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

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P. HEMMERSBACH, K.I. BIRKELAND, B. BJERKE, T. SAND, N. NORMAN:
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P. Hemmersbach, K. I. Birkeland, B. Bjerke, T. Sand and N. Norman

The use of Endocrine Parameters in the Evaluation of Doping Analysis Results

Hormone Laboratory, Oslo, Norway

Introduction

The International Olympic Committee has based its definition of doping on the establishment of forbidden drugs and methods. This means that a condition for punishing an athlete is the proof that he/she has either taken a banned drug or used a banned method. The biological fluid we have been using for providing analytical proofs is urine but some international federations as well as the IOC have now opened for using blood samples for this purpose.

As long as we are talking about exogeneous compounds, the identification of the banned drug and/or its metabolites is regarded as a direct proof for administration of the banned drug in question. The problems start when administration of endogeneous substances as testosterone, erythropoietin or growth hormone is to be proved or when the administration of anabolic androgenic steroids should be proved beyond the excretion period of the drug (by monitoring changes in the endogeneous steroid profile). In these cases we deal with indirect proofs normal range problems and statistical probabilities. Any additional criterium will enhance this indirect proof and improve the effectiveness of our controls.

The control of testosterone administration is today based on the ratio between testosterone and epitestosterone. Below 6 a sample is regarded as negative, between 6 and 10 the IOC recommends additional tests before making a decision and above 10 it is regarded as positive.
A lot of additional criteria have been discussed in connection with testosterone administration or long term use of anabolic steroids. All these criteria are based on endrocrinological influences of the use of anabolic androgenic steroids on the excretion of regulating hormones and steroid metabolites. The ratio between testosterone and luteinizing hormone (LH) has been proposed to indicate testosterone or HCG administration[1,2]. Steroid profiling[3] has shown reduced levels of endogeneous steroids after long term use of anabolic steroids and a test with ketoconazole administration is able to distinguish between endogeneous and exogeneous testosterone[4].

In this connection we have gathered some data on luteinizing hormone in urine. Examples from longitudinal follow ups are presented and the first experiences of additional information by analyzing blood samples in doping control are discussed.

Methods

Advances in immunological methods for gonadotrophine determination have decreased the limit of detection to levels in the low physiological range with accuracy. The method we use is derived from our routine method for analyzing LH in serum and was modified in collaboration with the company selling the kits (Pharmacia Diagnostics AB, Uppsala, Sweden: DELFIA, hLH Spec)[5-7]. It is a solid phase, two-site fluoroimmunometric assay based on the direct sandwich technique, in which two monoclonal antibodies are directed against two seperate antigenic determinants on the ß-subunit of the LH molecule (see Fig. 1). The standards have been calibrated against the WHO 2nd International Standard for pituitary LH for immunoassay (coded 80/552). The limit of detection is 0.02 U/l and the cross reactivity to HCG 1.5%. We observed in urine intra- and inter-assay coefficients of variation (C.V.) lower than 5% for three different levels of concentration. In an interlaboratory control we correllated our results with those of the IOC-accredited laboratory in Sweden, and we found for 42 urine samples (LH < 2 U/l) a correlation coefficient of 0,96[8].

Results

Fig. 2 shows the distribution of LH concentrations in 1030 routine urine samples (male athletes) compared with the distribution of LH concentrations in the controls of males taken in fitness studios and from the Norwegian Bodybuilder Federation. The values were corrected linearly (to 1,020 g/ml) for differences in urine density.

In Table 1 a follow-up of two positive testosterone cases are presented, where the additional samples were taken four and five weeks after the first test.

Athlete	Date	T/E ratio	LH (U/l)	T/LH ratio (nmol/U)
I	12.10.91	8,7	< 0,02	4700
I	8.11.91	3,1	1,2	35
I	15.11.91	2,8	1,1	42
II	15.10.91	6,7	6,8	56
II	15.11.91	6,6	4,3	97
II	22.11.91	7,4	7,1	42

Table 1: Measurement of LH in urine in following-up cases of increased T/E ratios.

