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Steroidprofile and Peptides Hormones in Urine of Argentine Athletes.

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INTRODUCTION

Administration of endogenous substances can be detected by alteration of known physiological variables (indirect markers). In the case of doping with endogenous steroids, the first step toward its detection is the exhaustive study of the steroidprofile parameters in urine of a given population, the same holds true for peptide hormones such as hCG and LH. Human Chorionic Gonadotrophin has been used by some athletes to stimulate the endogenous secretion of testosterone, in these cases T/E ratio remains constant but T/LH ratios show a grate increase because of the negative feedback on LH secretion [1]. A statistical evaluation of these results may indicate any deviation from their normal values and consequently a dope case.

Since 1999-second semester, this laboratory has determined the steroidprofile and hCG and LH quantification analysis of samples from Argentinean male athletes from sports as football, basketball, volleyball, rugby, cycling, etc. SterOIdprofiles from 3504 samples were measured, employing gas chromatography-mass spectrometry acquiring data in SIM mode. Values obtained were corrected to urine density of 1.020 g/cm^3. hCG (whole fraction) and LH, from 2350 and 2178 samples respectively were determined using Abbot IMx procedure.

This work presents the steroidprofile, hCG level and T/LH ratio descriptive statistics, which are compared with results obtained previously by other authors [2-5].

EXPERIMENTAL

Sample preparation. For the establishment of steroidprofile, urine (4.0 ml) was treated accordingly to the habitual screening procedure for anabolic steroids [2]. For quantification of hCG and LH all samples were subjected to a previous centrifugation step [4].

Chromatographic and mass spectrometric conditions. The chromatographic system consisted of a Hewlett-Packard (Palo Alto, CA, USA) 5890 Series II Plus gas chromatograph, equipped with an autosampler/autoinjector, coupled to a Hewlett-Packard 5972 Mass Selective Detector. The column used was an HP Ultra-1 (25 m x 0.2 mm x 0.11 μm) operated at a constant head pressure of 12 psi. Helium flow was 0.38 ml/min (190 °C). Injections were performed in the split mode and the split ratio was 10:1. The injector was maintained at 280 °C, and the interface at 310 °C. The temperature program used was: T1, 190 °C; rate1, 2 °C/min; t1, 0 min; T2, 235 °C; rate2, 25 °C/min; t2, 0 min; T3, 310 °C; t3, 5 min. The mass spectrometer worked under electron ionization conditions with an ionization energy of 70 eV, and a repeller voltage of 30 V. Data were acquired in the SIM mode. In table 1 are presented the endogenous steroids, which are monitored for steroidprofiling and their corresponding ions. All of them were acquired with a dwell time of 100 ms [6].

Table 1. Endogenous steroids monitored and their ions, which are measured to establish steroidprofiles normal value.

Substance	Abbreviations	Rt (min.)	Calibration standard (ng/ml)	*m/z*
Androsterone	And	16.17	2000	434
Etiocholanolone	Etio	16.39	2000	434
Epitestosterone	E	18.99	80	432
Testosterone	T	20.41	80	432
5α-Androstane-3α,17β-diol	5α-diol	16.66	100	241
5β-Androstane-3α,17β-diol	5β-diol	16.82	100	241
5β-Pregnane-3α,20α-diol	Pregnandiol	23.92	1000	117
5β-Pregnane-3α,17α,20α-triol	Pregnantriol	24.93	1000	255
Dehydroepiandrosterone	DHEA	18.21	100	432
Dihydrotestosterone	DHT	19.46	100	434
11β-Hidroxyandrosterone	11OHAnd	20.77	320	522
11β-Hidroxyetiocholanolone	11OHEtio	21.08	160	522

Microparticle enzyme immunoassay (MEIA) determinations. Peptide hormones measure were made using the Abbott IMx procedure (Abbott Park, Ill., USA), which determines the whole molecule associated β-subunit of human chorionic gonadotrophin and β-subunit of the luteinizing hormone. IMx system was calibrated using a hCG and LH reagent packs respectively.

Statistic determinations. The SPSS 9.0 for windows (SPSS Inc., Chicago, IL., USA) was used for statistical evaluation of the results.

RESULTS AND DISCUSSION

Steroidprofile descriptive statistics. Tables 2 and 3 show the statistics results of the concentrations and concentration ratios.

Table 2. Descriptive statistics of the concentration of endogenous steroids in male Argentinean athletes (n= 3504).

