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Growth Hormone distributions from a cohort of track and field Athletes

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Abstract

During the 2011 Athletic World Championship held in Daegu, our laboratory was deeply involved to provide blood passport analyses and to collect serum for Growth Hormone (GH) analyses. While blood passport results have been published elsewhere [1], the distribution of GH-related concentrations (REC and PIT, IGF-1 and P-III-NP) has not yet been presented. Our aim was to search for parameters influencing the two mainstream tests that can detect GH abuse - the GH Ratio (isoforms quantification) and the GH Score (secondary markers of GH abuse) - for a future implementation of a passport targeting GH-axis manipulation. Most pre-analytical and individual factors that could influence GH markers have been studied and published, but some doubts still persists about the influence of some of them (like ethnicity, type of sport or collection time) on the GH Ratio. Based on more than 1800 athletes, we present GH ratio, GH Score and their markers distribution depending on numerous factors, including time of collection, gender and type of sports. With as big a cohort as ours, statistics can point out significant differences that are not relevant from a technical perspective. Based on our results, the GH ratio is dependent only on Gender, while the type of sport seems to slightly influence the GH Score together with Gender and Age. The type of sport influence is difficult to ascertain as the absolute difference is close to the test uncertainty.

Introduction

While GH Ratio is used in most anti-doping laboratories, GH Score is still in implementation. For GH Ratio, recent Court of Arbitration in Sports (CAS) awards indicated a relative confidence in the DLs, but the claims by one of the athletes about the collection time, recent strenuous effort and type of sport are still not completely closed. For the GH Score, despite the publication of DLs [2] and two cases of GH abuse found, GH Score did not pass in front of the CAS. GH score is supported by a plethora of publications documenting a lack of influence form most of the known parameters, including ethnicity [3] or freeze-thaw cycle [4]. Use of GH administration studies, like the famous ones from the GH-2000 and GH-2004 studies or the 2011 study [5] give the capability to discriminate between doped and non-doped state in the volunteers. But these tools must be tested on a pool of athletes. Such athletes' population-based studies also give us the opportunity to improve our knowledge of the analytical methods and of the issues related to quantification of proteins [6]. They are also among the few possibilities to detect minority populations that must be treated in a different way [7]. The aim of this study is to search for the parameters that have an influence on the tools used by anti-doping community to detect GH abuse.

Experimental

A total of 1855 athletes were summoned for anti-doping purposes. Together with the collected samples, some individual data were also recorded: Gender, Age (in years), Type of sport (non-endurance or endurance; limit set at 3 minutes meaning 3000m is endurance), time of collection, if the athlete performed strenuous exercise less than 2 hours before collection and if the athlete spent time at more than 1000m of altitude within the last month. On the 1855 serum samples collected, 30 have been discarded because of incomplete information on Gender. Finally, we got a total of 858 women (Age range 16-47 (mean=25.9), 678 non-endurance) and 967 men (Age range 17-42 (mean=26.0), 741 non-endurance). On-site, samples were first centrifuged, and then stored at least 2 hours at 4°C, before a transfer at -20°C following provider instructions. Insulin-like growth factor I (IGF-1) was measured on Immulite 2000 (Siemens Healthcare Diagnostics SA, Switzerland), P-III-NP was measured on radioactive manual test (CisBio Bioassays, Codolet, France) and GH isoforms were tested first on

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Immulite 2000 to select only samples with GH concentration higher than 0.1 ng/mL (See results and discussions). Only these samples have been tested using one of the two GH isoform immunoassays (CMZ assay GmbH, Berlin, Germany). The choice of the assay was totally random. Data were pooled from the different sources in an Excel 2010 (Microsoft) file. Plots are made with Excel. Before any statistical analyses, all values lower than the test limit of detection were excluded. SPSS 20 (IBM) software was used to compare populations. For statistical comparison, results were split based on the Athlete-related factors. A limit was set to exclude any population smaller than 30 athletes to avoid any possibilities of recognition and to obtain meaningful results. The Bartlett's test for equal variance was then applied to determine if the population variances were significantly different or not. A second test was made; the non-parametric Kruskal-Wallis equality-of-population rank test. P-values calculated for both test must be lower than 0.05 for us to consider the differences between population as significant.

