Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (2)

M. Donike
H. Geyer
A. Gotzmann
U. Mareck-Engelke
(Editors)

Sport und Buch Strauß, Köln, 1995

M. Saugy, C. Cardis, L. Rivier, G. Brisson, C. Ayotte, P. Hemmersbach, E. Haug, J. Segura:
Urinary Human Growth Hormone (U-HGH): II. Measurements in Competition Tests: More the Expression of the Renal Function than the Endocrinological Status

In: M. Donike, H. Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in doping analysis (2). Sport und Buch Strauß, Köln, (1995) 223-233

M. Saugy[1], C. Cardis[1], L. Rivier[1], G. Brisson[2], C. Ayotte[2], P. Hemmersbach[3], E. Haug[3] and J. Segura[4].

Urinary Human Growth Hormone (U-HGH) : II. Measurements in competition tests: more the expression of the renal function than the endocrinological status .

1 Unité d'Analyse du Dopage, University of Lausanne, Switzerland
2 Montreal Anti-doping Laboratory, INRS-Santé, Pointe Claire,Canada
3 Hormone Laboratory, Aker Hospital, Oslo, Norway
4 Institut Municipal d'Investigacio Médica, Dpt de Farmacologia i Toxicologia, Barcelona, Spain.

Abstract

The urinary hGH was measured in samples provided by the anti-doping controls in major and minor competitions. Depending on the type of effort developped during the competition, the hGH concentration in urine could be dramatically increased. Although it is well known that exercise is increasing the circulant hGH in blood, the high concentration observed in some cases in urine could not be the expression of this phenomenon.
The post exercise proteinuria, quite common in humans, seems to be related to the intensity of exercise rather than its duration. After strenous work, the hGH seems to follow the same type of dynamic excretion. It is certainly due to a transient increased glomerular permeability and a partial inhibition of tubular reabsorption of macromolecules.
These observations show that any conclusion after hGH tests of competition urines could be risked or, at least depending on different parameters which should be also tested.
IGF-binding proteins and ß2-microglobulins in urine and/or in blood could be the necessary tools to investigate correctly on any hGH doping test procedure.

Introduction

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.
It has been recently shown that new progresses in ELISA technology make the detection of hGH in urine reliable, whereas the concentration is one thousand-fold lower than in blood (see part I in this book). These measurements has been shown to be very useful in the

investigations of abnormalities of GH excretion (1, 2). However u-hGH excretion depends not only on plasma GH concentrations, but also on the renal handling of the hormone (3). The kidney plays a major role in the removal of the circulating low molecular weight proteins and peptides (4) including peptide hormones such as GH. The mean hGH production in normal subjects ranges from 500 to 875 µg/day and about 0.01 - 0.001 % of that amount is excreted in the urine. This is due to extensive proximal renal tubular reabsorption and catabolism within the tubular epithelial cells (5). The tubular uptake process of GH seems to be an energy-dependent adsorptive endocytic process and is caracterized by a high capacity as compared with the normal filtred load of small proteins. Impairement of tubular uptake of proteins can give rise to massive peptiduria, up to 10'000-fold greater than in normal conditions. In diabetic population, the elevation in u-hGH was found to be 100-1'000 fold. This huge increase in u-hGH could not be attributed to the 2-3 fold elevation in plasma, but to a defect of the renal handling of GH in diabetic patients (3). The same authors found a stong positive correlation between u-hGH and u-ß2- microglobulin in the diabetic cases indicating that both factors are handled by the kidney in the same manner.

Strenous physical exercise proteinuria is a common phenomenon in humans. It seems to be related to the intensity of exercise rather than to its duration. This excretion of proteins in urine is transient with a half-time of approximatively 1 hour (6). It is depending on the type and the size of proteins and a correlation can be drawn between proteinuria and the intensity of exercise expressed by the formed lactate (7). The increased clearance of plasma proteins and peptides during strenous effort suggests an increased glomerular permeability and a partial inhibition of tubular reabsorption of these molecules. (6).

Aim of the study

Our laboratory has the mandatory to analyse the urines collected after major competitions from all kinds of sport in Switzerland. The aim of this part of the study was first to measure u-hGH concentration in urines provided by volonteers before and after competition to establish a reference values for athletes. It is known that the physical as well as the psychological stress influence the circulating hGH into plasma (8; 9). But the influence of sport practice on the urinary GH had not been investigated.

Secondly, some urines provided by the swiss anti-doping control system (at the national and international level) were analysed. Moreover, the Barcelona laboratory was able to provide urines collected for the controls during the Olympic games in Barcelona.

Material and Methods

Volonteers in competition

Before and after a well-known 17 km-long race, 130 male volonteers were given urines in order to measure the influence of effort on their urinary hormonal profile. Beside urine collection, several clinic observations were done which will not be reported here.

