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Comparison of hGH serum concentrations of Caucasian and African top athletes: an ethnicity study

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Abstract

Growth hormone is prohibited in sports and currently measured by a luminescence immunoassay developed by Bidlingmaier and Strasburger. The application of an immunoassay in doping control necessitates a deep evaluation of ethnical influences as well as many other parameters. This study was carried out to determine the differences between Caucasian and African athletes. The mean values of the ratios were significantly higher for Caucasian athletes. Additional interesting results included the fact that no significant differences were found between men and women. The current cut-off values of the assay may be adapted after deeper statistical evaluation of the data by the World Anti-Doping Agency.

Introduction

Growth hormone is prohibited in sports [1] due to its performance enhancing properties such as fat reduction and protein anabolism [2] and serum samples are currently measured by the differential luminescence immunoassay (LIA) developed by Bildingmaier and Strasburger [3-4]. Although several validation studies were already performed [4], the application of an immunoassay in doping control necessitates extensive studies concerning the variation of endogenous values in different ethnic groups and under different sampling conditions.

The test uses two immunoassays bearing a different antibody. The antibody of assay “rec“ mainly detects unmodified, 22 kDa growth hormone which is the main endogenous variant and identical to the homogenous recombinant growth hormone. In contrast, the antibody of the assay “pit” mainly binds to endogenous, pituitary variants [5]. Additionally, two kits with different sets of “pit” and “rec” antibodies are available for screening and confirmation (Kit 1 and 2). Cut-off values of the ratio rec/pit were determined to evaluate samples and will be adjusted according to further validation studies. The data presented here were produced to

compare rec/pit ratios of the currently used LIA assay of samples from African and Caucasian athletes.

Materials and Methods

Serum samples:

Serum samples of African athletes were doping control samples taken at the IAAF world championship 2009. Serum samples of Caucasian athletes were out-of-competition doping control samples of different sport categories from the German National Anti-Doping Agency where athletes gave their written consent to use the samples for research purposes. All samples were stored at -20°C over a maximum period of 2 years.

Luminescence Immunoassay:

All samples were prepared in duplicate using Kit 1 and Kit 2 according to the suppliers´ recommendations (CMZ, Berlin, Germany). Samples with a %CV < 15 were included in the evaluation and samples below the limit of detection (0.05 ng/ml for all assays) were excluded.

Results and Discussion

Kit 1

	African athletes			Caucasian athletes		
	male (n=45, n<LOD=12)			**male (n=109, n<LOD=31)**		
	concentration rec [ng/mL]	concentration pit [ng/mL]	**ratio**	concentration rec [ng/mL]	concentration pit [ng/mL]	**ratio**
mean	0.28	0.80	**0.41**	0.62	1.29	**0.49**
sd	0.43	1.19	**0.21**	1.21	2.58	**0.17**
	female (n=43, n<LOD=2)			**female (n=55, n<LOD=3)**		
	concentration rec [ng/mL]	concentration pit [ng/mL]	**ratio**	concentration rec [ng/mL]	concentration pit [ng/mL]	**ratio**
mean	1.47	2.49	**0.42**	1.26	2.34	**0.53**
sd	4.46	5.81	**0.18**	1.90	3.52	**0.17**

Kit 2

	African athletes			Caucasian athletes		
	male (n=48, n<LOD=9)			**male (n=117, n<LOD=23)**		
	concentration rec [ng/mL]	concentration pit [ng/mL]	**ratio**	concentration rec [ng/mL]	concentration pit [ng/mL]	**ratio**
mean	0.33	0.71	**0.47**	0.65	1.36	**0.53**
sd	0.57	1.04	**0.19**	1.34	2.84	**0.18**
	female (n=44, n<LOD=1)			**female (n=55, n<LOD=3)**		
	concentration rec [ng/mL]	concentration pit [ng/mL]	**ratio**	concentration rec [ng/mL]	concentration pit [ng/mL]	**ratio**
mean	1.62	2.69	**0.45**	1.44	2.46	**0.59**
sd	4.74	6.28	**0.16**	2.20	3.92	**0.18**

Figure 1: Summary of the mean values (concentrations and ratios) of the different groups for Kit 1 and Kit 2

The investigation of samples from African and Caucasian athletes resulted in a valuable set of data that will be further evaluated by the World Anti-Doping Agency. Figure 1 gives an overview on the number of samples measured as well as the mean concentration and the different ratios.

Comparison of African and Caucasian athletes: The mean values of the ratios were significantly higher in samples of Caucasian than in samples of African athletes (Figure 2 and 3).

