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Toward the Development of a Test for Growth Hormone (GH) Abuse: A Study of Extreme Physiological Ranges of GH-Dependent Markers in 813 Elite Athletes in the Postcompetition Setting

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There is a need to develop a test to detect GH abuse by elite athletes. Measured levels of GH in blood or urine, however, provide little information on the GH-IGF-I axis. Previous studies have identified a series of indirect markers of GH action that are markedly altered by the administration of GH, but to a lesser degree by acute exercise. This study was undertaken to determine the physiological range of these GH-dependent variables in elite athletes after a competitive event to determine whether such values differ from resting values in normal and athletic subjects and to establish whether any adjustments to this range are required on the basis of age, gender, demographic characteristics, or the nature of the exercise performed. Serum samples were collected from 813 elite athletes (537 males and 276 females; age range, 17–64 yr) from 15 sporting disciplines within 2 h of completion of a major competitive event. IGF-I, IGF-binding protein 2 (IGFBP-2), IGFBP-3, acid-labile subunit, and the bone and soft tissue markers, osteocalcin, carboxyl-terminal propeptide of type I procollagen, carboxyl-terminal cross-linked telopeptide of type I collagen, and procollagen type III were measured. Sporting category, gender, age, height, weight, body mass index (BMI), and racial group of the athlete were documented, and results were compared both to normative data and to values obtained from elite athletes under resting conditions. Forty-one percent of IGF-I values in male athletes and 41% of values in female athletes were above the upper limits of 99% reference ranges derived from resting values in a normal population. Postcompetition levels of all variables except carboxyl-terminal propeptide of type I procollagen and carboxyl-terminal cross-linked telopeptide of type I collagen differed from resting values. There was a consistent age-dependent fall in measured levels of all variables ($P < 0.0001$) with the exception of IGFBP-2, which increased with age ($P < 0.0001$). BMI, but not height, exerted a small, but significant, influence on several variables. After adjustment for age, there were no significant differences in the levels of any of the measured variables between sporting categories. IGFBP-2 and IGFBP-3 were lower in 35 black athletes compared with those in 35 white athletes matched for age, gender, height, BMI, and sporting category. We have demonstrated that there are predictable age-dependent levels of GH-dependent markers in elite athletes that are consistent even at the extremes of physical exertion and that these are independent of sporting category. Normative data applicable to white athletes are provided. This provides important groundwork for the development of a test for GH abuse, although these values may be specific for the reagents and assays used. (*J Clin Endocrinol Metab* 90: 641–649, 2005)

THERE IS AN increasing body of evidence that GH is widely abused by athletes and sportsmen as a performance-enhancing agent. Although there is no robust epidemiological data concerning the extent of GH abuse, anecdotal evidence as well as the seizure of GH supplies by police and customs officials suggests that it represents a significant problem in a number of sports, including track and field, swimming, and cycling (1, 2).

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Abbreviations: ALS, Acid-labile subunit; BMI, body mass index; CV, coefficient of variation; ICTP, carboxyl-terminal cross-linked telopeptide of type I collagen; IGFBP, IGF-binding protein; PICP, carboxyl-terminal propeptide of type I procollagen; PIIIP, procollagen type III; r-h, recombinant human.

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GH is a powerful metabolic hormone; its absence in GH deficiency leads to decreased lean body mass, increased body fat, decreased exercise capacity, and increased fatigue (3–5). GH replacement in GH-deficient adults improves exercise performance and strength. There is also evidence that supraphysiological GH exerts a protein anabolic effect in athletes, both at rest and during exercise, although it is not known whether this effect translates into improved performance. Despite this lack of evidence, there is an expectation among athletes that by attaining supraphysiological levels of GH, their physical performance may be enhanced.

Abuse of GH is undesirable because of unfair competition and also, as illustrated by the pathophysiological model of acromegaly, because the maintenance of supraphysiological levels of GH is detrimental to health (7). Although it is clearly essential to develop a detection strategy for GH abuse, sev-

eral factors complicate this task. Exogenous recombinant human GH (r-hGH) and endogenous GH have identical amino acid sequences, making chemical distinction impossible. GH is secreted in a pulsatile manner and is under the influence of stress, exercise, sleep, and food intake (8). This pattern of secretion results in serum concentrations that vary widely throughout the day and frequently overlap with measurements obtained after exogenous administration of GH. Measurement of urinary GH has been previously demonstrated to be insensitive as a marker of either GH administration or acromegaly (9, 10).

To overcome the problems that surround the direct measurement of GH, we have recently identified a series of markers of GH action, which includes components of the IGF/IGF-binding protein (IGFBP) axis and markers of bone and collagen turnover and which shows promise as the basis for a test for GH abuse (11, 12). These markers have been identified, by assessing the effects of acute exercise, GH administration, and GH withdrawal on a large number of physiological substances that are influenced by GH. The markers that we have selected are responsive to GH administration, but less sensitive to the acute effects of exercise (11–15). We have demonstrated that GH administration induces predictable modifications in these markers that persist after GH withdrawal and allow for clear discrimination to be made between placebo- and GH-treated groups (13, 15).

Before these markers of GH administration may be applied as a basis for a doping detection strategy, it is necessary to construct appropriate normative data against which an individual result may be compared. Where reference ranges exist for these markers, they have been constructed from nonathletic populations. Values in athletes may differ significantly from those in a nonathletic population, reflecting the effects of physical training, acute exercise, genetic background, and the athletic physique. In this study we have measured markers of the IGF/IGFBP axis and markers of bone and soft tissue turnover in serum samples collected from 813 elite athletes after a major competitive event, the situation in which a doping test is most likely to be applied. We have compared these results with values obtained from elite athletes under resting conditions and with previously published normative data (16). Finally, we have studied variables that might influence these normative data, such as age, gender, body habitus, sporting discipline, and racial origin, and used this information to construct reference ranges, which may be applied under resting conditions and in the postcompetition setting, to detect doping with GH.