Table 2 shows some quantitative results from the first international competition were blood and urine samples were taken in Norway. Additionally to the analyses mentioned in Table 2 it was measured FSH, 17-hydroxyprogesterone, hematocritt, hemoglobin and erythropoietin. All quantitative results of 4 male athletes were in the normal physiological ranges.

	S-LH (U/l)	S-Testosterone (nmol/l)	S-Estradiol (nmol/l)	SHBG (nmol/l)	U-LH (U/l)	U-T/E
Reference intervall	<12	0.3 - 2.8	0.11 - 1.85	30 - 90		
Athlete 1	4.7	4.9	0.13	28	2.6	5.4
Athlete 2	9.3	2.6	0.33	60	2.5	2.2
Athlete 3	1.4	1.4	0.06	46	1.3	3.9
Athlete 4	1.0	2.5	0.04	11	1.2	4.9

Table 2: Blood analyses in doping control. Results of 4 female participants.

Discussion and Conclusion

LH concentrations in spot-urine samples show a broad range of normal values. Nevertheless, LH values in the very low (< 1 percentile) range, strongly indicate long term use of anabolic androgenic steroids. Because our LH-assay is sensitive down to 0,02 U/l, it allows an evaluation of increased T/E ratios due to long term use of anabolic steroids, and not necessarily caused by the use of testosterone.

In the case of athlete I in Table 1 the results of the 1) LH measurements in the urine and 2) repeated testing, strongly support our suspicion that the elevated T/E ratio is a result of administration of anabolic-androgenic steroids.

The follow-up of athlete II did not give decisive conclusions and without further tests, e. g. by administering ketoconazole, it could not be excluded that the ratio was due to a normal variant of endogenous steroid synthesis and metabolism.

We conclude that measuring LH in urine on a routine basis or in cases with elevated T/E ratios and/or a suppressed steroid profile will give additional information which may indicate doping.

The main value of measuring endocrinological parameters in urine and blood will come from longitudinal studies of athletes. The intraindividual variations in endocrine parameters generally are far less than the normal variations between individuals, and longitudinal studies also circumvent some of the problems of harmonizing immunological methods between different laboratories. To accomplish such follow-up analyses on athletes, it is necessary for each laboratory to have a near contact to the sports federations conducting doping controls.

The use of blood analyses as a supplement to urine analyses in doping control, will give the opportunity to follow individual athletes with respect to several endocrine parameters. Already our first experiences with blood samples taken at an international competition in Norway indicate the importance of repeated sampling from individual athletes. The combination of laboratory values found in two of the four women may be seen in women with androgen excess due to overproduction of androgens (e.g. the polycystic ovary syndrome or adrenocortical enzyme deficiencies), but also could result from anabolic androgenic steroid use. We don't want to overinterprete these results but T/E ratios of > 4 and high serum testosterone should be an indication for further unanounced testing. However, it may be that these variants with relative androgen excess are overrepresented in the group of top female athletes.

The discussion on the use of blood samples in doping control has to be intensified, especially among the IOC accredited laboratories. Therefore we want to draw your attention to the Second International Symposium on Drugs in Sport in cooperation with IUPAC: Towards the Use of Blood Samples in Doping Control?, which will be held in Lillehammer, Norway, August 29th - 31th, 1993.

