Research article

Drug Testing and Analysis

Received: 22 June 2011 Revised: 16 August 2011 Accepted: 17 August 2011 Published online in Wiley Online Library: 6 December 2011

(wileyonlinelibrary.com) DOI 10.1002/dta.359

Detection of doping with rhGH: Excretion study with WADA-approved kits

Jing Jing,[a] Sheng Yang,[a] Xinmiao Zhou,[a,b†] Chunji He,[a] Lisi Zhang,[a] Youxuan Xu,[a] Minhao Xie,[b] Yi Yan,[b] Hao Su[b] and Moutian Wu[a]*

The detection of recombinant human growth hormone (rhGH) doping using the World Anti-Doping Agency (WADA) approved kits is reported in this research. Twenty-five young male students were selected and divided randomly into two groups with six belonging to the placebo and nineteen to the administration group. Thirteen volunteers in one group were administered with a Chinese preparation of rhGH while six volunteers included in the other group were given rhGH made in Switzerland. Both preparations were administered at a dose of 0.1 IU/kg body weight, one injection per day for 14 consecutive days. Blood samples were collected using WADA guidelines and all blood samples were analyzed with WADA-approved Kits 1 and 2.

The time window for detection of rhGH doping using WADA-approved kits and criteria are discussed. Based on the comparison of the data obtained from this excretion study and from our routine (Chinese population as reference), consideration of the recent WADA criteria for rhGH AAF (Analytical Adverse Findings) is reported statistically. A comparison of data obtained from the two sample groups administered with pharmaceutical preparations, one Chinese rhGH (GenHeal®, S19990019, 1.6 mg (4 IU), Shanghai, China) obtained from prokaryotic cells and the other (Saizen®, S20080036, 1.33 mg (4 IU), Laboratoires Serone S.A., Switzerland) from eukaryotic cells is reported and did not show any significant difference for the detection of doping with rhGH. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: anti-doping analysis; growth hormone; excretion study; differential immunoassay; isomers

Introduction

Detecting the use of recombinant human growth hormone (rhGH) in performance-enhancing doping is one of the challenges for anti-doping analysis. Reviews by Holt,[1] He,[2] Segura,[3] and Bidlingmaier[4] have summarized the most recent advances in the detection of rhGH doping. Peer reviewed publications have reported new methods for anti-doping analysis focusing on nanopartical technology,[5] mass spectrometry-based peptide analysis, their metabolites in urine[6] and blood[7] plus gene expression profiling.[8,9]

Presently there are two different methodologies for the detection of rhGH: (1) the isoform approach (22 KDa GH and non-22 KDa hGH)[10] and (2) the biomarkers approach (IGF-1 and P-III-P). The isoform approach was approved by the World Anti-Doping Agency (WADA) and has been implemented in the Olympic Games since Athens 2004. While the biomarkers approach was initiated by the GH 2000 project[11] and subsequently surpassed by the GH 2004 project,[12] it has not been officially approved by WADA. The biomarkers approach benefits from a more complex validation criterion with different considerations, such as injury,[13] gender, and age of different teams.[14,15]

WADA published the *Guideline of hGH Isoform Differential Immunoassays for Anti-Doping Analyses (version 1.0)* in June 2010[16] and *Guidelines for Blood Sample Collection (version 2.2)* in August 2010.[17] These publications report the detailed technical criterion for the detecting rhGH doping. Guidance includes 'direction on the sample pre-analytical preparation procedure, the performance of the test(s), and the interpretation of the test results'.

The isoform differential immunoassay is essentially based on the molecular nature of GH and GH variants (isoforms), which are present at constant relative proportions. In contrast, rhGH is only consists of the 22-KDa isoform. The administration of rhGH leads to an increase in the concentration of the 22-KDa isoform and a decrease of the non-22-kDa isoforms, thus the natural ratios established between these hGH isoforms can be changed. For the WADA-approved test, two separate kits with different anti-body clones (Kit 1 and Kit 2, supplied by CMZ-Assay GmbH, Beijing, China), are used for the measurement of the hGH isoforms. One of these two kits may be utilized for screening, whereas the other shall be used for confirmation analyses. Each kit contains one 're-combinant' and one 'pituitary' assay. In the recombinant (recGH, rec) assay, the coated capture antibody preferentially binds to the 22-kDa hGH, whereas the pituitary (pitGH, pit) assay employs a capture antibody that recognizes a variety of pituitary-derived hGH isoforms except 22 KDa hGH.

* *Correspondence to: Moutain Wu, China Anti-Doping Agency, 1st Anding Lu, Beijing, 100029, China. E-mail: moutianwu@yahoo.com.cn*

† *This paper is a part of Ms Xinmiao Zhou's PhD dissertation.*

a *China Anti-Doping Agency*

b *Beijing Sport University*

Experiments

Design of excretion study

The excretion study was double blind, randomized, placebo controlled, and officially approved by a panel for both ethics and scientific review. All volunteers were fully informed by seminars, written materials, and signed consent forms.