Subjects and Methods

Subjects and sample collection

Samples were collected from 813 volunteer elite athletes (athletes competing at the national or international level) within 2 h of a major competitive event at the regional, national, or international level. Demographic data are given in Tables 1 and 2.

The nature of the project was explained to the athletes before their participation in their event, and written informed consent was obtained immediately before blood sampling. All samples were taken by a member of the GH-2000 team and collected according to a standardized protocol at the sporting venue where the event had taken place. Before sample collection, the following demographic data were recorded: gender, race, height (self-reported), weight (measured using the Tanita TB7–305 bioimpedance analyzer, Tanita, Tokyo, Japan), age, and sporting category. During sampling, volunteers were seated, and blood was drawn from a vein in the antecubital fossa using a Vacutainer (BD Biosciences, Franklin Lakes, NJ) and 20-gauge needle into two 5-ml bottles containing SST clot activator gel. Samples were left to clot for 15 min at room temperature before being centrifuged at 1500 rpm in a portable centrifuge. Two-milliliter aliquots of serum were then transferred by pipette into small storage tubes (Cryotubes) and immediately placed on dry ice. They were subsequently transferred on dry ice to storage at −80 C in the four participating GH-2000 centers.

Results were compared with both previously published normative data (IGF-I only) derived from 400 healthy Swedish volunteers and resting samples (all variables) from 262 elite athletes, which were also collected as part of the GH-2000 project. All samples were measured in the same laboratory.

The West Lambeth Health Authority gave ethics approval for this study.

TABLE 1. Characteristics of elite male athletes according to the sports in which they participated

Sport	n	Age (yr)	Height (cm)	Weight (kg)	BMI (kg/m^2)
Power lifting	20	35 ± 5	170 ± 10	85 ± 19	29.8 ± 1.6
Basketball	27	25 ± 5	192 ± 8	91 ± 13	24.4 ± 0.4
Football	46	24 ± 4	178 ± 7	74 ± 8	23.3 ± 0.3
Swimming	100	22 ± 3	187 ± 39	81 ± 9	23.2 ± 0.3
Marathon	8	36 ± 12	173 ± 7	66 ± 6	22.0 ± 0.7
Canoeing	10	26 ± 6	183 ± 5	81 ± 5	24.1 ± 0.6
Rowing	73	27 ± 4	187 ± 8	82 ± 11	23.5 ± 0.2
Cross-country skiing	20	23 ± 2	181 ± 4	74 ± 6	22.6 ± 0.3
Alpine skiing	17	24 ± 3	183 ± 5	82 ± 6	24.4 ± 0.3
Weightlifting	10	29 ± 6	177 ± 6	79 ± 13	25.2 ± 1.2
Judo	26	27 ± 7	177 ± 7	74 ± 7	23.18 ± 0.4
Bandy	21	27 ± 4	180 ± 7	80 ± 7	24.7 ± 0.3
Ice hockey	38	26 ± 4	181 ± 5	85 ± 5	25.9 ± 0.2
Olympic handball	23	27 ± 3	189 ± 6	90 ± 12	25.1 ± 0.5
Track and field	98	26 ± 5	182 ± 8	78 ± 17	23.4 ± 0.4

Values are the mean ± SD.

Analytical methods

All samples were coded and kept at −80 C until analyzed at one of two central reference laboratories: Sahlgrenska Hospital (Gothenburg, Sweden) and Kolling Institute (Sydney, Australia). Serum IGF-I, procollagen type III (PIIIP), carboxyl-terminal cross-linked telopeptide of type I collagen (ICTP), and carboxyl-terminal propeptide of type I procollagen (PICP) were determined in Gothenburg, Sweden, and IGFBP-2, IGFBP-3, and acid-labile subunit (ALS) were measured in Sydney, Australia.

Serum GH was determined by immunoradiometric assay (Pharmacia

TABLE 2. Characteristics of elite female athletes according to the sports in which they participated

Sport	n	Age (yr)	Height (cm)	Weight (kg)	BMI (kg/m^2)
Power lifting	1	41	156	51	20.4
Basketball	14	21 ± 3	176 ± 6	68 ± 5	22.0 ± 1.4
Swimming	90	21 ± 4	173 ± 6	64 ± 7	21.5 ± 1.6
Marathon	8	36 ± 7	165 ± 6	54 ± 4	19.7 ± 2.4
Canoeing	4	34 ± 8	164 ± 8	58 ± 10	21.5 ± 1.3
Rowing	33	26 ± 4	174 ± 8	68 ± 10	22.4 ± 1.8
Cross-country skiing	14	23 ± 2	168 ± 4	59 ± 6	21.0 ± 1.7
Alpine skiing	19	24 ± 3	168 ± 6	62 ± 5	21.7 ± 2.2
Weightlifting	7	25 ± 5	162 ± 7	63 ± 14	23.9 ± 5.1
Olympic handball	35	24 ± 4	172 ± 5	67 ± 6	22.5 ± 1.6
Track and field	51	26 ± 5	171 ± 7	63 ± 12	21.2 ± 3.1

Values are the mean ± SD.

