Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (11)

W. Schänzer
H. Geyer
A. Gotzmann
U. Mareck
(Editors)

Sport und Buch Strauß, Köln, 2003

D. MARTINEZ, A. ALVAREZ, M.T. CORREA, A. RODRIGUEZ,
X. DE LA TORRE, R. DE LA TORRE :
Endogenous Steroids Profiles Preliminary Evaluation for a Cuban Athletes Population
In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck (eds.) Recent advances in doping analysis (11). Sport und Buch Strauß, Köln, (2003) 335-339

Martinez D.[1], Alvarez A.[1], Correa M.T.[1], Rodriguez A[1], de la Torre X[2]., de la Torre R[2].

ENDOGENOUS STEROIDS PROFILES PRELIMINARY EVALUATION FOR A CUBAN ATHLETES POPULATION.

[1] Laboratorio Antidopaje. Instituto de Medicina del Deporte. La Habana, Cuba
[2] Pharmacology Research Unit, Institut Municipal d' Investigació Mèdica, Universitat Pompeu Fabra, Barcelona, España.

Introduction:

The evaluation of urine steroid profile has been proposed as an additional tool in doping control to detect changes in steroids normal excretion patterns. This changes may be caused by exogenous testosterone misuse and/or synthetic anabolic steroids and some drugs like trimethoprim, ethanol and probenecid, etc.[1-3]

Different studies have already shown the limitations of the determination of the reference values for specific populations. Nevertheless several steroid profile parameters including ratios between some of them (e.g. Testosterone/Epitestosterone (T/E), Androsterone/Etiocholanolone (And/Etio) ratios, etc.) have shown enough stability in order to be used in doping control as indirect markers for the detection of anabolic agents' misuse.

This work shows the results obtained in one year after the implementation of the analytical procedure for the detection of steroids excreted in their free and conjugated forms.

2. Materials and Methods:

2.1 Sample:

Samples (n=630 for males and n=259 for females), were from national athletes included in the Cuban National Antidoping Program during 2002. All the samples included in this study were negatives at the control.

Profile parameters determined were: Testosterone (T), Epitestosterone (E), Androsterone (And), Etiocholanolone (Etio), 11β-hydroxy-androsterone (OHA), 11β-hydroxy-etiocholanolone (OHE), and T/E, And/Etio and 11β-hydroxy-androsterone/11β-hydroxy-etiocholanolone (OHA/OHE) urinary ratios.

2.2. Samples extraction and analysis:

A 2.5 ml volume of urine was extracted in XAD_2 column from matrix, followed by enzymatic hydrolysis with β-glucuronidase (*E. coli*) and a liquid-liquid extraction using t-butyl methyl ether. Internal standard was methyltestosterone (500ng/mL) and testosterone-d_3 (50 ng/mL). A mixture of MSTFA:NH_4I: 2-mercaptoethanol was used as derivatization agent.[4]

The instrumental analysis was done in an Agilent 5973, GC/MS quadrupole instrument. A calibration sample was used at the beginning of the batch in order to calculate response factors.

2.3 Statistical evaluation:

Statistical evaluation of the data was done using the SPSS for Windows v. 10. Mean, median, minimum, maximum and standard deviation were calculated for each studied parameter.

The 95% of the references values were calculated following the non-parametric percentile method.

3. Results:

Response factor for each studied steroid was calculated using a calibration sample injected at the beginning of each batch. These factors leading to determine the concentration of steroids in the sample. To determine the concentration of testosterone and epitestosterone, the Testosterone-d_3 was used as internal standard, while methyltestosterone was used to calculate concentration for the remaining steroid. Samples with specific gravity higher than 1.020 were corrected. Samples with specific gravity below 1.007 were eliminated.

