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Effect of plasma volume on the marker approach to detect Growth Hormone doping

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Abstract

Growth Hormone (GH) detection can be made by two distinct and complementary approaches: Differential immunoassays (GH Ratio) and quantification of proteins induced by GH (GH Score). While the GH Ratio has been used for years by anti-doping laboratories, GH Score was routinely used only during the London 2012 Olympic Games with successful results. The GH Score is calculated from 3 parameters: Athlete's age, IGF-1 concentration and P-III-NP concentration. Both concentrations are linked to plasma volume (PV). To establish the effect of PV, a study was established to mimic a cycling stage-race, forcing significant PV changes. The study demonstrated a strong correlation of Score with Plasma volume. Within a longitudinal approach, this correlation could hinder variation coming from doping origins. We were not able to calculate the variation related to the plasma volume neither were we able to compare this variation with the other variation observed during our study. Finally, even if the plasma volume is correlated with the GH Score, the GH Score variance has predominantly other origins.

Introduction

GH detection for the fight against doping is currently made thanks to the GH Ratio test [1]. The future GH Score is based on concentrations of IGF-1 and P-III-NP without including the plasma volume. Numerous published works were made to circumvent the factors that can influence the Score (see [2] and references cited therein) like physiology, ethnicity, injuries or sport type [3-6]. But no study was made to collect samples every mornings and every evenings during a stage-race of 9%nbspdays. During this study, we observed - and published [7] - that GH Score values were higher in the morning than in the evening - regardless of the type of exercise made during the day. As this could induce variations during a long-term follow-up that could wrongly be attributed to abnormal behavior, we used data from [7] to calculate if the variations induced by the plasma volume change are important compared to other variations we observed during this study.

Experimental

Detailed study protocol and laboratory tools have been published in [7]. Briefly, 15 healthy male Caucasian cyclists or triathletes were selected. Among the selection’s criteria was the request to train like an elite athlete, for example, riding at least 20,000 km per year. University of Freiburg’s (Germany) ethical committee accepted the study. This study consisted on a stage-race of 9 days in Doha (with financial gain to motivate the volunteers), including some recovery days before, during and after the race, like in real races. Samples were collected every day at 8:00 am (before breakfast) and at 6:00 pm (at least 2 hours after the exercise). Plasma volume (PV) was first measured by a CO re-breathing method before the start of the study, and then it was calculated using hematological parameters during the study. This gave us the possibility to obtain a normalization of all concentrations to a baseline PV. IGF-1 was measured with the Immulite 2000 (Siemens) and P-III-NP by manual RIA (CisBio assays). Analyses, plots and statistics were performed using Excel 2010 (Microsoft).

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Results and Discussion

We published that the Score is related to the hydration state of our volunteers [4], which is consistent with the use of concentration in the Score formulae. Thanks to the knowledge of PV, we can normalize concentrations to a baseline PV for each volunteer. The change of Score after correction is correlated (at 99.37%) with the PV change as shown on Figure 1. Figure 2 shows on the left the non-corrected GH Scores and on the right the corrected Scores for all volunteers. For each volunteer, we calculated the mean and the variance of its 29 Scores measured during the study with or without PV correction. The variance of the means gives the inter-individual variance while the mean of all variances gives an estimate of the intra-individual variance. The intra-individual variance is modified by the PV correction (0.163 without correction vs. 0.118 with) while the inter-individual variance is not significantly modified (0.298 without correction vs. 0.311 with). The impact of PV was predicted to impact mostly intra-individual values and this impact can be estimated as equivalent to a variance of 0.045. The repartition of the origin of these variances is shown on Figure 3. Approximately 2/3 of the variance is from inter-individual origin, which means that an individual follow-up would lead to a better sensitivity to detect GH-axis manipulation than a DL based on a population. This also implies that the DL based on population is very conservative for most individuals. Figure 4 shows the follow-up for 3 representative volunteers during the whole study. The curve obtained from the GH Score (filled circles) is very close to the curve obtained with the PV-corrected Scores (empty circles). It must be noted that analyses were made with only one batch of reagents, reducing technical variability.