Biotech, Uppsala, Sweden), with within-assay coefficients of variation (CVs) of 10%, less than 5%, and less than 5%, and between-assay CVs of 9.0%, less than 5%, and 7% at 10, 23, and 43 mU/liter, respectively.

Serum IGF-I was measured by RIA using a monoclonal antibody after acid-ethanol extraction (17), 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 104, 281, and 1324 ng/ml. Results were compared with previously published population-based reference data from the same laboratory (16). The analyses used on both occasions were carried out using the same technique and the same antisera.

IGFBP-2 (18), IGFBP-3 (19), and ALS (20) were assayed using in-house RIAs and polyclonal antibodies. Serum IGFBP-2 within-assay CVs were 2.8%, 2.8%, and 3.2% at 140, 275, and 595 μg/liter, and between-assay CVs were 14.1%, and 12.7% at 65 and 775 μg/liter, respectively.

Serum IGFBP-3 within-assay CVs were 6.2%, 5.5%, and 4.5% at 2.5, 5.7, and 12.6 mg/liter, and between-assay CVs were 11.9%, 14.5%, and 13.1% at 2.5, 5.7, and 12.6 mg/liter, respectively.

Serum ALS within-assay CVs were 3.4%, 3.3%, and 3.4% at 60, 245, and 502 nmol/liter, and between-assay CVs were 10.5%, 5.4%, and 6.5% at 62, 282, and 676 nmol/liter, respectively.

Serum osteocalcin was measured by RIA (OSTK-PR In Vitro Test Kit, CIS Biointernational, Oris Industries, Gif-sur-Yvette, France); the within-assay CVs were 3.7% and 3.0% at 3.8 and 24.7 μg/liter, and the between-assay CVs were 6.6% and 5.5% at 3.8 and 24.7 μg/liter.

Serum PICP was measured by RIA (Orion Diagnostica, Espoo, Finland); the within-assay CVs were 2.1% and 3.2% at 103 and 415 μg/liter, and the between-assay CVs were 4.1% and 4.0% at 105 and 435 μg/liter.

Serum ICTP was measured by RIA (Orion Diagnostica); within-assay CVs were 6.2% and 4.4% at 3.8 and 11.2 μg/liter, and the between-assay CVs were 7.9% and 6.5% at 3.3 and 10.5 μg/liter.

Serum PIIIP was measured by a two-stage sandwich RIA (CIS Biointernational; as described above); total assay CVs (within- plus between-assay) were 9.1%, 5.7%, and 6.8% at 0.62, 0.95, and 1.18 μg/liter.

Data analysis

Data analysis revealed that age was the single most important determinant of all eight variables. Therefore, we initially determined the form of the relationship between age and each of the markers and found that a linear relation between the logarithm of the markers and the reciprocal of age represented the best-fit relationship. Because our analysis disproved the hypothesis that both sexes have a common relationship between marker and age, the two sexes were treated separately.

Any independent influence of height, weight, and body mass index (BMI) was determined using stepwise multiple regression analysis after adjustment for the effects of age. The influence of sporting category on each of the variables was assessed before and after adjustment for age, using ANOVA. Resting and postcompetition samples were compared using unpaired *t* tests; although a few subjects provided resting and postcompetition samples, most subjects provided only one sample for this exercise, justifying the use of unpaired tests.

Using the model, $y = \alpha + \beta x +$ random error, where y represents the logarithm of a marker, and x represents the reciprocal of age; prediction intervals (reference ranges) were constructed for each marker. Regression analyses for each combination of marker and gender provided least squares estimates (a and b) of the intercept α and slope β of the relationship between y and x together with an estimate of the residual SD. Approximate lower (L) and upper (U) prediction limits for an observation on y at a given value of x are thus provided as: $L = a + bx - ts$ and $U = a + bx + ts$, where t is the appropriate percentage point of the t distribution. The value t is dependent upon sample size, and in our large dataset it is appropriate to replace t values with the equivalent normal values, 1.960 for a 95% range and 2.576 for a 99% range. Because the prediction intervals are expressed on a logarithmic scale, the limits for a marker in natural units are e^L and e^U.

To investigate any possible racial influence on analytes, each black athlete was pair-matched with the white athlete who was most similar with regard to age, gender, height, and weight, and sporting category. Black and white athletes were then compared using paired *t* tests. This method of analysis was performed in view of the small number of black athletes studied. All statistical comparisons were two-tailed. $P < 0.05$ was considered statistically significant.

Results

Postcompetition samples were collected from 813 athletes from 15 different sporting categories. Five hundred and thirty-seven of the samples collected were drawn from male athletes, and 276 were from female athletes. Male athletes were older (26.5 ± 5.3 *vs.* 25.1 ± 5.4 yr; $P < 0.02$), taller (182.3 ± 8.5 *vs.* 171.1 ± 7.2 cm; $P < 0.001$), and had a greater BMI (24.1 ± 3 *vs.* 21.8 ± 2.5 kg/m^2; $P < 0.001$) than female athletes. Age, height, and BMI varied widely between the sports, reflecting the different nature of each discipline (Tables 1 and 2). Resting samples were collected from 262 athletes. The distribution of age (26.5 ± 5.3 yr), gender, and BMI (23.2 ± 2.5 kg/m^2) in these subjects did not differ significantly from that in the postcompetition group.

