-healing rates. The side-effects of supraphysiological doses of rhGH include sodium and water retention (acute onset) and accelerated osteoarthritis, hypertension, cardiac failure, and an increased incidence of malignancies (delayed onset). Such concerns do not appear to deter abuse.

Administration of GH by athletes to enhance performance is banned by the International Olympic Committee and major sporting bodies, but there is currently no approved means of detection. Measurement of serum or urinary total GH itself

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Address all correspondence and requests for reprints to: Jennifer D. Wallace, Metabolic Research Unit, University of Queensland, Department of Medicine, Princess Alexandra Hospital, Brisbane 4102, Australia. E-mail: jwallace@medicine.pa.uq.edu.au.

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is unlikely to represent an optimal detection method because 1) exogenous rhGH and endogenous GH have identical amino acid sequences, making chemical or immunological distinction difficult; 2) normal serum GH concentrations fluctuate widely, reflecting endogenous pulsatility and responses to stress and exercise (4); 3) measurement of urinary GH is relatively insensitive as a marker of GH administration or acromegaly (5, 6); and 4) chemical tagging of rhGH by pharmaceutical manufacturers would not solve the problem of unlicensed manufacture. Therefore, markers of GH action are being investigated as a potential test for GH abuse.

GH exerts major regulatory influences over several components of the GH/insulin-like growth factor (IGF) system. For example, GH modulates its own receptor expression and the circulating form of the extracellular component of the receptor, the high affinity GH-binding protein (GHBP) (7). GH exerts powerful stimulatory regulation over IGF-I, a protein produced in many tissues, where it exerts anabolic and mitogenic actions (8), and to a lesser extent over IGF-II. The IGFs are transported in serum bound to a number of IGF-binding proteins (IGFBPs), the predominant form comprising the ternary complex of IGF-I, IGFBP-3, and acid-labile subunit (ALS) (8). Responses of the GH/IGF system to rhGH administration are well described in GH-deficient adults (9), but data in normal adults and athletes are limited (10–13). The ratio of IGF-I/IGFBP-2 at rest has been proposed as a test for GH abuse (14). Acute exercise, however, may also influence markers of GH action. For example, serum IGF-I (15–17) and IGFBP-1 (18, 19) both increase transiently after acute exercise. In developing a GH detection system, the rate of disappearance of changes on markers of GH action must be described, but data in athletes either before or after exercise are unknown.

We therefore aimed to define 1) the effects and time course of acute endurance-type exercise, 2) the effect of 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 the GH/IGF axis in a group of nonelite, athletic males.

## Subjects and Methods

### Subject selection

Selection criteria included male gender; age between 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 ( $\text{VO}_{2\text{max}}$ ) above 45 mL/kg-min; and no illnesses or medications known to impair exercise or to alter endocrine function.

### Study design

Subjects attended for seven visits (Fig. 1a). After screening (visit 1), three consecutive studies were performed to assess the effects of acute exercise, GH administration, and GH withdrawal on the GH/IGF axis. Each subject was studied after a 3-h fast in the late afternoon or evening at an identical time. An identical protocol was used on each visit, with the exception of a resting visit in study 1 in which exercise was omitted.

### Screening

A full physical examination was performed, and blood was taken for routine biochemistry, hematology, and serum testosterone,  $T_4$ , and  $T_3$

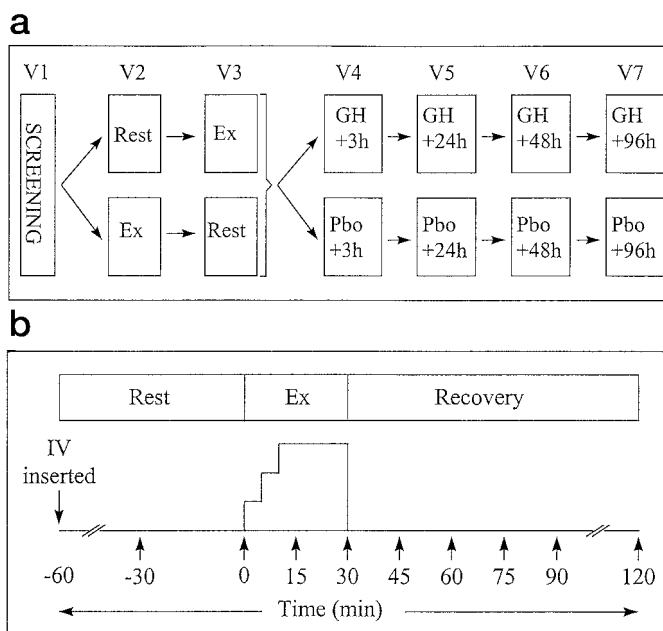


FIG. 1. Experimental design. a, Overall study design. b, Protocol for rest (visit 2 or 3 only) and exercise (visits 2–7) studies.

measurements. Urine samples were tested to exclude glycosuria. Skinfold thicknesses were measured with a Harpenden calliper at standard sites (biceps, triceps, subscapular, abdominal, and suprailiac), and percent body fat was calculated (20).  $\text{VO}_{2\text{max}}$  was measured by cycle ergometry and respiratory gas analysis (see screening exercise test).

### Study 1: effect of acute exercise on the GH/IGF axis

At visit 2, subjects were randomized to one of two conditions: rest day or exercise day. The alternative condition was performed on visit 3 within 48 h. Body weight was recorded in a hospital gown. At -60 min (see Fig. 1b) 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, and at 45, 60, 75, 90, and 120 min in a semirecumbent position during recovery. Subjects drank 250 mL water immediately after exercise and again at 60 min to replace predicted sweat loss. Chair-sitting subjects were given 50 mL water at these two times to ensure uniformity of fluid ingestion.

### Study 2: effect of GH administration on the GH/IGF axis at rest and during and after acute exercise

After study 1 subjects were rerandomized using a computer-generated code to a double blind, parallel study in which each subject received either recombinant human GH (Genotropin, Pharmacia-Upjohn, Stockholm, Sweden) or identical placebo at a dose of 0.15 IU/kg-day for 7 days. Treatment was self administered by sc abdominal injection at 2000 h for six doses, and the seventh dose was given 3 h before cannulation for the posttreatment visit (visit 4). The exercise day protocol of study 1 was repeated on visit 4. Data from visit 4 were compared with those from the identical pretreatment protocol from study 1 to assess the effect of GH/placebo treatment.

### Study 3: effect of withdrawal of GH administration on the GH/IGF axis at rest and during and after acute exercise

The parallel design of study 2 was continued after cessation of treatment for an additional three visits, visits 5, 6, and 7, which were per-

formed 24, 48, and 96 h after the posttreatment visit (visit 4). The exercise day protocol used in studies 1 and 2 was repeated.

