R.I.G. Holt[1)], I. Erotokritou-Mulligan[1)], C.M. McHugh[1)], E.E. Bassett[2)], C. Bartlett[3)], A. Fityan[1)], J.L. Bacon[1)], D.A. Cowan[3)], P.H. Sönksen[1)]

The GH-2004 project: ethnicity does not affect the response of IGF-I and procollagen III N-terminal propeptide to the administration of exogenous growth hormone (GH) in amateur athletes

[1)] Endocrinology and Metabolism, Developmental Origins of Health and Disease Division, University of Southampton School of Medicine, Southampton, UK

[2)] School of Mathematics, Statistics and Actuarial Science, University of Kent, UK

[3)] Drug Control Centre, Department of Forensic Science and Drug Monitoring, King's College London, UK

Abstract

Context: There is widespread anecdotal evidence that growth hormone (GH) is misused by athletes for its anabolic and lipolytic properties, despite its use being prohibited by the World Anti-Doping Agency (WADA). A method based on the measurement of IGF-I and procollagen III N-terminal propeptide (P-III-NP) has been proposed to detect exogenously administered GH. As previous studies involved mainly Caucasian athletes, it is important to assess whether there are ethnic differences in the response of these markers to rhGH.

Objective: To examine the change in serum IGF-I and P-III-NP and GH-2000 score in response to rhGH in non-Caucasian amateur athletes.

Design: Double blind placebo controlled rhGH administration study.

Setting: Wellcome Trust Clinical Research Facility, Southampton General Hospital

Subjects: 31 male and 13 female amateur athletes of different ethnicities.

Intervention: Treatment with placebo or 0.1 IU/kg/day (low dose) or 0.2 IU/kg/day (high dose) rhGH for 28 days. Blood was collected at baseline, weekly during treatment and on days 35, 42, and 84 during the wash-out period. Serum IGF-I was measured by the DSL-5600 ACTIVE® IGF-I IRMA and P-III-NP was measured by a two-stage sandwich RIA (CIS

Biointernational). GH-2000 discriminant function scores were calculated.

Results Mean IGF-I & P-III-NP concentrations & GH-2000 score rose in response to both low and high dose GH in both men and women. In men, the mean GH-2000 score was significantly higher in GH-treated subjects throughout the treatment period (low & high dose GH v placebo $p<0.0001$ on day 28). The mean score remained significantly higher in the high dose GH group throughout the washout period ($p=0.01$ on day 84) & for 14 days in the low dose GH group ($p=0.002$ on day 42). In women treated with high dose GH, the GH-2000 score was significantly higher than placebo throughout the treatment period but not in the wash-out phase ($p<0.0005$ v placebo on visit day 28). When the results were compared with the previous GH-2000 study conducted in Caucasian volunteers, there was no difference in the peak IGF-I or P-III-NP between studies. There was no difference between studies in the maximal change in IGF-I, P-III-NP & GH-2000 score in response to GH in either gender.

Conclusions: There was no significant effect of ethnicity on the response to GH. The GH-2000 detection method based on IGF-I and P-III-NP would be valid in all ethnic groups.

Introduction

There is widespread anecdotal evidence that growth hormone (GH) is misused by athletes for its anabolic and lipolytic properties, despite its use being prohibited by the World Anti-Doping Agency (WADA) (Holt & Sonksen 2008; World Anti-Doping Agency 2009). The GH-2000 project proposed a test based on the measurement of the GH-sensitive markers, insulin-like growth factor-I (IGF-I) and procollagen III N-terminal propeptide (P-III-NP) (Powrie et al. 2007). Both of these markers rise in response to GH administration in a dose dependent manner (Dall et al. 2000; Longobardi et al. 2000). Although a single marker could be used, by combining markers in conjunction with gender specific equations, "discriminant functions", the sensitivity and specificity of the test to detect GH abuse can be improved compared with single-marker analysis (Powrie et al. 2007). Following the presentation of the results of the GH-2000 project at an IOC workshop in Rome in March 1999 (Sonksen 2009), it was felt that several issues needed to be addressed before the test could be fully implemented at an Olympic games. The biggest issue related to potential ethnic variation in the effects of GH. The aim of the current study was to determine whether the response to GH in other ethnic groups is similar to that seen in the white European amateur athletes in the GH-2000 study (Dall et al2000; Longobardi et al 2000).