IGF-I/IGFBP axis

Forty-one percent of IGF-I values in males and 41% of values in females were above the upper limits of 99% reference ranges derived from normal subjects 25 yr and older under resting conditions (Fig. 1). When only subjects greater than 25 yr of age were considered, 24% of IGF-I values in males and 21% of values in females were above the upper limits of this range. The dependence of all components of the IGF-I/IGFBP axis on age was best represented through a model in which the logarithm of the marker depends linearly on the reciprocal of age. IGF-I, IGFBP-3, and ALS all declined with age, whereas IGFBP-2 increased with age (Fig. 2). The percent decline in IGF-I (35%/decade) was more marked

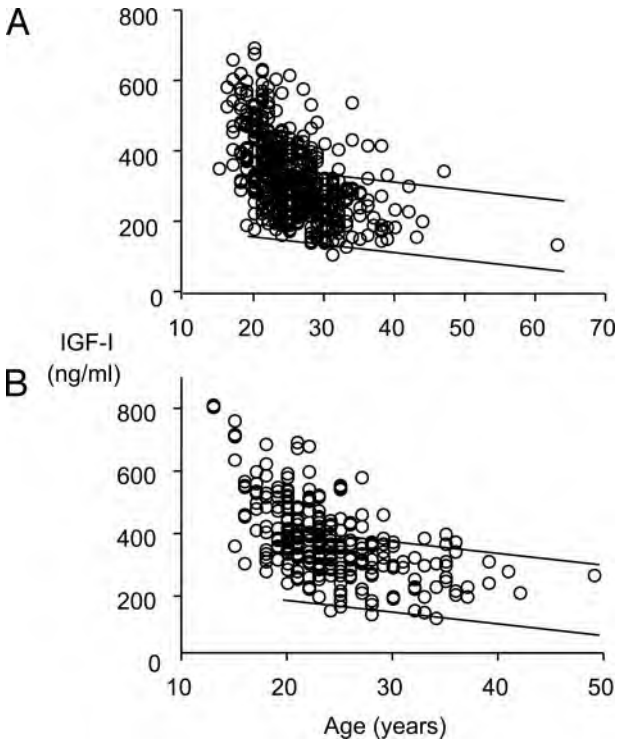

FIG. 1. Comparison of IGF-I levels (nanograms per milliliter) measured in the postcompetition setting in 537 elite male athletes (A) and 276 elite female athletes (B) with laboratory reference data obtained from a normal Swedish population.

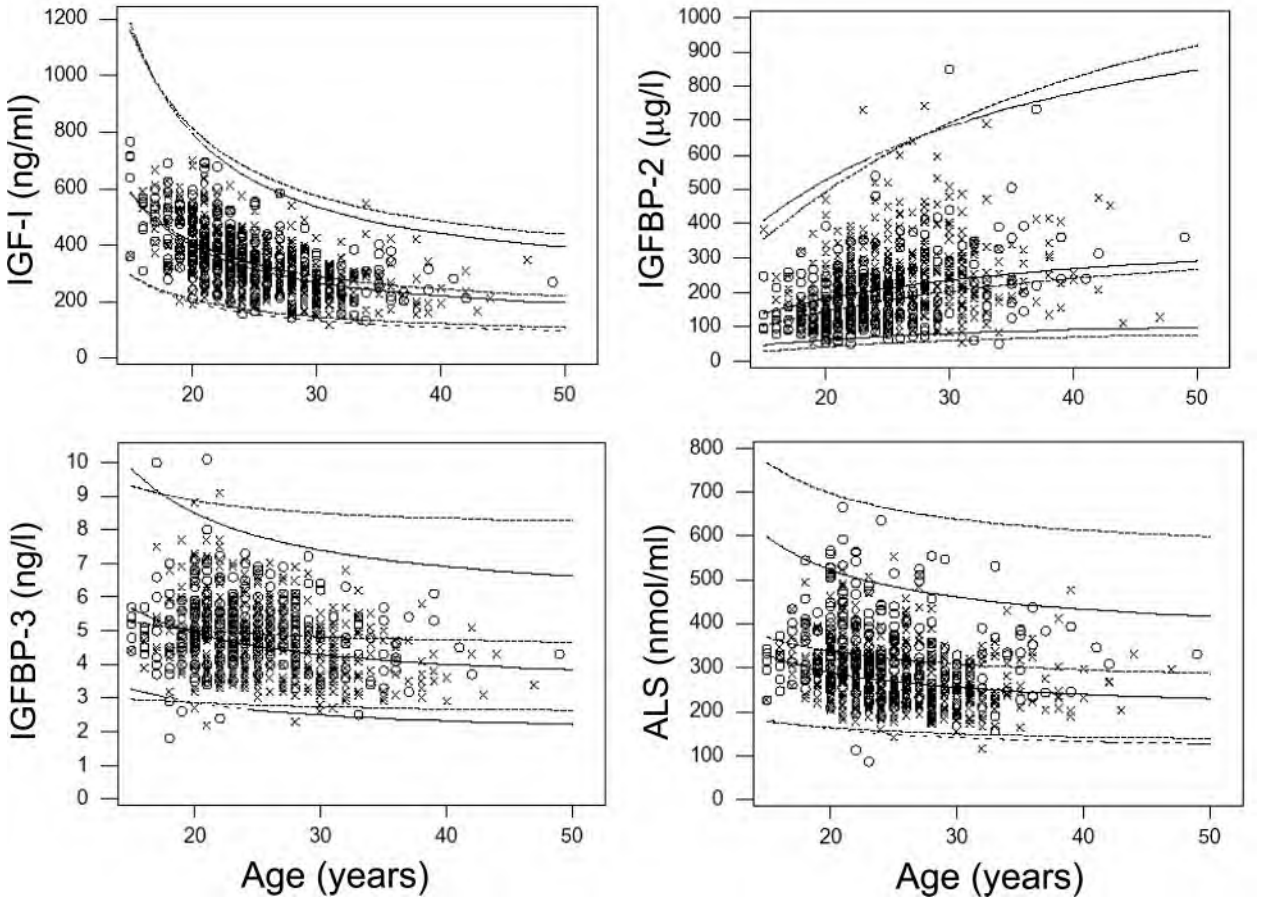

FIG. 2. Age-dependent change in components of the IGF/IGFBP system in 537 elite male (X) and 276 elite female (O) athletes. Reference ranges for male (*solid lines*) and female (*dashed lines*) athletes are shown.

than the percent decline in IGFBP-3 (9%/decade) and ALS (11%/decade).

