Pharmacodynamics of Growth Hormone Abuse Biomarkers and the Influence of Gender and Testosterone: A Randomized Double-Blind Placebo-Controlled Study in Young Recreational Athletes

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Context: IGF axis proteins and collagen peptides are promising markers of GH abuse.

Objective: Our objective was to investigate whether responses of serum IGF axis and collagen markers to GH differ between men and women, and are influenced by testosterone (T).

Design: This was a randomized, double-blind, placebo-controlled study of 8-wk treatment followed by 6-wk washout.

Setting: The study was performed at a clinical research facility.

Participants: A total of 96 recreationally trained healthy athletes (63 men, 33 women), aged 18–40 yr, were studied.

Intervention: All subjects received GH (2 mg/d sc) or placebo for 8 wk; men also received T (250 mg/wk im) or placebo for 5 wk.

Main Outcome Measures: Serum IGF axis proteins (IGF-I, IGF binding protein-3, and acid labile subunit) and collagen peptides (N-terminal propeptide of type I procollagen, C-terminal telopeptide of type I collagen, and N-terminal propeptide of type III procollagen) were measured.

Results: GH induced significant increases in IGF axis and collagen markers that were greater in men than women ($P < 0.001$). Of the IGF axis markers, IGF-I showed the greatest increase. The relative incremental responses of the collagen markers in general were greater than the IGF markers, especially for PIIINP. The collagen markers increased and decreased more slowly with most remaining elevated ($P < 0.01$) after 6 wk, in comparison to IGF markers, which returned to baseline within 1 wk. Addition of T to GH amplified the response of PIIINP by more than 1.5-fold but did not affect any other marker. T alone did not affect IGF axis markers but modestly increased collagen markers.

Conclusions: These markers of GH abuse are less responsive in women. The increases in collagen markers have a different time course to the IGF markers and extend the window of detection in both sexes. The response of PIIINP is increased by coadministration of T. **(*J Clin Endocrinol Metab* 93: 2213–2222, 2008)**

0021-972X/08/$15.00/0

Printed in U.S.A.

doi: 10.1210/jc.2008-0402 Received February 21, 2008. Accepted March 20, 2008.

First Published Online April 1, 2008

Abbreviations: ALS, Acid labile subunit; Cmax, maximum observed serum concentration; CV, coefficient of variation; ICTP, C-terminal telopeptide of type I collagen; IGFBP, IGF binding protein; PIIINP, N-terminal propeptide of type III procollagen; PINP, N-terminal propeptide of type I procollagen; T, testosterone; Tmax, time to reach maximum concentration.

GH administration increases circulating levels of IGF-I, IGF binding protein (IGFBP-3), and the acid labile subunit (ALS), which together form a circulating ternary complex (1, 2). GH also stimulates bone and connective tissue turnover, resulting in increased serum concentrations of specific collagen peptides, including N-terminal propeptide of type I procollagen (PINP), a marker of bone formation, C-terminal telopeptide of type I collagen (ICTP), a marker of bone resorption, and N-terminal propeptide of type III procollagen (PIIINP), a marker of connective tissue synthesis (3, 4). These GH-responsive proteins have been evaluated as potential markers of GH doping in sports (5–8). However, few studies have addressed the time course and the influence of gender on the responses of these markers to GH in young healthy adults. A GH doping test based on the suppression of endogenous GH isoforms by exogenous GH has recently been implemented. However, this test has a short time window of detection, so there is a need for a more robust test based on markers with longer detection windows, such as IGF axis proteins and collagen peptides (9).

There is strong anecdotal evidence that "polypharmacy" is often practiced, in which other agents such as anabolic steroids are abused concurrently with GH (10, 11). Web-based surveys have reported that up to 25% of androgen abusers also use GH, at doses ranging from 1–10 mg daily (12, 13). However, the effect of concurrent administration of androgens with GH on GH-responsive markers in young healthy adults is unknown.

The aims of this study were to establish the pharmacodynamics of serum IGF axis and collagen markers in response to GH in young recreational athletes, and to examine whether there is a gender difference and any influence of combined administration of testosterone (T) on the responses.

Subjects and Methods

Study subjects

Recreational athletes aged 18–40 yr, who undertook at least two exercise sessions per week and had done so for at least 1 yr, were recruited from university sports associations, gymnasia, medical and sports science faculties, and from the general public. The volunteers were screened to exclude any history of diabetes mellitus, cardiovascular, hepatic or renal disease, or known cancer. They were excluded if they had any self-reported abuse of performance-enhancing drugs at any time, and specifically excluded if they were competing at a state or national level or higher in any sport. At screening, all subjects underwent physical examination, and a detailed history was obtained. All subjects had normal biochemistry and hematology results, including prostate-specific antigen (PSA) in men, and a negative pregnancy test in women before randomization into the study. All subjects also had a negative urine screen for prohibited anabolic agents (The 2007 Prohibited List–World Anti-Doping Code. http://www.wada-ama.org/rtecontent/document/2007_List_En.pdf), using gas chromatography/mass spectrometry by the Australian Sports Drug Testing Laboratory.