Subjects and Methods

Subjects: 47 healthy amateur athletes (aged 18-35 yrs) of non-White European ethnic backgrounds were recruited by a poster campaign at the University of Southampton and local sports facilities. Subjects were asked to self assign themselves to one of three ethnic groups: 1) Indo-Asian (people whose ancestry was either the Indian Sub-continent or the Middle East), 2) Afro-Caribbean or 3) Chinese. All subjects were required to provide written assurance that they had never used performance enhancing drugs.

The study was approved by the Southampton and South West Hampshire Local Research Ethics committee, conducted in accordance with the ethical principles of the Declaration of Helsinki and was regulated by the Research and Development department of Southampton University Hospitals NHS Trust. All subjects gave written informed consent

The study was conducted in the Wellcome Trust Clinical Research Facility (WTCRF) at Southampton General Hospital. A medical history and physical examination were performed in all subjects and women underwent a pregnancy test. Two subjects dropped out for personal reasons. Data were therefore available for 31 men and 14 women (table 1).

Study design: For men and women separately, the subjects were randomly assigned to treatment with placebo or 0.1 IU/kg/day (low dose) or 0.2 IU/kg/day (high dose) rhGH (Saizen, Serono) in a double blind fashion. Subjects were taught to self-administer GH or placebo by daily subcutaneous injections at bedtime.

Treatment protocol and follow-up studies: The study protocol consisted of 28 days of treatment followed by 56 days of wash-out. Study visits were scheduled every week during treatment and on days 35, 42, and 84 during the wash- out period. Resting venous samples were obtained at baseline and at each visit. The samples were allowed to clot, and the serum was separated and stored at -80°C until analysis.

Analytical procedures: All samples were analysed in duplicate by the London WADA accredited laboratory. IGF-I was measured by the DSL-5600 ACTIVE® IGF-I IRMA. Intra-assay CVs were 3.4%, 3.0% and 1.5% at 9.4, 55.4 and 263.6 μg/L respectively. Inter-assay CVs were 8.2%, 1.5% and 3.7% at 10.4, 53.8, and 255.9 μg/L respectively. P-III-NP was measured

	High Dose	Low Dose	Placebo	P value
Men (n)	11	10	10	
Age	23.3 ± 0.52	22.6 ± 0.52	24.6 ± 0.52	0.42
Ethnicity				
Indo-Asian*	6	5	6	
Afro-Caribbean	4	4	3	
Chinese	1	1	1	
BMI (kg/m^2)	24.2 ± 0.58	23.4 ± 0.58	25.9 ± 0.58	0.34
IGF-I (μg/L)	545 ± 25.0	578 ± 25.0	554 ± 25.0	0.91
P-III-NP (U/ml)	0.36 ± 0.02	0.56 ± 0.02	0.40 ± 0.02	0.24
GH-2000 score	-1.7 ± 0.17	-0.9 ± 0.17	-1.2 ± 0.17	0.41
Women (n)	5	4	5	
Age	21.9 ± 0.28	22.1 ± 0.28	23.3 ± 0.28	0.43
BMI (kg/m^2)	22.4 ± 0.34	26.7 ± 0.34	23.1 ± 0.34	0.18
Ethnicity				
Indo-Asian*	1	2	1	
Afro-Caribbean	4	2	3	
Chinese	0	0	1	
IGF-I (μg/L)	457 ± 19	470 ± 19.4	555 ± 19.4	0.60
P-III-NP (U/ml)	0.34 ± 0.01	0.42 ± 0.01	0.32 ± 0.01	0.41
GH-2000 score	-1.5 ± 0.10	-1.2 ± 0.10	-1.6 ± 0.10	0.86

