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Influence of Demographic Factors and Sport Type on Growth Hormone-Responsive Markers in Elite Athletes

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Context: GH-responsive markers of the IGF system and of collagen turnover hold promise as the basis of a GH doping test.

Objective: The purpose of this study was to determine the influence of age, gender, body mass index (BMI), ethnicity, and sporting type on GH-responsive serum markers in a large cohort of elite athletes from different ethnic backgrounds.

Design: The study was designed as a cross-sectional study.

Participants: A total of 1103 elite athletes (699 males, 404 females), aged 22.2 ± 5.2 yr, from 12 countries and 10 major sporting categories participated in this study.

Main Outcome Measures: Serum IGF-I, IGF binding protein-3 (IGFBP-3), acid labile subunit (ALS), and collagen markers [N-terminal propeptide of type I procollagen (PINP), C-terminal telopeptide of type I collagen (ICTP), N-terminal propeptide of type III procollagen (PIIINP)] were measured.

Results: There was a significant negative correlation ($r = -0.14$ to -0.58, $P < 0.0005$) between age and each of the GH-responsive markers. Serum IGF-I, IGFBP-3, and ALS were all lower ($P < 0.05$), whereas the collagen markers PINP, ICTP, and PIIINP were higher ($P < 0.05$) in men than in women. Multiple regression analysis indicated that age, gender, BMI, and ethnicity accounted for 23–54% of total between-subject variability of the markers. Age and gender cumulatively accounted for 91% of the attributable variation of IGF-I and more than 80% for PINP, ICTP, and PIIINP. Gender exerted the greatest effect on ALS (48%), and BMI accounted for less than 12% attributable variation for all markers. The influence of ethnicity was greatest for IGFBP-3 and ALS; however, for the other markers, it accounted for less than 6% attributable variation. Analysis of 995 athletes indicated that sporting type contributed 5–19% of attributable variation.

Conclusions: Age and gender were major determinants of variability of GH-responsive markers except for IGFBP-3 and ALS. Ethnicity is unlikely to confound the validity of a GH doping test based on IGF-I and these collagen markers. **(*J Clin Endocrinol Metab* 91: 4424–4432, 2006)**

ALTHOUGH DOPING WITH GH is prohibited by the World Anti-Doping Code (www.wada-ama.org/rtecontent/document/code_v3.pdf), its use is suspected to be widespread (1); therefore, a reliable test is essential to detect GH abuse and enforce the code. One successful approach has been based on the measurement of GH isoforms produced by the pituitary. Specific immunoassays have been developed that distinguish between 22-kDa GH and total pituitary GH or specific isoforms such as 20-kDa GH (2, 3). Suppression of these isoforms by administration of 22-kDa GH via negative feedback regulation has been demonstrated (2, 4, 5). However, the time window of detection of this method is relatively short.

An alternate approach is based on serum GH-responsive proteins of the IGF system and markers of bone and connective tissue turnover. IGF-I is produced in response to GH, together with IGF binding protein-3 (IGFBP-3) and the acid labile subunit (ALS), which form the IGF-I ternary complex (6, 7). IGF-I mediates many of the anabolic actions of GH, including stimulation of bone and connective tissue turnover (8), resulting in increased serum concentrations of specific peptides related to collagen synthesis and degradation (9). These include the marker of bone formation, N-terminal propeptide of type I procollagen (PINP); the marker of bone resorption, C-terminal telopeptide of type I collagen (ICTP); and the marker of connective tissue synthesis, N-terminal propeptide of type III procollagen (PIIINP).

In placebo-controlled studies in recreational athletes, GH administration increased the serum concentrations of IGF-I, IGFBP-3, and ALS, as well as of PICP, ICTP, and PIIIP (10–12), indicating the potential of these GH-responsive proteins as markers for exogenous GH abuse. Many studies in the general population have reported that these GH-responsive proteins are influenced by age, gender, body mass index (BMI), and ethnicity (13, 14). However, little is known as to whether the influence of these factors is similar in elite athletes, which is important for the valid application of this approach to detect GH abuse in sport.

The aim of this study was to determine the influence of demographic factors including ethnicity and of sporting type on the serum concentrations of GH-responsive markers IGF-I, IGFBP-3, ALS, PINP, ICTP, and PIIINP. This was in-

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Abbreviations: ALS, Acid labile subunit; BMI, body mass index; CV, coefficient of variation; ICTP, C-terminal telopeptide of type I collagen; IGFBP-3, IGF binding protein-3; PINP, N-terminal propeptide of type I procollagen; PIIINP, N-terminal propeptide of type III procollagen.

vestigated in a unique sample set of over 1000 elite athletes of diverse ethnic backgrounds from a wide range of sports. The samples were collected at random with regard to competition or exercise, representing the out-of-competition setting.

Subjects and Methods

Study group

The study subjects (n = 1103) were elite athletes, defined as having competed at the state or regional level, or higher, during the previous 12 months. Athletes included were at least 14 yr of age and were required to declare by questionnaire that they had not taken GH or IGF during the previous 2 months. These samples were originally collected as part of a study to determine reference ranges for markers of altered erythropoiesis in elite athletes (15). The study was approved by the Ethics Committee of the Australian Institute of Sport and written informed consent was obtained. Samples and data provided for the study were coded and not personally identifiable.