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

### Exercise testing

Exercise testing was performed using an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Gronningen, 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  $\pm 1\%$  [zirconia oxide  $O_2$  analyzer (response time of  $< 80$  ms), and infrared  $CO_2$  analyzer (response time of  $< 130$  ms)]. Gas volume was measured with a bidirectional differential pressure preVent pneumotach (accuracy,  $\pm 3\%$ ). A 12-lead electrocardiograph was monitored during and after exercise.

### Screening exercise test

For screening purposes, the  $VO_{2max}$  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  $\sim 80\%$   $VO_{2max}$ ).

### Assays

Samples from visits 2–4 were assayed in the same run (except all IGFBPs, where all assays for the entire study were performed together); subsequent visits were assayed in batches for analytes considered to be potential markers of GH abuse (free IGF-I and GH-BP were not assayed after visit 4). Laboratory staff were blinded to the treatment code, which was broken after results were entered into a database.

Serum GH was assayed by immunoradiometric assay (Pharmacia Biotech, Uppsala, Sweden), with within-assay coefficients of variation (CVs) of 10%, less than 5.0%, and less than 5.0% and between-assay CVs of 9.0%, less than 5.0%, and 7.0% at 10, 23, and 43 mU/L, respectively. Serum GHBP was assayed by a fluoroimmunoassay for functional GHBP (21), with an assay sensitivity of 0.044 nmol/L, a within-assay CV of 3.44%, and between-assay CVs of 12% and 6.3% at 0.56 and 1.40 nmol/L, respectively. Serum IGF-I was measured by RIA using a monoclonal antibody after acid-ethanol extraction (22), with within-assay CVs of 6.6%, 4.4%, and 2.3% and between-assay CVs of 9.7%, 7.0%, and 4.6% at 13.6, 36.7, and 173 nmol/L, respectively. Serum free IGF-I was determined by ultrafiltration and centrifugation after reintroducing physiological temperature and pH in serum as previously described (23). IGFBP-1 (24), IGFBP-2 (25), IGFBP-3 (26), and ALS (27) were assayed using in-house RIAs and polyclonal antibodies. Serum IGFBP-1 within-assay CVs were 9.8%, 4.6%, and 6.4% at 17, 70, and 250  $\mu$ g/L, and between-assay CVs were 24.7%, 14.3%, and 11.5% at 17, 73, and 330  $\mu$ g/L, respectively. Serum IGFBP-2 within-assay CVs were 2.8%, 2.8%, and 3.2% at 140, 275, and 595  $\mu$ g/L, and between-assay CVs were 14.1% and 12.7% at 65 and 775  $\mu$ g/L, respectively. Serum IGFBP-3 within-assay CVs were 6.2%, 5.5%, and 4.5% at 2.5, 5.7, and 12.6 mg/L, and between-assay CVs were 11.9%, 14.5%, and 13.1% at 2.5, 5.7, and 12.6 mg/L, respectively. Serum ALS within-assay CVs were 3.4%, 3.3%, and 3.4% at 60, 245, and 502 nmol/L, and between-assay CVs were 10.5%, 5.4%, and 6.5% at 62, 282, and 676 nmol/L, respectively. Molar ratios were calculated assuming the molecular mass of IGFBP-3 was 43 kDa.

### Statistics

Differences in subject characteristics at baseline between GH and placebo groups were assessed with the 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 assess differences in washout characteristics after cessation of treatment, serum GH data were assessed in an identical manner, comparing pretreatment with posttreatment visits. Descriptions of disappearance half-times involved exponential curve fitting to the individual data in the GH-treated group for IGF-I, IGFBP-3, and ALS at visits 4–7 and 5–7. Simple linear regression analysis was used to assess relationships between variables. Results are reported as the mean  $\pm$  SEM.

## Results

### Subjects

We studied 17 males; one 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, assessed by counting used vials, appeared to be 100%. 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.

### Effect of acute exercise

Acute exercise increased mean serum total GH from  $5.2 \pm 2.2$  mU/L before exercise to a peak concentration of  $72.8 \pm 10.9$  mU/L (range, 14.2–93) at the end of exercise (effect of exercise,  $P = 0.0001$ ; see Fig. 2). There was no association between peak serum total GH and age, weight, fatness (percent body fat), fitness ( $VO_{2max}$  or  $VO_{2max}/kg$ ), or serum testosterone,  $T_4$ , or  $T_3$  concentrations. After the peak at the end of exercise, total serum GH declined in an exponential fashion, with a calculated disappearance half-time of  $20.0 \pm 1.7$  min ( $n = 16$ ). The serum GHBP concentration immediately before exercise was  $1.19 \pm 0.12$  nmol/L (range, 0.38–2.38; male adult reference range, 0.7–3.5 nmol/L). Serum GHBP increased in all patients in response to acute exercise, with a peak concentration at the end of exercise of  $1.44 \pm 0.14$  nmol/L (range, 0.53–2.94), and declined to basal values by the end of the observation period, with no change on the rest day (effect of exercise,  $P = 0.005$ ; see Fig. 2). There were associations between baseline and exercise-induced incre-

**TABLE 1.** Subject characteristics

	All subjects	GH group	Placebo group
Age	26.9 $\pm$ 1.5	28.3 $\pm$ 2.8	25.5 $\pm$ 1.5
Ht (cm)	176.9 $\pm$ 1.1	175.4 $\pm$ 1.0	178.2 $\pm$ 2.0
Wt (kg)	73.9 $\pm$ 2.2	69.5 $\pm$ 2.8	76.7 $\pm$ 2.8
BMI	23.6 $\pm$ 0.6	22.6 $\pm$ 0.7	24.2 $\pm$ 0.9
Fat (%)	17.3 $\pm$ 1.1	16.0 $\pm$ 1.8	18.5 $\pm$ 1.6
Waist/hip ratio	0.84 $\pm$ 0.02	0.83 $\pm$ 0.02	0.85 $\pm$ 0.02
$VO_{2max}$ (L/min)	4.09 $\pm$ 0.09	3.93 $\pm$ 0.11	4.26 $\pm$ 0.15
$VO_{2max}$ (mL/min·kg)	56.0 $\pm$ 1.2	57.0 $\pm$ 1.6	56.0 $\pm$ 1.5