Table 1: Characteristics of subjects by GH allocation at baseline. BMI: body mass index. Data are mean ± SEM. *Asian subjects: 14 of the men were from the Indian sub-continent and 3 were Arabic. All of the female Asian subjects were from the Indian sub-continent

by the CIS two-stage sandwich RIA. The intra assay CV at 0.8, 1.5, 4.0 U/mL is 2.9%, 2.9% and 4.0% respectively. The inter-assay CV at 0.25, 1.5 and 5.6 U/mL is 11.3%, 7.8% and 9.3%.

Data Analysis: All analysis was performed on the log-transformed values of IGF-I and P-III-NP. The concentrations of both biomarkers and the GH-2000 scores at each visit day were assessed against the clean observation values (baseline and placebo treated samples). The analysis was carried out using a mixed effects model with subject defined as a random effect. The mixed model was defined with each time point as a within subject factor, and treatment, ethnicity and age as between subject factors. The genders were analysed and presented separately. The analysis was performed separately for each treatment arm following an adjustment for the reciprocal of age of each subject. Results are expressed as the mean ± SEM.

Adjusting for IGF-I Assay differences: In the GH-2000 studies, serum IGF-I was determined by the Nichols hydrochloric acid-ethanol extraction RIA (Dall et al 2000). As this assay is no longer available, an alternative IGF-I assay was therefore needed. In order to compare the

results of the current study with the previous GH-2000 studies, the values of serum IGF-I measured in this study were converted to the GH-2000 scales using the following formula: GH-2000 RIA = 0.660 x DSL-5600 IRMA (Erotokritou-Mulligan et al. 2008).

The GH-2000 Detection method: The previously published GH-2000 discriminant function formulae are (Powrie et al 2007):

Male score = -6.586 + 2.905 x log(P-III-NP) + 2.100 x log(IGF-I) - 101.737/ age

Female score = -8.459 + 2.454 x log(P-III-NP) + 2.195 x log(IGF-I) – 73.666/age

Comparison with GH-2000 study: The results of the current study were compared with the previous GH-2000 GH administration study, the details of which are given elsewhere (Dall et al 2000; Longobardi et al 2000).

Results

Baseline Characteristics: 10 men and 4 women received low GH dose, 11 men and 5 women received the high dose and 10 men and 5 women received placebo (Table 1). The groups were well matched for age, body mass index, baseline IGF-I and P-III-NP & GH-2000 score.

Effect of GH on IGF-I: Serum IGF-I rose in response to low and high dose GH (figure 1a). In men, mean IGF-I concentrations were significantly higher in both GH treated groups throughout the treatment period (day 28 high dose GH $p<0.0001$, low dose GH $p=0.041$ v placebo). Mean IGF-I remained higher in the high dose GH group than placebo for up to 7 days after the cessation of GH ($p=0.003$). In women, mean IGF-I concentrations were significantly higher in the high GH group throughout the treatment period compared with placebo (day 28 high dose GH $p=0.0019$). In the low dose group, mean IGF-I levels were significantly higher than placebo at the visit on day 21 ($p=0.03$) but not day 28 ($p=0.09$). Mean IGF-I concentrations did not differ from placebo in the wash-out period.

Effect of GH on P-III-NP: P-III-NP concentrations rose in response to low and high dose GH (figure 1b). In men, mean P-III-NP was significantly higher in both GH treated groups during the treatment period (day 28 high dose & low dose GH $p<0.0001$ v placebo). In the high dose GH group, mean P-III-NP remained higher than placebo for up to 56 days after the end of the cessation of GH ($p=0.01$ on day 84). In the low dose GH group, mean P-III-NP remained higher than placebo for up to 14 days after the end of the cessation of GH ($p=0.006$ on day 42). In women, mean P-III-NP concentrations were significantly higher in the high

GH group from day 14 of the treatment period compared with placebo (day 28 high dose GH p=0.0001).