Correction was done according to [5]: $C_{corr} = (1.020-0.998)/(Dens-0.998)$

Where: C_{corr}: steroid corrected concentration, Dens: sample specific gravity, 0.998: water specific gravity

Means, median, minimum, maximum and standard deviation were obtained in descriptive statistical using the same statistical program. The 95% references values were calculated following the non-parametric percentile. Tables and graphics show the obtained results.

In spite of the heterogeneity of the sample, reference values obtained for most of the steroids and data distribution, they are quite similar to those described in the specialized literature [6-9].

Results of the study are shown on Tables 1 to 6 and Figures 1 to 18.

References:

1. Schänzer W. and Donike M., "Synthesis of deuterated for GC/MS Quantitation of endogenous steroids" in Recent advances in doping analysis (2), 1994, p.93
2. De la Torre X., Pascual J.A., Ortuño J. and Segura J., "Steroid Profile in Sports" in Recent advances in doping analysis (4), 1996, p.59
3. Geyer H., Schänzer W. Mareck-Engelke U, and Donike M. "Factors influencing the steroid profile" in Recent advances in doping analysis (3), 1995, p.95.
4. De la Torre X., Segura J., Yang, Z., Li Y., Wu M.: "Testosterone detection in different ethnic groups" in Recent advances in doping analysis (4), 1996, p.71
5. Donike M., Rauth S and Sample B. "Excretion of ephedrine and endogenous steroids under conditions of controlled water diuresis", in 10th Cologne Workshop on dope analysis, 1992, p.163.
6. Donike M., et al. "Detection of Dihydrotestosterone (DHT doping: alterations in the steroid profile and reference ranges for DHT and its 5α-metabolites." J. Sports Med Phys Fitness, 1995, 35,p. 235:50.
7. Donike M., Rauth S. und Wolansky A., "Reference ranges of urinary endogenous steroids determined by Gas-Chromatography/Mass spectrometry" in 10th Cologne Workshop on dope analysis, 1992, p.69.
8. Donike M., "Steroid profiling in Cologne" in 10th Cologne Workshop on dope analysis, 1992, p.47.
9. Mareck-Engelke U., Geyer H. and Donike M., "Stability of steroid profiles" in 10th Cologne Workshop on dope analysis, 1992, p.87.

Table 1. Descriptive statistics of endogenous steroids concentrations for females.

Steroid	Mean	Median	Minimum	Maximum	Std. Dev
Testosterone	6.7	4.9	0.7	52.4	6.8
Epitestosterone	13.4	9.8	0.6	96.0	12.5
Androsterone	2060.9	1475.4	9.8	16785.4	2019.1
Etiocholanolone	1871.9	1541.1	168.4	9450	1546.6
11ß-OH-Androst.	167.5	113.2	2.0	1183.4	178.3
11ß-OH-Etioc.	28.6	12.3	1.0	525.0	57.7

Table 2. Descriptive statistics of endogenous steroids concentrations ratios for females.

Ratios	Mean	Median	Minimum	Maximum	Std. Dev
T/E	0.761	0.628	0.016	4.627	0.661
And/Etio	1.173	1.078	0.019	2.925	0.560
OHA/OHE	10.165	8.43	0.489	39.324	7.067

Table 3. Percentiles of endogenous steroids concentrations and its ratios for females.

	Percentile	
	5%	95%
Testosterone	0.85	19.81
Epitestosterone	2.04	40.92
Androsterone	298.85	6126.74
Etiocholanolone	403.05	5027.81
11ß-OH-Androst.	22.75	507.22
11ß-OH-Etioc.	2.80	102.30
T/E	0.049	2.109
And/Etio	0.425	2.236
OHA/OHE	2.153	24.622

Table 4. Descriptive statistics to endogenous steroids concentrations for males.