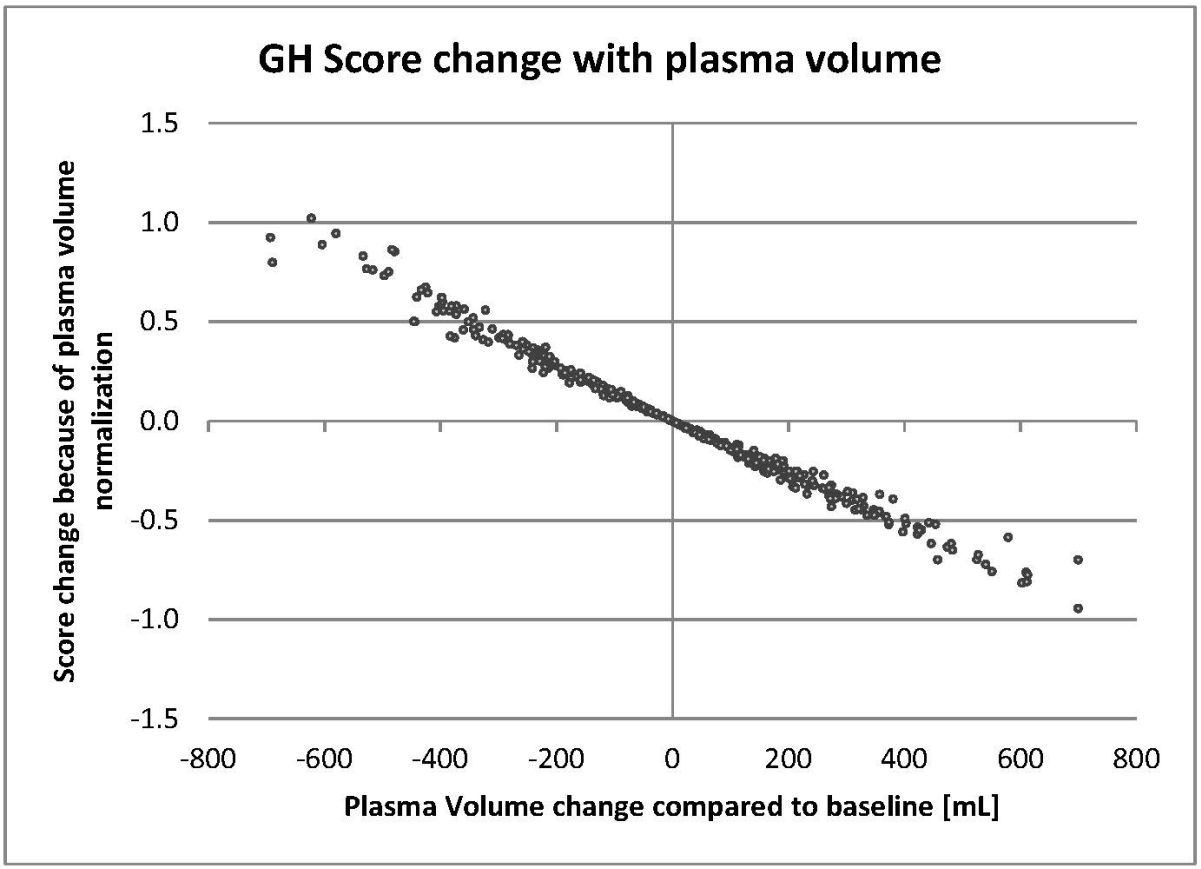

Figure 1: Changes of plasma volume compared to the baseline is strongly correlated to the Score variation after the plasma volume correction.