Anonymous samples from the anti-doping control system.

U-hGH was measured in urines provided by anonymous athletes practising 5 different sports where competitions were organised at international level: track and field, cycling, rowing, volleyball and weightlifting.

U-hGH analysis and other tests

All the samples were analysed with the Norditest (graciously offered by Novo-Nordisk) already presented in the first part of this text. Some comparative analyses were performed with the Delfia system (from Pharmacia) based on a two-site fluoroimmunometric assay . Urinary IGFBP-3 and ß2-microglobulin were both radio immunoassays graciously obtained from Nichols Institute Diagnostics (Geneva, Switzerland).

Results and discussion

Urinary hGH before and after 17 km race

130 athletes (a mixed population of national and regional level of runners) were tested for u-hGH before and after a 17 km-long race. Because of the differences of hydration status of each individual, the results are normalised with the creatinine content of urine. The results show (Figure 1) that after the race, most of the athletes exhibit an elevated concentration of u-hGH. The mean values of each population are of course significantly different :

Before the race:	**12.6 ± 1.3 ng/g creat (± S.E.)** ***4.0 ± 0.2 ng/l***
After the race:	**65.2 ± 10.4 ng/g creat (± S.E.)** ***62.8 ± 7.9 ng/l***

If the mean value (4.0 ng/l) obtained before the race is not different from that measured on the untreated volonteers in the first part of the protocol (see part I in this book), after the race, the concentration of hGH reached by some of the runners is close to the level measured after hGH treatment. (30 urines showed concentration between 100 ng/l and 600ng/l, which is almost the range of peak concentration after subcutaneous injections of 12 IU r-hGH).