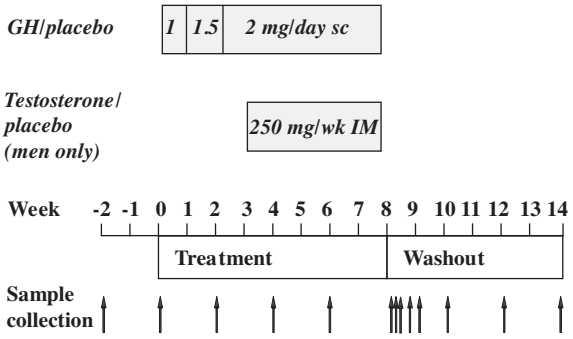

FIG. 1. Study protocol. Men and women were randomized to GH/placebo at the final dose of 2 mg/d sc for an 8-wk treatment period, followed by 6-wk washout. Men were also randomized to T/placebo, 250 mg/wk im for the last 5 wk. Serum samples were collected before treatment (wk −2 and 0), during treatment (wk 2, 4, 6, and 8), and after treatment (corresponding to 1, 2, 4, and 7 d, then 2, 4, and 6 wk after the last GH/placebo injection).

All subjects provided written informed consent. The study was approved by St. Vincent's Hospital Human Research Ethics Committee and registered with the Australian New Zealand Clinical Trials Registry (ACTRN012605000508673, www.anzctr.org.au).

Of 106 subjects that underwent screening visits, 103 were randomized, with three screened subjects not proceeding to randomization due to scheduling difficulties. A total of 97 subjects completed the protocol. Of the six who discontinued the study, five were unable to continue for personal or work-related reasons, and one withdrew due to polyarthritis and a rash, though a relationship to the study medication was considered unlikely. One further subject was excluded from the analysis due to noncompliance. Subjects were undertaking endurance (*e.g.* running, tria-

TABLE 1. Baseline characteristics of 96 participants

	Women			Men				
	Placebo (n = 16)	GH (n = 17)	*P* value	Placebo (n = 16)	GH (n = 15)	T (n = 16)	GH + T (n = 16)	*P* value (ANOVA)
Age (yr)	27.8 ± 1.3	29.7 ± 1.6	0.36	28.9 ± 1.3	25.2 ± 1.4	29.0 ± 1.5	26.8 ± 1.3	0.18
Height (m)	164.3 ± 1.6	169.3 ± 1.5	0.03	186.2 ± 1.3	177.4 ± 1.7[a]	180.2 ± 2[a]	180.6 ± 1.3[a]	0.003
Weight (kg)	61.6 ± 2.3	65.8 ± 2.5	0.24	90.5 ± 3.1	75.3 ± 2.8[a]	83.3 ± 4.9	79.5 ± 2.6	0.023
BMI (kg/m^2)	22.8 ± 0.8	22.9 ± 0.7	0.97	26.1 ± 0.8	23.8 ± 0.7	25.4 ± 1	24.4 ± 0.7	0.23
IGF-I (μg/liter)	136.8 ± 10.0	124.4 ± 7.9	0.34	110.4 ± 9.6	128.3 ± 9.7	127.5 ± 9.5	113.0 ± 10.3	0.43
IGFBP-3 (mg/liter)	4.1 ± 0.2	3.7 ± 0.2	0.09	3.6 ± 0.1	3.7 ± 0.2	3.7 ± 0.2	3.6 ± 0.2	0.88
ALS (nmol/liter)	295.6 ± 15.9	289.6 ± 14.8	0.79	242.2 ± 11.1	248.9 ± 9.4	258.4 ± 16.4	250.3 ± 15.7	0.86
PINP (μg/liter)	62.3 ± 9.3	53.2 ± 4.1	0.37	68.9 ± 6.6	98.0 ± 13.8	68.3 ± 5.1	79.4 ± 7.2	0.07
ICTP (μg/liter)	4.4 ± 0.3	4.3 ± 0.3	0.96	4.0 ± 0.3	5.1 ± 0.3	4.5 ± 0.5	4.3 ± 0.2	0.19
PIIINP (μg/liter)	4.5 ± 0.3	3.7 ± 0.2	0.03	3.4 ± 0.2	4.2 ± 0.4	4.1 ± 0.3	4.0 ± 0.2	0.17

Data shown as mean ± SEM. BMI, Body mass index.

[a] Group significantly different from placebo ($P < 0.05$).

thlon, cycling, n = 22), power (*e.g.* weight lifting, sprint, boxing, n = 10), and mixed training (*e.g.* ball sports, aerobics, n = 64). The reported amount of training per week was 2–4 h (n = 27), 4–10 h (n = 58), and more than 10 h (n = 11).

Study protocol

The study was double-blind placebo-controlled. Women were randomized to 2 mg/d GH (n = 17) or placebo (n = 16), and men to 2 mg/d GH (n = 15), 250 mg/wk T (n = 16), GH plus T (n = 16), or double placebo (n = 16) for a treatment period of 8 wk, followed by 6-wk washout (Fig. 1).

GH (Somatropin, 1 mg/ml) and matched GH placebo were provided by Novo Nordisk (Bagsvaerd, Denmark). GH or placebo was self-administered sc, with subjects instructed to administer the injection at night. To minimize side effects, the dose was increased from 1 mg/d (first week) to 1.5 mg/d (second week), then to the final dose of 2 mg/d for 6 wk. Participants attended the Clinical Research Facility at the end of each week, when the injection cartridge was changed, and compliance was checked by verbal reports from the subject and the volume remaining in the cartridge.

Men were also randomized to 250 mg T esters (Sustanon; Organon, Oss, Holland) or placebo saline, administered once per week im for 5 wk, from the end of the third week until the end of the seventh week. Injections were administered by a nurse who was not otherwise involved in the study, and who was not involved in clinical assessment of subjects, including side effects.