Over the main age range for the data available here, IGF-I, IGFBP-3, and ALS were greater, on the average, in women, whereas IGFBP-2 was greater, on the average, in men. There was no significant influence of height on any of these variables in either males or females, whereas BMI exerted a small, but significant, independent positive effect on IGFBP-2 in both males ($r^2 = 0.109$) and females ($r^2 = 0.030$). BMI had no significant effect on any other marker.

Markers of bone and collagen turnover

The relationship between age and each of the bone markers was also best fitted using a linear model between the logarithm of the marker and the reciprocal of age. There was a decline in all markers of bone turnover with age (Fig. 3). Osteocalcin declined by an average of 15%/decade, PICP by 5%/decade, ICTP by 38%/decade, and PIIIP by 19%/decade.

Over the main age range for the data available here, osteocalcin, PICP, ICTP, and PIIIP were greater, on the average, in men. BMI exerted a small, but significant, independent positive influence on PICP in male athletes only ($r^2 = 0.01$). Height had no significant effect.

Comparison of resting and postcompetition values and influence of sporting category

In male athletes, IGF-I, IGFBP-2, IGFBP-3, ALS, ICTP, and PIIIP were all higher, whereas osteocalcin was lower, in the postcompetition setting than under resting conditions (Table 3). In female athletes, IGFBP-2, IGFBP-3, ALS, and PIIIP were all higher, and osteocalcin was lower, in the postcompetition setting than under resting conditions (Table 3). There was a strong trend ($P = 0.054$) for IGF-I to be higher in female athletes postcompetition compared with resting conditions. The influence of sporting category on each of the variables was assessed before and after adjustment for age. Initial analysis suggested wide variation in all variables between sporting categories, but after correction for age, there were no major differences in any of the variables between sporting categories. It can be seen in Fig. 4 that after age adjustment, intersport variability was much reduced. Differences between sports were not significant once the age-adjustment had been performed.

Influence of race

Mean levels of IGFBP-2 and IGFBP-3 were significantly lower in black athletes compared with pair-matched white athletes. There were no significant differences in any other