Demographic information and information on sport and ethnic group (self-reported) was obtained. The athletes represented 10 major sporting categories and were from 12 countries, classified into four major ethnic groups (Table 1), namely Caucasian, Asian, African, and Oceanian and others, which included those who reported mixed ethnic origin and those who declined to report ethnic origin. Blood samples were collected from volunteers on a casual basis, that is at random with regard to the time of day, food intake, exercise, and competition, as previously described (15). Three venous samples on average were collected from each athlete over a 2- to 3-wk period.

Measurement of GH-responsive markers

Serum samples were stored at −80 C before analysis. IGF-I was measured by RIA after acid-ethanol extraction (16). IGFBP-3 and ALS were measured using polyclonal antibodies (17, 18). The intraassay coefficients of variation (CVs) were: IGF-I 6.1%, IGFBP-3 5.0%, and ALS 5.8%. The markers 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 in serum using competitive RIAs (Orion Diagnostica, Espoo, Finland), using the same batch and serial analyses.

Statistical analysis

To assess the contribution of demographic parameters to the between-subject variation in the GH-responsive markers, multiple linear regression was used. The specific model considered was $y = \beta_0 + \beta_1 age^{-1} + \beta_2 age^{-2} + \beta_3 sex + \beta_4 BMI + \beta_5 ethnicity + \varepsilon$, where y is a marker, β_0 is the intercept, β_i (i = 1, 2, 3, 4) are regression parameters associated with each predictor, and ε is the random error term, which is assumed to be independently normally distributed with mean 0 and a constant variance. The model parameters were estimated by the least squares method by PROC GLM in the SAS system. The usual assumptions of regression analysis (*e.g.* normal distribution, independence, and constancy of variance) were checked by residual analysis, and it was found that the assumptions for all models were satisfactory. In this analysis, all markers were first transformed by the natural logarithmic scale to stabilize the variance and ensure the normal distribution of the data. However, the final results of comparison among subgroups were based on the original unit of measurement (after back-transformation).

The sequential type I sum of squares was used to determine the relative contribution of each predictor to the variation in the marker values. This sequential type I sum of squares is a measure of the amount of variation in each marker attributable to a determinant in the model, after adjusting for the effects of preceding factors. The order of the predictors was age, sex, followed by BMI and ethnicity. As there were four ethnicities in the study, there were six possible pairwise comparisons for each marker, and type I error could significantly be inflated beyond the nominal significance level of 0.05. To control for type I error, the Tukey's Honestly Significant Difference Test was used for each comparison.

Based on the multiple linear regression analysis, reference intervals were established for each marker using the approach described by Wright and Royston (19). This approach estimates the expected value and SD for each individual based on the individual's covariates (namely age, BMI, sex, and ethnicity). The 95% and 99% reference intervals were then estimated as the expected value ± 1.96 × SD and ± 2.57 × SD, respectively. Any value outside this interval was classified as an "extreme value."

Results

Demographic characteristics of the study group

There were 1103 elite athletes (699 males and 404 females) in the study group with mean age 22.2 ± 5.2 (Table 2). Age, height, weight, and BMI were significantly higher in men. The majority of men and women were of Caucasian ethnicity (53%), followed by Asian (32%), African (10%), and Oceanian and others (5%). There were significant differences in age, height, and weight between the ethnic groups. The Asian group was younger (20.0 ± 3.9 yr, mean ± SD), shorter (169.0 ± 8.9 cm), and weighed less (62.8 ± 11.4 kg) than the other groups; whereas, the African group was older (24.5 ± 5.5 yr) and the Oceanian and others group was heavier (75.7 ± 18.0 kg) than the other groups.

TABLE 1. Ethnicity and sporting categories for athletes

		Male (n)	Female (n)
Ethnic group	Ethnic origin[a]		
Caucasian	European, North African, South West Asian, Arabian, Persian, and Indian	354	236
Asian	Chinese, Korean, and Japanese	223	126
African	Central and Southern African	82	27
Oceanian and others	Australian Aboriginal, Melanesian, and Papuan	40	15
Sporting category	Sports		
Aesthetic sports	Gymnastics, diving	11	18
Athletics	Track and field	56	27
Combat sports	Boxing, wrestling, judo, karate, fencing	150	58
Endurance sports	Cycling, marathon running, triathlon, orienteering	80	34
Power sports	Weightlifting, hammer, discus	37	14
Power/endurance	Rowing, swimming, track cycling	103	80
Racket sports	Tennis, badminton, squash	42	27
Team ball sports	Basketball, netball, soccer, hockey	202	136
Skill sports	Table tennis, shooting, archery	7	7
Multiple	Modern pentathlon, decathlon, heptathlon	7	2
Not stated		4	1

[a]Ethnic origin was self-reported. "Others" includes mixed or unreported background.

