Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (7)

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Sport und Buch Strauß, Köln, 1999

A. KNIEß, R.K. MÜLLER, J. KRATZSCH:
Quantitation of Human Growth Hormone, Insulin-like Growth Factors I and II (IGF-I and II) and IGF Binding Protein-2 and -3 in Athletes [„Blood Study" of the German Antidoping Commission (ADK)]
In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in doping analysis (7). Sport und Buch Strauß, Köln, (1999) 365-369

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Quantitation of Human Growth Hormone, Insulin-like Growth Factors I and II (IGF-I and II) and IGF Binding Protein-2 and -3 in Athletes
["Blood Study" of the German Antidoping Commission (ADK)]

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This work was founded by the Bundesinstitut für Sportwissenschaften, Köln, Germany, (VF 0414/03)

Introduction

The study is intended to be a pilot study contributing to improve the analytical detection of hGH-administration in doping control.

The total concentration of hGH in serum does not permit a significant distinction between a physiological increase and an exogenous administration, as hGH is secreted in a pulsatile fashion by pituitary gland. Furthermore, the hGH concentration in serum scatters very strongly. Both, amplitude and frequency of the hGH secretory pulses, depend on age and individual circadian rhythm of the hGH secretion. In addition, the hGH secretion is stimulated by physical stress and many other factors [1-4]. Following the application of physiological or supraphysiological doses of rhGH (subcutaneous, intramuscular), hGH concentrations in serum or urine reach a maximum after 3-6 h and decrease to the basal value in less than 24 hours [5-7].
IGF-I and IGFBP-3 are controlled by the hGH action. Endogenous as well as exogenous hGH cause increases of IGF-I and IGFBP-3 in blood, with strong interindividual variations [8,9,14]. IGFBP-3, the major binding protein for both IGF-I and IGF-II in circulation represents a depot factor for small peptide molecules, impeding their fast renal clearance.

Aim of the study

The aim of our study was to find reference ranges for hGH, IGF-I, IGF-II and IGFBP-3 in a special group of athletes. Relationships between these parameters as well as associations with age and physical stress should be investigated.

Materials and methods

Subjects

224 athletes (males, females, age 15-37 years, mean 22 y.) were included in the study (Fig.1). Blood samples were withdrawn on a voluntary base prior to as well as after stress during training or competition. Athletes declared written informed consent. The performance of the trial and the time schedule in blood sampling was not standardized.

Age (years)	Anzahl (n)	Alter (Jahre)	Anzahl (n)	Alter (Jahre)	Anzahl (n)
15	2	22	17	29	8
16	11	23	13	30	3
17	22	24	13	31	3
18	22	25	14	32	3
19	22	26	12	33	1
20	17	27	9	37	1
21	25	28	6		

Hormone measurements

Serum hGH was analysed using an chemiluminescence immunometric assay (Nichols Institute Diagnostica, Bad Nauheim, Germany).

Serum IGF-I was determined by two immunoassays based on different methods for the separation of interfering IGF-binding proteins. In a competitive assay (Kratzsch et al. (1993) [10]), that separation step was performed by the extraction with acid-ethanol [11]. Thereafter, sample IGF-I competetes with biotinylated IGF-I for the polyclonal anti-IGF-I antibody raised in rabbits. The detection step was performed by europium-labelled streptavidine and fluorescence measurement. The further assay (Nichols, Bad Nauheim, Germany) was a fully automated, commercially available, binding-protein blocked chemiluminescence technique.

Serum IGF-II and serum IGFBP-3 were measured in duplicates by ELISA and IGFBP-2 was measured by RIA using kits purchased from DSL Inc. (Webster, Texas, USA).

Results and Discussion

The hGH determinations show concentrations between 0 and 5 ng/ml in 80 % of the subjects. Values above 10 ng/ml have been partially observed after trials, levels of more than 60 ng/ml may be observed only during extended and intensive exercises. This response of hGH to individual exercise demonstrated a considerable variance. Due to the fast decrease of serum hGH the values vary in the time interval between exercise and sampling (the peak was detected 10-35 min after exercise, it returns to basal level within 60 min, depending on type and duration of the stress [2-4]). hGH-concentrations above 10 ng/ml, which were determined in an exercise free period, indicate that a circadian secretion peak was detected accidentally.

