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RECENT ADVANCES IN DOPING ANALYSIS (2)

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Urinary Human Growth Hormone (U-HGH) : I. Measurements in Non-Athletes volunteers after intra-muscular or subcutaneous hGH injections.

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Abstract

The evaluation of urinary growth hormone excretion was shown to be useful in the monitoring of hGH therapy for growth retardation and in many other clinical situations.
The unlimited availability of authentic biosynthetic hGH and the pronounced anabolic effect of the hormone has led athletes (especially body-builders) to use it as a very efficient agent to increase their lean body mass. The misuse of growth hormone in sport is deemed to be unethical and dangerous because of various adverse effects and thus, it has been put in the IOC list of banned substances.
Until now, the very low concentration of hGH in the urine made it difficult to measure using the classical methodology. Indeed, for a large scale use in routine diagnostics, only plasma measurements were available.
However, unlike blood samples, urine is generally provided in abundant quantities and is, the time being, the only body fluid allowed to be analysed in sport. With a recently developed immunoassay (Norditest^{TM}) , it is now possible, without any extraction, to measure urinary-hGH in a dynamic range of 2-50 ng hGH/l .

In this protocol, untreated and treated non-athlete volunteers were followed. They received for one week therapeutical doses of recombinant hGH (Norditropine) either intramuscular (3 increasing doses) or subcutaneous (12 IU every day). The urinary hGH excretion after treatment showed dramatic increases up to 50-100 times the basal values and returned to almost the mean normal level after 24 hours. They were significantly higher than the upper values from most of the athletes after competition. But after some types of competition, the level of hGH measured was either much higher.

Introduction

The main international sport federations and the International Olympic Committee (IOC) have instituted comprehensive control procedures which are directed, beside the classical drugs (stimulants, analgesic,...), mainly towards the eradication of anabolic steroid use. As

the efficiency of detection increases, it seems that some athletes stopped steroids in favour of other anabolic agents. Non-official reports indicate that human growth hormone (hGH) is the current choice for many athletes. There are no reliable or scientific evidence so far on the use of hGH by athletes, but only indirect or anecdotal indications. It is the case in american football, track and field and body-building. HGH is placed on the IOC list of banned substances in sports. By the moment, urine being the only biological sample routinely available for doping tests, the anti-doping laboratories should control hGH in urine.

Human growth hormone is produced naturally by the pituitary gland. In human, hGH is heterogeneous, the major component being a single-chain peptide of 191 amino acids stabilised by two disulphide bridges, with a molecular weight of approximately 22,000 Daltons (22K).
Biosynthetic hGH, produced by recombinant DNA technology, has an amino acid sequence identical to pituitary derived one.

There are two main reasons to believe that the use of hGH in sports could soon become widespread:

1) The promotion of biotechnology products has drastically increased the supplies and the availability of recombinant hGH (r-hGH).

2) Recent reports suggest an increasing non-muscular fat-free mass (1, 2) and thus increased resistance against tendon rupture in young athletes treated with supra physiological doses of hGH (3).

Aim of the study

The main purpose of this pilot study is analytical. An immunoassay (ELISA) is evaluated for the screening detection in urine of any hGH doping. Several groups are concerned: non-treated and treated non-athlete volunteers., known trained athletes and anonymous trained athletes (spot urines)(see part II in this book).

U-hGH measurements

Any test used to measure hGH in urine should be very sensitive and specific since the concentration of the hormone is usually very low in this biological fluid. For a mean integrated plasma concentration of 3.5 ng/ml, 400 µg GH would be filtered per 24 hours (4).

However, only a small proportion will finally appear in urine since most is absorbed and metabolised in the renal tubules. The urinary fraction of GH is around 0.001-0.01% of the circulating amount.
For this reason, a sensitive enzyme immunometric assay has been developed (5), which is now available through Novo Nordisk (NordiTest™) .

Material and Methods

Protocol

18 healthy male volunteers (age: 18-50; weight: 65-80; non-athletes) were followed during three days before the treatment. They were then treated in the morning with r-hGH (Norditropine ®) following the protocol defined below:

First treatment*:
5 (6 in one case) subcutaneous injections of 12 IU hGH (one every day)
Second treatment*:
3 intra-muscular injections (every two days) with increasing dosage:(6, 12 and 24IU resp.)