In total, 25 non-athlete male students from the Beijing Sport University were selected. Before induction to the study, all of the volunteers undertook a normal health check screening with a blood test for GH, FSH, LH, Testosterone, TSH, T_4, T_3 and ECG. The norm ranges obtained from the health check served as inclusion criteria.

Before each blood collection and rhGH/placebo administration, clinical doctors confirmed that the cohort displayed physical conditions suitable for further sampling. Abnormal ranges of the parameters determined by the doctors constituted the exclusion criteria. During the excretion study, no one was excluded.

The physiological conditions of these volunteers are listed in Table 1. Among them, 19 volunteers were randomly selected as the administration group, while the remaining 6 volunteers were determined as the placebo cohort. Thirteen volunteers were administered with rhGH made in China (GenHeal®, S19990019; code of Chinese State Food and Drug Administration approval (SFDA), 1.6 mg (4 IU), Shanghai, China). The information provided by the manufacture reported that the rhGH preparation with identical 191 amino acids as 22 KDa hGH was obtained from prokaryotic cells. Six volunteers were administered with rhGH made in Switzerlan (Saizen®, S20080036 (code of SFDA approval), 1.33 mg (4 IU), Laboratoires Serone S.A., Switzerland). The information provided by the manufacture reported that the rhGH preparation contained identical 191 amino acids as 22 KDa hGH was obtained from eukaryotic cells. The same schedule and dose for both cohorts was administered for the double blind excretion study. The placebo cohort of six volunteers was administered saline with same volumes and schedule as the non-placebo growth hormone cohort.

The doses were administered at 0.1 IU/kg by one subcutaneous injection on the different areas of abdomen. This was repeated for 14 days in succession. The first administration took place at 8 am with all subsequent administrations at 9 pm. All volunteers were injected on the same area of their abdomen. The time for the first injection was recorded as 0 h. Before the second injection on day 2, six blood samples were collected 2, 4 8, 12, 23, and 34 h after the first injection and all blood samples were collected either 10 h or 21 h after each injection. The last blood samples were collected 826 h after the first injection. Blood samples were collected and stored following WADA guidelines.[16,17]

Figure 1 shows us the date and the schedule for injection and blood collection.

Sample analysis

All blood samples (2 x 5 ml for each subject) were drawn into a tube that has an inert polymeric serum separator gel and a clotting activation factor (BD Vacutainer® SST II, EU ref 367955). The tubes were gently inverted upside down five (5) times and centrifuged for 10 min at 1500 *g* as required in WADA guideline mentioned above.

The serum of each sample was separated and stored into five vials (each vial with around 1 ml of serum) at −70°C till analysis.

All samples were analyzed following our routine standard operating procedure with Kit 1 and Kit 2.

Based on WADA guidelines,[16] all quality control samples were stored for long-term quality comparison without exception (Inter-assay CV not higher than 20%). If the CV% of duplicates was higher than 10%, the sample was reanalyzed.

Results and discussion

Quality control for the excretion study

As required by the WADA guideline,[16] the long term quality comparison is showed in the following Tables. Usually in our procedure Kit 1 (former Kit B) is used for screening and Kit 2 (former Kit A) is used for confirmation. As fewer cases require confirmation, less data with Kit 2 (former Kit B) in routine testing has been accumulated. Some data with Kit 2 (former Kit B) research has been reported at the 2011 Cologne Workshop.[18] Table 2 reports the statistic results of control 1

Table 1. Physical information of the volunteers

n		Age (Y)	Stature (cm)	Weight (kg)	BMI	Fat%
n = 13	Mean	24.0	171.2	64.9	22.2	18.3
	SD	1.35	3.17	7.18	2.26	3.46
	Median	24.0	171.0	62.0	21.5	17.1
	Max	26.0	177.0	81.0	26.8	23.2
	Min	22.0	165.0	58.0	19.3	14.2
n = 6	Mean	23.7	171.3	62.8	21.4	16.7
	SD	2.16	3.72	5.42	1.66	3.52
	Median	24.0	171.5	62.0	21.7	17.5
	Max	26.0	175.0	72.0	23.6	20.8
	Min	20.0	165.0	56.0	19.3	10.4
n = 6 (Placebo)	Mean	23.5	174.2	74.8	24.8	22.2
	SD	2.88	5.19	9.87	2.80	6.48
	Median	23.5	175.5	77.5	25.8	25.0
	Max	28.0	179.0	84.0	27.2	27.0
	Min	20.0	164.0	62.0	20.9	11.1

(negative control) and control 2 (positive control) measured with Kit 1 (former Kit B) in routine over the last three years. Table 3 reports the quality control data measured with Kit 2 (former Kit A). These data are obtained from both our routine and research results. These data confirm that the Kits, at least Kit 1 (former Kit B) with large numbers of tests, are adequately stable, even when utilized to determine very low concentrations of GH in less than 1 ng/ml. Data from our previous presentation at the 2010 Cologne Workshop (unpublished, see Table 2) confirmed the stability of the quality controls measured with Kit 2 (former Kit A). All CV% of concentrations were less than 10%, while CV% of ratios of about 10%.