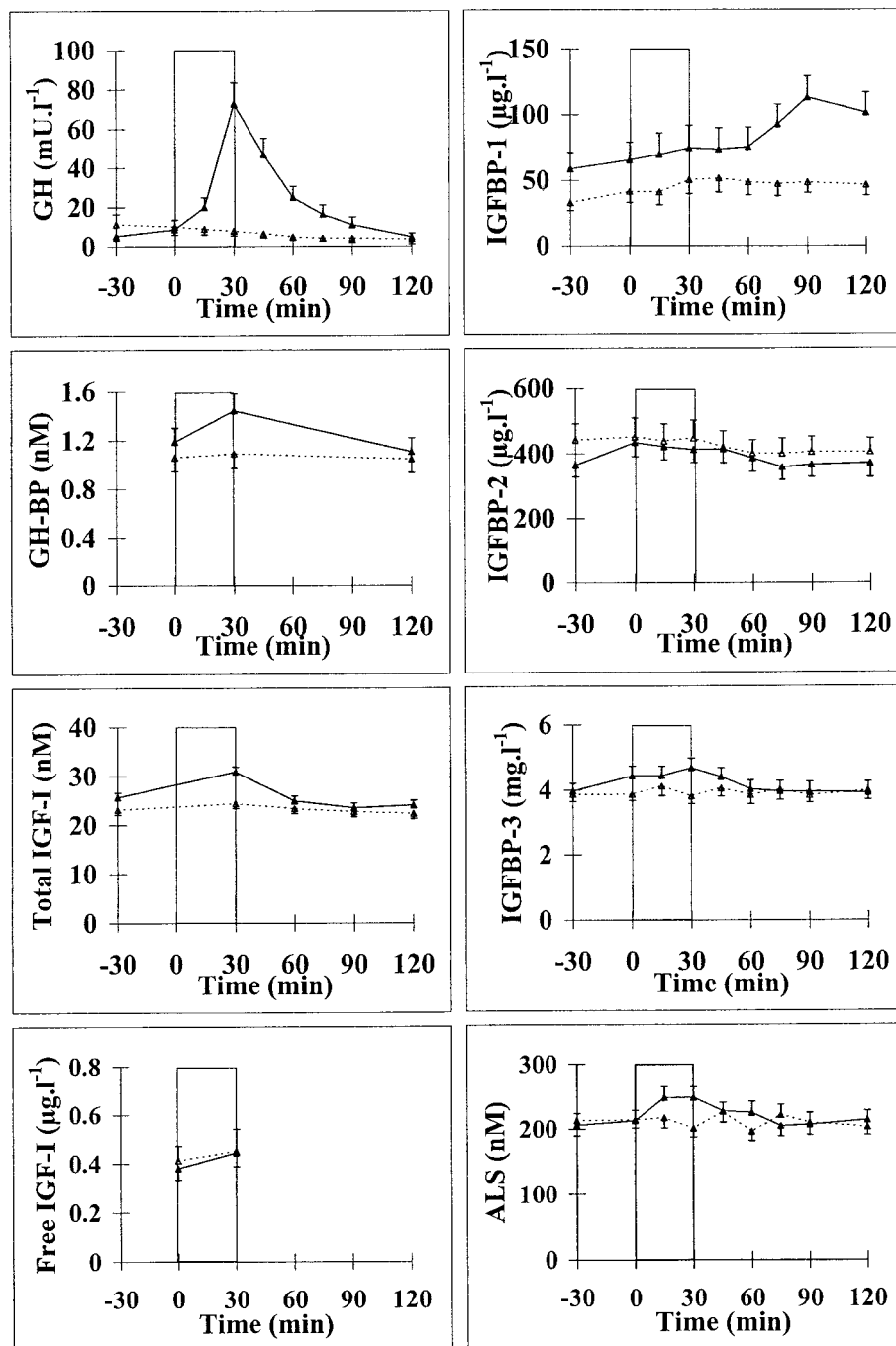


FIG. 2. The effect of acute exercise on the GH/IGF axis. Subjects ( $n = 17$ ) underwent a random sequence exercise study [closed triangles; semirecumbency from  $-30$  to  $0$  min; exercise from  $0$ – $30$  min with 10-min two-stage warm-up and 20 min at 65% of the workload at  $VO_{2max}$  (oxygen consumption,  $\sim 80\%$   $VO_{2max}$ ); semirecumbency from  $30$ – $120$  min] or a rest study (open triangles; upright posture during equivalent exercise period indicated by box).

ment in GHBP and fatness (percent body fat;  $r = 0.68$ ,  $P = 0.003$  and  $r = 0.46$ ,  $P = 0.06$ , respectively) and fitness ( $VO_{2max}$  in milliliters per min/kg;  $r = -0.65$ ;  $P = 0.005$  and  $r = -0.56$ ;  $P = 0.02$ , respectively).

The serum total IGF-I concentration at rest on the exercise day was  $25.2 \pm 0.2$  nmol/L (range, 7.1–36.3; adult reference range, 15–45 nmol/L). Acute exercise increased serum total IGF-I to  $30.3 \pm 0.2$  nmol/L (range, 9.7–46.7) at the end of exercise, declining to baseline values approximately 30 min after the end of exercise; there was no change on the rest day (effect of exercise,  $P = 0.0001$ ; see Fig. 2). The serum free IGF-I concentration immediately before exercise was  $0.38 \pm 0.05$

$\mu\text{g/L}$ . There was an order effect ( $P = 0.007$ ), but no significant effect of exercise over that of rest for serum free IGF-I ( $P = 0.8$ ; see Fig. 2).

Serum IGFBP-1 concentrations were quite variable among individuals, with a resting mean of  $58.8 \pm 12.4$   $\mu\text{g/L}$  (range, 5.2–170; adult reference range, 70–110  $\mu\text{g/L}$ ). These values are higher than those reported in some other studies, because the antibody used detects a variety of phosphorylated forms of IGFBP-1. In response to exercise, serum IGFBP-1 was unchanged during exercise, but a prominent postexercise increment was observed, starting 30 min after the end of exercise, peaking 30 min later at  $74.6 \pm 17.3$   $\mu\text{g/L}$  (range,