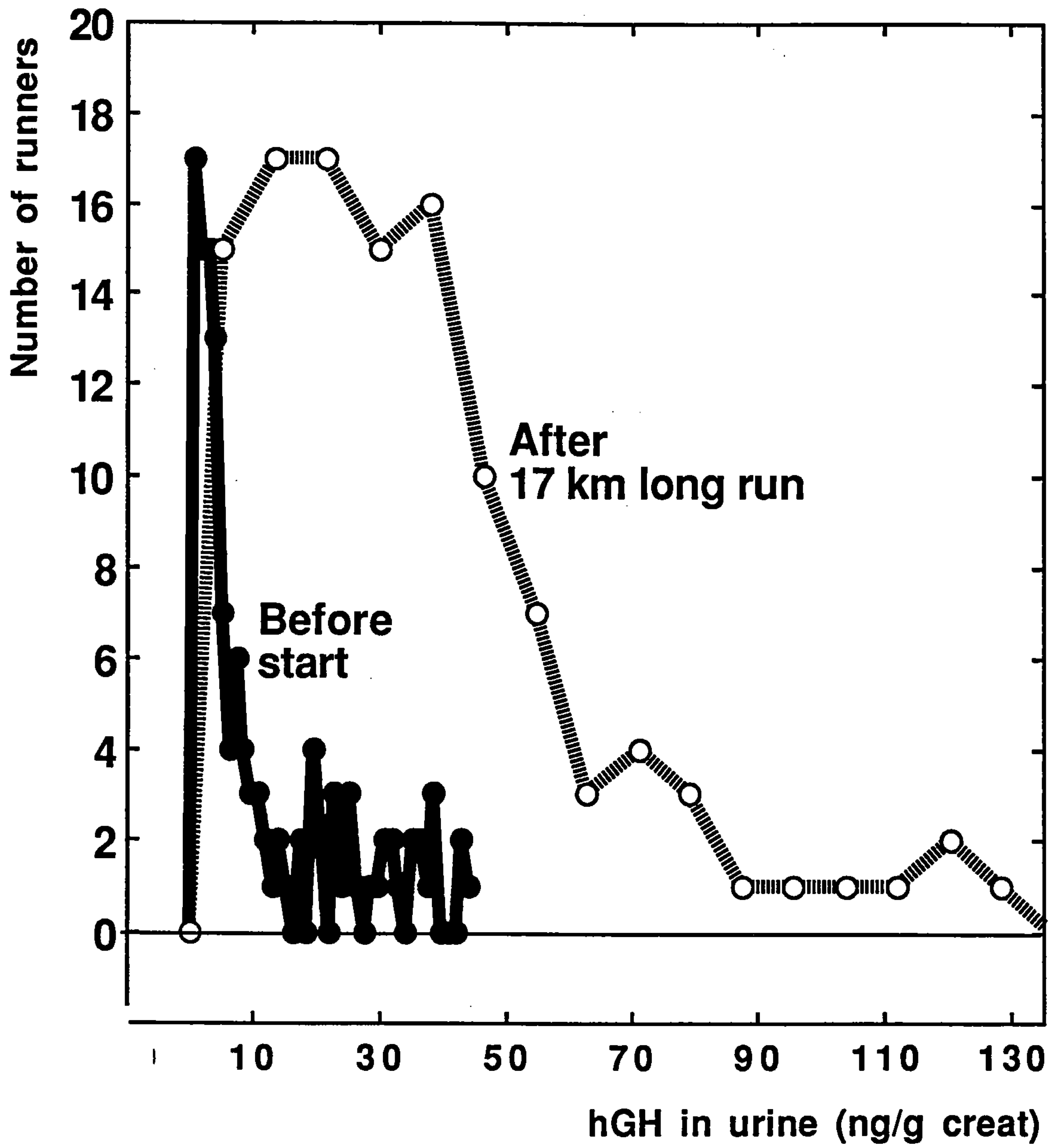

Figure 1: Frequency distribution of u-hGH (in ng/g creat) in a population of 130 athletes before and after a competition. In the after competition values, ten high values (between 130 and 1000 ng/g creat) are not shown to better read the figure.

Anonymous samples

U-hGH was measured in anonymous spot urines collected after competitions of cycling (Tour de Suisse) and track and field at the international level held in