TABLE 2. Demographic characteristics and serum concentrations of IGF and collagen markers in male and female elite athletes

Characteristic/analyte	Males (n = 699)	Females (n = 404)	*P* value[a]
Age (yr)	22.6 ± 5.4	21.4 ± 4.4	<0.0001
Weight (kg)	75.0 ± 14.6	60.3 ± 8.5	<0.0001
Height (cm)	178.0 ± 9.2	167.1 ± 8.2	<0.0001
BMI (kg/m^2)	23.5 ± 3.3	21.6 ± 2.2	<0.0001
IGF-I (μg/liter)	154.8 ± 45.6	161.9 ± 52.4	0.020
IGFBP-3 (mg/liter)	3.6 ± 0.6	3.8 ± 0.6	<0.001
ALS (nmol/liter)	283.7 ± 47.9	324.7 ± 58.8	<0.001
PINP (μg/liter)	121.0 ± 90.0	85.2 ± 54.9	<0.001
ICTP (μg/liter)	6.5 ± 3.0	5.6 ± 1.6	<0.001
PIIINP (μg/liter)	5.4 ± 2.3	5.1 ± 1.5	0.003

Values are means ± SD. All data are normally distributed.
[a] Significantly different between females and males by *t* test.

Correlation between markers

There were significant correlations between the markers, both within the IGF and collagen groups and between the groups (Table 3). There was a modest correlation among the IGF markers and a stronger correlation among the collagen markers. There were significant correlations between IGF-I and each of PINP, ICTP, and PIIINP; however, there was no significant correlation between IGFBP-3 or ALS and any of the collagen markers.

Age

There was a negative correlation between age and all the IGF markers and collagen markers (Fig. 1). For IGF-I, the relationship with the reciprocal of age was best fitted by a quadratic function ($r = -0.41$, $P < 0.0001$). For both IGFBP-3 and ALS, linear relationships with the reciprocal of age were the best fit and were also significant ($P = 0.0005$). The influence of age was considerably higher for IGF-I ($r = -0.41$) than for IGFBP-3 ($r = -0.14$) and ALS ($r = -0.25$). All collagen markers were strongly correlated with age, and the relationship was best fitted by a quadratic relationship with the reciprocal of age ($P < 0.0001$). The contribution of age to the variability was greatest for ICTP ($r = -0.58$), followed by PIIINP ($r = -0.45$) and PINP ($r = -0.44$).

Gender

There were differences between women and men for all markers, with the IGF markers higher in women and the collagen markers higher in men in general (Table 2). The difference was greater for ALS (14% higher in women, $P < 0.001$) than for IGFBP-3 (6%, $P < 0.001$) and for IGF-I (5%, $P = 0.02$). Differences were considerable for PINP (42% higher in men, $P < 0.001$) and ICTP (16%, $P < 0.001$); whereas, PIIINP was moderately higher (6%, $P = 0.003$). The differences between genders were still significant after correction for differences in age.

TABLE 3. Correlations between IGF axis and collagen markers

	IGF-I	IGFBP-3	ALS	PINP	ICTP	PIIINP
IGF-I	1.00	**0.36**	**0.39**	**0.23**	**0.30**	**0.21**
IGFBP-3		1.00	**0.72**	−0.01	0.00	0.01
ALS			1.00	−0.02	0.03	0.02
PINP				1.00	**0.81**	**0.74**
ICTP					1.00	**0.79**
PIIINP						1.00

Correlation coefficients (r) are shown, with statistically significant correlation coefficients ($P < 0.05$) indicated in ***bold***.

BMI

The relationships between BMI and the GH-responsive markers were weak in general. IGFBP-3 was positively correlated with BMI; however, the correlation was weak ($r = 0.06$, $P = 0.047$) and there was no correlation between BMI and IGF-I or ALS. All collagen markers were negatively but weakly correlated with BMI (PINP, $r = -0.16$, $P < 0.001$; ICTP, $r = -0.15$, $P < 0.001$; PIIINP, $r = -0.16$, $P < 0.001$).

Ethnicity

Comparison of the mean concentrations of each marker indicated some differences between the ethnic groups (Fig. 2). In general, the unadjusted mean concentrations were lower in Africans for IGF markers and higher in Asians for collagen markers. However, there were significant differences between the ethnic groups for both age ($P < 0.001$) and BMI ($P < 0.001$), and also differences in the proportion of males in each ethnic group (Caucasian 60%, Asian 64%, African 75%, Oceanian and others 73%). The strong correlations of the markers with these variables already described could confound the influence of ethnicity; therefore, the data were adjusted for age, gender, and BMI.

After this adjustment, some of the differences between the ethnic groups were no longer significant (Fig. 2). For IGF-I, PINP, and ICTP, there were no significant differences between individual groups in the adjusted data. However, there remained significant differences between groups for IGFBP-3 and ALS, which were both higher in Caucasians and lower in Africans. After adjustment, IGFBP-3 remained higher in Caucasians than in Asians and Africans (by 8 and 18%, respectively, $P < 0.001$), and lower in Africans ($P < 0.001$), compared with each of the other groups. ALS was higher in Caucasians than in Asians and Africans (by 5 and 15%, respectively, $P < 0.001$), and lower in Africans ($P < 0.001$), compared with each of the other groups. Some significant differences also remained for PIIINP, which was higher in Asians than in Caucasians by 8.5% ($P < 0.0001$).

Multivariate analysis

The data were next analyzed by multiple linear regression, which indicated that age, gender, BMI, and ethnicity exerted independent effects on all the GH-responsive markers. Collectively, these factors accounted for 23–26% of total variation of the IGF axis markers and 37–54% of total variation of the bone turnover markers (Tables 4 and 5). Age accounted for the largest proportion of the attributable variation, accounting for 20–52% of total variation in IGF-I and the three collagen markers, equivalent to more than 80% of the attributable variation in these markers. The contribution of gender varied from 0.6–12.5%, with the greatest effect on ALS, equivalent to 48% of the attributable variation. BMI made a small contribution to the total variation of 0.02–3%, equivalent to less than 12% of the attributable variation of all the markers.

