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The GH-IGF-2012 project: the use of growth hormone (GH)-dependent markers in the detection of GH abuse in sport: Intra-individual variation of IGF-I and P-III-P

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Introduction

Growth hormone (GH) is a naturally occurring endogenous peptide hormone produced by the pituitary gland. It has strong anabolic properties regulating body composition and is widely accepted as being a major drug of abuse in sport. Its use is banned by the International Olympic Committee (IOC) and it appears on the World Anti-Doping Agency (WADA) list of prohibited substances.

The detection of exogenously administered GH is challenging, as it is almost identical to the GH produced naturally by the pituitary gland [1]. Furthermore the pulsatile secretion of GH leads to wide variations in circulating GH concentrations, not least in the post-competition setting where exercise acts as a potent stimulus for GH secretion.

The methods for detecting the abuse of androgenic anabolic steroids and related substances measured by mass spectrometry are highly sophisticated but no such methods have been developed for testing for abuse with GH. Immuno-assays and blood sampling are required for the detection of these substances and, because they are rapidly degraded in the body, urine analyses are not an option.

Two approaches for the detection of GH doping have been developed. The first approach is based on the measurement of different GH isoforms. When rhGH is administered as a single 22K isoform, it inhibits endogenous pituitary production of the multiple GH isoforms by negative feedback regulation, resulting in suppressed concentrations of other GH isoforms and their ratio to 22K GH [2]. This method is able to detect administered rhGH within a short ‘window of opportunity’ of up to about 36 hours after the last injection but has several limitations: it will not detect any injection of pituitary-derived GH (that is readily available) and its sensitivity and specificity have not been fully assessed. Furthermore this test is unable to detect abuse with GH secretagogues.

The second approach is based on the measurement of GH-dependent protein markers, such as insulin like growth factor-I (IGF-I), IGF binding proteins and bone and soft tissue markers, such as pro-collagen type III (P-III-P) [3-5]. The administration of rhGH leads to a significant rise in these markers, the magnitude and duration of elevation of which is dependent on the dose of rhGH given, gender and the individual marker [6-9].

As these proteins occur physiologically, detection of GH abuse must rely on detecting levels in excess of those found in an established reference range. Although these markers are more stable in serum than GH and are relatively insensitive to the effects of exercise, they can vary widely among individuals, depending on age, gender, body weight, habitual physical activity, diet and androgen or oestrogen use [3,10-12]. This makes it more difficult to define cut-off levels beyond which GH abuse could be proven [3]. To address this issue and in order to improve the sensitivity and specificity of any test compared with single marker analysis, the GH-2000 team, after considering combinations of up to eight IGF binding proteins and bone markers, proposed a test based on the measurement of IGF-I and P-III-P in conjunction with specific equations, “discriminant functions”, derived from the observed changes of these markers during a double-blind placebo-controlled rhGH administration study.

Although the GH-2000 and GH-2004 projects have shown that the marker method can detect individuals taking rhGH with high sensitivity and specificity, there are a number of potential disadvantages. There is a wide inter-individual variation which could potentially mean that those with lower baseline marker concentrations are harder to catch. In practice there is no correlation between pre-GH and post-GH concentrations and there is a weak non-significant correlation between baseline marker concentration and the post GH increase. More genuine concerns are the need for age correction and the fact that women are harder to catch than men. GH secretion falls by 14% per decade after the mid-20s and there is a corresponding decline

in marker concentration. It is well recognized that women are more resistant to the action of GH, as reflected by the higher doses required to treatment women with GH deficiency.

Recently there have been discussions about the possible use of a biological “athlete passport”. As it is likely that there are smaller differences in intra-individual variation of marker concentrations, the use of a “passport” may improve the sensitivity of detection.

The aim of this study was to assess the intra-individual variation of IGF-I and P-III-P in elite and amateur athletes.