Substance	Mean (ng/ml)	2.5 per0c. (ng/ml)	97.5 perc. (ng/ml)	Median (ng/ml)	Std. deviation (ng/ml)	Skewness
And	2877	610.2	7413	2512	1708	1.31
Etio	2366	601.9	6300	2023	1456	1.68
E	47.3	7.3	144.2	36.5	38.3	2.32
T	52.2	3.9	171.5	42.0	44.1	2.40
5α-diol	75.2	17.9	212.5	62.9	50.0	2.09
5β-diol	160.4	20.1	523.2	122.0	140.7	2.64
Pregnanediol	409.7	65.9	1359.1	308.8	340.8	2.31
Pregnanetriol	61.8	11.4	168.7	51.8	41.5	1.71
DHEA	8.9	0.8	34.5	5.4	8.8	1.72
DHT	6.0	0.7	19.2	4.4	6.1	6.69
11OHAnd	1042.4	167.1	2659.0	923.1	639.8	1.43
11OHEtio	149.5	14.4	533.1	104.1	147.5	2.25

Concentration corrected by density.

Peptide hormones descriptive statistic- human chorionic gonadotrophin.

The distribution of hCG whole fraction for urine samples obtained from August 1999 to July 2000 was not normal. Therefore, we calculate a "far outside value" of 1.32 mIU/ml as proposed by Delbeke et. al [3]. This value is defined as [(3 x IQR) +Q3], where IQR is the interquartile range and Q3 is the third interquartile line. For this distribution the median value was 0.23 mIU/ml and the mean 0.30 mIU/ml (range 0 to 37.59 mIU/ml).

Table 3. Descriptive statistics of the concentration ratios of endogenous steroids in male Argentinean athletes (n= 3504).

Ratio	Mean	2.5 perc.	97.5 perc.	Median	Std. deviation	Skewness
And/Etio	1.34	0.48	2.86	1.23	0.61	1.33
T/E	1.53	0.11	5.63	1.11	1.42	2.31
And/T	106.9	13.6	5.58	59.2	171.5	7.50
And/E	90.4	14.9	291.9	67.9	74.8	2.31
Etio/T	91.1	11.0	489.8	50.6	148.9	6.41
Etio/E	77.7	12.0	276.7	55.2	74.7	3.13
5α-diol/5β-diol	0.63	0.18	1.65	0.52	0.39	1.67
DHT/Etio x 1000	4.99	0.29	22.6	2.78	6.01	2.72
DHT/E	0.32	0.01	1.67	0.15	0.44	3.01

Transforming this "no normal" values into the square root, we obtained a gaussian distribution. A threshold level of 1.46 mIU/ml was calculated adding 4 standard deviation to the mean (table 4). That transformation was obtained after neglecting all zero values (data under the detection limit) [4].

Table 4. Descriptive statistics of hCG (whole fraction) in urine of Argentinean athletes.

	N	Mean (mIU/ml)	2.5 perc. (mIU/ml)	97.5 perc. (mIU/ml)	Median (mIU/ml)	Std. deviation (mIU/ml)	Skewness
SQRT hCG	2350	0.543	0.533	0.553	0.538	0.229	1.446

Peptide hormones descriptive statistic-Luteinizing hormone.

LH and testosterone levels in 2178 urines from male athletes were measured and T/LH ratios were calculated. Testosterone levels were not corrected to density urine to perform this calculation. As this data were not normal, we transform them into square root to obtain a gaussian shape. Descriptive statistics is shown in table 5. We calculate a threshold level of 369 mmoles/IU adding five standard deviation to the mean to compare our data with values reported previously by Cowan et al. [5]

Table 5. Descriptive statistics of T/LH ratio in urine of Argentinean athletes.

	N	Mean	2.5 perc.	97.5 perc.	Median	Std. deviation	Skewness
SQRT T/LH	2178	5.694	5.581	5.808	5.412	2.701	1.044

CONCLUSIONS

Steroid profiles measured in urine of argentinean male athletes collected during the period august 1999-july 2000, are quite similar to the data reported previously by the Cologne laboratory, except that Argentine concentration data are more disperse, concentration ratios are less disperse, and skewness are smaller than Cologne data [2].

Delbeke et al. [4] proposed a threshold value of 5 mIU/ml for hCG level in urine. Our apparent threshold level of 1.46 mIU/ml -and our " far outside" value of 1.32 mIU/ml- means that using the threshold value of 5 mIU/ml we are absolutely sure of not having any false positive. Cowan et al. [5] applied the square root transformation to the not normal T/LH data obtaining a cut off level of 340 nmoles/IU. We obtain a very similar cut off level (369 nmoles/IU) after applying the same transformation to our data.

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