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A hGH Application Study: Issues related to valid Detection Parameters

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Introduction

Human Growth Hormone (hGH) is abused as an anabolic hormone among athletes to enhance their physical performance. There are two different ways to detect doping with rhGH: the "direct method", using specific antibodies to determine isoform ratios [1] and the "indirect method", using markers of GH action. The final result of the GH 2000 project (indirect method) were discriminant functions including insulin-like growth factor I (IGF-I) and N-terminal propeptide of type III procollagen (PIIINP) [2-4]. The aim of our hGH treatment study was to evaluate some hGH markers with special respect to their intra and inter individual variation and the response to low doses of hGH. Furthermore we wanted to prove the concept of a discriminant function in doping control for distinguishing between hGH and placebo treated subjects with an independent set of samples.

Subjects and Methods

Study Design: 15 non-competitive male athletes (age range:21-33 years, mean 24 years, 4 to 6 exercise sessions / week, 10 hGH treated with 0.06 IU/kg BW/day for 14 days, 5 placebo) were included in the single blind, placebo-controlled study. All subjects gave written informed consent to their participation. The study protocol was approved by the Ethic Committee.

Analytical Procedures: Serum IGF-I levels were analysed after HCl-ethanol extraction using a competitive fluorescence immunoassay. The IGF Binding Protein 3 (IGFBP-3) was determined by ELISA (DSL Inc. Webster, Texas, USA). PIIINP and PINP were analysed by specific RIAs (Orion Diagnostica, Espoo, Finland). Serum osteocalcin was measured using a luminescence immunoassay (Brahms Diagnostica, Hennersdorf, Germany)

Results

At baseline no significant differences were found between the data of the hGH treatment and the placebo group for all measured parameter. Furthermore, no significant change of the mean value of any serum parameter was observed within the placebo group during the whole study. As shown in Fig. 1, the IGF-I levels increased rapidly after the start of hGH treatment.

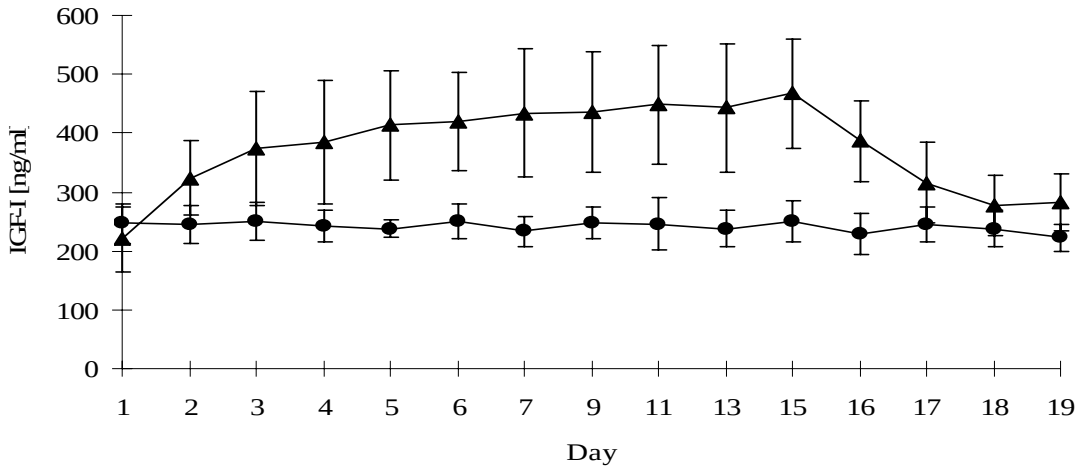

Fig. 1 Effects of hGH treatment (0.06 IU/kg ^{per} day up to day 14) and withdrawal on serum IGF-I concentrations (mean +/- SD) ▲ hGH treatment group; ● placebo

PIIINP is a marker of the soft connective tissue collagen metabolism. Fig. 2 shows the response of PIIINP to hGH treatment. From day 7 up to day 22 (hGH application ended on day 14) serum PIIINP concentrations in the group receiving hGH were significantly increased compared to the basal level. Both time course and magnitude of the response varied inter-individually.