7.0–261; effect of exercise,  $P = 0.001$ ; see Fig. 2). Plasma insulin did not change significantly with exercise ( $P = 0.2$ ), but declined from a preexercise concentration of  $10.1 \pm 2.4$  to  $6.0 \pm 0.8$  mU/L at the end of exercise and rebounded after exercise. The serum IGFBP-2 concentration before exercise was  $364 \pm 35$   $\mu$ g/L (range, 120–656; four values were above the adult reference range of 70–450  $\mu$ g/L). There was no significant effect of acute exercise on serum IGFBP-2 ( $P = 0.7$ ; see Fig. 2). The serum IGFBP-3 concentration before exercise was  $3.97 \pm 0.23$  mg/L (range, 1.68–5.93; five values were above the adult reference range of 2.2–4.6 mg/L). In response to acute exercise, serum IGFBP-3 rose to a peak of  $4.68 \pm 0.29$  mg/L (range, 2.46–7.20) at the end of exercise and declined to resting values approximately 45 min later (effect of exercise,  $P = 0.005$ ; see Fig. 2). The serum ALS concentration at rest was  $206 \pm 17$  nmol/L (range, 80–330; three values were above the adult reference range of 112–253 nmol/L). In response to acute exercise, serum ALS rose to a peak of  $249 \pm 18$  nmol/L (range, 153–422) at the end of exercise and declined to resting values approximately 45 min later (effect of exercise,  $P = 0.001$ ; see Fig. 2). Considering two time points at which it may be practical to sample athletes in competition (–30 min before exercise and 30 min after the end of exercise), there was no statistically significant change in the molar ratios of IGF-I/IGFBP-3 ( $0.246 \pm 0.012$  and  $0.255 \pm 0.021$ , respectively;  $P = 0.51$ ), IGF-I/ALS ( $0.121 \pm 0.016$  and  $0.110 \pm 0.014$ , respectively;  $P = 0.29$ ), or IGFBP-3/ALS ( $0.496 \pm 0.069$  and  $0.442 \pm 0.053$ , respectively;  $P = 0.11$ ).

Hematocrits before and after upright posture on the rest day were  $36.8 \pm 0.5\%$  (range, 33.5–40.4) and  $37.7 \pm 0.6\%$  (range, 32.5–41.6), respectively, and those before and after acute exercise were  $39.2 \pm 0.7\%$  (range, 35.8–46.3) and  $40.9 \pm 0.6\%$  (range, 36.3–47), respectively. There was no significant effect of exercise on hematocrit ( $P = 0.3$ ).

#### Effect of GH administration

The serum total GH responses to acute exercise before and after placebo treatment were almost identical, whereas rhGH administration caused an elevation of the preexercise serum total GH concentration and a marked attenuation of the response to acute exercise. After rhGH administration, the serum GH concentration declined slowly throughout the observation period (see Fig. 3), with individual data confirming compliance before the visit. The effects of treatment ( $P < 0.0001$ ) and the nature of the response to exercise (visit  $\times$  treatment  $\times$  time point interaction;  $P < 0.0001$ ) were statistically significant.

The serum total IGF-I responses to acute exercise were almost identical before and after placebo treatment, whereas rhGH administration resulted in a marked increase in the serum IGF-I concentration throughout the observation period ( $P = 0.0001$ ) and an augmentation in the acute response to exercise (visit  $\times$  treatment  $\times$  time point interaction  $P = 0.028$ ; see Fig. 3). The mean preexercise concentration in the rhGH group increased from  $24.5 \pm 3.2$  nmol/L (range, 7.1–36.3) before rhGH treatment to  $69.6 \pm 8.1$  nmol/L (range, 39.7–104) on the visit immediately after rhGH treatment (visit 4); similarly, the mean total IGF-I concentration 30 min after the end of exercise increased from  $23.6 \pm 3.5$  nmol/L (range, 8–39.6) to  $73.5 \pm 9.3$  nmol/L (range, 43.6–107). After rhGH administration, free IGF-I increased from  $0.31 \pm 0.08$  (range, 0.08–0.72) to  $1.71 \pm 0.34$  (range, 0.36–3.15)  $\mu$ g/L immediately before exercise, and from  $0.44 \pm 0.11$   $\mu$ g/L (range, 0.06–0.84) to  $2.00 \pm 0.40$   $\mu$ g/L (range, 0.54–3.55) at the end of exercise (effect of treatment,  $P = 0.001$ ; visit  $\times$  treatment  $\times$  time point interaction,  $P = 0.5$ ).

Serum IGFBP-1 concentrations were variable before and after placebo treatment and before rhGH treatment (see Fig.

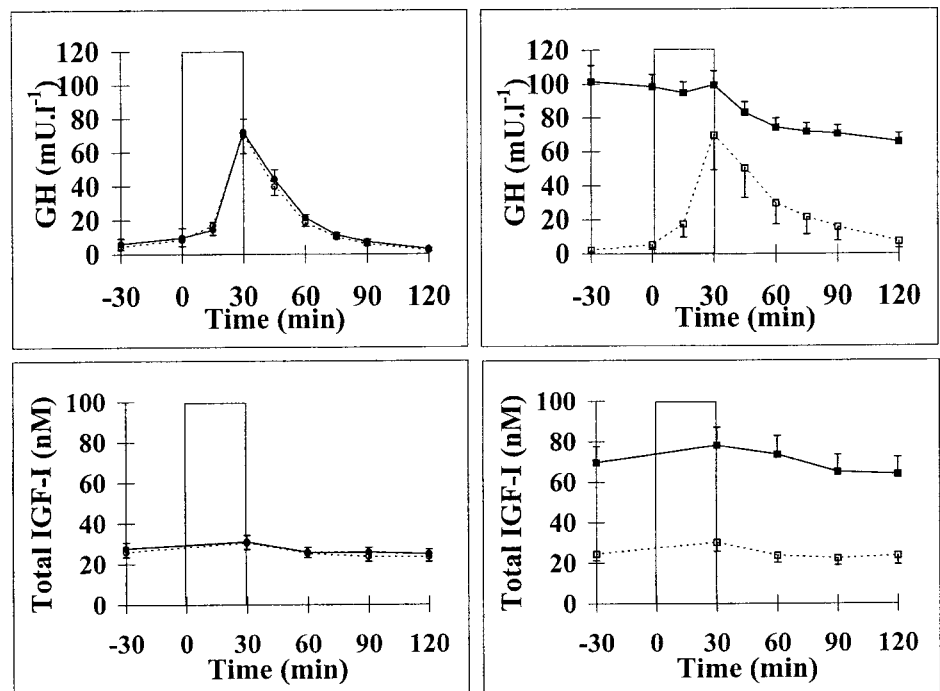


FIG. 3. The effect of rhGH treatment on serum GH and total IGF. Subjects underwent the exercise tests described in Fig. 2 before (open symbols) or after (closed symbols) randomization to treatment with placebo (circles; left panel) or rhGH (0.15 IU/kg-day; squares; right panel) for 7 days. The final sc dose of rhGH was administered 3 h before the study.