Switzerland. The frequency of distribution of hGH concentrations are shown in the Figure 2. The results obtained after competition in cycling are quite similar to those after a middle distance run (see Fig 1), whereas in track and field, some urines are showing huge content of hGH.

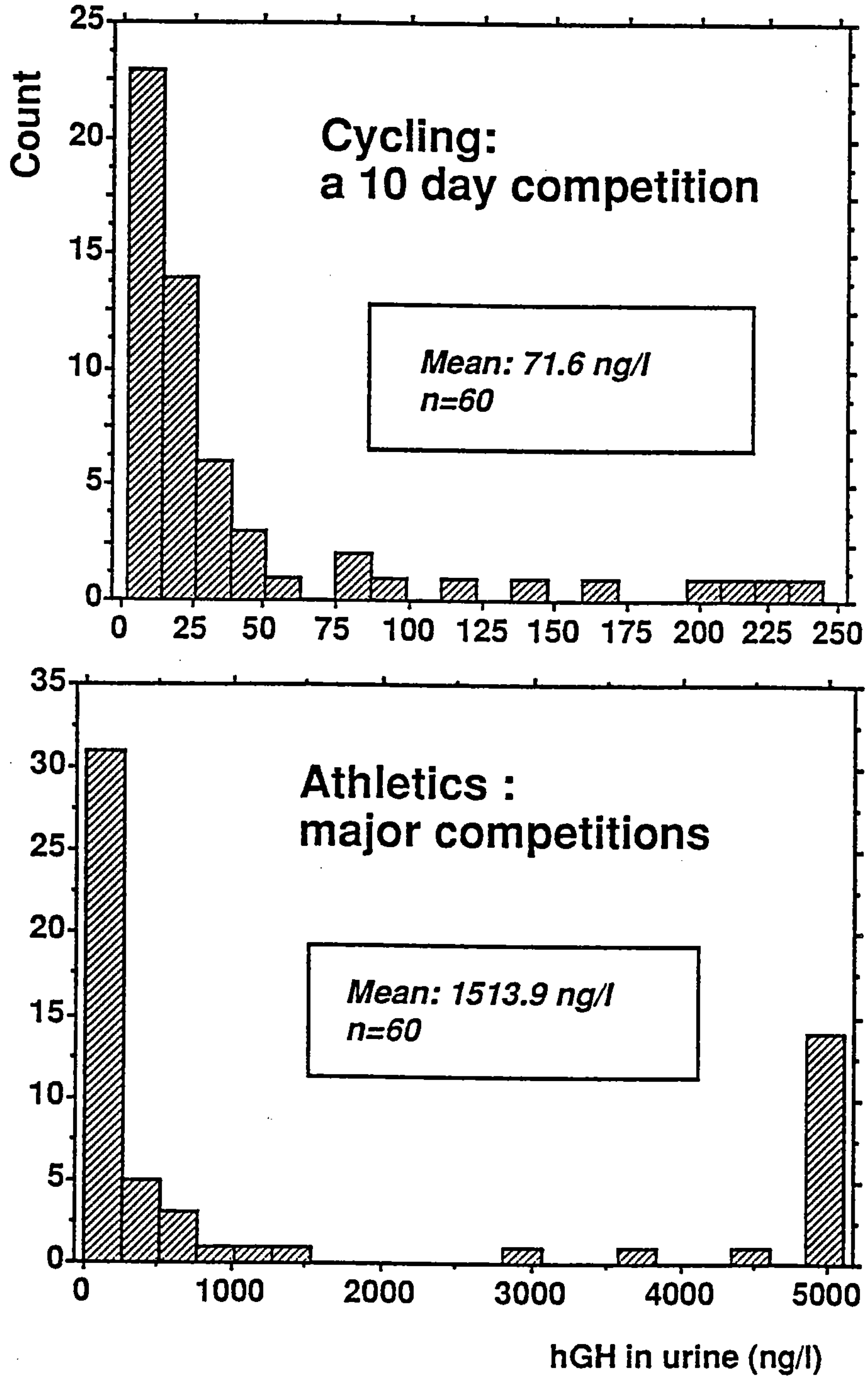

Figure 2: Frequency distribution of u-hGH values of samples from the "Tour de Suisse"(cycling) and major competition in track and field.