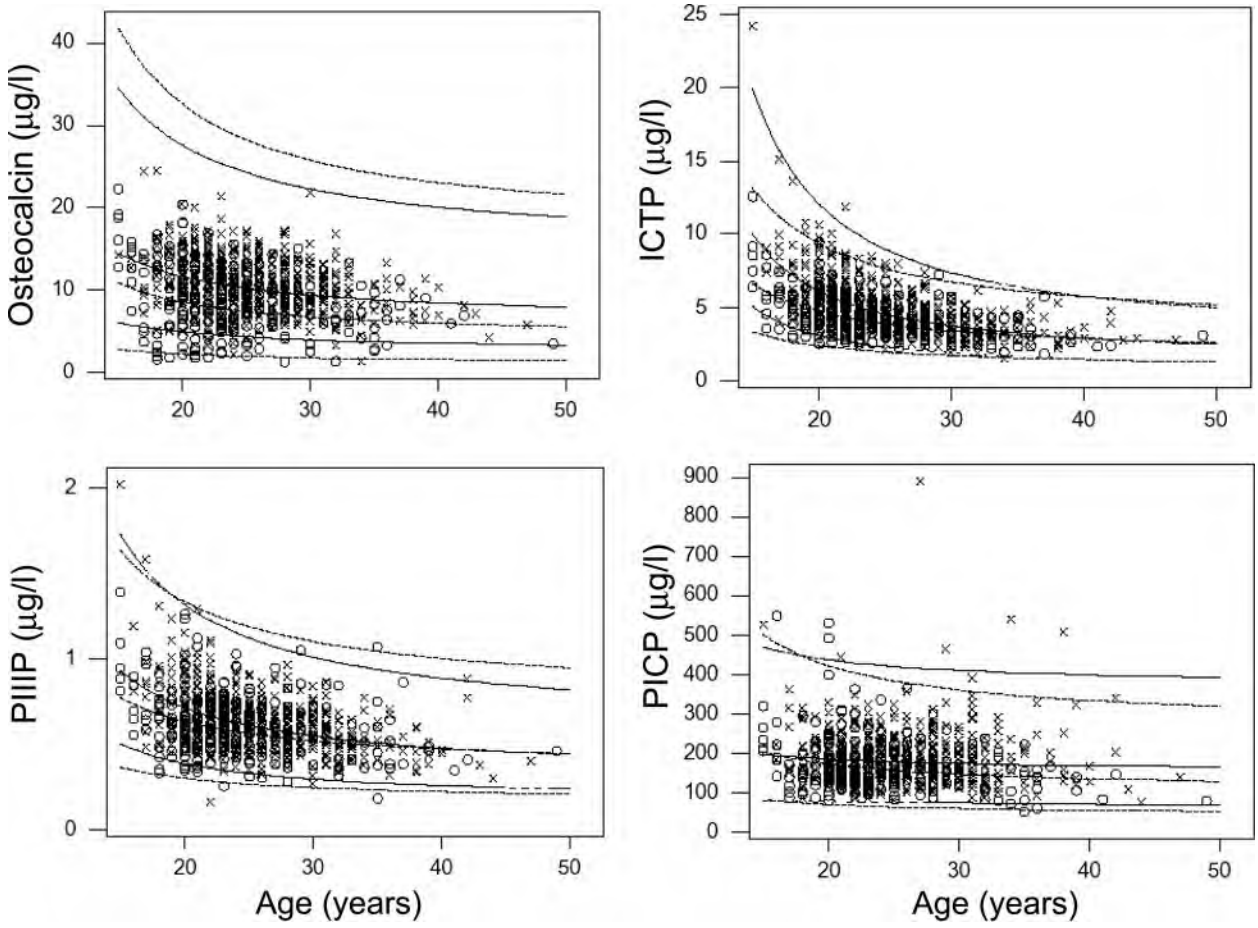

FIG. 3. Age-dependent change in markers of bone turnover in 537 elite male (X) and 276 elite female (O) athletes. Reference ranges for male (*solid lines*) and female (*dashed lines*) athletes are shown.

variables (Table 4). Analysis was repeated after removal of the two female subjects in each group, with no significant effect on the findings.

Reference ranges

Because age and gender proved to be the principal determinants of each of the markers, age-related reference ranges were constructed for male and female athletes individually (Figs. 2 and 3).

Table 5 displays the values *a*, *b*, and *s* derived from the least squares analysis for each of the eight markers. The 99% reference range at any given age will be given by e^L and e^U, where $L = a + b/\text{age} - 2.576$, $U = a + b/\text{age} + 2.576$. *a* and *b* are the least squares estimates of the relationship between *y*, the log (marker), and *x*, the reciprocal of age, and *s* is the residual SD. This approximation is valid because the number of data points is large, and as long as no extrapolation is attempted, the limits given will be good approximations for ages in the range from about 16–45 yr. The following examples illustrate the use of these values to generate 99% prediction intervals for ICTP.

Male, age 16 yr. On a log scale, $0.339 + 29.5/16 \pm 2.576 \times 0.2614$ gives the approximate limits. Hence, L = 1.51 and U = 2.86, and the 99% reference range is (e^L, e^U), or (4.52, 17.39 µg/liter).

Female, age 16 yr. The corresponding limits are $0.566 + 19.8/16 \pm 2.576 \times 0.2615$ on the log scale, giving (3.10, 11.91 µg/liter) for ICTP in its natural scale.

Discussion

This cross-sectional study demonstrates that age is the most important determinant of postexercise serum levels of components of the IGF-I/IGFBP system and markers of bone and collagen turnover in elite athletes. As shown clearly in Figs. 2 and 3, gender has a much smaller effect than age and produces a reference range that is age-related, but, encompassing both genders, differs very little from the gender-specific reference range. Once adjustment has been made for age and gender, then demographic characteristics and the nature of the exercise performed exert little or no influence on any of these variables. These results have allowed development of age- and gender-specific normative data, and from this the construction of appropriate reference ranges, which can be applied in the development of a test for GH abuse by athletes.

All components of the IGF-I ternary complex (IGF-I,

TABLE 3. Comparison of results of GH-dependent variables in resting and postcompetition samples from elite athletes

	Male		Female	
	Resting (n = 267)	Post competition (n = 813)	Resting (n = 267)	Post competition (n = 813)
IGF-I (ng/ml)	308 (93)	333 (108)[a]	352 (104)	381 (123)
IGFBP-2 (μg/liter)	216 (82)	239 (103)[a]	180 (91)	207 (109)[a]
IGFBP-3 (mg/liter)	4.2 (0.8)	4.7 (1.0)[a]	4.7 (0.8)	5.1 (1.1)[a]
ALS (nmol/liter)	241 (50)	277 (68)[a]	298 (85)	340 (95)[a]
Osteocalcin (μg/liter)	12.7 (3.3)	10.9 (3.4)[a]	10.2 (3.4)	8.8 (3.9)[a]
PICP (μg/liter)	202 (74)	190 (73)	182 (57)	175 (68)
ICTP (μg/liter)	4.6 (1.5)	4.9 (2.0)[a]	4.3 (1.2)	4.4 (1.5)
PIIIP (μg/liter)	0.64 (0.18)	0.57 (0.13)[a]	0.62 (0.19)	0.56 (0.15)[a]

All values are means (±SD).
[a] $P < 0.01$ *vs.* resting values.

IGFBP-3, and ALS) declined with age, whereas IGFBP-2 increased with age. GH exerts powerful stimulatory regulation over IGF-I, and age-related changes in IGF-I closely correspond to age-related changes in integrated 24-h GH secretion. Interpretation of age-related changes in GH-IGF-I activity, however, is complicated by concurrent age-related changes in body composition and physical fitness, variables that also influence GH secretion. It has been hypothesized that the age-related fall in GH-IGF-I activity may occur secondary to age-related changes in body composition or physical fitness (21), rather than being an inherent part of the ageing process (6). A recent study, however, that compared older and younger subjects matched for lean body mass and fat mass provided evidence that the age-related decline in GH secretion is independent of changes in body composition (22). Consistent with this finding, the age-related decline in IGF-I observed in elite athletes in the current study is at least as marked as previous reports of the age-related decline in sedentary subjects (16, 23, 24).