Elite Athletes

GH-2000 study

Methods

Up to 4 fasting blood samples were obtained from 175 male and 83 female elite athletes over a period of up to 1 year. The athletes were recruited at national or international sporting events. The vast majority of the athletes were white European with only 4 Afro-Caribbean and 1 Oriental subject. The mean age of the men and women was 25.9 ± 0.4 yrs and 24.7 ± 0.5 yrs respectively.

After collection of the blood sample, it was allowed to clot and then centrifuged for 15 minutes at approximately 1200g before the serum was separated and frozen at -80C prior to analysis. Serum IGF-I was analysed after hydrochloric acid-ethanol extraction by radioimmunoassay using authentic IGF-I for labeling (Nichols Institute Diagnostics, San Juan Capistrano, CA) with intra-assay CV of 10.1%, 6.3% and 5.7% at serum concentrations of 61.5, 340.8 and 776.9 ng/mL respectively. Serum P-III-P concentration was determined by a RIA (International CIS, Gif-sur Yvette, France) with intra-assay CVs of 5.7%, 9.1%, and 6.7% at serum concentrations of 0.95, 0.62 and 1.18 U/mL, respectively.

Results

The intra-subject variation of IGF-I and P-III-P is shown in figure 1.

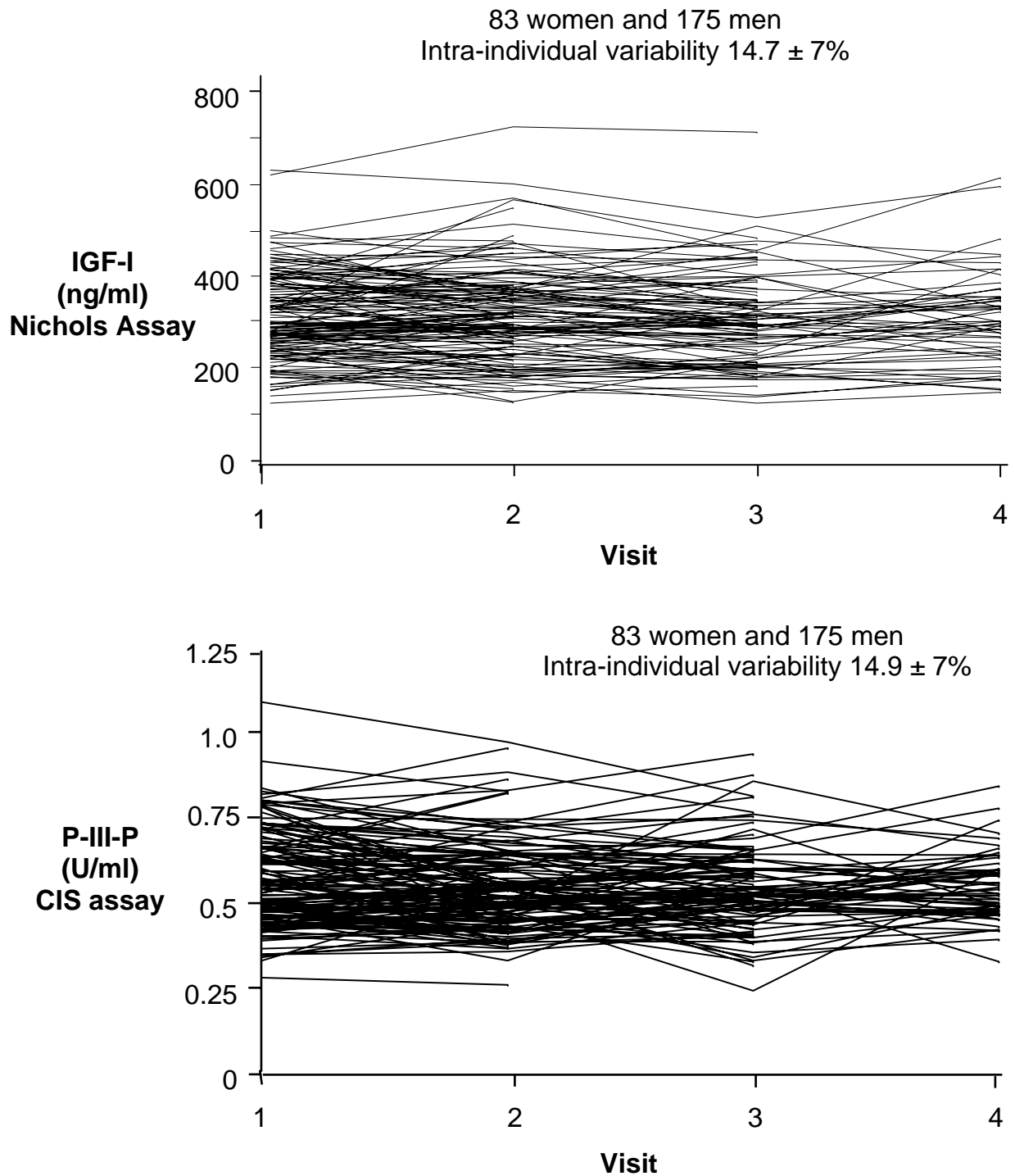


Figure 1: Intra-individual variation in IGF-I and P-III-P in 175 male and 83 female elite athletes.

Statistical analysis showed that the intra-individual variation was $14.7 \pm 7.0\%$ for IGF-I and $14.9 \pm 7.0\%$ for P-III-P. When the GH-2000 formula was applied to these data, the intra-variability of the male formula was $15 \pm 4\%$ and female formula was $25 \pm 6\%$.

Italian Elite Athletes

IGF-I and P-III-P had been previously measured in a longitudinal follow-up study of 25 male and 22 female elite Italian athletes from 9 different sporting disciplines [13,14]. The mean age of the men and women was 22.6 ± 0.2 yrs and 22.5 ± 0.2 yrs respectively. Four blood samples had been taken over a six month period. IGF-I concentrations were determined by using a commercial immunoassay kit (Mediagnost GmbH, Tübingen, Germany). The intra- and inter-assay coefficients of variation were 3.5% and 7% for IGF-I, respectively. P-III-P levels were determined using the Orion Diagnostica RIA kits (Oy, Espoo, Finland). Intra- and inter-assay coefficients of variation were 4.3% and 5.3% for P-III-P; the sensitivity was 0.2g/l for P-III-P.