Results and Discussion

No statistical test indicated a significant differences between men and women in terms of age and type of sport, but endurance sports tends to be made by older athletes than non-endurance ones as shown in Figure 1. On the 858 women samples, 114 (13.3%) were not-analyzed after our pre-assay as the GH concentration was too low to produce significant ratio. On the 339 samples analyzed on Kit1, 14 (4.1%) were not-valid (REC<0.1 ng/mL) and on the 405 samples analyzed on Kit 2, 26 were not valid (6.4%). For the 967 men samples, 567 (58.6%) were removed from our workflow. 160 samples were analyzed on Kit 1, with 8 not valid (5%) and 240 were analyzed on Kit 2 with 33 not valid (13.7%). This pre-assay strategy made by measuring GH concentration on the Immulite, despite not being described on the Guidelines, is something to keep in mind to reduce the costs for such big events. But this pre-assay has to be made in such a way that all samples with REC higher than 0.1 ng/mL are analyzed. The limit we choose (0.11 ng/mL) was high enough as we still got non-valid samples with the GH Ratio tests despite the removal of some samples.

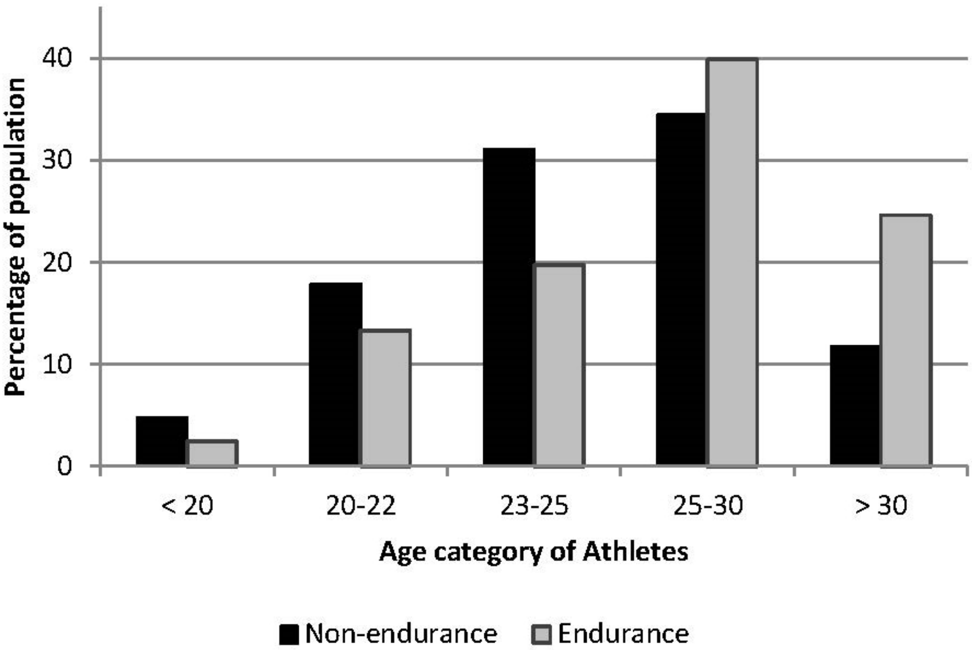

Figure 1: Age distribution of the endurance (grey) vs. non-endurance (black) population. The ages were not used as continuous parameters as the population for the high and low extremes would have been too small and not significant.

In Figure 2, the cumulative distributions of frequencies (cdf) of the ratios indicate that gender has an impact on ratios, this impact being greater for Kit 2 than Kit 1. We then separated the ratios by type of sport (endurance vs. non-endurance) and Figure 3 shows the results for the Men in Kit 1 and for the women in Kit 2. The apparent differences for men in Kit 1 are non-significant (they are graphic artefacts linked to the relatively small numbers of linked samples). Thus, the type of sport has no influence on the GH ratio determination. None of the tested parameters have any significant influence on the GH ratio, except the Gender. Time of collection, type of sport, altitude training, intense exercise, none impact the GH ratio. The variation of GH Ratio induced by Gender is soon taken into account as DLs are Gender-specific.