If any side effects occurred that affected daily functioning, the dose of GH or placebo was reduced to the previous dose during the initial treatment period, and treatment was discontinued if effects persisted more than 2 wk. In men, if side effects occurred with T or placebo, the dose was reduced by half and also discontinued if effects persisted more than 2 wk. Study medication was changed due to side effects in only three subjects, who were able to resume treatment at the full dose, except for one man in whom T was discontinued for the last 2 wk.

Serum and urine samples were collected at screening (wk −2), baseline (wk 0), during treatment (wk 2, 4, 6, and 8), and during the washout period, corresponding to 1, 2, 4, and 7 d, then 2, 4, and 6 wk after the last GH/placebo injection (Fig. 1).

Side effects were monitored by clinical assessment at weekly visits. Safety monitoring was performed at screening (wk −2), during treatment (wk 4 and 8), and at the end of washout (wk 14) by biochemistry, hematology, liver function tests, and measurement of prostate-specific antigen (men).

Assays

Serum samples were stored at −80 C before batch analysis, undertaken when all subjects had completed the protocol. All samples for any individual were measured in the same assay run for each analyte. IGF-I [intraassay and interassay coefficients of variation (CVs), < 4% and < 9%, respectively] was measured by RIA after acid-ethanol extraction (14), using iodinated des(1–3)IGF-I (GroPep, Adelaide, South Australia) as radioligand. IGFBP-3 (intraassay and interassay CVs, < 5%) and ALS (intraassay and interassay CVs, < 4%) were measured by RIA using polyclonal antibodies (15, 16). Serum ICTP (intraassay and interassay CVs, < 10%), PINP (intraassay and interassay CVs, < 9% and <12%, respectively), and PIIINP (intraassay and interassay CVs, < 7% and < 12%, respectively) were measured by RIAs (Orion Diagnostica, Espoo, Finland) using the same assay batch. Serum total T, SHBG, LH, and FSH were measured by Immulite automated chemiluminescent immunoassays (Siemens Medical Solutions Diagnostics Ltd., Gwynedd, UK), with CVs of 7.1% at 13.8 nmol/liter (T), 6.1% at 89.1 nmol/liter (SHBG), 3.3% at 11.2 IU/liter (LH), and 4.3% at 16.2 IU/liter (FSH).

Statistical analysis

Comparisons between genders and between treatment groups at baseline, and comparisons of reproductive hormones between treatment groups were performed using *t* tests and ANOVA. Statistical analysis of treatment effects, including effects at a single time point (wk 8) and at all time points, was performed using a linear mixed effects model on log-transformed data. The linear mixed effects model allows each individual to have a different baseline and each treatment group to have a different profile across time for the comparisons between groups when considering multiple time points. For each individual treated with GH, the time (Tmax) to reach the maximum observed serum concentration (Cmax) was determined using time points during treatment (2, 4, 6, and 8 wk) and washout, and groups compared by unpaired *t* tests. The frequencies of adverse effects reported were compared separately for men and women using Fisher's exact test.

Results

Participant characteristics

The 96 participants ranged in age from 18–40 yr (Table 1). There were no significant differences in age or body mass index between placebo and GH treatment groups in women, or between placebo, GH, T, and GH plus T treatment groups in men.

IGF axis markers

There were no significant differences in mean IGF-I, IGFBP-3, or ALS concentrations between treatment groups, in men or in women at baseline (Table 1). At baseline, mean ALS was significantly higher in women than men (292.5 ± 10.7 *vs.* 250.0 ± 6.7 nmol/liter, mean ± SE; $P < 0.005$).

In placebo-treated subjects, there was variation in all the markers across treatment and washout periods (Fig. 2A). Between-subject CVs for placebo subjects (14–30% in men; 15–32% in women) were greater than within-subject CVs (8–14% in men; 10–16% in women) for each analyte.

GH treatment increased mean IGF-I, IGFBP-3, and ALS concentrations (Fig. 2A) significantly compared with placebo at wk 8 ($P < 0.005$) (Table 2), and when analyzed using all treatment period time points ($P < 0.0001$). The percent increase from baseline at wk 8 was approximately 3-fold greater for IGF-I than IGFBP-3 or ALS (Table 2).

The mean time (Tmax) to reach the Cmaxs was 5.2–5.7 wk for IGF axis markers, and there were no significant differences in Tmax between men and women (Table 3). The mean concentrations of all IGF axis markers started to plateau by wk 4. There was a rapid decline in these markers after withdrawal of treatment at wk 8, with levels no longer significantly higher than those in the placebo group by wk 9, except for IGF-I, which was elevated slightly longer in men.

The increases in IGF-I, IGFBP-3, and ALS concentrations were all significantly greater in men than women when analyzed using all time points in the treatment period ($P < 0.001$). The changes in IGF-I and ALS at wk 8 compared with baseline were approximately 1.6-fold greater in men (Table 2).