The contribution of ethnicity to the variation in IGF axis and bone turnover markers in the multiple regression analysis was

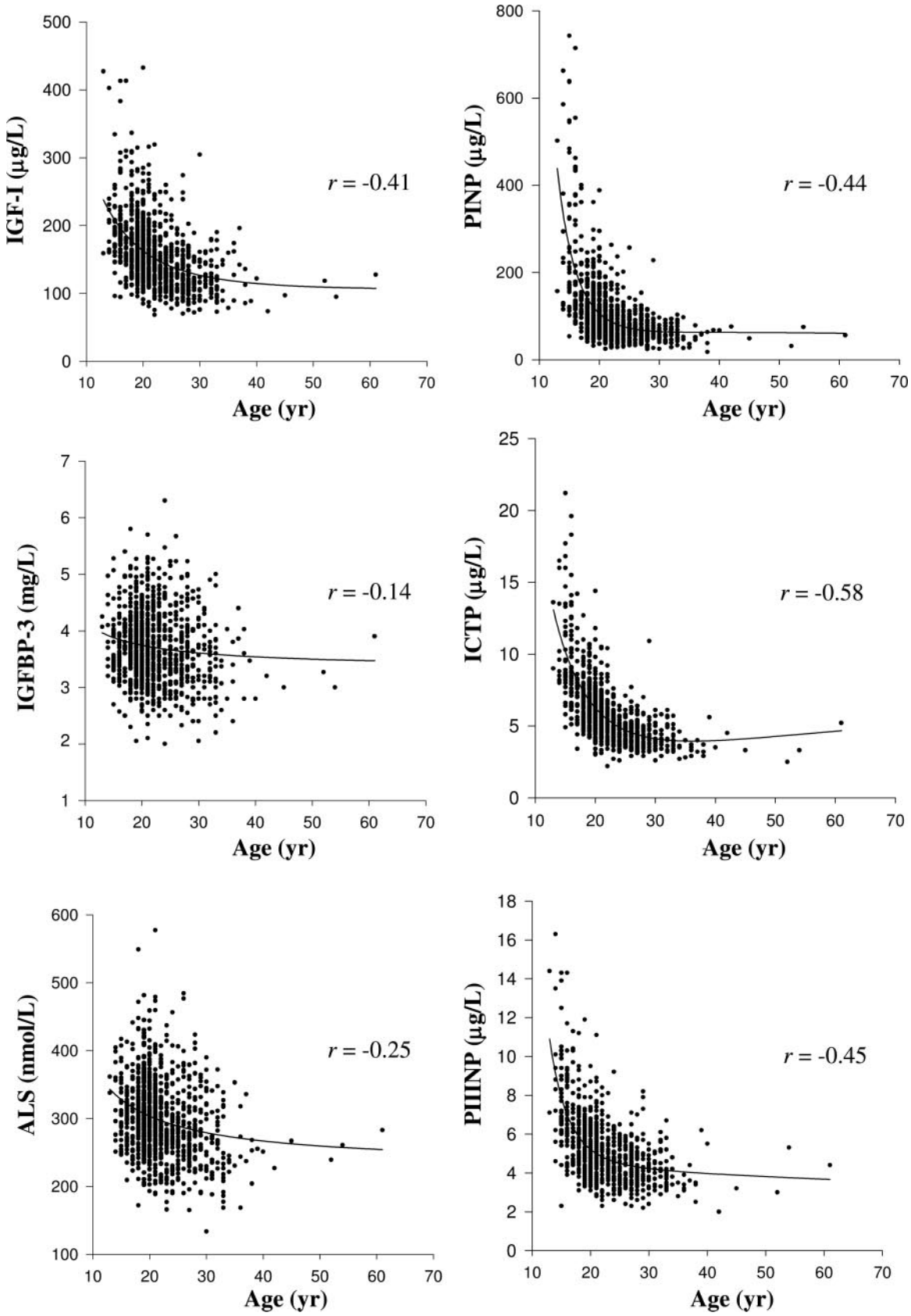

FIG. 1. Relationship between age and IGF axis and bone turnover markers. The mean measurement for each individual is plotted against age for IGF-I, IGFBP-3, and ALS (*left panels*), and PINP, ICTP, and PIIINP (*right panels*).