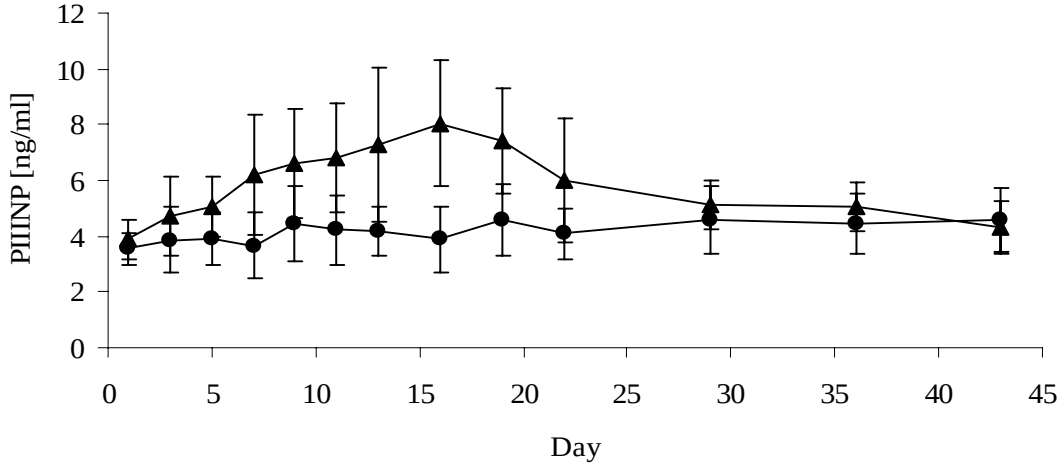

Fig. 2 Effects of hGH treatment on serum PIIINP concentrations (mean +/- SD)

Discriminant Function

One marker of GH action alone is not sufficient to prove doping with hGH. Therefore we calculated a discriminant function (SPSS program, SPSS Inc., Chicago, USA) using the pre-treatment and placebo data as negative class and the data of the hGH group from day 7 up to day 16 as positive class of 5 markers in total.

By means of the resulting discriminant function **F= -13.465 + 0.0272xIGF-I + 0.0398xIGFBP-3 + 1.367xPIIINP-0.00271x(IGF-IxPIIINP)** we were able to separate our data sets obtaining no false positive and 3 false negative results (day 7 up to day 16, cut off for discrimination F=0, Fig. 3)

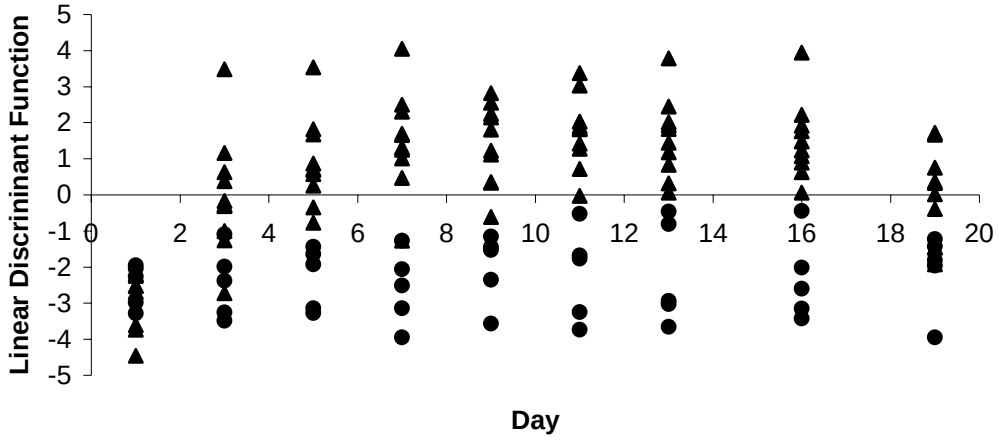

Fig. 3 Effects of hGH administration on the values of the discriminant function;
▲ hGH treatment group; ● placebo

To confirm our discriminant function we determined the f values of 60 untreated athletes. We found a clear age dependence of the values until the age of about 20 because of the known strong age dependence in particular of IGF-I. Athletes, older than 20, did not show false positive values.

Discussion and Conclusions

The results of this study show that 1) IGF-I, PIIINP and IGFBP-3 show a significant response to hGH applications even to the low doses applied in this study; 2) markers of the bone turnover are less useful in doping control because of their large inter individual scattering compared to a small response to hGH; 3) a discriminant function combining different markers of hGH action should contribute to solve the problem of hGH doping analysis; 4) the

discriminant function calculated in this study is particular useful in detecting applications of low doses of hGH; 5) the markers of hGH action depend very strong on age [5], therefore the cut off for f values has to be age dependent.

References

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