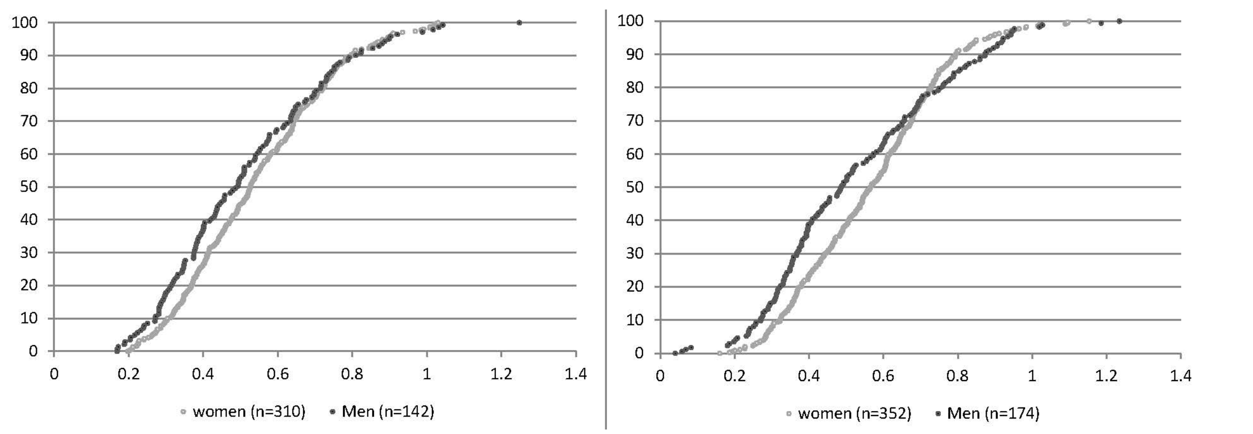

Figure 2: Cumulative distribution of frequencies (cdf) for the Ratios separated by Gender. On the left are the ratio distributions from Kit 1; on the right the distributions from the Kit 2. Men are represented by black dots while women are light grey circles.

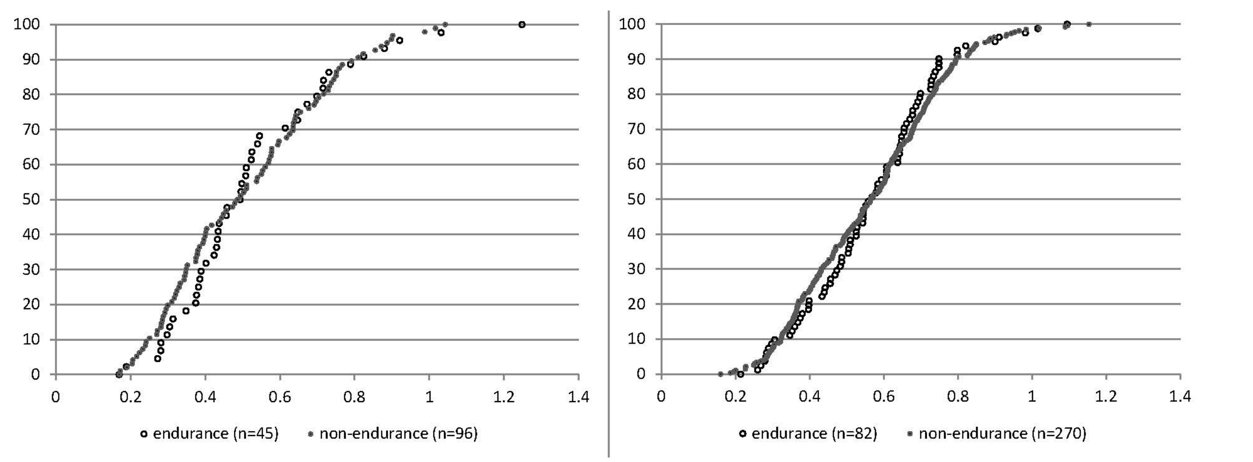

Figure 3: Cumulative distribution of frequencies (cdf) for the Ratios separated by the type of sport. Endurance sport is represented by empty circles while non-endurance sports are filled circles. Only the cdf for men measured in Kit 1 (left) and for women measured in Kit 2 (right) are given for clarity.

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Figure 4 shows that for the GH Score, the Gender has a serious impact, mostly on the IGF-1. Figure 5 present the cdf for the GH Score separated by Age categories. Differences between the lines are seen and globally, there is a significant impact of the Age on the Score. These two influences were predicted as these two parameters are included in the formulae and in the DLs.