Time window of the detection after one dose

As reported by Bidlingmaier *et al.*,[10] in a cohort of 20 recreational athletes, ratios (median SD) increased after a single injection of rhGH, reaching 350% (73%) (recA/pitA) and 400% (93%) (recB/pitB) of baseline ratios respectively, and at a moderate dose (0.033 mg/kg), mean recA/pitA and recB/pitB ratios remained significantly increased for 18 h in men, while after high-dose rhGH (0.083 mg/kg), mean rec/pit ratios remained increased for 32 h (recA/pitA) and 34 h (recB/pitB). Following the International Standard of rhGH used for the kits, 2.5 IU/mg, the moderate and high-dose should be equivalent to the dose of 0.0825 and 0.208 IU/kg

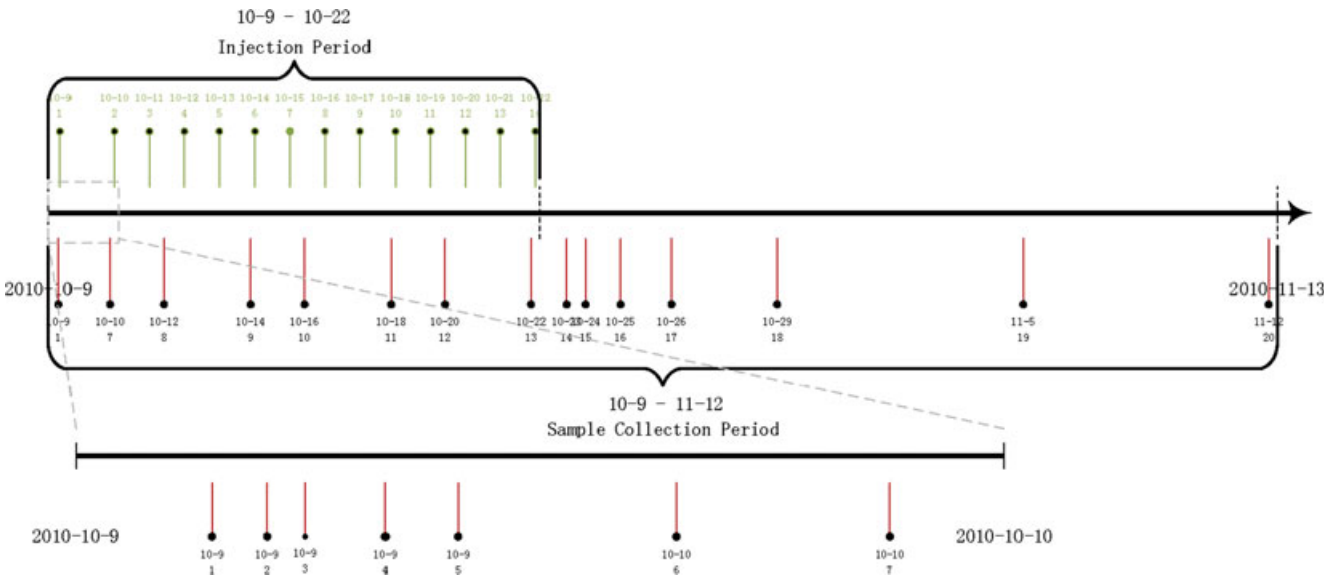

Figure 1. The date and schedule of injection and blood sample collection.

Table 2. Statistics of quality control in routine with Kit 1 (former Kit B) for screen

		Control 1			Control 2		
Year	Items	Rec (ng/ml)	Pit (ng/ml)	Ratio	Rec (ng/ml)	Pit (ng/ml)	Ratio
	Mean	0.84	1.02	0.83	3.47	1.09	3.20
	SD	0.06	0.07	0.06	0.26	0.10	0.34
	CV%	6.73%	6.45%	7.00%	7.49%	9.33%	10.74%
2008–2010	Median	0.84	1.01	0.84	3.50	1.10	3.20
	Max	1.05	1.20	0.97	4.07	1.36	4.08
	Min	0.65	0.86	0.69	2.55	0.88	2.41
	n	106	106	106	106	106	106