4). rhGH administration resulted in a reduction in resting IGFBP-1 concentration (pre-GH,  $84.8 \pm 19.2$ ; post-GH,  $28.2 \pm 7.9 \mu\text{g/L}$ ; treatment effect,  $P = 0.04$ ), with preservation of the late postexercise increase (visit  $\times$  treatment  $\times$  time point interaction,  $P = 0.4$ ; see Fig. 4). After rhGH administration, the plasma insulin concentration before exercise was markedly elevated ( $23.1 \pm 6.2 \text{ mU/L}$ ; overall effect of treatment,  $P = 0.018$ ), and the suppression during and the rebound after exercise were exaggerated (visit  $\times$  treatment  $\times$  time point interaction  $P = 0.001$ ; data not shown). The serum IGFBP-2 responses to acute exercise were almost identical before and

after placebo treatment, whereas rhGH administration resulted in a small reduction both before and after exercise (see Fig. 4). The mean preexercise concentration in the rhGH group decreased from  $426 \pm 53 \mu\text{g/L}$  (range, 164–656) to  $375 \pm 47 \mu\text{g/L}$  (range, 208–597); similarly, the mean IGFBP-2 concentration 30 min after the end of exercise decreased from  $452 \pm 65 \mu\text{g/L}$  (range, 244–819) to  $327 \pm 35 \mu\text{g/L}$  (range, 204–474). The effect of treatment was statistically significant ( $P = 0.015$ ), but the visit  $\times$  treatment  $\times$  time point interaction was not ( $P = 0.6$ ). The serum IGFBP-3 responses to acute exercise were almost identical before and after placebo treat-

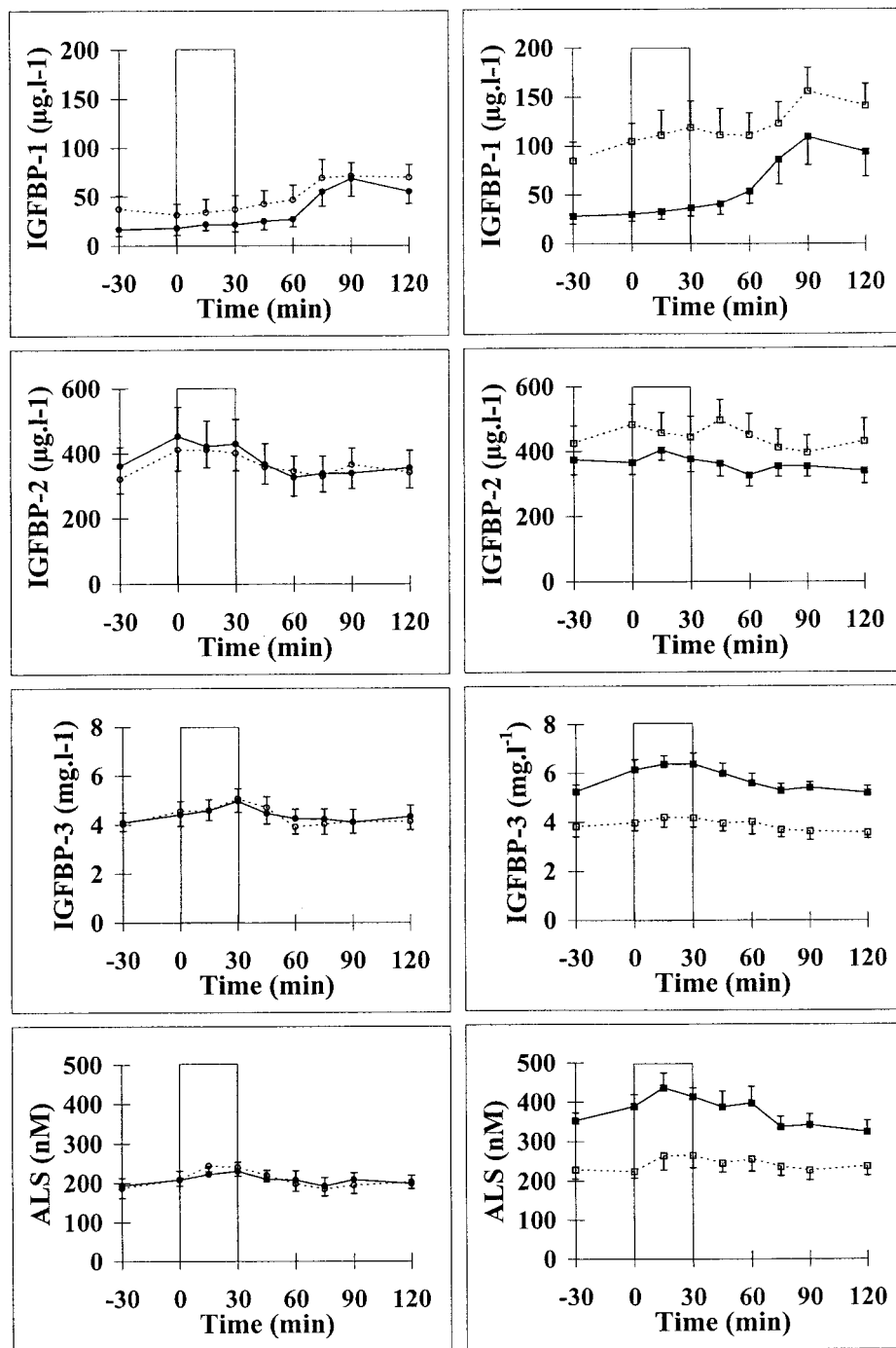


FIG. 4. The effect of rhGH treatment on IGFBP-1, -2, and -3 and ALS. Subjects underwent the exercise tests described in Fig. 2 before (*open symbols*) or after (*closed symbols*) randomization to treatment with placebo (*circles*; *left panel*) or rhGH (0.15 IU/kg-day; *squares*; *right panel*) for 7 days.