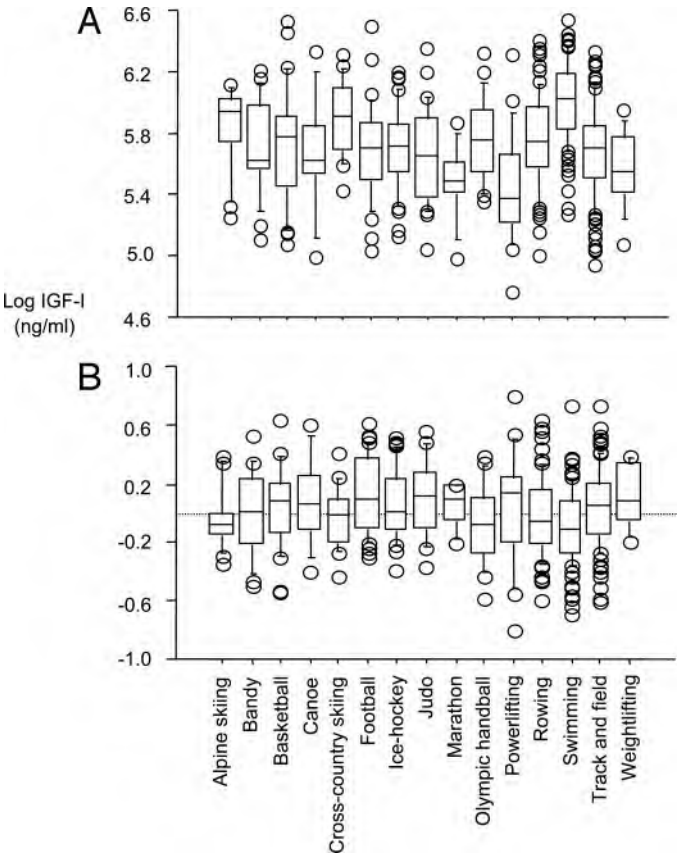

FIG. 4. A, Logarithmically transformed IGF-I levels in elite male athletes from 15 different sports. B, Box plots of age-adjusted residuals obtained by regressing these logarithmically transformed IGF-I levels on the reciprocal of age.

Postexercise levels of all components of the IGF/IGFBP axis were greater than resting values. This finding is consistent with our previous report that acute exhaustive exercise induces an increase in all of these variables that is maximal by the end of exercise and has returned to baseline within 2 h of completion of exercise (14). Approximately 40% of IGF-I levels were above a 99% reference range previously derived in normal subjects 25 yr of age and older, in which a linear relationship between age and IGF-I was assumed (16). Detailed analysis of the data in the current study confirmed the nonlinearity of that relationship, as previously demonstrated by Juul *et al.* (25). Notably, many of elevated IGF-I levels were derived from younger subjects, consistent with the very high levels of IGF-I that occur during and after puberty. It is likely that the discrepancy between the postcompetition values recorded in this study and the reference range derived by Landin-Wilhelmsen (16) reflects a combination of the acute effects of exercise, clearly demonstrating the need for reference data specifically applicable to athletes, the inappropriate assumption of a linear relationship between age and IGF-I in that report, and possibly recent pu-

TABLE 4. Comparison of results of GH-dependent variables in samples from 35 black athletes and 35 white athletes, pair-matched for demographic characteristics

	White	Black
Male/female	33/2	33/2
Age (yr)	25.8 (0.8)	25.6 (1.0)
Weight (kg)	78.0 (2.3)	75.6 (2.4)
Height (cm)	182.3 (1.3)	179.9 (1.5)
IGF-I (ng/ml)	317 (19)	321 (16)
IGFBP-2 (μg/liter)	296 (25)[a]	222 (16)
IGFBP-3 (mg/liter)	4.83 (0.21)[a]	4.32 (0.15)
ALS (nmol/liter)	254 (12)	236 (7)
Osteocalcin (μg/liter)	10.7 (0.5)	11.0 (0.6)
PICP (μg/liter)	182 (17)	151 (5)
ICTP (μg/liter)	5.17 (0.29)	5.47 (0.38)
PIIIP (μg/liter)	0.69 (0.03)	0.71 (0.04)

All values are means (±SEM).
[a] $P < 0.05$ *vs.* black athletes.

TABLE 5. Values of intercept, slope, and SD for prediction intervals for markers for male and female athletes treated separately

Marker	Sex	A	B	S
IGF-I (ng/ml)	M	4.80	23.7	0.2681
	F	4.97	20.9	0.2623
IGFBP-2 (μg/liter)	M	5.99	−15.6	0.4097
	F	5.99	−20.2	0.4691
IGFBP-3 (mg/liter)	M	1.18	8.37	0.2099
	F	1.49	2.58	0.2172
ALS (nmol/liter)	M	5.29	7.68	0.2280
	F	5.56	5.33	0.2781
Osteocalcin (μg/liter)	M	1.81	12.9	0.3326
	F	1.44	14.2	0.5140
PICP (μg/liter)	M	5.04	3.78	0.3303
	F	4.67	9.62	0.3427
ICTP (μg/liter)	M	0.339	29.5	0.2614
	F	0.566	5.33	0.2781
PIIIP (μg/liter)	M	−1.13	15.9	0.2357
	F	−1.03	11.6	0.2854

The numbers in the table are not directly in the units given (see text for further details).

berty in some of the younger subjects. Additional data are needed before the results in this study can be safely used in athletes who are peripubertal or recently postpubertal.