Table 3. Quality controls measured with Kit 2 (former Kit A) in research

Control	Item	Concentrations/Ratios	SD (n = 7)	CV%
Control 1 (negative)	Rec	0.855 ng/ml	0.034	4.0
	Pit	0.872 ng/ml	0.036	4.1
	Ratio	0.982 < Criteria	0.053	5.4
Control 2 (positive)	Rec	3.526 ng/ml	0.101	2.8
	Pit	1.312 ng/ml	0.078	5.9
	Ratio	2.692 > Criteria	0.092	3.4

respectively. The dose in our excretion study was 0.1 IU/kg between the moderate and high-dose levels, but closer to the moderate-dose of Bidlingmaier's. In our excretion study both with Chinese (n = 13) or Swiss (n = 6) rhGH preparation, the median values were very close to the mean values measured with both Kit 1 (former Kit B) and Kit 2 (former kit A) as seen in Figures 2 and 3. The mean values remained over the WADA criteria for Adverse Analytical Finding (AAF) for 12 h with Chinese rhGH preparation and 18 h with Swiss rhGH preparation. The detection window for a single injection of 0.1 IU/kg dose as described with the mean values is between 12 and 18 hours. When looking at the max and min values obtained in our excretion study (as showed with dashed lines), the possible maximum detection window can reach 21 h with a possible minimum of 4–6 h less than the detection window defined by mean values.

It is apparent that the recGH concentrations between volunteers with Chinese and Swiss preparations measured by Kit 1 and Kit 2 are not so different. The concentrations of recGH in the cohort administered with the Chinese preparations are generally higher than that in the cohort administered with the Swiss preparations, but the pitGH concentrations with the Swiss preparations measured by both Kit 1 and Kit 2 were often lower. This could explain why the detection window with the ratio of recGH/pitGH shows some difference between these two groups administered with different preparations (Figures 2–5).

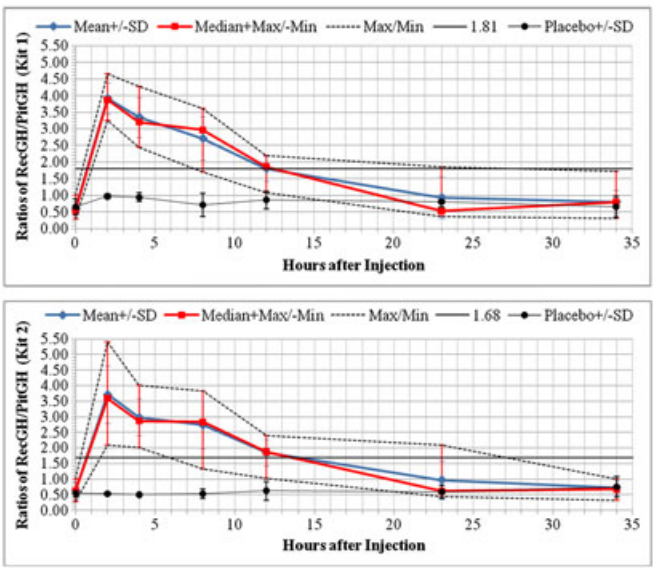

Figure 2. Time course of Ratios after Single Injection of Chinese rhGH Preparation (Upper: measured with Kit 1(n = 12). Lower measured with Kit 2(n-13)).

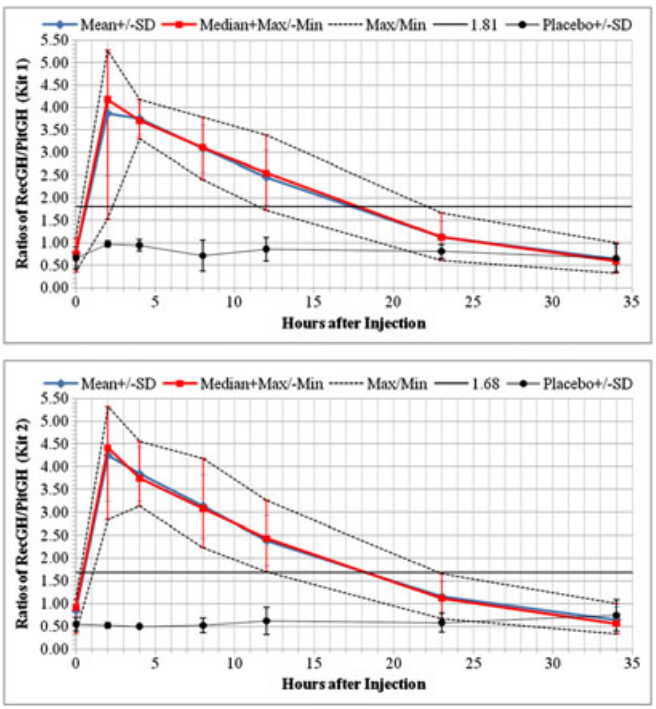

Figure 3. Time Course of Ratios after Single Injection of Swiss rhGH Preparation (n = 6) (upper: measured with Kit 1, lower: measured with Kit 2).