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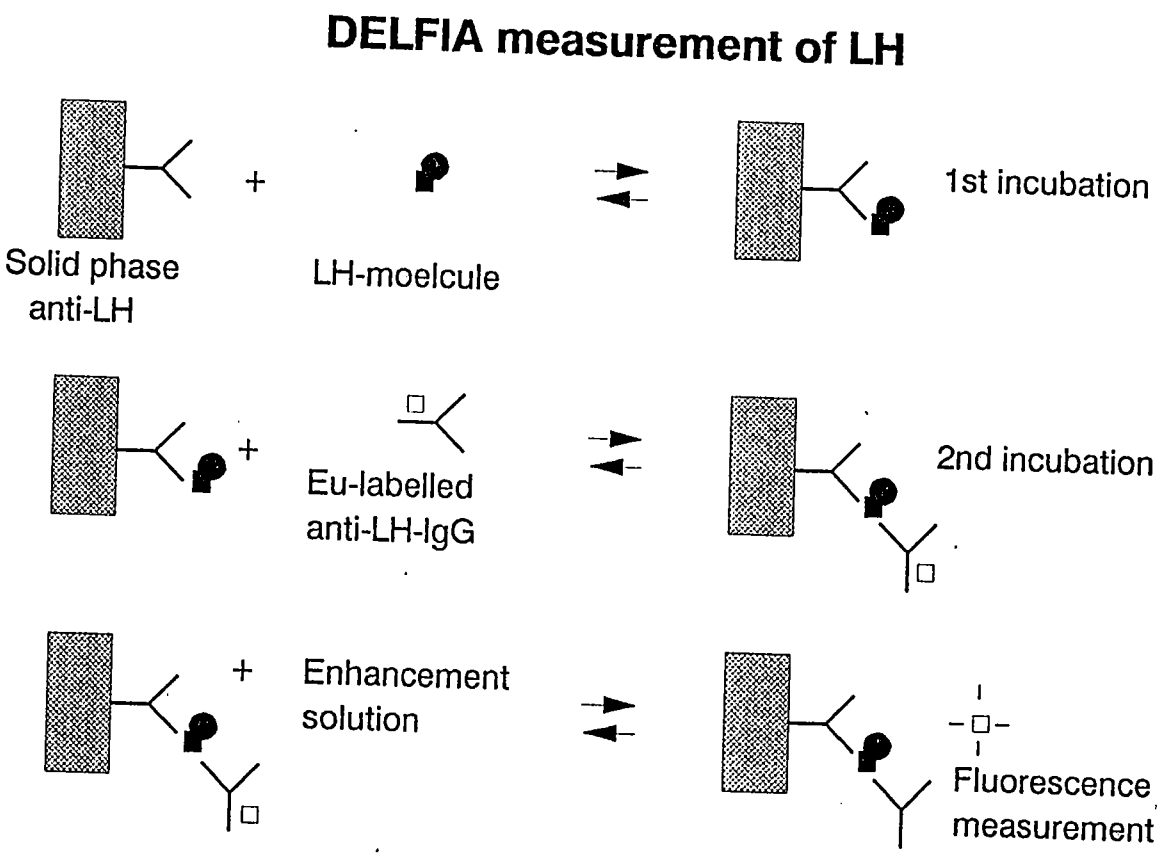

Fig. 1 Principles of the LH measurement with DELFIA (Dissociation Enhanced Lanthanide Fluorescence ImmunoAssay)

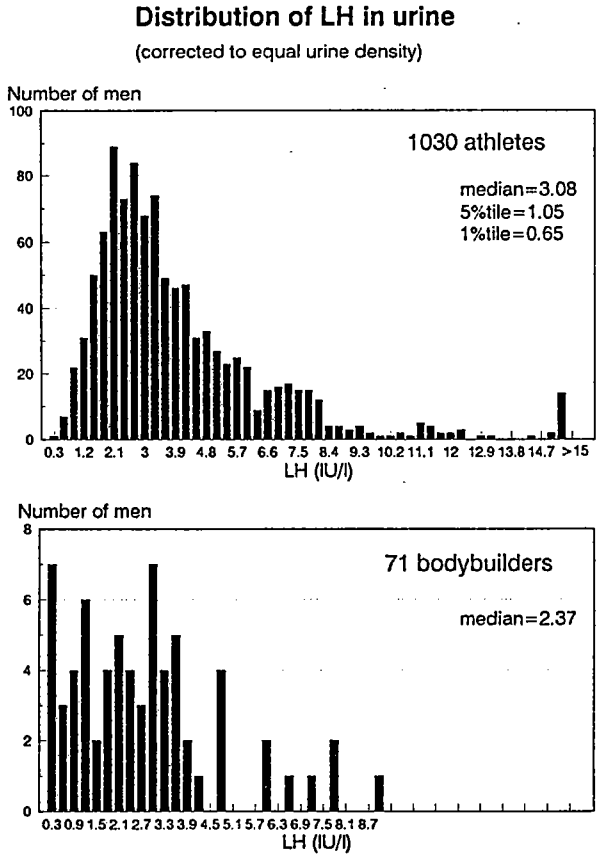

Fig. 2 Distribution of LH concentrations in urine.