Our analysis shows that the intra-individual variation in this cohort is consistent with the data from the GH-2000 study ($16 \pm 8\%$ for IGF-I and $18 \pm 9\%$ for P-III-P).

Amateur Athletes

Methods

The GH-2000 and GH-2004 projects have undertaken two double blind placebo controlled GH administration studies [8,9]. In these studies, GH was administered for 28 days and then subjects were follow-up for a further 56 days during the wash-out period. During the 3 month study, the subjects had up to 7 blood samples in the GH-2000 study and 9 samples in the GH-2004 study. The placebo treated subjects therefore provide us with an opportunity to study the intra-individual variation of IGF-I and P-III-P in amateur athletes. The GH-2000 study included 18 women (24.3 ± 0.9 years) and 21 men (26.0 ± 0.9 years) who received placebo. All subjects were white European while the GH-2004 study investigated a more ethnically diverse group. The GH-2004 study included 5 women (23.3 ± 1.01 years) and 10 men (24.7 ± 1.3 years) who received placebo.

After collection of the blood samples, they were allowed to clot and then centrifuged for 15 minutes at approximately 1200g before the serum was separated and frozen at -80C prior to analysis. In the GH-2000 study, serum IGF-I was analysed after hydrochloric acid-ethanol

extraction by radioimmunoassay using authentic IGF-I for labeling (Nichols Institute Diagnostics, San Juan Capistrano, CA) with intra-assay CV of 10.1%, 6.3% and 5.7% at serum concentrations of 61.5, 340.8 and 776.9 ng/mL respectively. In the GH-2004 study, IGF-I was measured by the DSL-5600 ACTIVE[®] IGF-I IRMA (Diagnostic Systems Laboratories, Inc., Webster, TX). The intra-assay precision of the assay was 3.4%, 3.0% and 1.5% at 9.4, 55.4 and 263.6 ng/mL respectively. The inter-assay precision of the assay was 8.2%, 1.5% and 3.7% at 0.9, 0.8 and 9.6 ng/mL respectively. Serum P-III-P concentration for both studies was determined by a commercial RIA (International CIS, Gif-sur Yvette, France) with intra-assay CVs of 5.7%, 9.1%, and 6.7% at serum concentrations of 0.95, 0.62 and 1.18 U/mL, respectively.