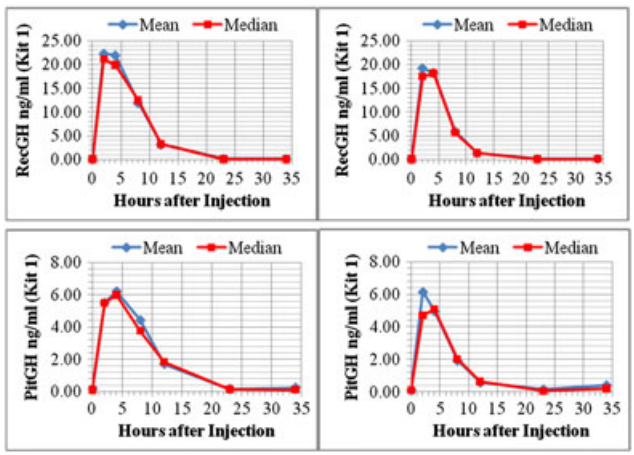

Figure 4. The time course of GH concentrations after Single Injection measured by Kit 1 (left: with Chinese Preparation, right: with Swiss Preparation).

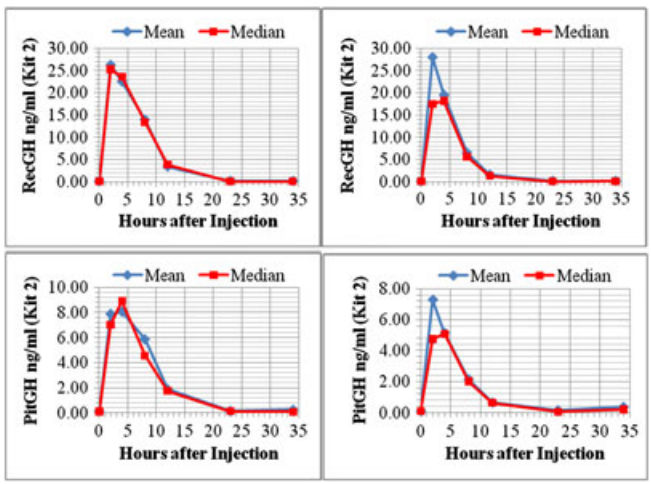

Figure 5. The time course of GH concentrations after Single Injection measured by Kit 2 (left: with Chinese Preparation, right: with Swiss Preparation).

Time window of the detection after multi-doses

As reported in Figures 6a and 6b the volunteers have been administrated with GH for continuous 14 days. The blood samples were collected 10 h or 21 h after their latest injection. The mean ratios of all volunteers (administered with Chinese preparation (Figure 6a) or with Swiss preparation (Figure 6b)), 10 h after the latest injection, measured either by Kit 1 or Kit 2, were always significantly over the detection limit (DL) values set by the WADA technical document. While the mean ratios of all volunteers 21 h after the latest injection, measured either by Kit 1 or Kit 2, were never significantly over the DL values, statistically, in such situations, doping with recGH can be detected no longer than 21 h after injection. The ratios of bloods taken 10 and 21 h after the latest injection were symmetrically located in DL line. With multi-doses, as compared to single dose, no significant change on the detection window was observed. This may be a result of the short half-life of recGH in the human body.