An aliquot of all the urines emitted were collected separately and stored at 4°C.

Assay

The NordiTest™ U-hGH assay is an enzyme immunometric assay (EIA) for quantitative determination of human growth hormone in urine. It incorporates two antibodies -one polyclonal guinea pig antibody labelled with alkaline phosphatase and one monoclonal mouse antibody adsorbed to the solid phase in the microtest plate format- and an enzyme amplification stage (AMPAK™). This later increased considerably the sensitivity compared to other types of tests. The method is applicable for direct assay of hGH in urine (i.e. unconcentrated, undialysed urine) and detect mainly 22K-hGH, the most abundant form. Its high specificity ensures that the naturally occurring forms of hGH are determined as well as the both commercially available hGH (r-hGH and methionine-hGH), but not structurally related hormones (6)

Non-athlete volunteers. without treatment

This control group was followed during 80 hours and each urine produced was collected. The frequency distribution of the concentrations (Figure 1, 18 volunteers, 262 determinations) shows that most values are lower than 10 ng/l, the mean value being 4.3 ng/l (S.D.= 4.8 ng/l).

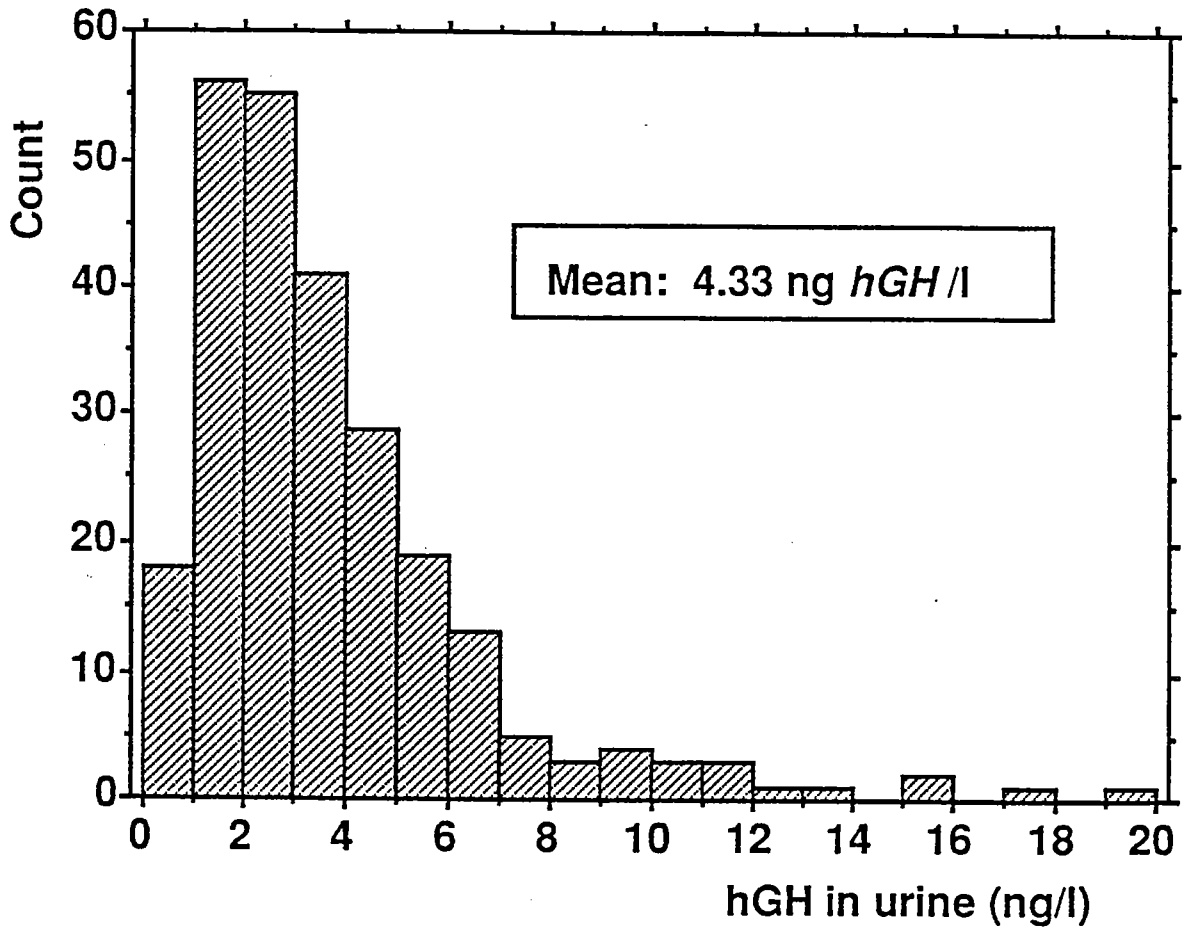

Figure 1: Frequency distribution of U-hGH in 18 healthy volonteers (n=262).