Results

The intra-individual variation was $13.9 \pm 7.0\%$ and $14.0 \pm 7.0\%$ for IGF-I in the GH-2000 and GH-2004 studies respectively. The intra-individual variation was $12 \pm 6\%$ and $19 \pm 9\%$ for P-III-P in the GH-2000 and GH-2004 studies respectively. When the GH-2000 formula was applied to the GH-2000 data, the intra-variability of the male formula was $12 \pm 2\%$ and female formula was $25 \pm 3\%$.

Discussion

The results from these four studies show remarkably consistent results, with no apparent difference between amateur and elite athletes. The intra-individual variability for IGF-I varies between 13.9 – 16% while the variability for P-III-P varies from 12 – 19%. These data suggest that the sensitivity of a test for GH based on markers might be improved by the concept of an athlete “passport” or “profiling”.

The GH-2000 project proposed a test based on the two GH-dependent markers, insulin-like growth factor-I (IGF-I) and type 3 pro-collagen (P-III-P) [8,9] in conjunction with discriminant function analysis [15]. IGF-I and P-III-P are ideal candidate markers because they exhibit little diurnal or day-to-day variation and are largely unaffected by exercise or gender [1]. For example in one study, subjects underwent exercise tests before, during and after GH administration. IGF-I (20%) and P-III-P (10.2%) rose following exercise but this rise was small in comparison with the larger 300% increase in the markers with GH [6,7].

Although this test provides good sensitivity and specificity to detect those abusing GH, there are some limitations. Like many endocrine tests, there is a trade-off between sensitivity and specificity. The usual laboratory ranges of mean plus or minus two standard deviations are not applicable in doping analysis because 5% of individuals would lie outside this range by definition and so the risk of a false positive is unacceptably high. Although the specificity of the test has not been determined, it is thought that a false positive risk of 1 in 10000 is likely to be acceptable. At this level of specificity, the GH-2000 formula will correctly identify 79% of men receiving GH but only 36% of women. The sensitivity improves with dose of GH in women and deteriorates in both sexes after the discontinuation of GH [15].

The major challenge for the marker approach is to ensure harmonisation between the different assays used to measure P-III-P and IGF-I. Although there are no established methodologies to adjust measured P-III-P and IGF-I from one assay to another, this problem is not insoluble as a similar problem has arisen for many assays including glycosylated haemoglobin [16]. The establishment of international reference preparation and quality control schemes has led to harmonisation of assays within the clinical arena. As there is no international reference preparation for P-III-P, an alternative is to use adjustment factors based on the values measured for normal individuals by different assays.

A further disadvantage of the marker approach is that the formula needs to include an adjustment for age because both GH secretion and markers fall with age after the 2nd decade of life. Although there are good data about the effect of age on GH markers in elite athletes [10,17], the need for the correction could potentially reduce the sensitivity because this assumes an average rate of fall in markers which may vary from one individual to another.

Recently there have been discussions about the possible use of a biological “athlete passport”. As it is likely that there are smaller differences in intra-individual variation of marker concentrations than inter-individual variation, the knowledge of previous measures of IGF-I and P-III-P may improve the sensitivity of detection. Furthermore there would be no need for an age correction or assay adjustment if the subsequent samples were taken with 1 year of the first sample and measured by the same assay.

This study has shown that the intra-individual variation of IGF-I and P-III-P in elite and amateur athletes is small and not much greater than assay variability. These data raise the possibility that an athlete passport or profiling might improve the sensitivity of the GH-2000

test but further work, for example examining the variables that affect the intra-individual variation of IGF-I and P-III-P, is needed.

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