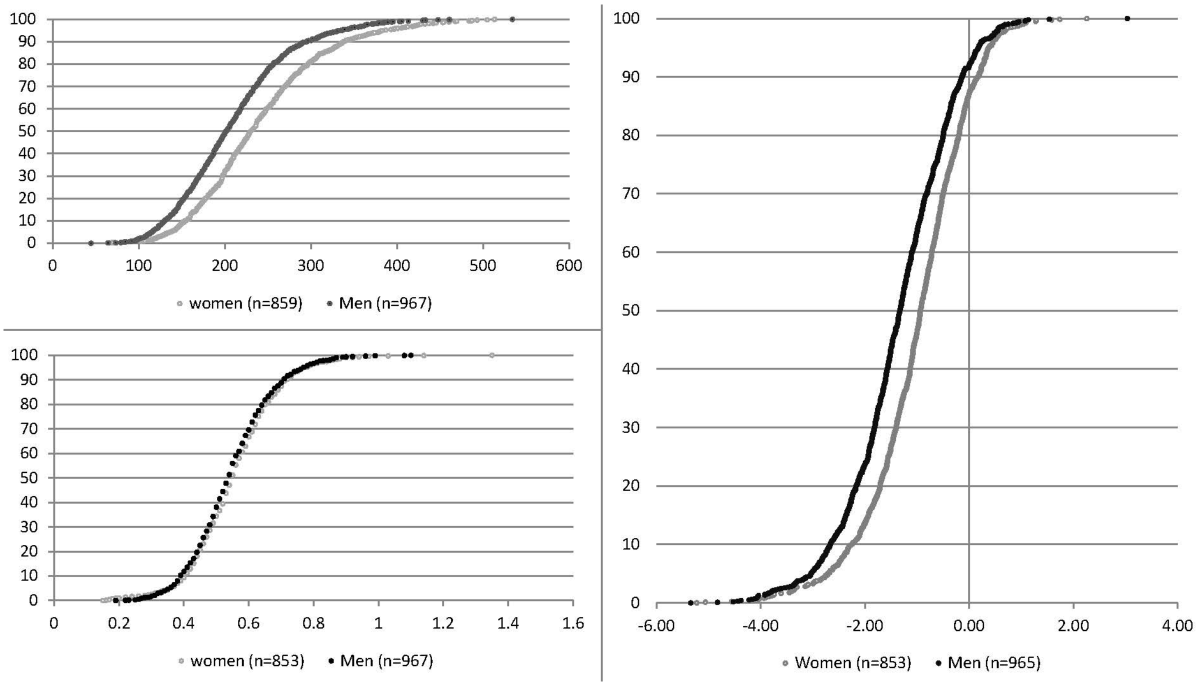

Figure 4: Cumulative distribution of frequencies (cdf) for the GH Score parameters. Igf-1 (top left) and P-III-NP (bottom left) distributions are shown with the Score (right) distributions. Men are represented by black dots while women are light grey circles.

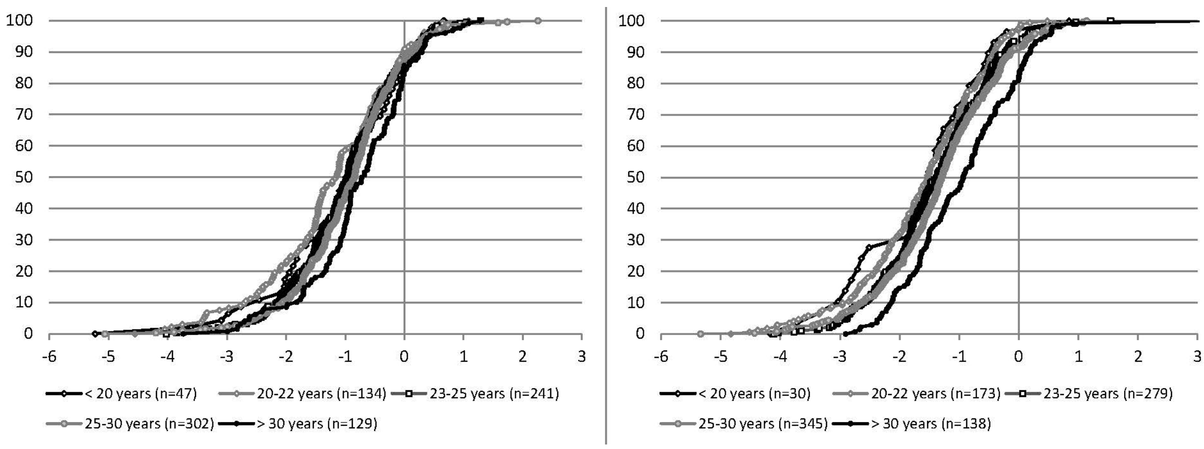

Figure 5: Cumulative distribution of frequencies (cdf) for the GH Score separated by age categories. The age categories are: less than 20 years old, between 20 and 22 years old, between 23 and 25 years, between 25 and 30 years and older than 30 years. The GH Score distributions for the women are on the left, for the men on the right.