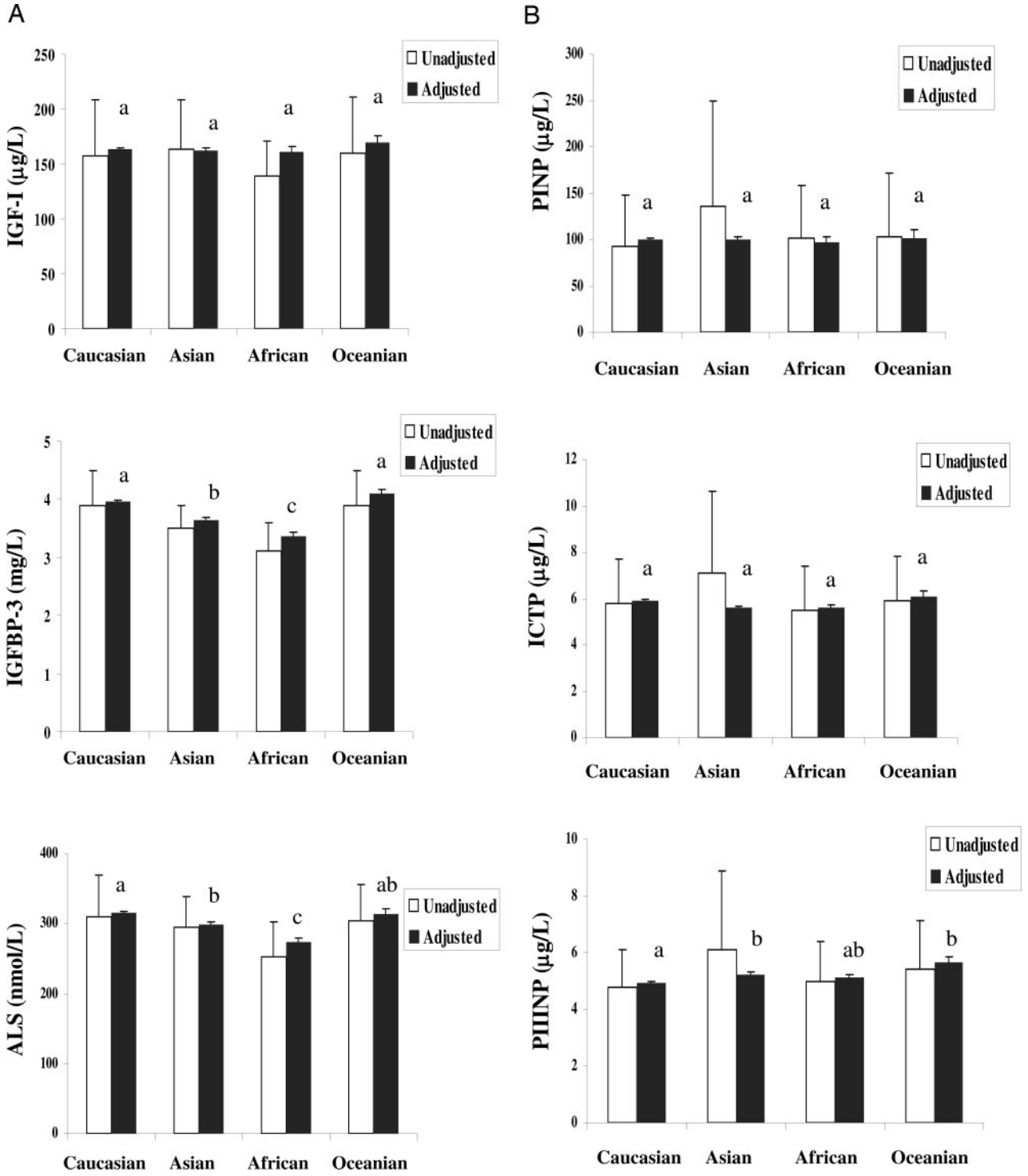

FIG. 2. IGF axis and collagen markers for different ethnic groups. The IGF axis markers and collagen markers for each of the four ethnic groups: Caucasian (n = 590), Asian (n = 349), African (n = 109), and Oceanian and others (n = 55) are shown unadjusted (mean ± SD) and adjusted for age, gender, and BMI (mean ± SE). Significant differences are shown for the adjusted data and have been adjusted for multiple comparisons. Groups with *different letters* are significantly different, $P < 0.005$.

small (≤2% of total variation, equivalent to <6% of the attributable variation), except for IGFBP-3 and ALS, where ethnicity accounted for 14.7 and 5.7% of total variation, respectively (equivalent to 65 and 22% of the attributable variation of IGFBP-3 and ALS).

All possible two-way interactions between the predictors were examined, and none were statistically significant. A sequential analysis in which the contribution of age, then gender, then BMI, and finally ethnicity are accounted for in a cumulative manner, indicated age and gender to be the major contrib-

TABLE 4. Multiple regression analysis: estimates of regression parameters from the multiple linear regression analysis

Marker	Parameter	Estimate	SE
LogIGFI	Intercept	4.007	0.153
	Sex: male	0.031	0.017
	1/Age	13.231	5.874
	$1/Age^2$	28.345	61.521
	BMI	0.014	0.003
	Ethnicity: Asian	−0.022	0.018
	Ethnicity: African	−0.036	0.028
	Ethnicity: Others	0.020	0.038
LogIGFBP-3	Intercept	0.966	0.091
	Sex: Male	0.065	0.010
	1/Age	6.530	3.481
	$1/Age^2$	−36.318	36.453
	BMI	0.006	0.0016
	Ethnicity: Asian	−0.099	0.011
	Ethnicity: African	−0.200	0.016
	Ethnicity: Others	0.016	0.022
LogALS	Intercept	4.972	0.099
	Sex: Male	0.133	0.011
	1/Age	14.738	3.795
	$1/Age^2$	−100.159	39.745
	BMI	0.010	0.002
	Ethnicity: Asian	−0.051	0.012
	Ethnicity: African	−0.162	0.018
	Ethnicity: Others	−0.010	0.025
LogPINP	Intercept	4.342	0.240
	Sex: Male	−0.414	0.027
	1/Age	−22.559	9.185
	$1/Age^2$	632.062	96.197
	BMI	−0.004	0.004
	Ethnicity: Asian	0.061	0.028
	Ethnicity: African	0.108	0.043
	Ethnicity: Others	0.028	0.059
LogICTP	Intercept	0.937	0.137
	Sex: Male	−0.134	0.015
	1/Age	6.431	5.273
	$1/Age^2$	217.418	55.225
	BMI	0.002	0.002
	Ethnicity: Asian	−0.027	0.016
	Ethnicity: African	0.003	0.025
	Ethnicity: Others	0.063	0.034
LogPIIINP	Intercept	1.393	0.144
	Sex: Male	−0.063	0.016
	1/Age	−9.545	5.521
	$1/Age^2$	273.683	57.821
	BMI	0.001	0.003
	Ethnicity: Asian	0.079	0.017
	Ethnicity: African	0.061	0.026
	Ethnicity: Others	0.142	0.036