Addition of T did not significantly affect the increases in IGF-I, IGFBP-3, or ALS induced by GH in men (Table 2). For IGFBP-3, there was if anything a reduced response to combined GH and T compared with GH alone, however, this was not significant. There were no significant differences for the IGF axis markers between Tmax for combined treatment compared with

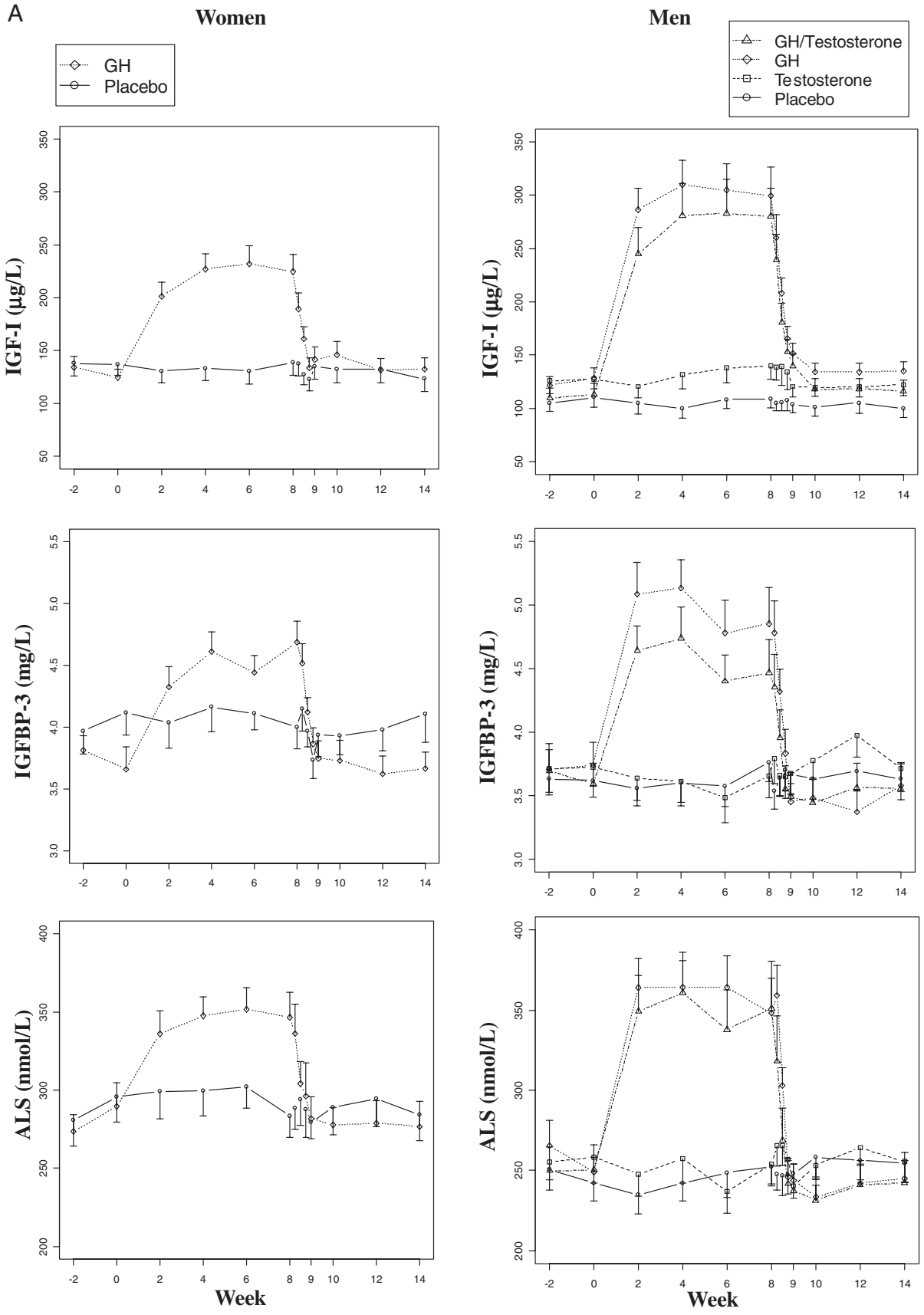

FIG. 2. Response of IGF axis and collagen markers to GH and T. Serum concentrations of markers, in women and in men, before treatment (wk −2, 0), during treatment (wk 2, 4, 6, and 8), and during the washout period (corresponding to 1, 2, 4, and 7 d, then 2, 4, and 6 wk after the last GH/placebo injection). Data shown as mean ± SEM for each treatment group. A, IGF-I (µg/liter), IGFBP-3 (mg/liter), and ALS (nmol/liter). B, PINP (µg/liter), ICTP (µg/liter), and PIIINP (µg/liter).