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Figure 6 presents surprising results, the type of sport also has an influence on the GH Score. The difference is significant and higher than the method uncertainty (median diff = 0.25, Uc = 0.15 for women, median diff = 0.34, Uc = 0.22 for men). Similar differences between type of sports were soon observed [8], but the impact on the DLs is not clear. As shown in Figure 1, the age distribution is not the same for the Endurance sports than for the non-endurance sports. Older athletes tending to have higher Score, the endurance scores should be higher than the ones from non-endurance. The Figure 6 indicates the reverse situation. Behind endurance and non-endurance are also injuries, altitude training, national preferences, physiology and other parameters which could impact the GH Score. But this impact would be very small, as the numerous studies soon published by the GH-2000 team did not perceived such influences in any of their results. In any case, the fact that the type of sport has an impact on the GH Score means that there would be a widening of the global population distribution. Therefore, there is no risk of false positive as the DL will only be over-estimated due to these potentials variations of GH Score due to type of sport. Individual variation of the Score has been published [9] to be smaller than the population variation, meaning that a passport approach would be beneficial for the detection of GH-axis manipulation using the GH Score.

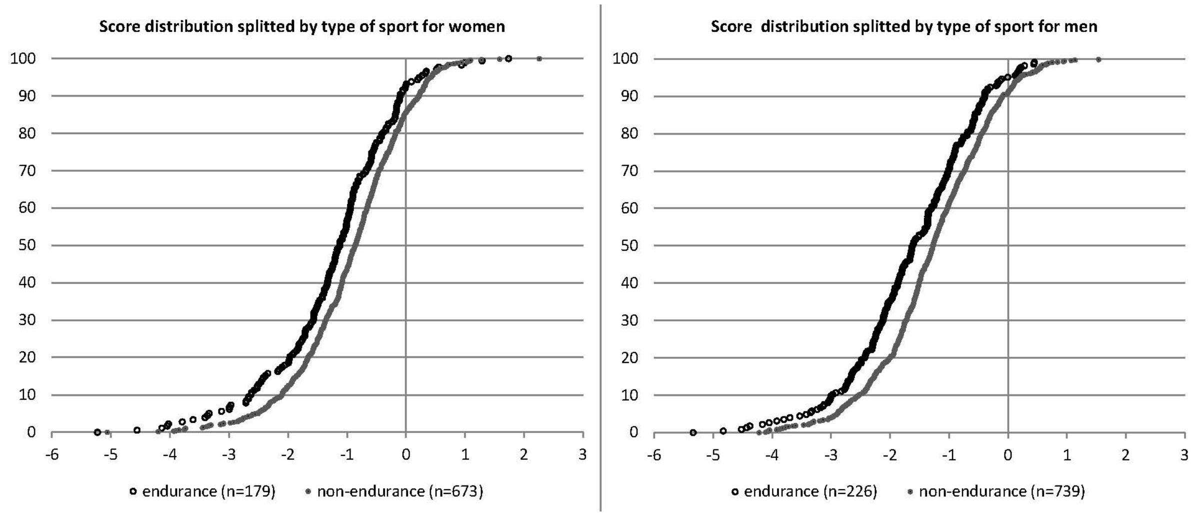

Figure 6: Cumulative distribution of frequencies (cdf) for the GH Score separated by Type of discipline. Disciplines whose effort duration was below 3 minutes were considered as non-endurance. The endurance Scores are given by empty circles while the non-endurances are given by filled circles. The distributions on the left are from female athletes and on the right from male athletes.

Conclusions

The GH Ratios are very robust; only the Gender had a small influence on the Ratios distributions and this was clearer on the Kit 2 than on the Kit 1. The Score is influenced by Gender and Age, factors that have been included in the Formula used to calculate the Score [2]. We observed that the type of sport also induced a Score distribution change, but it can be related to other factors. There are known differences between the endurance or non-endurance athletes that could explain the distribution shift. To reach the maximal efficiency for GH abuse detection, we propose to measure both Ratio and Score with robust methodologies and to include them in a longitudinal follow-up.

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