ment, whereas rhGH administration resulted in a marked increase in the serum IGF-1 concentration throughout the observation period ( $P = 0.001$ ) and an augmentation of the acute response to exercise (visit  $\times$  treatment  $\times$  time point interaction,  $P = 0.046$ ; see Fig. 4). The mean preexercise concentration in the rhGH group increased from  $3.82 \pm 0.42$  mg/L (range, 1.68–5.93) to  $5.24 \pm 0.29$  mg/L (range, 3.97–6.02) on the visit immediately after rhGH treatment (visit 4); similarly, the mean IGF-1 concentration 30 min after the end of exercise increased from  $4.01 \pm 0.50$  mg/L (range, 1.92–6.78) to  $5.60 \pm 0.39$  mg/L (range, 3.69–6.76). The serum ALS responses to acute exercise were almost identical before and after placebo treatment, whereas rhGH administration resulted in a marked increase in the serum ALS concentration throughout the observation period ( $P = 0.001$ ) and a trend toward an augmentation of the acute response to exercise (treatment  $\times$  time point interaction,  $P = 0.08$ ; see Fig. 4). The mean preexercise concentration in the rhGH group increased from  $229 \pm 23$  nmol/L (range, 141–330) to  $353 \pm 20$  nmol/L (range, 294–427) on the visit immediately after rhGH treatment (visit 4); similarly, the mean ALS concentration 30 min after the end of exercise increased from  $255 \pm 30$  nmol/L (range, 147–380) to  $397 \pm 43$  nmol/L (range, 269–627). There were significant changes in the molar ratios of components of the IGF ternary complex in response to rhGH administration. The IGF-1/IGFBP-3 ratio increased from  $0.240 \pm 0.019$  (range, 0.187–0.344) to  $0.566 \pm 0.051$  (range, 0.355–0.779) at rest (values before and after rhGH, respectively;  $P = 0.0003$ ;  $n = 8$ ), and from  $0.231 \pm 0.025$  (range, 0.167–0.390) to  $0.556 \pm 0.046$  (range, 0.385–0.797) at 30 min after the end of exercise ( $P = 0.0001$ ;  $n = 8$ ). The IGF-1/ALS ratio increased from  $0.095 \pm 0.011$  (range, 0.050–0.139) to  $0.197 \pm 0.020$  (range, 0.112–0.276) at rest ( $P = 0.0003$ ;  $n = 8$ ) and from  $0.084 \pm 0.009$  (range, 0.044–0.126) to  $0.193 \pm 0.027$  (range, 0.119–0.346) 30 min after the end of exercise ( $P = 0.002$ ;  $n = 8$ ). The IGF-1/ALS ratio, however, remained unchanged from  $0.394 \pm 0.034$  to  $0.349 \pm 0.021$  at rest ( $P = 0.17$ ;  $n = 8$ ), and from  $0.381 \pm 0.047$  to  $0.342 \pm 0.028$  30 min after the end of exercise ( $P = 0.32$ ;  $n = 8$ ).

Hematocrits immediately before exercise pre- and post-placebo were  $39.7 \pm 1.1\%$  and  $38.7 \pm 0.6\%$ ; pre- and post-GH values were  $36.9 \pm 0.7$  and  $37.8 \pm 0.6\%$ , respectively. Hematocrits at the end of exercise pre- and postplacebo were  $41.3 \pm 1.0\%$  and  $40.4 \pm 0.7\%$ , respectively; pre- and post-GH values were  $40.1 \pm 0.8\%$  and  $40.2 \pm 0.7\%$ , respectively. There was no significant response to rhGH administration (effect of treatment,  $P = 0.2$ ; visit  $\times$  time point  $\times$  treatment interaction,  $P = 0.4$ ).

#### Disappearance kinetics

After cessation of rhGH administration, the serum GH response to exercise was inhibited. Compared to the pre-treatment peak GH concentration in the GH group alone of  $69.8 \pm 20.4$  mU/L (range, 14.2–193.1), peak concentrations at visits 5, 6, and 7 were  $23.2 \pm 9.5$  (range, 0.4–71.5),  $31.3 \pm 12.2$  (range, 1.2–110.4), and  $27.9 \pm 4.2$  (range, 7.8–42.0) mU/L, respectively. The serum GH response to exercise was significantly reduced at visits 5 (27 h after the last dose) and 7 [99 h after the last dose; effect of treatment (visit  $\times$  treatment),

$P = 0.004$  and  $P = 0.039$ , respectively; nature of the response (visit  $\times$  treatment  $\times$  time point interaction),  $P = 0.015$  and  $P = 0.001$ , respectively]. Several missing data points at visit 6 reduced the power to resolve a difference at this intervening time after treatment.

After the cessation of rhGH administration, the preexercise serum total IGF-I concentration in the GH group remained elevated 24 h later ( $69.6 \pm 8.1$  and  $70.3 \pm 8.8$  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. The preexercise serum IGF-1 concentration in the GH group increased slightly 24 h later ( $5.24 \pm 0.29$  and  $5.56 \pm 0.35$  mg/L at visits 4 and 5, respectively) and returned to basal values by 96 h after the cessation of rhGH. The preexercise serum ALS concentration in the GH group declined marginally 24 h later ( $352 \pm 20$  and  $323 \pm 11$  nmol/L at visits 4 and 5, respectively) and approached basal values between 48–96 h after cessation of rhGH. Disappearance half-times calculated from individual curves for both pre- and postexercise data were as follows: IGF-I,  $89.5 \pm 4.2$  and  $79.3 \pm 2.8$ ; IGF-1,  $176 \pm 11$  and  $171 \pm 17$ ; and ALS,  $119 \pm 14$  and  $110 \pm 12$  h, respectively. Although half-times calculated from visits 4–7 and visits 5–7 were not statistically different, the latter allowed better curve fitting (data not shown). The equations describing the disappearance of analytes based on group mean data for visits 5–7, at rest and 30 min after the end of exercise, respectively, were as follows: total IGF-I =  $76.857e^{-0.0087x}$  ( $r^2 = 0.966$ ) and  $81.198e^{-0.0097x}$  ( $r^2 = 0.982$ ); IGF-1 =  $5.6637e^{-0.004x}$  ( $r^2 = 0.876$ ) and  $5.8949e^{-0.0045x}$  ( $r^2 = 0.954$ ); and ALS =  $361.79e^{-0.0064x}$  ( $r^2 = 0.829$ ) and  $344.27e^{-0.0049x}$  ( $r^2 = 0.943$ ).  $x =$  time (min) where  $e =$  the exponential constant (2.71828).