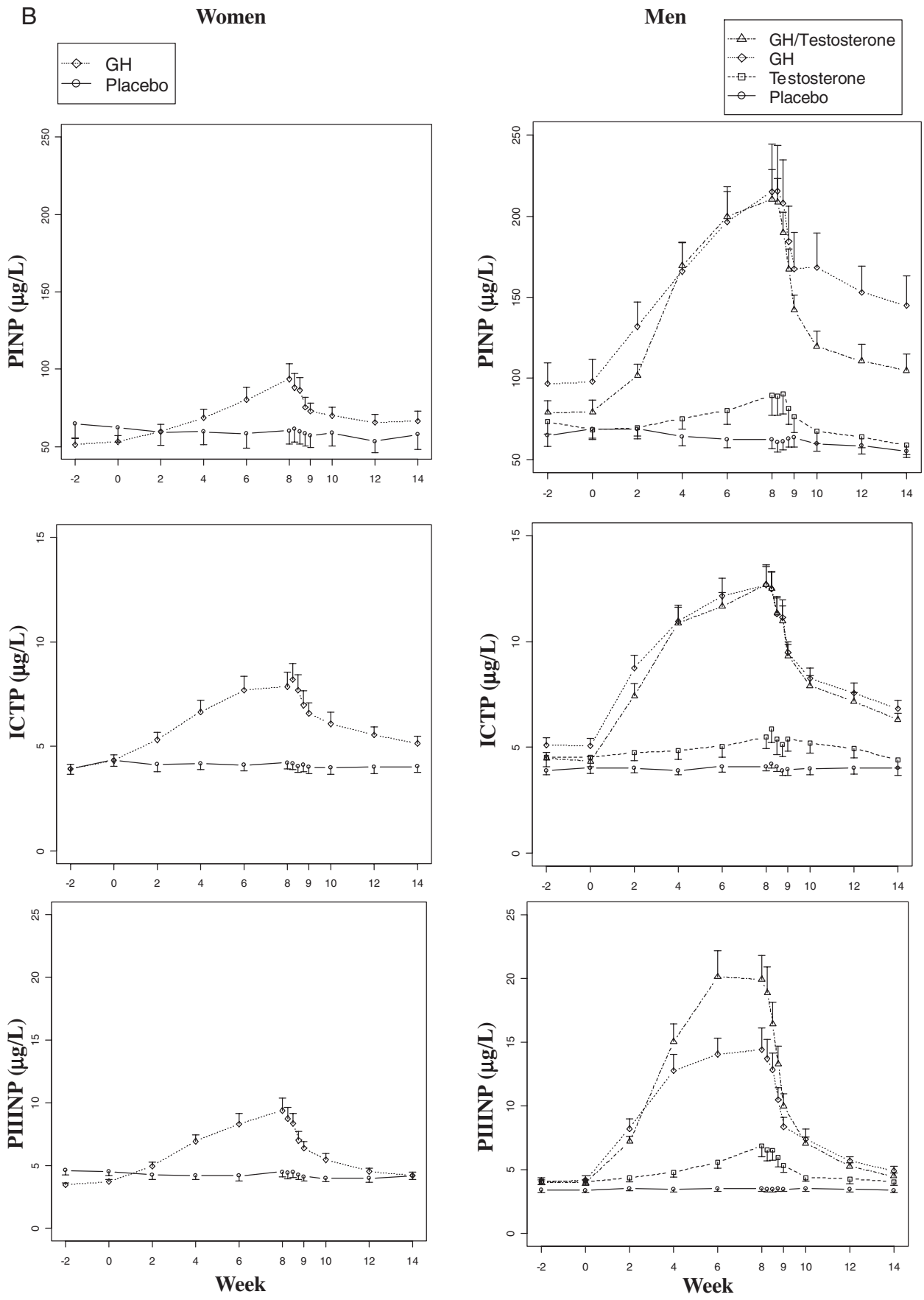

FIG. 2. Continued.

TABLE 2. Percent changes in IGF axis and collagen markers at the end of 8-wk treatment

	Women		Men			
	Placebo	GH	Placebo	GH	T	GH + T
Δ IGF-I (%)	3 ± 8	86 ± 12[a]	3 ± 7	144 ± 23[a]	9 ± 4	160 ± 24[a]
Δ IGFBP-3 (%)	−2 ± 3	33 ± 8[b]	4 ± 3	30 ± 5[b]	−1 ± 4	24 ± 3[b]
Δ ALS (%)	−3 ± 3	24 ± 7[b]	5 ± 3	41 ± 9[b]	2 ± 6	40 ± 6[b]
Δ PINP (%)	−2 ± 4	85 ± 23[a]	−9 ± 4	134 ± 26[a]	28 ± 11[b]	185 ± 27[a]
Δ ICTP (%)	−2 ± 4	87 ± 16[a]	4 ± 4	168 ± 23[a]	22 ± 4[b]	196 ± 19[a]
Δ PIIINP (%)	−1 ± 4	155 ± 26[a]	5 ± 4	253 ± 39[a]	70 ± 17[b]	419 ± 56[a,c]

Means ± SE of the change in each maker at wk 8 compared with wk 0, expressed as a percentage of wk 0.

[a] Groups significantly different from placebo ($P < 0.0001$).

[b] Groups significantly different from placebo ($P < 0.005$).

[c] GH plus T is significantly different from GH ($P < 0.01$).

GH alone (Table 3). There was no significant effect of T treatment alone on IGF axis markers.

Collagen markers

Baseline values of PINP, ICTP, or PIIINP did not differ between treatment groups, in men or in women (Table 1), except for PIIINP in women, which was slightly (but significantly) higher in the placebo group. Mean PINP at baseline was higher in men than women (78.3 ± 4.5 *vs.* 57.6 ± 5.0 μg/liter, mean ± SE; $P < 0.005$).

In placebo-treated subjects, there was variation in all the markers across treatment and washout periods (Fig. 2B), with greater between-subject variation in the collagen markers (CVs: 20–34% in men; 27–56% in women) than that observed in IGF axis markers. Within-subject CVs (10–14% men; 11–15% women) were comparable to the IGF axis markers.

GH treatment increased PINP, ICTP, and PIIINP concentrations (Fig. 2B), which were all significantly increased compared with placebo at wk 8 ($P < 0.005$) (Table 2), and when analyzed using all treatment period time points ($P < 0.0001$). The most marked response was for PIIINP. The percent increase in PIIINP at wk 8 was approximately 1.7-fold greater than that of PINP, ICTP, or IGF-I (Table 2).