The potential predictors considered in the multiple regression model (using GLM in SAS) were age, sex, BMI, and ethnicity. The model considered was: $y = \beta_0 + \beta_1 age^{-1} + \beta_2 age^{-2} + \beta_3 sex + \beta_4 BMI + \beta_5 ethnicity + \varepsilon$, where y is a marker, β_0 is the intercept, β_i ($i = 1, 2, 3, 4$) are regression parameters associated with each predictor, and ϵ is the random error term, which is assumed to be independently normally distributed with mean 0 and a constant variance.

utors to the between-subject variation of IGF-I, PINP, ICTP, and PIIINP. Age and gender considered cumulatively account for 91% of the attributable variation for IGF-I and for more than 80% of the attributable variation for PINP, ICTP, and PIIINP.

Reference intervals

Reference intervals were established, taking into account the influence of age, BMI, gender, and ethnicity. The values outside the 95 and 99% reference intervals were classified as extreme values for each marker and were analyzed for concordance. For 95% reference intervals, there were 163 individuals with one extreme value, 42 subjects with two extreme values, and 15 subjects with three different markers that were extreme values. For 99% reference intervals, there were 91, 19, and 9 individuals with one, two, and three extreme values, respectively. There were no individuals with more than three markers that were extreme values using the 95 or 99% intervals. For all the individuals with three extreme values using the 99% intervals, the markers were either all from the IGF system or all collagen markers, but not both.

TABLE 5. Multiple regression analysis: contributions of age, sex, BMI, and ethnicity to the variability in markers: "percent explained" in the multiple regression analysis

Factor	IGF-I	IGFBP-3	ALS	PINP	ICTP	PIIINP
Age	20.2	1.2	4.8	41.4	51.9	34.3
Sex	0.6	4.1	12.5	4.8	1.8	0.4
BMI	1.9	2.6	2.9	0.8	0.02	0.1
Ethnicity	0.4	14.7	5.7	0.3	0.2	2.0
Total	23.1	22.6	26.0	47.3	53.9	36.8

Effect of sporting type

Analysis was performed of a subset (n = 995) of seven sporting categories, namely athletics, combat, endurance, power, power/endurance, racket, and team ball sports. Esthetic, skill sports, and multiple sports, where numbers were low or from a single ethnic group, were excluded from the analysis.

The data were again adjusted for age, gender, BMI, and ethnicity to avoid the confounding effects of these variables and adjusted means compared (Fig. 3). IGF-I was significantly lower in team ball than in power or power/endurance sports (by 22 and 19%, respectively, $P < 0.005$) and also lower in combat sports than in power or power/endurance sports (by 21 and 19%, respectively, $P < 0.005$). IGFBP-3 and ALS were also significantly lower in combat sports than endurance, power, or power/endurance sports (by 11–14% for IGFBP-3 and 6–8% for ALS, $P < 0.005$). The collagen markers were all significantly higher ($P < 0.005$) in combat sports than in most of the other sporting groups, by 29–77% for PINP, by 11–40% for ICTP, and by 13–28% for PIIINP. In a multiple linear regression model with age, gender, BMI, and ethnicity for this subset of athletes, sporting type accounted for 2–5.5% of the total variation of IGF and collagen markers, equivalent to 5–19% of the attributable variation (Fig. 4).

Discussion

In this study of 1103 elite athletes from a wide range of ethnic backgrounds, age, gender, BMI, and ethnicity accounted for up to 54% of the total variability of the GH-responsive markers: IGF-I, IGFBP-3, ALS, PINP, ICTP, and PIIINP. Age, which was negatively correlated with all the markers, exerted the greatest effect for IGF-I and for all the collagen markers. The contribution of gender was smaller than that of age, except for IGFBP-3 and ALS. BMI made a minor overall contribution. After adjustment for age, gender, and BMI, the differences between ethnic groups were small apart from IGFBP-3 and ALS. Analysis of a subset of athletes (n = 995) indicated that sporting type exerted a modest influence on variability.