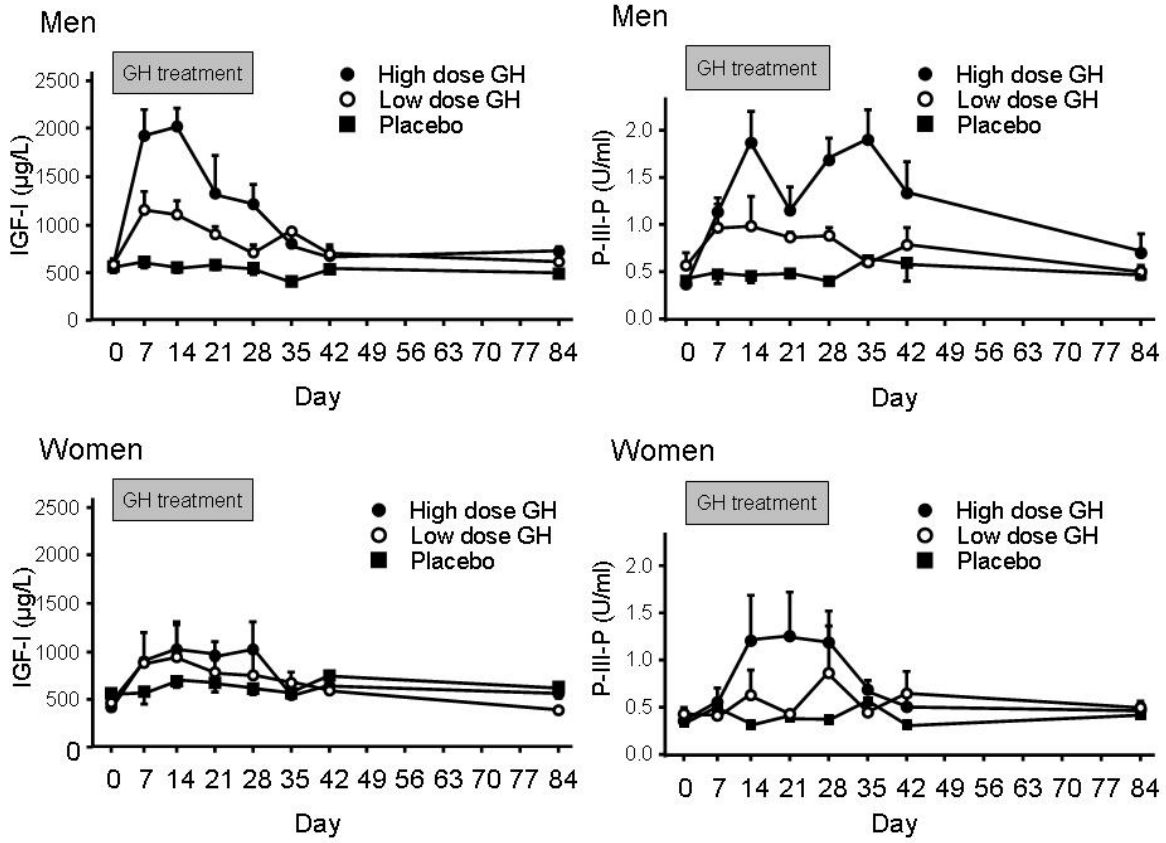

Figure 1a: Change in serum IGF-I (left) and P-III-NP (right) in response to GH 0.2 Units/kg/ day (high dose), 0.1 Units/kg/day (low dose) or placebo. Upper figure: men, Lower figure women. Data are mean ± sem.

There was no difference in P-III-NP between the high dose GH group and placebo during the wash-out period. The low dose GH group did not differ from placebo.