The increases in collagen markers were less rapid than those of IGF axis markers and did not achieve a plateau by wk 8 (Fig. 2B). The Tmax values for collagen markers at 7.1–7.7 wk for the whole group were greater than for the IGF axis markers (Table 3). The decline in the collagen markers after withdrawal of treatment was slower than for IGF axis markers. The elevation in all collagen markers was sustained until the end of the washout period, with all markers significantly higher in the GH group compared with placebo at wk 14 ($P < 0.01$), except for PIIINP in women, which was elevated at 12 wk, but not at 14 wk.

The increase in all collagen markers in response to GH was significantly greater in men than women ($P < 0.001$), with the percent increase at 8 wk approximately 2-fold greater in men.

Addition of T to GH did not significantly affect the increase in PINP and ICTP in response to GH alone at wk 8 (Table 2), and there was no effect on Tmax for any of the collagen markers, with no significant differences between the GH and GH plus T groups (Table 3). However, for PIIINP, combined T and GH resulted in a greater increase that was 1.65-fold that of GH at wk 8 ($P < 0.001$) (Table 2) and was significantly greater than the effect of GH from wk 4 onwards ($P < 0.0001$).

There was a small but significant effect of T treatment alone in men on all collagen markers ($P < 0.005$ compared with placebo), of smaller magnitude than that of GH; the percent increase in PINP, ICTP, and PIIINP at wk 8 was 20, 10, and 30%, respectively, of the response to GH (Table 2).

TABLE 3. Cmaxs and Tmax for subjects treated with GH

	Women		Men				Women and men	
	GH (n = 17)		GH (n = 15)		GH + T (n = 16)		GH, GH + T (n = 48)	
	Cmax	Tmax (wk)	Cmax	Tmax (wk)	Cmax	Tmax (wk)	Cmax	Tmax (wk)
IGF-I (μg/liter)	259.4 ± 15.7	6.2 ± 0.6	345.9 ± 25.3[a]	5.2 ± 0.5	311.4 ± 28.7	5.5 ± 0.5	303.8 ± 14.3	5.7 ± 0.3
IGFBP-3 (mg/liter)	5.1 ± 0.1	6.4 ± 0.6	5.4 ± 0.2	5.0 ± 0.7	5.1 ± 0.2	4.6 ± 0.5	5.2 ± 0.1	5.4 ± 0.3
ALS (nmol/liter)	396.6 ± 13.9	5.8 ± 0.5	407.1 ± 19.4	5.1 ± 0.7	391.4 ± 25.4	4.8 ± 0.5	398.2 ± 11.3	5.2 ± 0.3
PINP (μg/liter)	98.8 ± 9.5	8.0 ± 0.5	241.1 ± 28.9[a]	8.1 ± 0.5	231.4 ± 16.7[a]	7.0 ± 0.4	187.5 ± 14.5	7.7 ± 0.3
ICTP (μg/liter)	8.9 ± 0.7	7.4 ± 0.3	13.6 ± 0.8[a]	6.7 ± 0.5	13.4 ± 0.9[a]	7.6 ± 0.3	11.9 ± 0.6	7.2 ± 0.2
PIIINP (μg/liter)	10.0 ± 0.9	7.3 ± 0.3	16.3 ± 1.5[a]	6.6 ± 0.5	22.4 ± 2.0[a,b]	7.4 ± 0.2	16.1 ± 1.1	7.1 ± 0.2

Data shown as means ± SEs. For Tmax there were no significant differences between women and men on GH, or between men on GH and men on GH plus T.

[a] For Cmax, men on GH were significantly different from women ($P < 0.005$).

[b] For Cmax, men on GH plus T were significantly different from men on GH ($P < 0.05$).

TABLE 4. Serum concentrations of reproductive hormones before and after treatment, and after washout

	Women			Men				
	Placebo (n = 16)	GH (n = 17)	*P* value	Placebo (n = 16)	GH (n = 15)	T (n = 16)	GH + T (n = 16)	*P* value (ANOVA)
T (nmol/liter)								
wk 0	1.4 ± 0.2	1.2 ± 0.2	0.56	21.9 ± 1.9	25.3 ± 2.1	23.5 ± 2.0	23.1 ± 1.3	0.64
wk 8	1.5 ± 0.2	1.4 ± 0.2	0.63	21.3 ± 1.5	24.6 ± 2.7	32.2 ± 2.5[a,b]	31.2 ± 1.7[a,b]	0.001
wk 14	1.3 ± 0.1	1.2 ± 0.1	0.83	20.5 ± 1.7	22.7 ± 1.7	21.1 ± 2.3	21.4 ± 1.5	0.85
SHBG (nmol/liter)								
wk 0	120.7 ± 24.9	130.8 ± 24.7	0.78	45.0 ± 4.2	53.9 ± 5.9	51.2 ± 4.6	51.4 ± 5.3	0.64
wk 8	156.5 ± 38.9	140.1 ± 25.6	0.72	46.2 ± 4.7	51.9 ± 4.8	38.3 ± 5.7[b]	40.7 ± 4.2[c]	0.21
wk 14	135.4 ± 29.3	106.9 ± 17.2	0.40	38.4 ± 3.2	45.7 ± 4.1	57.0 ± 8.2	46.7 ± 4.7	0.12
FSH (IU/liter)								
wk 0	4.3 ± 1.0	4.5 ± 0.6	0.89	5.3 ± 0.9	3.9 ± 0.5	3.2 ± 0.4	3.0 ± 0.4	0.03
wk 8	4.5 ± 0.9	4.0 ± 0.7	0.63	5.1 ± 0.7	3.6 ± 0.5	0.1 ± 0.01[a,b]	0.2 ± 0.07[a,b]	0.000
wk 14	4.7 ± 0.8	4.3 ± 0.6	0.70	5.6 ± 0.9	3.8 ± 0.5	3.1 ± 0.3	3.2 ± 0.5	0.016
LH (IU/liter)								
wk 0	6.1 ± 1.6	4.4 ± 0.9	0.35	5.8 ± 0.7	5.2 ± 0.5	4.8 ± 0.5	4.8 ± 0.4	0.47
wk 8	6.0 ± 1.3	5.0 ± 1.8	0.65	4.8 ± 0.5	4.4 ± 0.6	0.1 ± 0.04[a,b]	0.2 ± 0.1[a,b]	0.000
wk 14	6.1 ± 1.6	3.8 ± 0.6	0.18	5.9 ± 0.8	5.1 ± 0.5	3.7 ± 0.3	4.3 ± 0.5	0.06