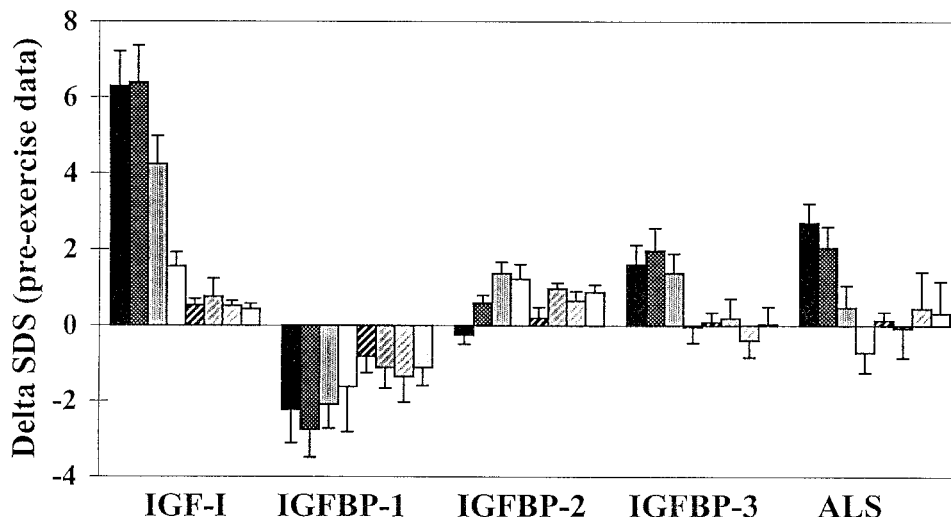
#### Relative responses

Data for individual analytes were converted into SD scores, using the means and SDs of resting, pretreatment values as reference data. The following changes in response to 7 days of rhGH administration in the GH group were observed: IGF-I,  $+6.3 \pm 0.9$ ; IGF-1,  $-2.2 \pm 0.9$ ; IGF-2,  $-0.3 \pm 0.2$ ; IGF-3,  $+1.6 \pm 0.5$ ; and ALS,  $+2.7 \pm 0.5$  SD score, compared to respective changes in the placebo group of  $+0.5 \pm 0.2$ ,  $-0.8 \pm 0.4$ ,  $+0.2 \pm 0.3$ ,  $+0.1 \pm 0.3$ , and  $+0.1 \pm 0.2$  SD score (see Fig. 5). Using the lack of overlap of individual data points to separate the groups, serum IGF-I allowed correct identification of rhGH-treated individuals at visit 4 in 87.5% and 100% of cases for pre- and postexercise time points, respectively, and in 75% and 87.5% of cases at visit 5 (27 h after the last dose of rhGH). Similarly, serum ALS allowed correct identification in 100% and 87.5% for pre- and postexercise time points, respectively, and 100% and 50% at visit 5. Serum IGF-1 was less sensitive at each visit.

#### Discussion

Novel findings from this study include 1) acute exercise transiently increased all components of the IGF ternary complex (IGF-I, IGF-1, and ALS); 2) GH preadministration further augmented this response to acute exercise; 3) total IGF-I, IGF-1, and ALS were markedly increased by supraphysiological doses of GH and appear to be potential

FIG. 5. The relative responses to GH treatment. Data have been transformed into SD scores (SDS) using mean and SD from the pretreatment group and pre-exercise data for the entire study group. Results report the differences in the SD score (mean  $\pm$  SEM) from the 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 the placebo group (black, dark gray, midgray, and light gray oblique stripes, respectively).



markers for the detection of exogenous GH abuse; and 4) the time course of these markers in relation to acute exercise and cessation of GH abuse has been defined, allowing construction of a detection strategy.

The study assessed very fit, but not elite, male athletes because 1) highly trained individuals may respond to exercise or GH administration differently from the untrained, because intense endurance training may reduce serum IGF-I concentrations (28, 29); 2) it was considered unethical to administer a banned substance to an elite athlete; and 3) although the problem of GH abuse in sport is unlikely to be confined to males, assessment of follicular and luteal phase effects would have greatly increased the complexity of the study. Submaximal exercise of comparable relative intensity was chosen to 1) induce a substantial serum GH increment, 2) permit individuals to complete the repeated nature of the study, and 3) permit comparisons of hormonal responses between individuals, as stress hormone responses are known to be proportional to the percentage of maximal exercise (4, 30, 31). Strict randomization of the GH/placebo administration was employed; the rest-exercise component of the study employed an arbitrary randomization. The dose of GH used in this study was clearly supraphysiological, as the mean serum total IGF-I more than doubled, but the doses used by sportspersons is unknown. Physiological replacement doses of rhGH in GH-deficient adult males are approximately 1/10th to 1/5th the dose administered in this study (32, 33). Despite the known side-effects of GH administration to adults with GH deficiency (32), our subjects reported a low prevalence of such effects, which did not result in unblinding of the experimental design. Compliance was assessed by counting empty vials and appeared to be complete. The duration of GH treatment permitted a plateau in serum IGF-I increments (9).

#### Responses to exercise

The serum total GH response was comparable to that in previous reports (4), with peak concentrations at the end of exercise, disappearing exponentially with an estimated disappearance half-time similar to that reported by others (34–36).

The main findings in response to acute exercise were that all components of the ternary complex, IGF-I, IGFBP-3, and ALS, increased acutely. The constancy of molar ratios suggested that intact ternary complexes were increasing, a conclusion supported by the lack of change in free IGF-I across exercise. Others have shown similar transient exercise-related increments in serum IGF-I and IGFBP-3 (15–17), but the finding of increased ALS in response to exercise is novel. Changes in components of the ternary complex exceeded those expected from hemoconcentration (expected increases of 7 and 4% across exercise and rest periods, respectively) (37, 38). The known disappearance times for individual components of the ternary complex (39–42) and those found in this study exclude transient alterations in known clearance pathways as an explanation of the rapid fluctuations in IGF-I, IGFBP-3, and ALS concentrations. The concurrent increment in serum GH is unlikely to increase IGF-I secretion. We propose a novel mechanism of changes in IGF concentrations: that sequestered ternary complexes enter and leave the central circulation in response to exercise, from vascular-related reservoirs, possibly from the hepatic sinusoidal system, fed by hepatocyte synthesis of IGF-I and ALS, and hepatic Kupffer cell synthesis of IGFBP-3 (43, 44). The biological effect of an exercise-induced change in IGF ternary complexes may be to enhance postexercise reparative processes, such as protein anabolism, as IGF-I bound to IGFBP-3 may have enhanced biological activity in certain tissues (8).

Another novel finding was the acute increase in serum GHBP in response to acute exercise. The physiological role of GHBP at rest is thought to act as a damper on GH oscillation, as a reservoir for later release (49, 50), and potentially to augment GH's biological activity (51) and GH receptor expression (52). We speculate that similar functions may operate during exercise, where a postexercise increment in GHBP concentrations may prolong the GH signal, increasing the GH-mediated signal for postexercise protein synthesis, tissue repair, and muscle glycogen replenishment, or assisting in metabolic adaptations during prolonged exercise to provide fuel via lipolysis. The increment in the serum GHBP concentration may represent either increased synthesis from the liver (53, 54) or reduced clearance.