Effect of GH on GH-2000 score: The GH-2000 score increased in response to both doses of GH. In men, the mean GH-2000 score was significantly higher in GH-treated subjects throughout the treatment period (low and high dose GH v placebo $p<0.0001$ on visit day 28). Compared with the placebo group, the mean score remained significantly higher in the high dose GH group throughout the washout period ($p=0.01$ on day 84) and for 14 days in the low dose GH group ($p=0.002$ on day 42). In women treated with high dose GH, the mean GH-2000 score was significantly higher than placebo throughout the treatment period but not in the wash-out phase ($p<0.0005$ v placebo on visit day 28). There was no significant difference

in GH-2000 score between the low dose GH group and placebo group throughout the study.

Comparison with the GH-2000 study: The subjects in the GH-2000 study were slightly older than the GH-2004 subjects (men 25.7 ± 0.6 vs 23.5 ± 0.6 years, p=0.018; women 25.6 ± 0.6 vs 22.5 ± 0.5 years, p=0.006). In keeping with the younger age of the subjects in the GH-2004 study, the mean assay adjusted baseline and placebo treated IGF-I concentrations were higher in the GH-2004 study in the men (p=0.001) (table 2). There was no difference in the women (p=0.57). By contrast the GH-2004 baseline and placebo treated P-III-NP concentrations were lower in both men (p=0.01) and women (p=0.001), as was the GH-2000 score was lower in the GH-2004 study (men p=0.005, women p=0.004).

Post GH administration, peak IGF-I or P-III-NP did not differ between the studies (table 2). The peak post-GH GH-2000 score was lower in the men of the GH-2004 study but there was no difference in women. There was no difference between studies in the maximal change in IGF-I, P-III-NP and GH-2000 score in response (calculated as difference between peak and baseline) to GH in either men or women. There was no significant effect of ethnicity on response to GH even when both treatment groups were combined.

	IGF-I (μg/L) Placebo and Baseline	**Peak IGF-I (μg/L) Post-GH**	**Delta Maximal change in IGF-I**	**P-III-P (U/ml) Placebo and Baseline**	**Peak P-III-P (U/ml) Post-GH**	**Delta Maximal change in P-III-P**	**GH-2000 score Placebo and Baseline**	**Peak GH-2000 score Post-GH**	**Delta Maximal change in GH-2000 score**
Men									
GH-2000	297 ± 10	871 ± 40	548 ± 191	0.53 ± 0.02	1.71 ± 0.11	1.15 ± 0.52	-0.7 ± 0.1	4.9 ± 0.2	5.1 ± 1.5
GH-2004	361 ± 18	888 ± 88	518 ± 382	0.43 ± 0.04	1.49 ± 0.17	1.00 ± 0.73	-1.3 ± 0.2	3.4 ± 0.4	4.7 ± 2.2
P value	p=0.001	p=0.85	p=0.72	p=0.01	p=0.26	p=0.41	p=0.005	p=0.01	p=0.41
Women									
GH-2000	320 ± 12	647 ± 40	348 ± 181	0.50 ± 0.01	1.03 ± 0.08	0.54 ± 0.39	-0.6 ± 0.1	2.6 ± 0.3	3.4 ± 1.6
GH-2004	335 ± 23	701 ± 103	377 ± 304	0.37 ± 0.02	1.06 ± 0.29	0.73 ± 0.95	-1.4 ± 0.2	1.7 ± 0.8	3.2 ± 2.5
P value	p=0.57	p=0.64	p=0.74	p=0.001	p=0.92	p=0.40	p=0.004	p=0.29	P=0.88

Table 2: Baseline, delta and peak IGF-I, P-III-P and GH-2000 scores in the men and women who participated in the GH-2000 and GH-2004 GH administration studies

Discussion

The study has confirmed that there is a dose dependent increase in mean serum IGF-I and P-III-NP concentration, and GH-2000 score in response to GH administration in healthy amateur athletes of non-Caucasian ethnic origin. The gender differences observed in the GH-2000 study were also seen in the current study (Dall et al 2000; Longobardi et al 2000; Powrie et al 2007). Although there were baseline differences in mean IGF-I and P-III-NP between the GH-2000 and GH-2004 studies, there was no measurable difference in the peak IGF-I and P-III-NP or maximal change in these markers following GH administration. This indicates that ethnicity should not significantly affect the performance of the GH-2000 detection method.