Data shown as means ± SEs. The data at wk 8 refer, in the case of T for the T and GH plus T groups in men, to treatment actually initiated in the fourth week.

[a] Significantly different at wk 8 from placebo ($P < 0.001$).

[b] Significantly different at wk 8 from baseline by the paired *t* test ($P < 0.005$).

[c] Significantly different at wk 8 from baseline by the paired *t* test ($P < 0.02$).

Reproductive hormones

There were no significant differences at baseline between treatment groups, in men or in women, in total T, SHBG, FSH, or LH (Table 4). As expected, in men at wk 8 after 5-wk T administration, serum total T increased significantly in T and GH plus T groups ($P < 0.005$), and SHBG significantly decreased (T: $P < 0.001$; GH plus T: $P < 0.02$) compared with baseline (Table 4). Concentrations of FSH and LH were strongly suppressed in T and GH plus T groups at wk 8 compared with baseline ($P < 0.0001$).

Adverse effects

No subjects discontinued the study due to adverse effects related to the study medication. Minor adverse effects were reported by subjects in all treatment groups, including placebo (Table 5). Swelling was reported by more subjects on GH than placebo (men: 67% *vs.* 25%, $P = 0.02$; women: 65% *vs.* 31%, $P = 0.06$). More joint pain, and pins and needles were reported by subjects treated with GH, however, the frequencies were significantly different from placebo only in men (60% *vs.* 19%, $P = 0.02$; 40% *vs.* 6%, $P = 0.03$; respectively). Swelling was reported by more men on T (63%) and on combined treatment (88%) than on placebo (25%) ($P = 0.04$ and $P < 0.001$, respectively), and more men on combined treatment reported muscle pain than placebo (81% *vs.* 31%; $P = 0.006$).

Discussion

In this double-blind, placebo-controlled study in 96 young recreational athletes, the IGF axis markers IGF-I, IGFBP-3, and ALS, and collagen markers PINP, ICTP, and PIIINP all significantly increased in response to GH compared with placebo, and the response of all markers was greater in men than

TABLE 5. Adverse events

	Women			Men					
	Placebo (n = 16)	GH (n = 17)		Placebo (n = 16)	GH (n = 15)		T (n = 16)		GH + T (n = 16)	
	No.	No.	*P* value	No.	No.	*P* value	No.	*P* value	No.	*P* value
Swelling	5	11	0.06	4	10	0.02	10	0.04	14	<0.001
Joint pain	3	6	ns	3	9	0.02	5	ns	6	ns
Muscle pain	2	3	ns	5	4	ns	10	ns	13	0.006
Pins and needles	2	3	ns	1	6	0.03	3	ns	5	ns
Acne	0	2	ns	3	3	ns	5	ns	7	ns
Mood changes	0	0	ns	3	2	ns	2	ns	2	ns
Others	7	6	ns	8	8	ns	7	ns	7	ns
Total	19	31		27	42		42		54	

P values (*vs.* placebo) were calculated with the Fisher exact test. "Others" include bruising from sc injections, breast tenderness, hunger, headache, and increased sweating. ns, Not significant.

women. Of the IGF axis markers, IGF-I showed the greatest increase in response to GH. The relative incremental responses of the collagen markers in general were greater than those of the IGF axis markers, especially for PIIINP in which the percent increase at wk 8 was nearly 2-fold greater that of IGF-I. The collagen markers increased more slowly than IGF axis markers, as reflected by later Tmax values. Collagen markers also decreased more slowly, with most remaining elevated 6 wk after 8-wk treatment ($P < 0.01$), in comparison to IGF markers, which in general returned to baseline within 1 wk. Addition of T to GH did not change the time course of any marker, however, it significantly enhanced the response of PIIINP by 1.65-fold. T treatment alone did not affect IGF axis markers, however, it modestly increased all the collagen markers. Thus, this study has revealed differences in the pharmacodynamics of the IGF axis markers and collagen markers, a gender difference in the responses of all the markers, and an amplifying effect of T on one collagen marker, namely PIIINP.

A greater response to GH in men than in women for IGF-I, IGFBP-3, and collagen peptides has been observed in GH-deficient and older subjects (17–20) and in young healthy adults (5, 6). These sexually dimorphic responses to GH are likely due to regulation of GH action by sex steroids (21, 22). Our study in young healthy adults also demonstrated a greater response of IGF axis and collagen markers to GH in men, even though the body weight adjusted dose was lower in the men [0.024 ± 0.003 mg/kg·d (mean ± SD)] than the women (0.032 ± 0.004 mg/kg·d).