Serum IGFBP-1 concentrations increased after the end of exercise. The expression of IGFBP-1 messenger ribonucleic acid is inversely regulated by insulin, with an insulin-responsive promoter site being described on the IGFBP-1 gene (59, 60), but the change in peripheral venous insulin concentrations, although appropriate in direction, did not reach statistical significance. The increment in serum IGFBP-1 may therefore reflect intracellular nutrient deprivation (61) or reduced clearance. The former is consistent with greater IGFBP-1 increments to maximal, exhaustive exercise using the same assay (58). Although the source of circulating IGFBP-1 production in response to exercise is probably the liver (62), several other tissues show messenger ribonucleic acid expression (40, 63, 64). We speculate that the physiological role of the postexercise increase in IGFBP-1, given IGFBP-1's inhibition of IGF-I's metabolic actions (65), is to prevent late hypoglycemia.

Serum IGFBP-2 concentrations did not change significantly in response to exercise. IGFBP-2, a potential alternative carrier for the IGFs when IGFBP-3 levels are insufficient (58), has been shown to respond to severe fasting with an increase over several days, but the acute metabolic stress of exercise appears unable to elicit a change in its production or clearance (66).

#### *Responses to GH administration*

The posttreatment serum total GH response to exercise at visit 4 showed elevated resting concentrations and an absent response to exercise, suggesting 1) a profile consistent with absorption after a sc dose, where a peak is expected 3–4 h after a dose (9); and 2) a small deviation from a perfectly even absorption profile at the end of exercise, representing either reduced hepatic blood flow resulting in decreased hepatic GH clearance and/or hemoconcentration.

All components of the ternary complex were significantly increased after GH administration. The response to exercise for each of these was, somewhat surprisingly, augmented (although marginally for ALS). It seems unlikely that the augmented IGF-I response to exercise after rhGH could be explained by up-regulation of GH receptor expression (50), as serum GHBP may reflect tissue GH receptor expression (7), and GHBP did not change in response to rhGH administration. Previous studies of GH treatment in GH-deficient adults and children have shown little or no response in GHBP (67). Augmentation of the exercise-induced increments of individual components of the IGF ternary complex after rhGH administration further supports our contention that preformed ternary complexes are released to the systemic circulation during exercise; GH pretreatment may increase IGF-I, IGFBP-3, and ALS accumulation in the microcirculation of the liver or other tissues, with subsequent release in response to vascular, metabolic, or hormonal signals during exercise.

Serum free IGF-I increased markedly after rhGH administration despite a clear increase in other IGFbps in serum. This observation is consistent with other studies in humans administered GH and in patients with acromegaly (68). The molar ratios of the components of the ternary complex changed significantly after rhGH treatment, with the ratio of

total IGF-I/IGFBP-3 increasing from 1:4 to 1:2 immediately after rhGH treatment, and total IGF-I/ALS increasing from 1:10 to 1:5, suggesting that there is considerable ALS excess at all times. The increase in free IGF-I as a percentage of total serum IGF-I from 0.20 before to 0.36 after rhGH treatment implies that the capacity of IGF-I binding to the ternary complex may have been exceeded.

Serum IGFBP-1 concentrations were suppressed after GH treatment, a finding consistent with GH-induced insulin resistance and resultant hyperinsulinemia (69). Our novel finding of the preservation of the postexercise IGFBP-1 increment after GH pretreatment suggests a noninsulin-mediated process, such as diminished intracellular glucose supply (70).

#### *Disappearance kinetics*

Little is known about the disappearance kinetics of many of the components of the GH/IGF axis in humans, and no data have been published regarding these questions for the postexercise period in trained individuals. The serum GH response to exercise was significantly reduced in the GH-treated group for several days after the cessation of GH treatment, which is most likely due to IGF-I-mediated negative feedback on the pituitary and/or hypothalamus (72). The diminished GH response appeared to persist up to 96 h after the last GH dose, at a time when serum IGF-I was returning to pretreatment levels.

Serum total IGF-I, IGFBP-3, and ALS declined, with disappearance half-times of approximately 80, 170, and 120 h, respectively. Although disappearance half-times are descriptive of the rate of disappearance only and do not represent true clearance, such data may assist in the development of a detection strategy. Comparisons with disappearance kinetics derived from experiments using exogenously administered binding proteins or IGF-I are not comparable with the current approach, as free analytes are cleared more rapidly than intact ternary complexes (73, 74).

#### *The physiological model of the GH/IGF axis as a test for GH abuse*

We propose that total IGF-I, IGFBP-3, and ALS might be suitable markers of GH abuse based on the following considerations: 1) each analyte is GH regulated (8); 2) elevations of serum IGF-I, IGFBP-3, and ALS can only occur in a limited number of physiological conditions, namely puberty, late pregnancy, and acromegaly (8); 3) serum concentrations remain relatively stable throughout the day, with minor reductions during the early hours of the morning (75); 4) each changes only modestly and transiently during acute exercise; 5) these three show substantial increments after rhGH administration (greatest for IGF-I and least for IGFBP-3); and 6) disappearance kinetics suggest that detection might be possible for several days after cessation of GH treatment. Alterations of the normal physiological molar ratios of components of the IGF ternary complex may also allow detection of rhGH administration.

Alternatively, measurement of serum total GH may appear to allow detection of exogenous GH administration by 1) assessment of disappearance half-times (20 min for endogenous and prolonged for exogenous, as for visit 4) or 2)

suppression of serum total GH response to an event (as for visits 5–7). The sensitivity, specificity, and logistics of these approaches would need to be critically assessed. The use of free IGF-I as a marker for GH abuse depends on the development of a simpler assay technique. The variability of IGFBP-1 makes it an unlikely marker of GH treatment. Serum IGFBP-2 fell in response to rhGH treatment. Others have suggested that IGFBP-2 may be a potential marker for GH treatment (14), but the small magnitude of change, the large degree of overlap between individuals before or after exercise, and the unexpected rebound to values higher than baseline after the cessation of GH treatment mean that IGFBP-2 will not be a useful marker.

There are two general ways of applying these data in the development of a test: 1) comparison of an athlete's values against appropriate reference data, and 2) comparison of changes over time in an individual athlete's values. Reference data need to be obtained from elite athletes, taking into account variables that might influence concentrations of these analytes, such as pubertal status and age (such studies are underway). Secondly, rapid or substantial alterations in an athlete's values, which exceed day to day variability of such stable markers, may improve sensitivity and be applicable in repeated out of competition testing. Comparison between the modest GH-mediated increment in IGFBP-3 concentrations (which overlaps substantially with the placebo-treated group) and the substantial IGFBP-3 SD score increment (which depends on the within-individual increment) highlights this final point. Such assertions need to be tested in larger scale studies. Finally, our data suggest that in the selection of a marker, consideration needs to be given regarding the timing of sampling in relation to exercise. Additional testing strategies may also involve 1) mathematical or statistical combinations of markers from the GH/IGF axis as presented, and 2) markers of bone turnover, which may allow detection of GH abuse long after cessation.

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