Several controls on the specificity of the test were performed at that time because of this very high response in some specific disciplines. Biochemical investigations did not reveal any unspecified reaction of the test in case use of competition sample. Moreover, the samples from major "Grand Prix" track and field events were measured in parallel by using two commercial immunoassays systems, based on two different antibodies and detection reaction (Norditest® from NovoNordisk and Delfia® from Pharmacia). The comparison between these two systems performed by two laboratories (Oslo and Lausanne) gave similar results and confirmed the high concentrations obtained in several disciplines (data not shown).
The Figure 3 is summarising the mean values obtained in several routine competition samples analysed in our laboratory.

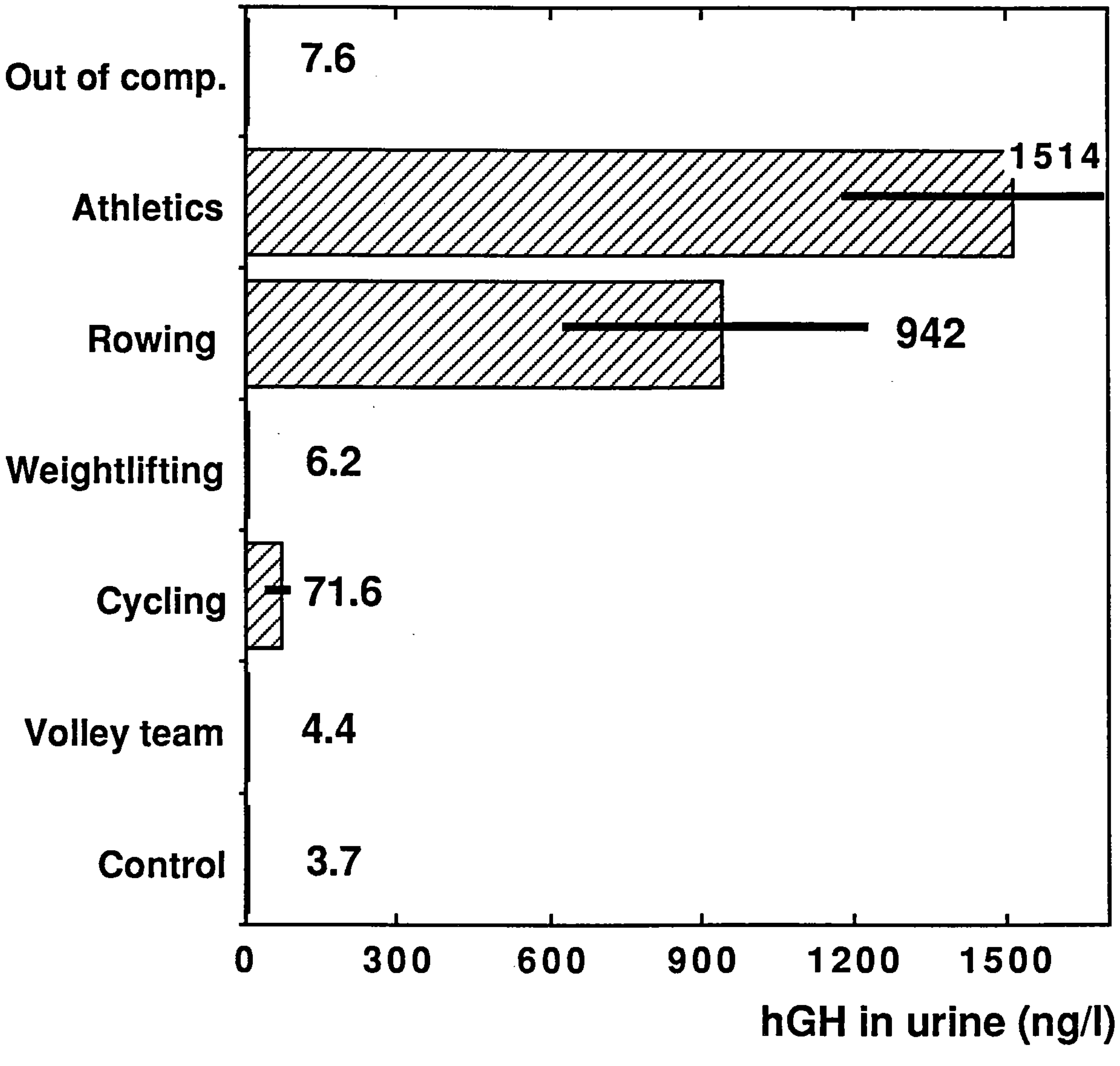

Figure 3: mean values of U-hGH measured in anonymous urines collected after major competitions in different sports. Number of samples per groups were: 50 for *out of competition* . 60 for *athletics;* 30 for *rowing;* 30 for *weightlifting;* 60 for *cycling;* 179 for *volley team;* 114 for *controls*