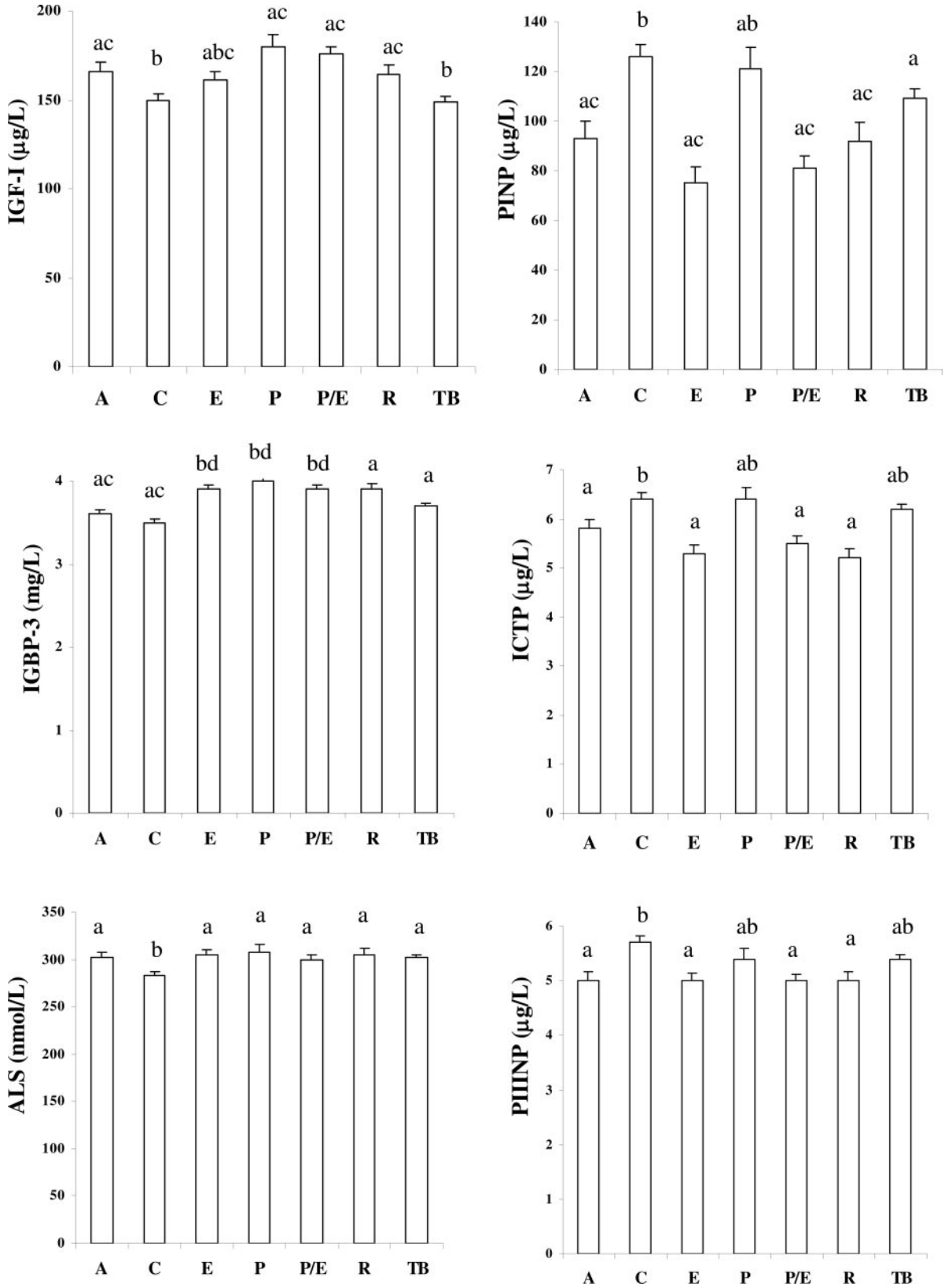

FIG. 3. IGF axis and collagen markers for different sporting types. The mean ± SE is shown for IGF-I, IGFBP-3, and ALS (*left panels*), and PINP, ICTP, and PIIINP (*right panels*) for the seven sporting groups (as defined, Table 1): athletics (A), combat (C), endurance (E), power (P), power/endurance (P/E), racket (R), and team ball (TB) sports. The data are adjusted for age, gender, BMI, and ethnicity. Significant differences are shown for the adjusted data and have been adjusted for multiple comparisons. Groups with *different letters* are significantly different, $P < 0.005$.

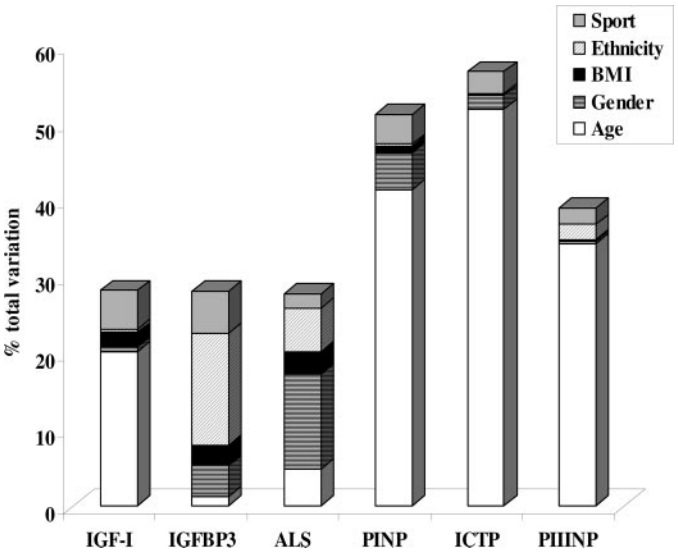

FIG. 4. Multiple regression analysis for sporting type analysis. The contribution of the age, gender, BMI, ethnicity, and sporting type to the total variation is shown for each marker, for the analysis of the 995 athletes from seven sporting groups.