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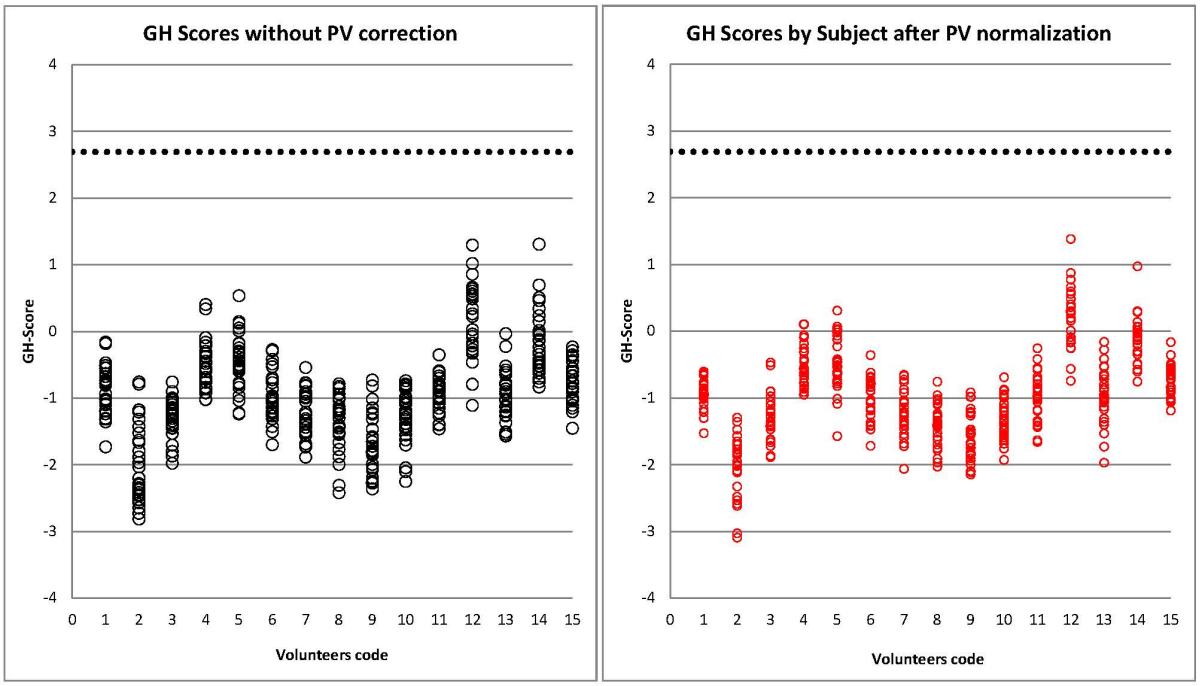

Figure 2: Correction of all Scores from each volunteers do not erase variations. The inter-individual variation are clear and the intra-variation is still significant.

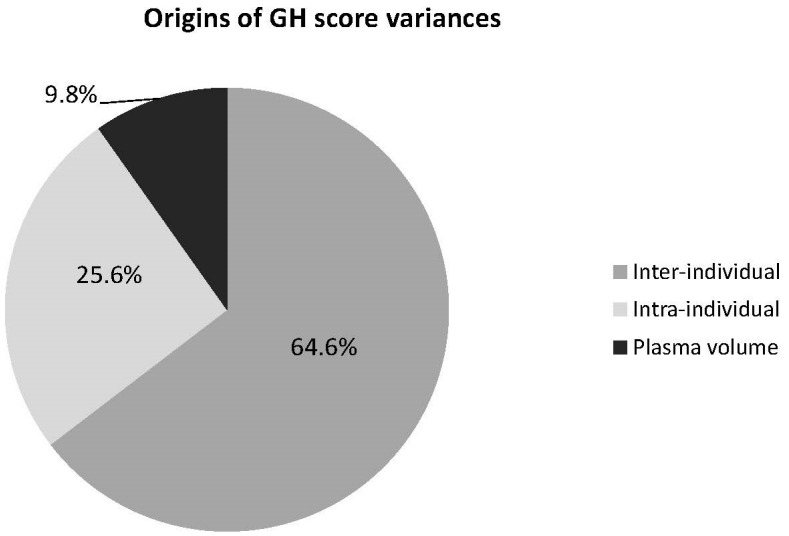

Figure 3: Sources of variance, in grey, the inter-individual variance. In light grey and black, the intra-individual variance, the black part is related to the plasma volume.