The variability in urinary-hGH (U-hGH) concentration is however quite important . Some of the subjects exhibit changes from 1 to 20 ng/l and this variability is generally not depending on the urine specific gravity or creatinine content (data not shown here, see 7) . There is no correlation between the urinary hGH concentration and specific events of normal life (sleep, meal, psychologic stress, etc.) as observed in blood (4).

Non-athlete volunteers treated with Norditropine®

Two types of treatments were applied to the volunteers. Usually, for therapeuthical purposes, hGH is applied subcutaneously (s.c.) 6 days a week for a long period. In the present case, beside s.c. applications, intramuscular (i.m.) injections were also applied in order to use a common habit spread over different risk populations (especially body-builders).

Subcutaneous treatment

After subcutaneous injection, the hGH concentration in urine rises quite rapidly to reach a peak concentration between 50 and 100 fold the initial value. The subjects present the same kind of hGH profile, whatever would be the parameter used for expression of the hGH excretion (direct concentration, total excretion, corrected by the creatinine content; data not shown). The table 1 shows the mean values of the peak urine concentrations for 9 volunteers receiving 5 subcutaneous injections during a 5 days period. If there is no significant differences between the days of treatment, it can be seen that the intervariability is quite important.

Injections	Mean hGH in urine (± Std De)
Before treatment	**4.3 ± 4.1**
1st day.	**136 ± 84**
2nd day	**185 ± 83**
3rd day	**147 ± 67**
4th day	**182 ± 73**
5th day	**202 ± 121**

Table 1: Mean values (n=9) of the peak concentration after 12 IU r-hGH s.c. injections

The figure 2 shows typical profile for one volunteerwho has received 6 times the 12 IU subcutaneous treatment. It appears that after each s.c. application, U-hGH rises to a value at least 100-fold higher than the normal level. But already 24 h later, the concentration returns back to its initial level.