IGF-I, IGFBP-3, and ALS were higher, on the average, in females, whereas IGFBP-2 was higher in males. The influence of gender on GH-IGF-I activity is extremely complex. There is a sexually dimorphic pattern of GH secretion (26). Parenteral testosterone increases IGF-I in normal men (27). Orally administered estrogen reduces the IGF-I response to GH, but also leads to increased GH secretion, probably at least partly secondary to reduced negative feedback by circulating IGF-I (28). Orally administered estrogen also reduces levels of IGFBP-3 and ALS (29). An exploration of the moderating effect of oral contraceptive usage on the data collected from the female athletes was beyond the scope of this study and requires additional investigation.

After adjustment for age and gender, the relationship between BMI and IGF-I/IGFBP activity was weak, although there was a positive correlation with IGFBP-2. In normal population groups, GH secretion is influenced by body fat distribution. Increased visceral fat is associated with lower serum IGF-I concentrations (30, 31) and decreased spontaneous (28) and exercise-induced (32) GH release. However, the relationship between axiological characteristics and components of the IGF-I/IGFBP axis may differ in athletic populations, because, firstly, body fat is much lower than in a normal nonathletic cohort, and secondly, athletes are likely to have an increased lean body to fat mass ratio, even when compared with normal subjects with a similar BMI.

IGFBP-2 and IGFBP-3 were lower in black athletes compared with a closely matched population of white athletes. Reduced IGFBP-3 despite increased integrated 24-h GH secretion has previously been documented in normal black men (33). To our knowledge, reduced IGFBP-2 in a black population has not been previously described. The physiological consequences of these changes are not known, but may be important. However, in the context of development of a test for doping with GH, these findings may be of major importance and warrant additional investigation of the GH-IGF-I axis in different racial groups. It should be emphasized that these are preliminary data and require validation in a much larger population.

The markers of bone formation reported in this study reflect different stages of osteoblastic cell function (34). Osteocalcin is generated during bone mineralization. Earlier phases of the bone-remodeling process involve the deposition of collagen scaffolding, which generates PICP. ICTP is a marker of bone resorption. 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 (35, 36). All of the markers of bone turnover declined with age. Although a large body of literature exists concerning the effect of age on bone turnover in postmenopausal and elderly subjects, little work has been carried out in younger subjects. In one of the few studies that included subjects of a similar age to those in the current study, Fatayerdi and Eastell (6) demonstrated that the influence of age on PICP, osteocalcin, and ICTP was best described by quadratic functions, with a decline in younger subjects, a nadir in the sixth decade, and an increase thereafter. This is compatible with our findings. Although we found no advantage in using a quadratic compared with a log-linear function, this is probably explained by the fact that we did not study subjects beyond the fifth decade and thus did not observe a leveling out of these markers.

The observation that postcompetition levels of some of the variables differed from resting data are compatible with our previous observations that exercise exerts a small, but significant, effect on both the IGF/IGFBP system and markers of bone and soft tissue turnover (11, 12). However, after adjustment for age, despite major differences in the nature and duration of the exercise performed, there were no substantial differences between sports for either components of the IGF/IGFBP system or markers of bone and soft tissue turnover. This finding is clearly of importance when developing a test for GH doping, because it implies that expected values will not differ depending on the nature of the sport performed. In addition to the acute effects of exercise, it is possible that postcompetition levels of some analytes are influenced by precompetition training practices. Generally, athletes will taper for a period of time (depending on the sport) before an event, which consists of doing a smaller volume of training but still training at a high intensity. How this affects components of the IGF/IGFBP system is not known.

The reference ranges quoted are specific to the assays and reagents used in this project. It is the nature of immunoassays that measurements are not absolute and inevitably a function of the reagents and conditions used in the assay. If immunoassays are to be used in a test for GH abuse that may lead to sanctions on an elite athlete, it will be necessary for the laboratory concerned to be able to demonstrate validation of their assays with the results reported here. Furthermore, in the development of a widely available test for GH abuse, it will be essential to develop assays using specific reagents that can be accredited to an international standard and regularly monitored by the International Olympic Committee

and the World Anti Doping Agency. The introduction of specific mass spectrometric assays for these protein hormones now seems feasible, and these may, in turn, replace immunoassays (at least for confirmation of the results of a screening test) and facilitate international standardization. The results presented here can only be used in the context of white elite athletes. Additional research is needed on the sensitivity of other ethnic groups to exogenous r-hGH.

The markers described in this study represent one of the most promising approaches to detection of GH abuse by athletes. An alternative approach makes use of the observation that although GH circulates predominantly as a 22-kDa isoform, there are also measurable amounts of other isoforms, including 17- and 20-kDa isoforms present in human serum. In contrast, r-hGH is entirely composed of the 22-kDa isoform, and therefore, it is possible that the ratio of other isoforms to 22-kDa GH in serum may differentiate endogenous and exogenous GH.

In summary, we have demonstrated that age is the most important predictor of serum levels of markers of GH drawn both from markers of GH action and from markers of bone and collagen turnover, reflecting the fall in GH secretion that normally occurs with ageing (somatopause). Results differ significantly between male and female athletes, although the impact of gender is less important than that of age. Using this information, we have constructed age-related reference ranges appropriate to elite white male and female athletes that are suitable for use under resting conditions and in the postcompetition setting. Because neither sporting category nor demographic characteristics were important predictors of levels of the selected markers, this range is applicable to athletes of all sporting disciplines. Establishment of appropriate reference data against which the results of an individual athlete may be compared is an important step in the development of a doping detection strategy to combat GH abuse. Assay validation will be a key step in the introduction of a robust test to detect GH abuse.

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