Previous studies of non-athletic populations have shown that mean serum IGF-I tend to be slightly higher (~10%) in Caucasians (DeLellis et al. 2003; McGreevy et al. 2005), although this may reflect differences in body composition (Bagg et al. 2006). Two studies have examined IGF-I concentrations in elite athletes (Erotokritou-Mulligan et al. 2009; Nelson et al. 2006). An Australian study of 699 males and 404 female elite athletes found no significant difference in mean IGF-I between African, Asian, Oceanian and Caucasian athletes. The same study also reported that mean P-III-NP was approximately 8.5% higher in Asians compared with Caucasians but overall the contribution of ethnicity to the variation of IGF-I and P-III-NP was <2% (Nelson et al 2006). The second study, which was undertaken by the GH-2004 project, included 242 male and 62 female elite athletes from 50 different nationalities, found small differences in IGF-I and P-III-NP between athletes of different ethnicities but almost all the observations were below the upper 99% prediction limits derived from Caucasian athletes (Erotokritou-Mulligan et al 2009). In keeping with these findings, the baseline and placebo treated P-III-NP values were lower in the GH-2004 study compared with the Caucasian subjects in the GH-2000 study. By contrast the baseline and placebo treated serum IGF-I were slightly higher in the male GH-2004 subjects which may reflect their younger age (Toogood 2003). Consequently the baseline and placebo treated mean GH-2000 score was lower in the GH-2004 study. Although this may reflect the ethnic difference between studies, it may also reflect differences in the levels of training and fitness between the studies.

Despite these small baseline differences in markers, there was no measurable difference in the peak IGF-I and P-III-NP or maximal change in these markers following GH administration, indicating that people of different ethnicities respond to GH in a similar manner. This is

important for the implementation of the GH-2000 methodology as an anti-doping test. For practical reasons it is not easy to assess accurately 'ethnicity' in an anti-doping setting as many athletes have parents or grandparents from differing ethnic backgrounds.

Following GH administration, mean GH-2000 score rose significantly in both men and women, to a similar extent to that observed in the original GH-2000 study. The post-GH GH-2000 score was lower in the men in the current study, possibly reflecting the lower baseline score. It is possible that this may affect the performance of the test but in reality this study is unsuitable for any formal estimation of 'sensitivity'. We cannot estimate the sensitivity of the test in the anti-doping arena where the doses may be 5-10 fold higher than those used in this study and regimens of GH administration are unknown. Higher doses and longer periods of administration are likely to result in further increases in IGF-I and P-III-NP, particularly in women who are less sensitive to the actions of GH. Nevertheless a high proportion of men taking the higher dose of GH were detected. For the same reasons, it is not possible to measure 'window of opportunity' accurately but it is interesting to note that 56 days after discontinuing GH one man who had received high dose GH had a score >3.7.

This study has a number of limitations; it did not examine ethnic differences in response over the entire treatment and washout periods because differences in timing of sampling precluded area under the curve analysis. The small numbers in each individual ethnic group reduced the power to detect differences between the three ethnicities. Not all the individuals attended for all their scheduled follow-up visits or were fully compliant with treatment.

In conclusion, this study has shown that there is no evidence that non-Caucasian athletes respond differently to GH than white European athletes, at least in terms of the peak and maximal change in IGF-I and P-III-NP. While the study cannot exclude small differences between ethnic groups, these data indicate that ethnicity should not have a major impact on the performance of the GH-2000 detection method.

Declaration of Interest: None.

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