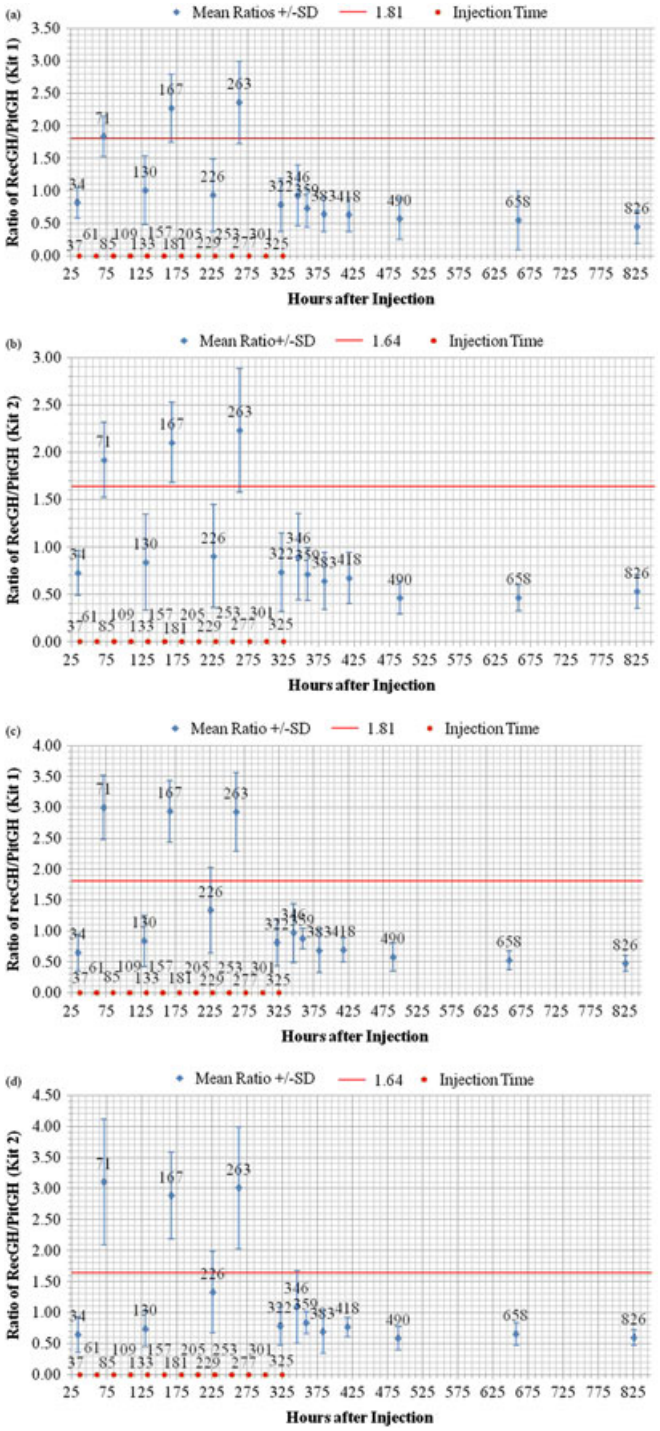

Figure 6. The time course of ratios after muli-doses with Chinese and Swiss Preparations ((a)/(b) Chinese preparation measured with Ki1 (n = 12)/Kit 2(n = 13); (c)/(d) Swiss preparation (n = 6) measured with Kit 1/Kit 2.

Decision limit for detection of doping with rhGH

The DLs for detection of doping with rhGH have recently changed. Before 2010 the DLs of Kit 1/Kit 2 were 1.64/2.17 and 1.19/1.68 for male and female, respectively. After 2010, these became 1.81/1.68 and 1.46/1.55. Some values have increased while others have decreased and gap between DLs between genders is closing.

As required by the WADA guidelines for the detection of doping with rhGH,[16] all samples with confirmed mean values of recGH below 0.1 ng/mL are to be reported as negative irrespective of the corresponding values of pitGH and the resulting kit ratio. In Table 4 (last line), samples that were not included provide data reporting that more than half of the male samples contain growth hormone below than 0.1 ng/ml. In our procedure the samples were tested only with Kit 1/Kit B for screening purposes without suspicion. As such there was no need for confirmation with Kit 2/Kit A. Rarely are routine samples tested with Kit 2/Kit A. Statistical analysis was carried out confirming that no significant difference between these mean values over the three years ($p > 0.05$) as demonstrated in Table 4. The mean ratio of all available data from our routine of three years is around 0.53 with SD 0.19 (n = 421).

In accordance with the WADA technical document TD2010DL,[18] if the Mean + 3*SD from the population based reference mentioned above could be taken as the threshold of a corresponding Kit for males, the threshold would be around 1.2. The assay measurement uncertainty (MU) should not be higher than 14% (relative) as Umax, so the DL of Kit 1 for males would be around **1.5** (threshold + 1.65 × Umax × threshold = 1.2 + 1.65 × 0.14 × 1.2 = 1.48). When 1.5 would be used as the DL for Kit 1, instead of 1.81, the time window of the detection is some hours longer than discussed before as indicated in this excretion study; however, more data are needed before the established criteria may be modified.

Table 4. Population based reference of males from routine (RecGH ≥ 0.1 ng/ml + PitGH ≥ 0.05 ng/ml)

Ration(KitB/1)	Year = 2008	Year = 2009	Year = 2010
Sample No.	159	134	128
Mean	0.514	0.533	0.551
SD	0.212	0.223	0.248
Median	0.490	0.487	0.490
Max.	1.168	1.228	1.254
Min.	0.087	0.200	0.186
No. that not included	179	166	158

Higher variation of ratios after administration of rhGH

The error bar (SD) in Figures 2 and 3 indicates clearly that the variation of the ratios for the placebo cohort is less than that of the administration group for both the Chinese and Swiss preparations during the administration period. The error bar (SD) in Figures 6a and 6b confirmed a phenomena wherein the variation of ratios for the administered period (with the time for injection, red point in the X axis) is much higher than that for washout period without the time for injection, 25 h after the last injection at 325 h). This could imply two hypotheses as follows:

First, the response of pituitary GH excretion with 22 KDa GH and non-22 KDa GH to the administration of rhGH can be quite different between individuals so that the variation of the ratios was significantly higher than that of placebo cohort or than that of the administration group in washout period.