Summarizing our hGH data of athletes we were not able to reveal any statistic significant association with the age of our subjects or with the type of the performed trial.
Serum IGF-I correlates with hGH status (24 h secretion) and exerts no circadian rhythm. The single data vary in a wide range, but obviously, the mean values of age groups decrease with increasing age (Fig. 1), e.g. 16 y. (n = 11) mean 410 +/- 89 ng/ml, 24 y. (n = 13) mean 277 +/- 88 ng/ml. The measured IGF-I levels were in the expected range of the age-dependent reference range, most of these values were between 50 th and 95 th percentile [12]. About 10 % of all IGF-1 data were determined above the 95 th percentile.

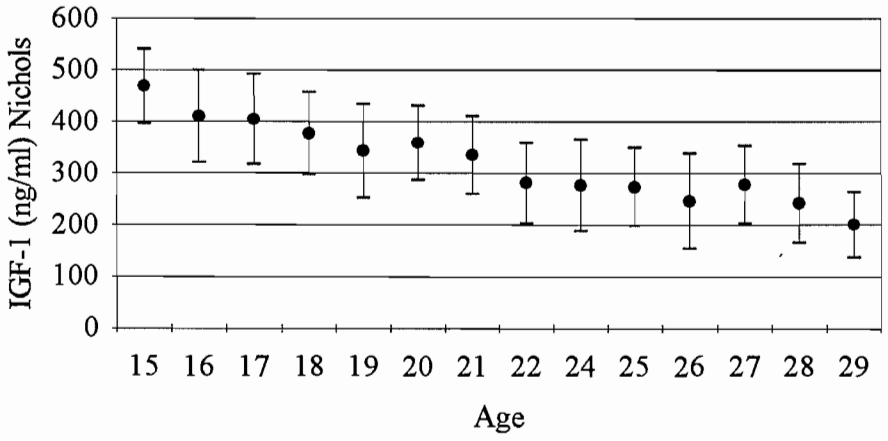

Fig. 1 Serum IGF-I measured by Nichols Advantage assay,
results are expressed as means +/- SD

Fig. 2 shows a lack of correlation for the comparison between IGF-I and random hGH data with or without exercise.

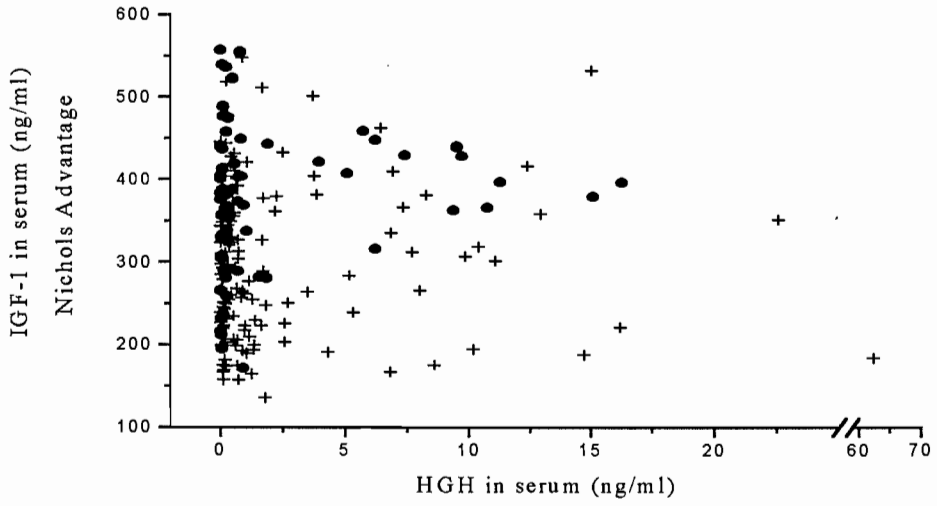

Fig. 2 Dependence of serum IGF-I levels on random serum hGH concentrations;
• sampling prior to stress; + sampling after stress

The comparison of both IGF-I assays shows a moderate relationsship between the results (r = 0,71; P < 0.0001, Fig.3). However, we have to consider that the techniques are substantially different (different IGFBP separation, antibodies, calibrators).