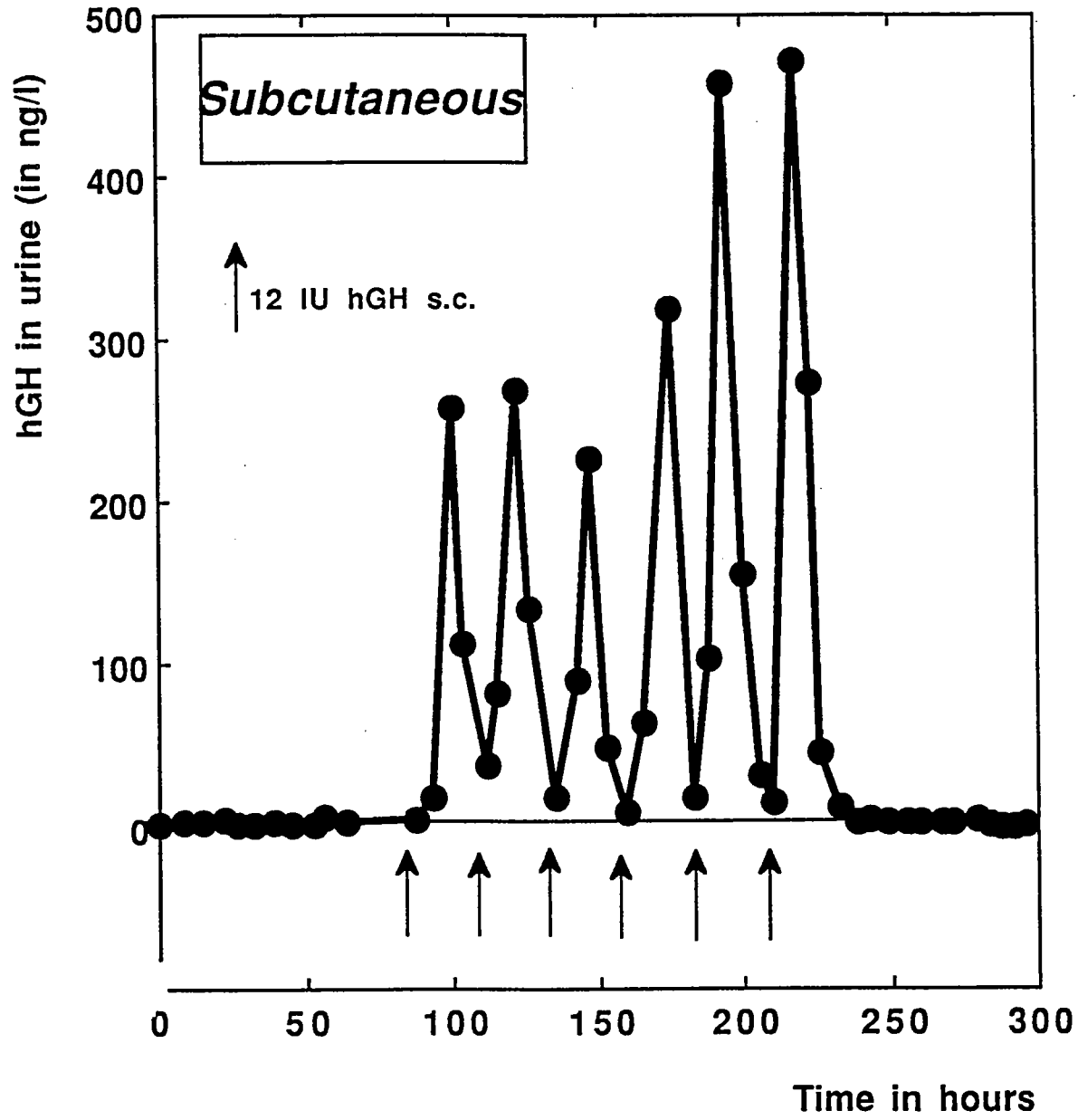

Figure 2: Urinary human growth hormone concentration (ng/l) before and during a subcutaneous treatment. The volunteer was receiving every day during 6 days 12 IU r-hGH s.c in the morning. The arrows are showing when the hormone was applied. during the experiment.

Intramuscular treatment

The intramuscular application was repeated three times with a 48h period interval. From 6 IU the first day, the dosage was increased to 12, then 24 IU in the following days of treatment. The figure 3 shows the results obtained for one of the volunteers .