Comparison of men and women: The mean values of the concentrations were higher in women than in men but the rec/pit ratios of female and male samples were similar within an ethnic group and within a kit, as demonstrated in Figure 2. This is an interesting observation as the current reference limits are different for the different genders.

Comparison of Kit 1 and Kit 2: The ratios of Kit 1 and Kit 2 were significantly different (Figure 2 and 3).

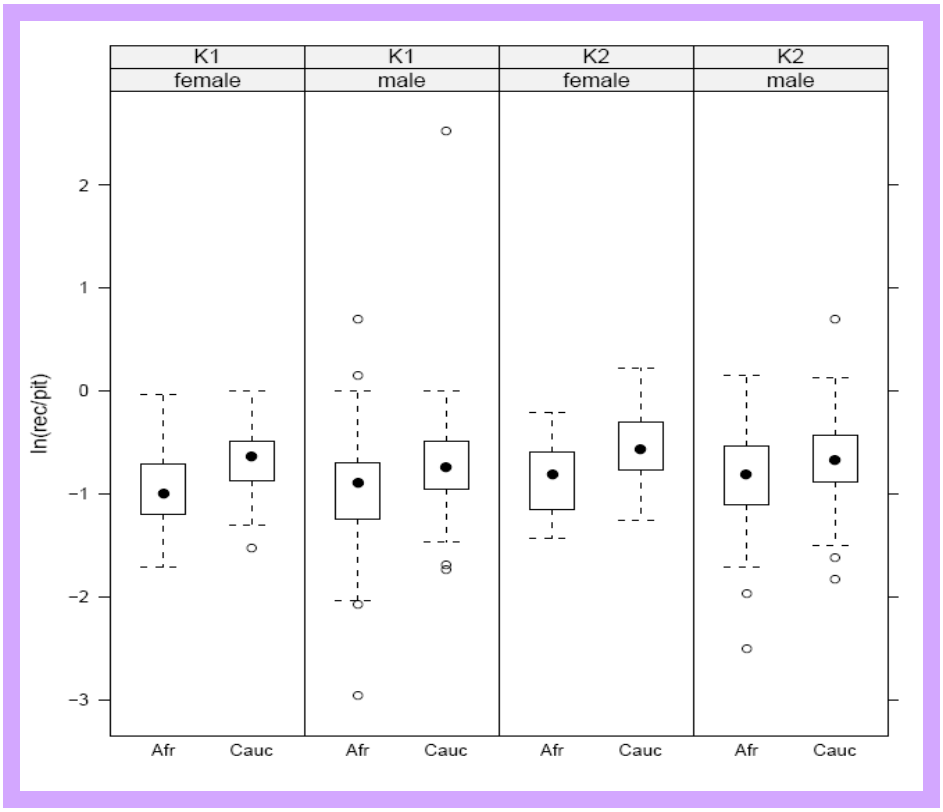

Figure 2: Box-plot of different sample groups indicating differences between African and Caucasian samples.

The results of this study will be evaluated more detailed by the World Anti-Doping Agency and the cut-off limits may be adapted after deeper statistical analysis. It is important to note that the different sport categories and in- or out-of-competition sampling need to be considered in the evaluation to avoid differences between the groups which are based on basic differences in sampling instead of real differences between the ethnicities.

Random effects

	Intercept	Residual
StdDev:	0.357	0.234

Fixed Effects

	Value	Std.Error	DF	t-value	p-value
Intercept	-0.920	0.0403	298	-22.86	<0.001
Ethn Cauc	0.218	0.0481	298	4.52	<0.001
Kit K2	0.076	0.0193	296	3.97	<0.001

Figure 3: Estimated parameters of the final model (linear mixed effects models)

Acknowledgements

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References

1. World Anti-Doping Agency. The 2010 prohibited list. International Standard, Montreal (2010) http://www.wada-ama.org/Documents/World_Anti-Doping_Program/WADP-Prohibited-list/WADA_Prohibited_List_2010_EN.pdf (access date 10.01.2010.
2. Sönksen PH. (2001) Insulin, growth hormone and sport. *J Endocrinol* **170**, 13-25.
3. Bidlingmaier M, Wu Z, Strasburger CJ. (2000) Test method: GH. Baillieres Best Pract *Res Clin Endocrinol Metab* **14**, 99-109.
4. Bidlingmaier M, Suhr J, Ernst A, Wu Z, Keller A, Strasburger CJ, Bergmann A. (2009) High-Sensitivity Chemiluminescence Immunoassays for Detection of Growth Hormone Doping in Sports. *Clin Chem* **55**, 445-453.
5. Baumann GP. (2009) Growth hormone isoforms. *Growth Horm IGF Res* **19**, 333-340.