Data show that in competitions of track and field and rowing, the mean concentration of the hormone is very high and differs considerably from other sports and from the controls. Looking into the different disciplines in track and field, it seems that middle distance runners are providing the highest results. This type of effort can be compared to rowing in the fact that it is a relatively short (between 1 and 15 min) but very intense effort.

Because the Olympic games are generally providing a large number of samples from different types of sports during a short period and in the same condition of collection, 100 samples from Barcelona 1992 were analysed to verify this discipline-dependent increase in u-hGH. the results are summarised in Figure 4.

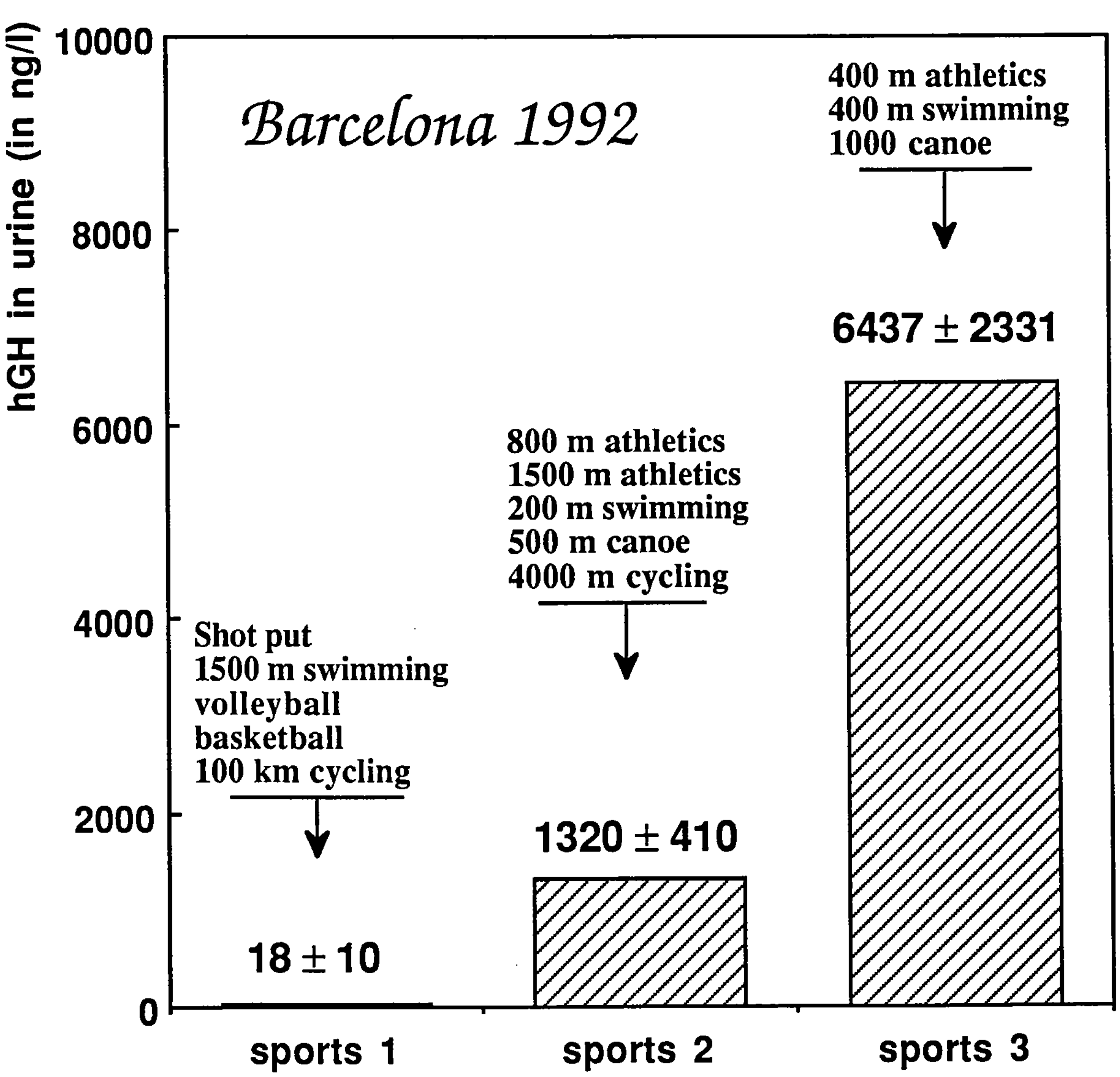

Figure 4: mean values of u-hGH measured in urines collected during the Barcelona Olympic games. Total number of samples: 100.