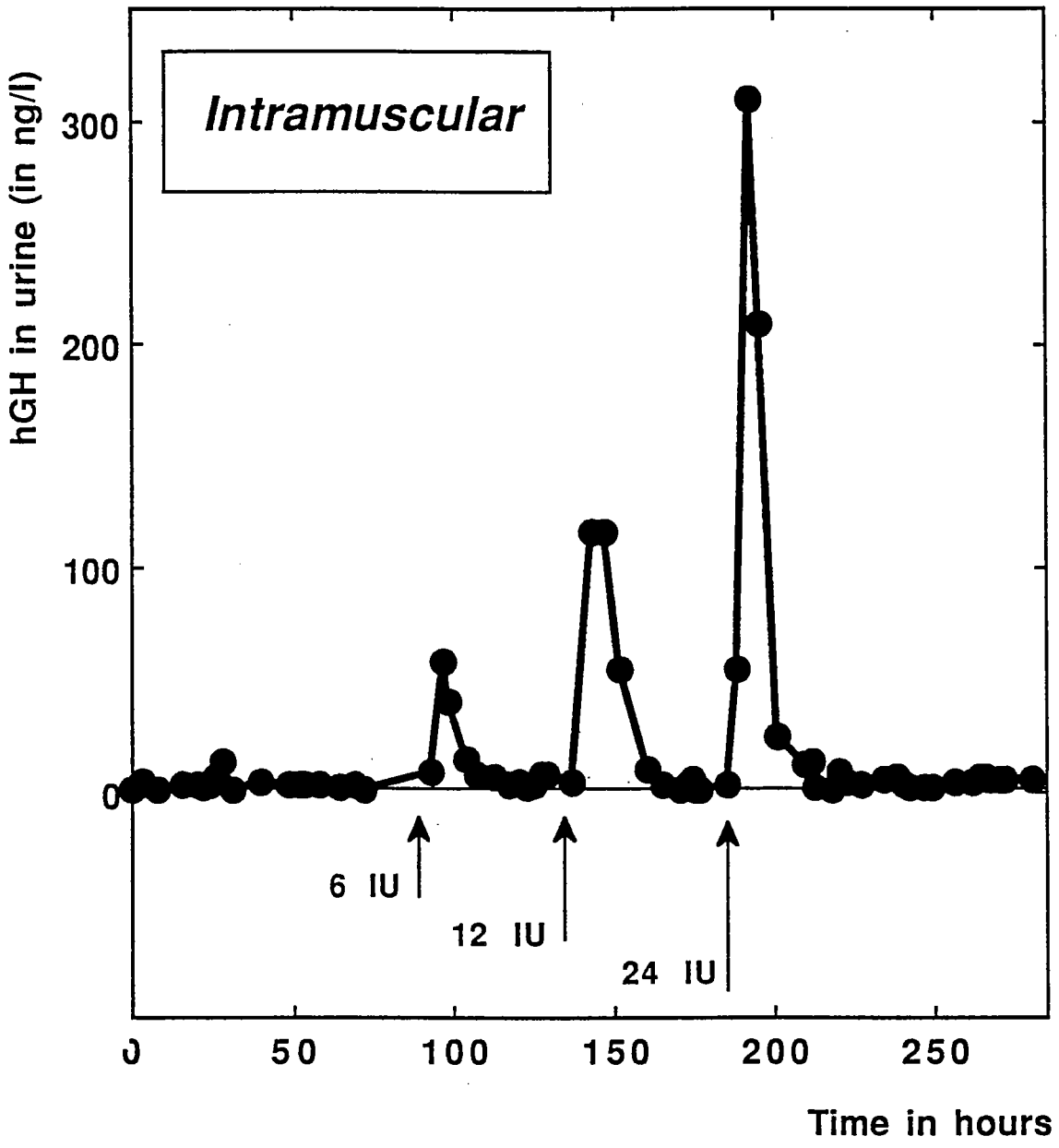

Figure 3: Urinary human growth hormone concentration (ng/l) before and during an intra-muscular treatment. The volunteer was treated in the morning every two days. He received 3 increasing doses of r-hGH i.m., respectively 6, 12 or 24 IU by day. The arrows are showing when the hormone was applied during the experiment.

For this volonteer, as for most of the subjects, the values of the maximum concentration are increasing with the dosage. Like in the s.c. treatment, the peaks are appearing almost 10-12 hours after the injection to disappear completely after 24 h. In that case also, the intervariability is high as it can be seen in the Figure 4 which shows the maximum values of hGH concentration of eight volonteers after intramuscular injections .