This is the first comprehensive study of the effect of ethnicity on these GH-responsive markers in elite athletes. There has been only one study in elite athletes that compared a small group of 35 black athletes with matched white athletes (20). IGFBP-3 was lower in black than in white athletes, in agreement with our study; however, the authors did not observe the difference we detected in ALS between African and Caucasian elite athletes (20). In the general population, there is evidence for ethnic differences in IGF axis markers, although some is inconsistent. IGF-I and IGFBP-3 have been found to be lower in African Americans (21), whereas no difference in IGFBP-3 was shown between black and white American women (22). A comparison of normal young adult Asians and Caucasians showed no significant difference in IGF-I; however, IGFBP-3 was higher in the Caucasian subjects (23), as seen in our study. In the general population, there have been some reports of genetic effects on type I collagen markers (24, 25) and of ethnic differences in bone turnover markers (14). After adjustment for the confounding influences of age, gender, and BMI, our data in elite athletes showed a trivial effect of ethnicity except for IGFBP-3 and ALS, which were both lower in Africans and higher in Caucasians. These differences appear similar to those reported in the general population.

The highly significant influence of age on GH-responsive markers in elite athletes is similar to that observed in normal subjects. IGF-I, IGFBP-3, and ALS increase with pubertal maturation in early adolescence, then decrease thereafter in the general population (17, 18, 26). The decrease with age in the IGF axis markers in the elite athletes in this study occurred despite their high level of fitness. In the general population, bone and connective tissue turnover markers also increase in early adolescence in association with growth spurts, then decrease with age (27–30). The elite athlete cohort in this study, although encompassing a wide age range, did not include subjects sufficiently young to demonstrate such increases in early adolescence. In multiple regression analysis, age remained the major contributor to variability for IGF-I, consistent with observations in the general population (31) and for all the collagen markers; however, age exerted only a modest influence on IGFBP-3 and ALS. The dissociation of the effect of age between IGF-I and its binding proteins IGFBP-3 and ALS, has not been observed in the general population (13). This could represent a true difference between the elite athlete and normal populations; however, it could have occurred because previous studies did not account for confounders such as gender and ethnicity.

IGF-I, IGFBP-3, and ALS were all significantly higher in women, whereas PINP, ICTP, and PIIINP were higher in men after adjustment for age, and these differences were also observed in a largely Caucasian group of elite athletes (20). Multivariate analysis performed in our study showed that the contribution of gender was smaller than that of age, except for IGFBP-3 and ALS. Varying effects of gender on IGF axis markers have been reported in the general population. In general, no effect of gender on IGF-I has been observed, as reviewed (13), although a recent study of a large multiethnic cohort reported lower IGF-I concentrations in females and higher IGFBP-3 concentrations in males (32).

BMI made a minor contribution to variability of the markers in elite athletes in this study. The small effect of BMI in general has also been reported in elite athletes, with a significant effect observed for PICP in males only (20). In these studies of elite athletes, the BMI fell within a relatively narrow range; therefore, it may not have had the power to detect the inverse associations that have been shown in the general population between both IGF-I and IGFBP-3, and BMI (32). The negative correlations between the bone turnover markers and BMI in this study are consistent with previous observations of a negative trend in serum osteocalcin, another marker of bone formation (33).

In this analysis of sporting type, incorporating adjustment for the influence of age, gender, BMI, and ethnicity, IGF markers were lower in combat sports and higher in power and power/endurance sports in general. In contrast, the collagen markers were higher in combat sports, which could reflect increased bone and connective tissue turnover in response to minor injuries. The contribution of sporting type was fairly modest, compared with that of age and gender, accounting for 2–6% of total variation in this study of elite athletes in the out-of-competition testing. In another study, no major differences were found between sporting categories after correction for age in elite athletes in the postcompetition setting (20). Therefore, the results of both of these studies indicate that sporting type need not be considered a potential confounder in establishing reference ranges for these markers for a GH doping test.

The potential for using serum IGF axis markers and collagen markers in a GH doping test has been shown by GH administration studies, which have indicated that IGF-I and PIIINP, in particular, are promising serum markers for a GH doping test (10–12, 34). In this current study, the extreme values tended to cluster in either the IGF axis group or the collagen marker group but not both, suggesting that our population did not harbor individuals using exogenous GH. The analysis also indicated that no individual in this population had an extreme value outside the 99% reference interval both for IGF-I and for the collagen markers, and thus, markers from the two different groups would not be elevated in nonabusing athletes. Therefore, a test based on two markers (one from each group) will

improve the accuracy of identifying GH administration in an individual, which supports the combination of IGF-I with a collagen marker such as PIIINP previously proposed (11, 12).

The key findings of this study are the major influence of age on IGF and collagen markers, a significant influence of ethnicity only on IGFBP-3 and possibly ALS, a smaller influence of gender and sporting type, and a minimal influence of BMI. The important implication of these findings is that tests for GH doping based on IGF-I and on collagen markers must have clearly defined age-stratified reference ranges, and that ethnicity need not be considered for these markers. Gender and ethnicity are more major considerations for IGFBP-3 and ALS, whereas age has only a minor influence. These findings provide the foundation for defining robust, demographically relevant reference ranges that are essential for establishing a GH doping test.

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