Those results are similar to those obtained with previously (see Fig.3). The high excretion in case of sports 2 and 3 could not be simply explained by an increase, due to the exercise, of the circulating hGH in the body. It is sure that under these conditions, hGH excretion in urine is increased because a modification of the renal function in that kind of effort.

It was demonstrated that both glomerular filtration and tubular reabsorption are implicated a higher clearance of proteins by a strenuous exercise (6). Proteinuria is induced and is mostly depending on the intensity of the effort rather than duration. The size of the protein is also determinant: while the clearance of albumin (M.W.: 69'000 daltons) increase nearly 30-fold under the influence of strenuous exertion, lysozyme (15'000 daltons) and ß2-microglobulin (11'500 daltons) increases 160-fold as compared to resting values. It can be assumed that hGH is affected in the same way. The kidney plays a major role in the removal of the circulating low molecular weight proteins and peptides (4) including peptide hormones such as GH. Turner et al (4) already found such huge increases of u-hGH in diabetic patients presenting a defect in the renal handling of hGH.

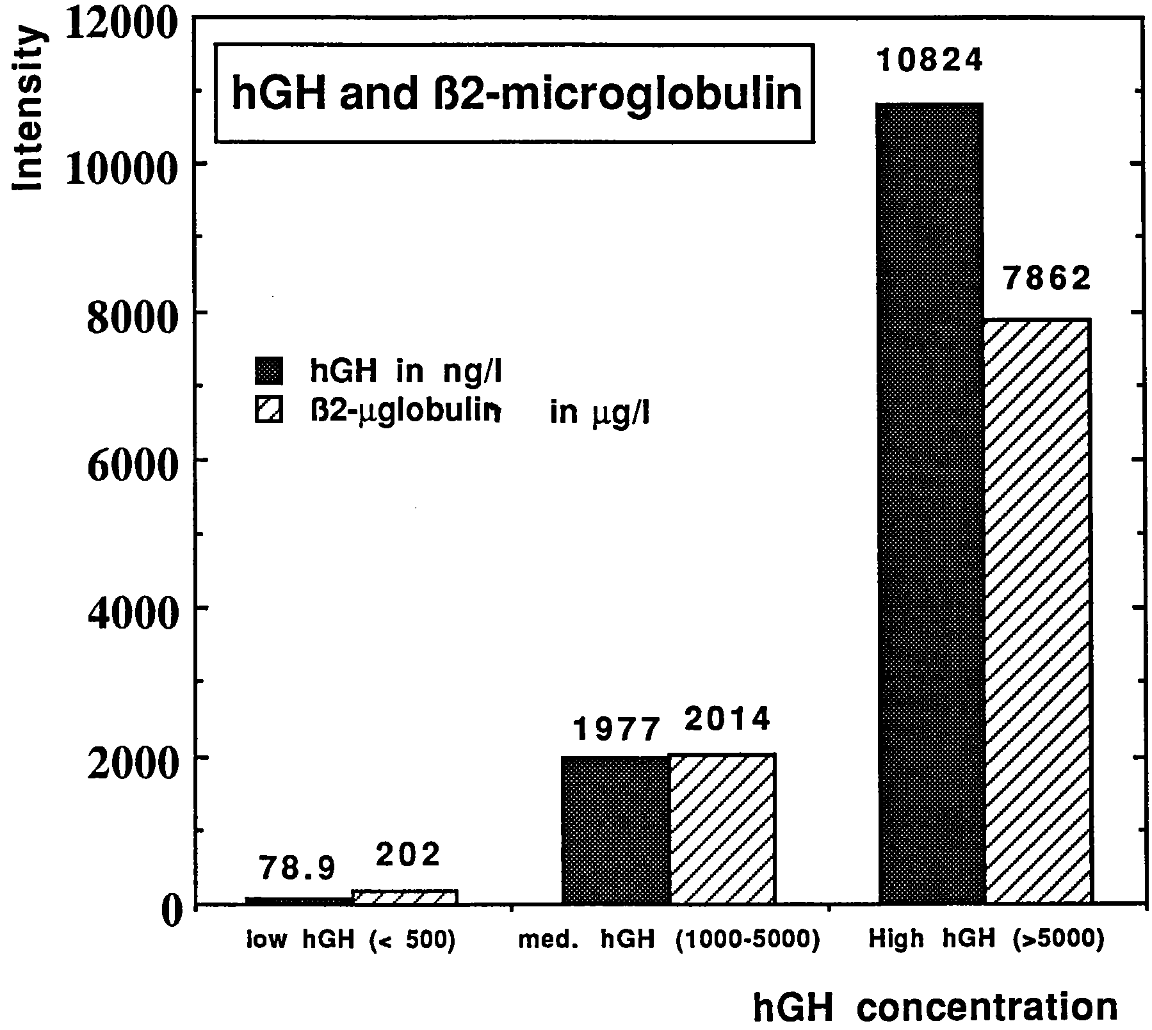

Figure 5: mean values of U-hGH and ß2-microglobulin measured in urines collected during the Barcelona Olympic games. Total number of samples: 60

Those authors suggest that ß2-microglobulin and hGH excretion are very similar, because of the strong correlation between these two parameters obtained in diabetes mellitus cases.
This hypothesis was also tested on these Olympic samples and the results are drawn on the Figure 5. Data shown here are preliminary results. In fact, the stability of both ß2-microglobulin and hGH with time in urine samples stored at -30°C has not been investigated. But, by forming three groups of low, medium and high values of hGH, there is a clear relation between these two parameters. Normal value for healthy man of urinary ß2-microglobulin is lower than 200 µg/l (10). This clearly indicates that the excretion of this peptide is dependent on physical exercise. Furthermore, one could suggest that ß2-microglobulin could be taken into account to control that kidney function is intact or not.

IGFBP-3

Recent development of specific radio immunoassays for IGFBP-3 have permitted the accurate quantification of IGFBP-3 in several biological fluids (11). The measurement of this binding