Second, the ratios were calculated by the concentrations of 22 KDa GH and non-22 KDa GH, which are recognized by different monoclonal anti-bodies. For the measurement of 22 KDa GH, it is quite easy to compare different commercial available kits which are widely implemented in clinical diagnostics. It is difficult to investigate the cross activity to all isoforms of non-22 KDa GH and to ascertain any other unexpected or unwanted cross activity in different physiological or pathological conditions because non-22 KDa GH is still a group of GH variants. The biological and immunological properties of GH variants are complex and beyond the scope of this article however some peer reviewed papers reported on the functional differences among GH isoforms.[19,20]

A special case with irregular ratio

A case with irregular high ratios of recGH/pitGH was observed. During the excretion study, only one volunteer with some samples measured by Kit 1 showed unusual GH ratios. This time course of ratios is presented in Figure 7 alone and this is why in Figure 2, n = 12 for Kit 1 while n = 13 for Kit 2.

Some concentrations of certain samples are listed in Table 5. With comparison of recGH and pitGH concentrations it was found that the (recGH) concentrations measured by Kit 1 were comparable with Kit 2 but the certain concentrations of pitGH by Kit 1 were significant lower than that by Kit 2 which may result in the increase of the ratios of recGH/pitGH (red values in Table 5). This phenomenon with Kit 1 was confirmed by repeating different tests however the reason for these elevated ratios with lower pitGH is yet for us to explain.

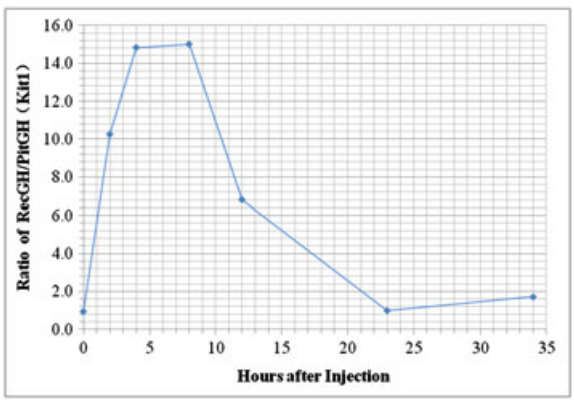

Figure 7. A special case with abnormally high ratios.

Table 5. Some concentrations of GH in a special case

Hours after Inj.	RecGH(ng/ml)	PitGH(ng/ml)	Ratio
0	0.084/0.087	0.105/0.307	0.95/0.28
2	31.79/35.0	3.10/16.79	10.26/2.09
4	27.14/23.60	1.83/9.25	15.83/2.55
8	14.38.13.03	0.98/4.42	15.01/2.95
12	2.67/2.27	1.28/1.28	2.09/1.77
23	0.041/0.042	0.038/0.088	1.0/0.57

ng/ml: kit1/kit2.
If the measured concentration was<0.05 ng/ml, then replaced with 0.05 for calculating the ratios.

Conclusion

The following points can be concluded from the results of this excretion study:

1) The kits proved by WADA are adequately stable and can detect doping with rhGH but the time window of detection is short.
2) The two rhGH preparations did not show significant difference in the time course of the ratios measured by the Kits though they were obtained from prokaryotic and eukaryotic cells respectively.
3) The criteria suggested by the WADA guidelines should be improved in order to broaden the time window of detection with more excretion studies and population based data.
4) After administration of rhGH, the ratios measured with WADA-approved kits varied significantly more than those of the placebo group, even more than that of the administration group during the washout period.
5) The reason for the special case observed in this excretion study is not clear so far. Thus further study for the specificity of these antibodies used for the kits is needed.

Acknowledgements

We give special thanks to Prof. Dr Zimeng Jin in the Department of Endocrinology of Peking Union Hospital for his valuable consultation, to Shanghai United Cell Biotechnology Co. Ltd for the donation of rhGH preparations and to Dr Ganglin Zhang in the Clinic of Beijing Sport University for the medical service during the excretion study.

References

[1] R. I. G. Holt. Detection growth hormone abuse in athletes. *Anal. Bioanal. Chem.* **2011**, DOI: 10.1007/s00216-011-5068-2
[2] C. He, M. Wu. Detection of doping with recombinant human growth hormone. *Bioanalysis* **2009**, *1*, 953.
[3] J. Segura, R. Gutiérrez-Gallego, R. Ventura, J. A. Pascual, J. Bosch, G. Such-Sanmartin, Z. Nikolovski, A. Pinyot, S. Pchini. Growth hormone in sport: Beyond Beijing. **2008**. *Ther. Drug Monit.* **2009**, *31*, 3.
[4] M. Bidlingmaier, C. J. Strasburger. Growth hormone. *Handb. Exp. Pharmacol.* **2010**, *195*, 187.
[5] C. Fredolini, D. Tamburro, G. Gambara, B. Lepene, V. Espina, E. Petricoin 111, L. A. Lotta, A. Luchini. Nanoparticle technology: Amplifying the effective sensitivity of biomarker detection to create a urine test for hGH. *Drug Test Analysis* **2010**, *1*, 447.
[6] M. Okano, M. Sato, A. Ikekita, S. Kageyama. Determination of growth hormone secretagogue pralmorelin (GHRP-2) and its metabolite in human urine by liquid chromatography/ electrospray ionization tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 2046.