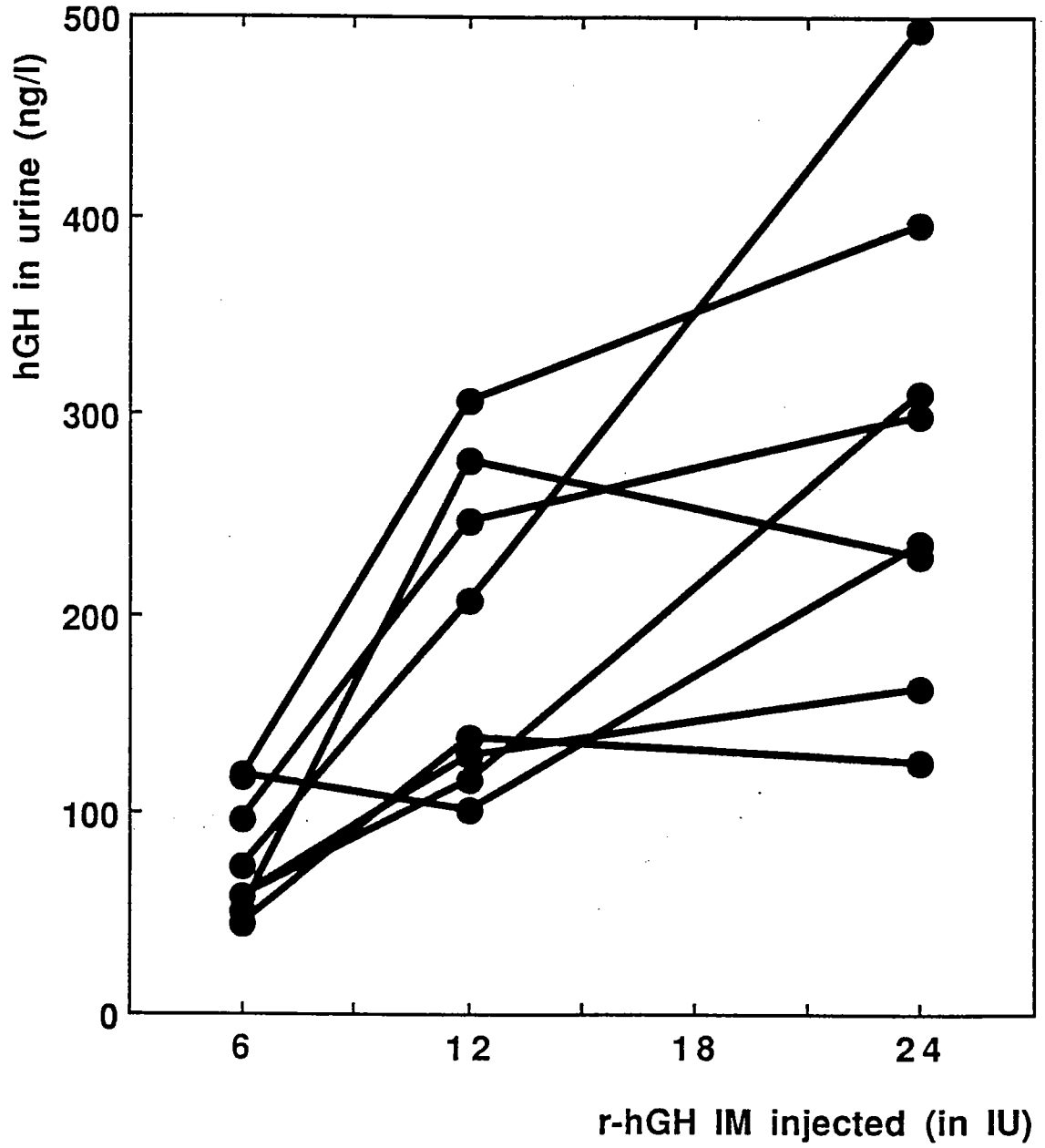

Figure 4: u-hGH peak concentration of 8 volonteers after receiving increasing dosages of r-hGH (6, 12 and 24 IU) by intramuscular injections. the interval of time between the injections is 48 h.

Conclusion

Sensitivity and specificity

The ELISA test used in the present study can achieve the measurements of U-hGH in control and athlete subjects in rest condition. The main advantage of this kit, allowing the measurements without any preparation of the sample (no concentration, no dialysis), is due to the high sensitivity of the detection system. Several tests of specificity (data not shown here) demonstrated that this kit was not cross-reacting with similar types of hormones and that no urine compounds were affecting the detection system.

U-hGH after application

After both intra-muscular and subcutaneous injections, the urinary hGH concentration increases significantly by a factor depending on the dosage. In both cases, the baseline is recovered after less than 24 hours. It can be concluded from this experiment that any doping with hGH in that range of dosage could be detected for a short period of time if the athlete was not competing before the urine collection. It is necessary then to investigate further by the study other parameters involved in that process. The measurements of insulin-like growth factor 1 (IGF-1) -this one being induced by hGH- in those urines are now in process.

Is U-hGH screening usable for the detection of hGH doping?

Of course, the answer could not be definitive. Several experiments to complete this study are still under investigation (see part II following). IGF-1, IGFBPs and other parameters implicated in growth processes are now studied on the same urine samples.
But it can be already postulated that U-hGH measurements in competition tests are not valuable for the detection of doping in some sports -due to the changes in the excretion processes in case of strenuous effort. However, an urine collection in the morning before the competition or out of competition could be considered to be in a normal range of hGH concentration. In that case, if the injection was performed the day before the urine collection (less than 12 to 18 hours), an elevated concentration of growth hormone should be detected.

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