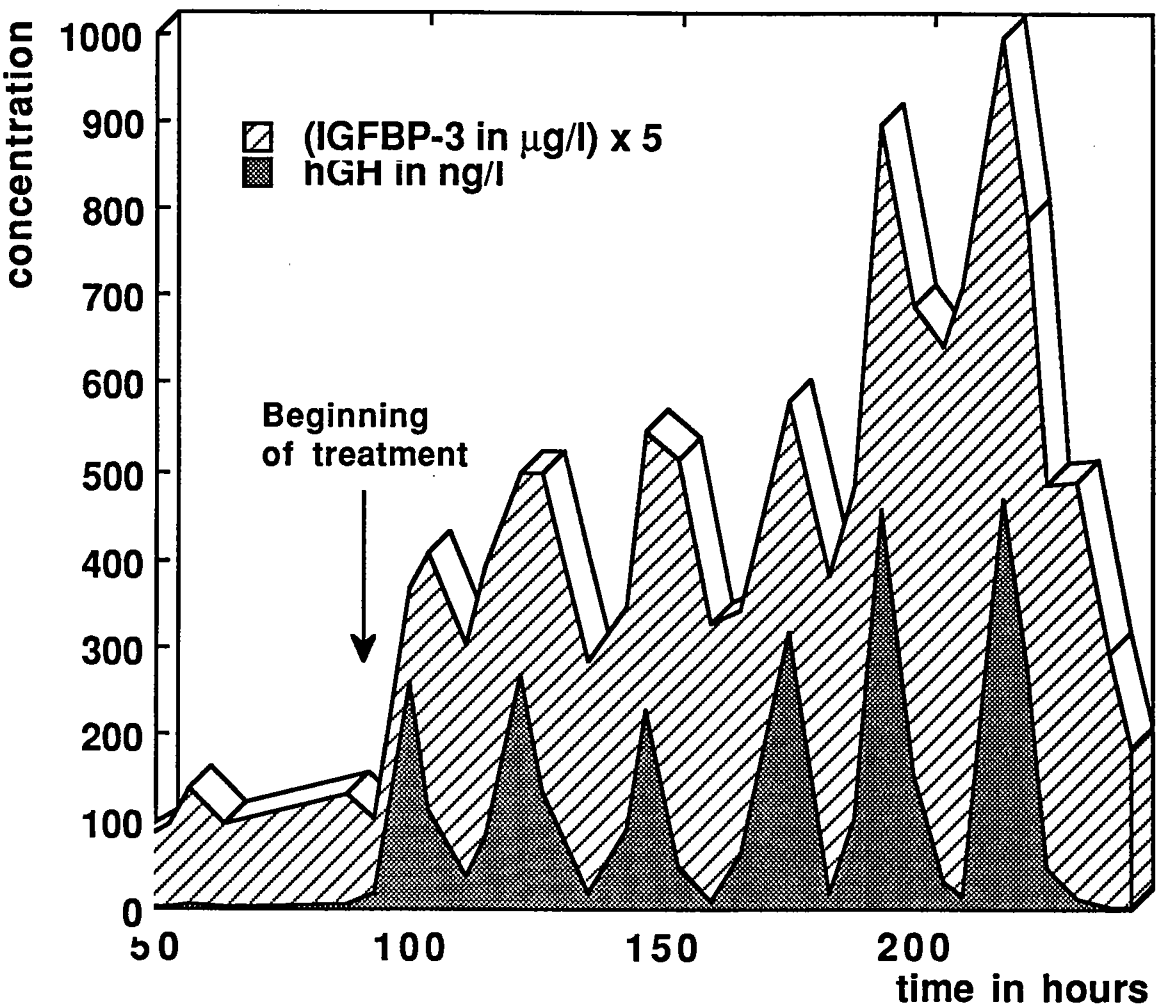

Figure 6: hGH and IGFBP-3 concentrations in urine from a r-hGH subcutaneously treated volunteer (6 sc inj., 12 IU rhGH)

protein, seems to be now more useful clinically than the value of the growth factor itself (insulin-like growth factor 1: IGF-1, which is produced under the control of hGH). The knowledge of both hGH and IGFBP-3 levels in serum has already been described to be used for diagnosis of GH deficiency (11).

This binding protein has been measured in urine samples from a recombinant hGH treated volunteer (one subcutaneous injection of 12 IU r-hGH every day for six days; see part I in this book) and the results are shown in Fig 6. They show that IGFBP-3 is increasing during the treatment. The difference between normal level (before the treatment) and the maximum peaks after the injections are not as marked as it is the case for hGH, but the normal level seems to be reached only 36 hours after the last injection. Further experiments are now under investigations in our laboratory to test the effect of a long term treatment of r-hGH on the IGFBP-3 level in urine.
The same samples were also tested for ß2-microglobulin and the results (not shown on the graph) indicate a normal value (< 200 µg/l) for all the samples during the treatment.

Conclusion

Proposal for hGH doping detection

Based on the results presented in this paper, some important parameters should be taken into account in order to propose a screening procedure for hGH doping:

1) Urines should be out of competition samples
2) ß2-microglobulin should be analysed to attest that no alteration of the kidney function occurred (due to strenuous effort or any kind of kidney dysfunction).
3) Urinary hGH should be analysed and if the concentration is higher than 100 ng/l, further investigations could take place:
- follow up of the athlete (take other urine samples)
- analysis of secondary parameters: binding proteins and IGF-1 in urine and blood.

To attest the efficiency of such strategy, further experiments are planed to work with real cases in body building, a sport especially exposed to the use of hGH.

Acknowledgements:

We would like to thank the Novo Nordisk company (in Denmark) and especially Mrs Suzanne Landolt and Mr Bertrand Picard (Novo Switzerland) and Nichols Institute in Geneva

and Wijchen Netherlands for providing graciously their constant technical support in this protocol .
All the technicians and the administrative staff from the UAD is gratefully acknowledged for their intense and meticulous work.

References

1. Tanaka T, Yoshizawa A, Miki Y et al . Clinical usefulness of urinary growth hormone measurement in short children. Acta Paediatr Scand , 366 (suppl.), 155-158, 1990.

2. Evans AJ, Willis DS and Wood PJ The assay of urinary growth hormonein normal and acromegalic adults. Clin Endocrinol , 35, 413-418, 1991.

3. Turner G, Coates P, Porter S, Peters JR and Woodhead JS. Urinary growth hormone measurements as a marker of renal tubular function in diabetes mellitus. Clin Chem Acta, 220, 19-30,1993.

4. Rabkin R and Kitaji J. Renal metabolism of peptide hormones. Mineral Electrolyte Metab, 9, 212-226, 1983.

5. Maak T, Johnson V, Kau ST, Figueiro J and Sigulem D. Renal filtration transport and metabolism of low molecular weight proteins. A Review. Kidney Int. 16, 251-270, 1979.

6. Poortmans JC. Exercise and renal function. Sports medicine 1: 125-153. 1984

7. Poortmans JC, Labilloy D, Niset G and Sellier M,. Relationship betweenpost-exercise proteinuria and venous lactate. Med Sci Sports Exercise 13, 84, 1981

8.Macintyre JG. Growth hormone and athletes. Sports Medicine. 4, 129-142. 1987

9. Felsing NE, Brasel JA and Cooper DM. Effect of low and high intensity exercise on circulating growth hormone in men. J. Clin. Endocr. Metab. 75 (1), 157-162. 1992.

10. Wibell L The serum level and urinary excretion of ß2-microglobulin in health and renal disease. Path. Biol, 26, 295-301, 1978

11. Blum WF, RankeMB, Kietzmann K, Gauggel E, Zeisel HJ and Bierich JR. A specific radioimmunoassay for the growth hormone (GH)-dependant somatomedin binding protein: its use for diagnosis of GH deficiency. J. Clin Endocrinol Metab, 70, 1292-8, 1990.