[7] M. Bredehöft, W. Schänzer, M. Thevis. Quantification of human insulin-like growth factor-1 and qualitative detection of its analogues in plasma using liquid chromatography/ electrospray ionisation tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2008**, *22*, 477.
[8] C. J. Mitchell, A. E. Nelson, M. J. Cowley, W. Kaplan, G. Stone, S. K. Sutton, A. Lau, C. M. Lee, K. K. Ho. Detection of growth hormone doping by gene expression profiling of peripheral blood. *J. Clin. Endocrinol. Metab.* **2009**, *94*, 4703.
[9] A. Paparini, F. Impagnatiello, A. Pistilli, M. Rinaldi, G. Gianfranceschi, E. Signori, A. M. Stabile, V. Fazio, M. Rende, V. Romano Spica. Identification of candidate genes and expression profiles, as doping biomarkers. *Ann. Ig.* **2007**, *19*, 303 (Article in Italian).
[10] M. Bidlingmaier, J. Suhr, A. Ernst, Z. Wu, A. Keller, C. J. Strasburger, A. Bergmann. High-sensitivity chemiluminescence immunoassays for detection of growth hormone doping in sports. *Clin. Chem.* **2009**, *55*, 445.
[11] I. Erotokritou-Mulligan, E. Eryl Bassett, D. A. Cowan, C. Bartlett, P. Milward, A. sartorio, P. H. Sonksen, R. I. Holt. The use of growth hormone (GH)-dependent markers in the detection of GH abuse in sport: Physiological intra-individual variation of IGF-I, type 3 pro-collagen (P-III-P) and the GH-2000 detection score. *Clin. Endocrinol (Oxf).* **2010**, *72*, 520.
[12] R. I. Holt, I. Erotokritou-Mulligan, C. McHugh, E. E. Bassett, C. Bartlett, A. Fityan, J. L. Bacon, D. A. Cowan, P. H. Sonksen. The GH-2004 project: the response of IGF1 and type III pro-collagen to the administration of exogenous GH in non-caucasian amateur athletes. *Eur. J. Endocrinol.* **2010**, *163*, 45.
[13] I. Erotokritou-Mulligan, E. E. Bassett, C. Bartlett, D. Cowan, C. McHugh, R. Seah, B. Curtis, V. Wells, K. Harrison, P. H. Sonksen, R. I. G. Holt. The effect of sports injury on insulin-like growth factor-I and type 3 procollagen: implications for detection of growth hormone abuse in athletes. *J. Clin. Endocrinol. Metab.* **2008**, *93*, 2760.
[14] A. E. Nelson, K. K. Ho. Demographic factors influencing the GH system: Implications for the detection of GH doping in sport. *Growth Horm. IGF Res.* **2008**, *19*, 327.
[15] T. V. Nguyen, A. E. Nelson, C. J. Howe, M. J. Seibel, R. C. Baxter, D. J. Handelsman, R. Kazlauskas, K. K. Ho. Within-subject variability and analytic imprecision of insulin-like growth factor axis and collagen markers: implications for clinical diagnosis and doping tests. *Clin. Chem.* **2008**, *54*, 1268.
[16] WADA. Detection of Doping with Human Growth hormone. Available at: http://www.wada-ama.org/en/World-Anti-Doping-Program/Sports-and-Anti-Doping-Organizations/Model-Rules--Guidelines/Guidelines/ [6 June 2011].
[17] WADA. Blood sample collection. Available at: http://www.wada-ama.org/en/World-Anti-Doping-Program/Sports-and-Anti-Doping-Organizations/Model-Rules--Guidelines/Guidelines/ [6 June 2011].
[18] WADA. Decision limits for the confirmatory quantification TD2010DL. Available at: http://www.wada-ama.org/en/World-Anti-Doping-Program/Sports-and-Anti-Doping-Organizations/International-Standards/Laboratories/ [6 June 2011].
[19] V. Popii, G. Baumann. Laboratory measurement of growth hormone. *Clin. Chim. Acta* **2004**, *350*, 1.
[20] E. F. De Palo, V. De Filippis, R. Gatti, P. Spinella. 67 Growth hormone isoforms and segments/fragments Molecular structure and laboratory measurement. *Clin. Chim. Acta* **2006**, *364*, 67.