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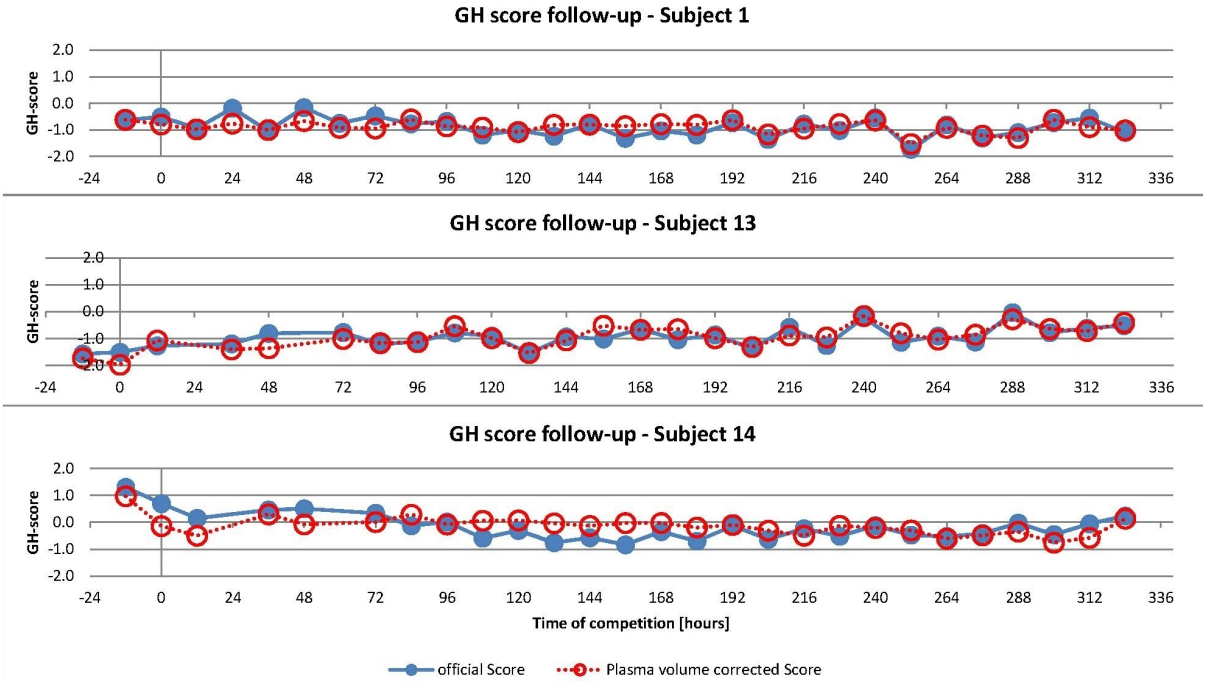

Figure 4: Representative follow-up of athletes during this study. Horizontal axis is the time after the start of the stage-race. Morning samples are at 0h, 24h, 48h and so forth. The in-between points are for evening samples. Filled circles represent uncorrected GH Score as published by GH-2000 team and supported by WADA. Empty circles are corrected score to remove any effect of Plasma volume variations.

Conclusions

PV-related variations are only a small part of the variations included in the data used to determine the DL for the GH Score. A PV correction is not required for anti-doping analyses. For passport, PV counts for less than 1/3 of the total variations within an individual Score follow-up. Illnesses, technical variations of these manual analyses will be the preeminent sources of variations. Physiology (not only the PV) and adaptation to the sport discipline could have some impacts on a GH Score passport but probably a minor one and the answer is beyond the scope of this study. For a future GH passport, both GH Ratio and Score could be included to increase the sensitivity and the deterrent effect.

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