The novel observation that the PIIINP response to GH was markedly enhanced by T indicates that its sensitivity as a marker of GH abuse is increased by concurrent T use in men. It is also possible that the sensitivity of GH markers may be increased in women administering androgens, although this could not be addressed in this study. There is strong evidence that androgens and GH interact in an independent and additive manner to regulate positively many biological processes, such as metabolism and body composition (23–25). Our study indicates that, for the collagen markers, the combined effects of GH and T seem to differ between tissues and cells primarily expressing type I collagen (bone), and type III collagen (connective tissue). Although T increased both type I (PINP, ICTP) and type III (PIIINP) collagens, it only amplified the effect of GH on type III collagen. This suggests that there may be a strong modulating action of T on type III collagen expression in response to GH because there did not appear to be any effect on clearance.

Combined GH and T did not significantly affect the response of IGF axis markers to GH alone, as previously observed in healthy older men (20, 26). In our study the increase in IGFBP-3 in response to GH appeared reduced by combined treatment with T, though not significantly. However, a small uncontrolled study observed in bodybuilders using large and variable doses of androgens (0.2–2.4 mg/d·kg) that there was no increase in IGFBP-3 in response to addition of GH (27).

In response to T alone, we observed no significant changes in IGF axis markers, similarly to studies in hypogonadal men (23) and in healthy older men using low or standard T doses (20). In healthy young men, increased IGF-I has been reported using a high T dose (600 mg/wk) (28), whereas reduced IGF-I and IGFBP-3 levels were described in a small study of men using high and variable doses of androgens (27). A metaanalysis indicated a slight reduction on bone resorption markers and no significant effect on bone formation markers by T administration in middle-aged men (29). Increased PIIINP has been reported in response to androgen administration in postmenopausal women (30). In our study we used a relatively high T dose as confirmed by the profound suppression of LH and FSH, as well as the significant reduction in SHBG. Despite this relatively high dose, the increases we observed in PINP, ICTP, and PIIINP in response to T, which suggested a general anabolic effect in young subjects, were relatively small, approximately 20% of the GH responses.

The application of GH-responsive markers for a doping test requires normative data in elite athletes to identify factors influencing their serum concentrations. A cross-sectional study from our group of elite athletes has shown that age and gender were the major determinants of variability for IGF-I and the collagen markers (31), supporting other data from elite athletes in the post-competition setting (32). Importantly, we also demonstrated that ethnicity is unlikely to confound the validity of a test based on IGF-I and the collagen markers (31). We have further shown that administration of human erythropoietin did not change IGF axis and collagen markers in young healthy male athletes, therefore, should not affect the validity of a GH doping test using these GH-responsive markers (33).

The different pharmacodynamic profiles of IGF axis and collagen markers shown in this study suggest that they have complementary attributes as potential diagnostic markers. The IGF axis markers, in particular IGF-I, increased rapidly after administration of GH, therefore, may be more sensitive early during administration. The collagen markers on the other hand remained elevated up to 6 wk after the end of 8-wk treatment, so may be more useful in the washout period. Of the collagen markers, PIIINP showed the greatest response, which was also enhanced by coadministration of T, and ICTP was more highly elevated at the end of washout. Combinations of IGF axis and collagen markers offer a promising approach to maximize the detection window for GH abuse (34). If one member of each system was to be represented, our data would suggest the use of IGF-I and PIIINP or ICTP, due to the sustained elevation of these collagen markers during washout. Our previous cross-sectional study in elite athletes also indicated that no individuals had extreme values (outside the 99% reference interval) both for IGF-I and for the collagen markers, therefore markers from both groups should be elevated only in athletes who are abusing GH (31). Therefore, the combination of IGF-I and a collagen marker potentially provide both high sensitivity and specificity for a GH doping test.

In conclusion, at the doses and duration of treatment used in this study, the IGF axis and collagen markers were more responsive markers of GH doping in men than in women. Coadministration of T in men does not affect the sensitivity of these markers for detection of GH, except for PIIINP, which

showed an increased response. The increases in collagen markers have a different time course to the IGF markers and extend the window of detection in both sexes up to 6 wk from cessation of treatment. This suggests that using both IGF-I and a collagen marker may provide the greatest discriminatory power for a doping test both during GH administration and withdrawal.

Acknowledgments

We thank all our volunteers for their participation in the study. We also thank Angela Peris, Shanley Rainsberry, and Vita Birzniece for clinical assistance, Kevin Hardman, Sri Meka, and James Modzelewski for their technical contribution with the assays, and Mr. Jim McBride for information technology support. We thank Novo Nordisk and Organon for the supply of medication. We also thank Mr. Kenneth Graham, New South Wales Institute of Sport, for his advice with participant recruitment. We thank the following for their assistance in the recruitment of study participants: the University of New South Wales (UniGym, Sports Association and the Faculties of Sports Science and Medicine); University of Sydney (Sydney University Sport and the Faculty of Medicine); and the Australian College of Physical Education.

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This work was supported by the World Anti-Doping Agency and by the Australian Government through the Anti-Doping Research Program of the Department of Communications, Information Technology and the Arts. The study was registered with the Australian New Zealand Clinical Trials Registry, (ACTRN012605000508673, www.anzctr.org.au).

Disclosure Statement: The authors have nothing to disclose.

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