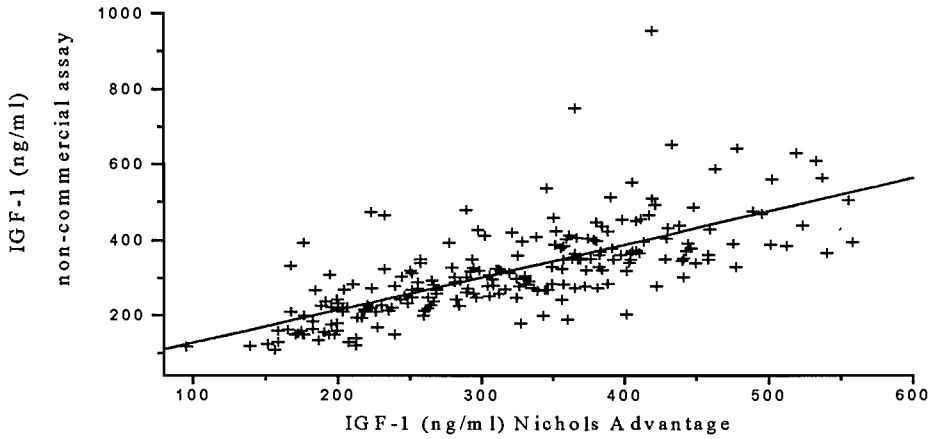

Fig. 3 Correlation of IGF-I levels measured by competitive non-commercial immunoassay and by the Nichols Advantage assay

No relationships were observable between serum levels of IGF-II or IGFBP-3 and the age of our athletes (data not shown). The measured serum values are within the age dependent reference range.

In Fig. 4 it is visible that only IGF-I permits to recognise a tendency to higher IGFBP-3 levels with increasing IGF-I concentrations (r = 0.53; P < 0.0001).

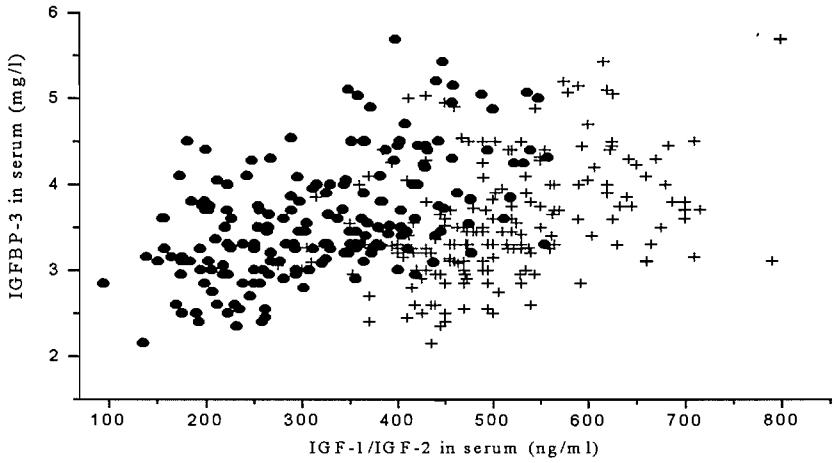

Fig. 4 Relationship between serum IGFBP-3 and IGF-I (Nichols Advantage) (•) or IGF-II (+)

In contrast, the sum of IGF-I and IGF-II correlates sufficiently with serum IGFBP-3 (r = 0,68; P < 0.0001) (Fig. not shown) [13].

To test the hypothesis of significant changes in the ratio of IGF-I/IGFBP-2 and IGFBP-3/IGFBP-2 dependent on GH (hGH-administration for 3 days, 0.15 IU/kg/d) (Kicman et al., 1997 [14]) serum IGFBP-2 was measured in 39 samples. However, the calculated relationships between theses parameters did not reach statistical significance in our study. The concentration ratios of IGF-I to IGFBP-2 and IGFBP-3 to IGFBP-2 demonstrated an important variance: IGF-I [ng/ml]/ IGFBP-2 [ng/ml]: 0.08 to 2,98; IGFBP-3 [µg/l] / IGFBP-2 [µg/l]: 2,4 to 28). These ranges overlap with those described in literature [14]. A changed relationsship between IGF-I or IGFBP-3 levels with IGFBP-2 is no serious proof for hGH-administration as the interindividual variation of that coefficient in an heterogenic population is to high.

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