Steroids	Mean	Median	Minimum	Maximum	Std. Dev
Testosterone	47.3	40.5	0.2	186.9	35.5
Epitestosterone	43.8	34.7	0.6	191.8	34.1
Androsterone	3106.2	2352.3	153.7	20407.2	2619.0
Etiocholanolone	2413.9	1847.2	69.3	16851.3	2110.7
11ß-OH-Androst.	371.6	204.6	6.3	2356.3	418.9
11ß-OH-Etioc.	72.6	21.3	0	1717.0	131.1

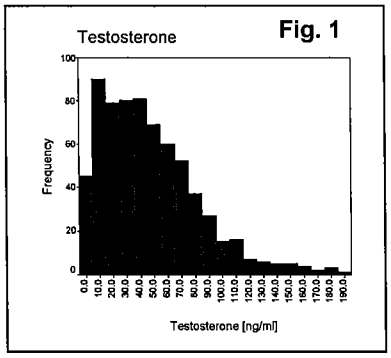
Table 5. Descriptive statistics to endogenous steroids concentrations ratios for males.

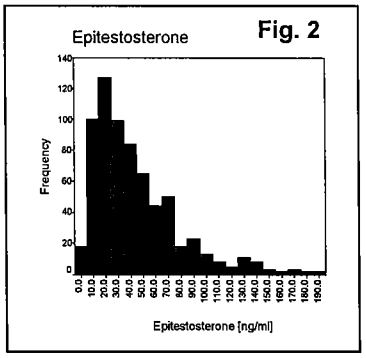
Ratios	Mean	Median	Minimum	Maximum	Std. Dev
T/E	1.467	1.186	0	5.795	1.165
And/Etio	1.523	1.356	0.015	8.691	0.848
OHA/OHE	10.110	7.830	0.342	43.333	7.954

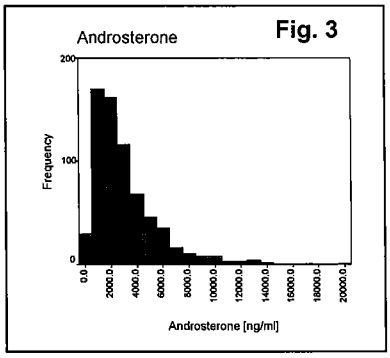
Table 6. Percentiles of endogenous steroids concentrations and its ratios for males

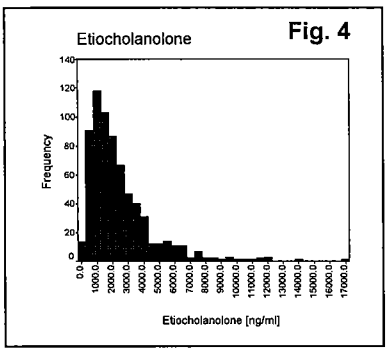
	Percentile	
	5%	95%
Testosterone	4.200	114.44
Epitestosterone	7.523	117.16
Androsterone	548.6	8326.29
Etiocholanolone	344.03	6574.08
11ß-OH-Androst.	38.8	1332.00
11ß-OH-Etioc.	4.00	328.27
T/E	0.096	4.006
And/Etio	0.504	3.032
OHA/OHE	1.747	27.311

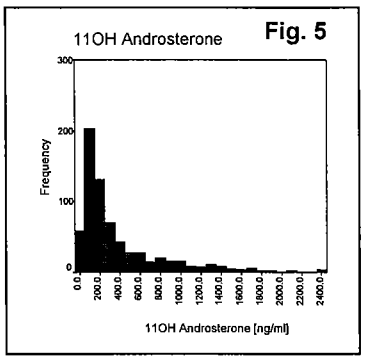
Figures 1 to 9: Endogenous Steroids distribution for males.

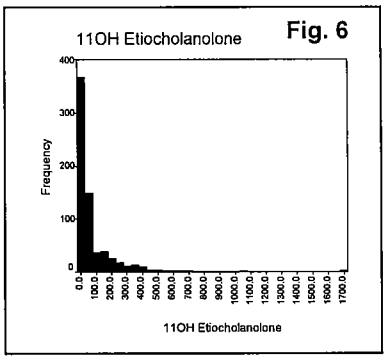


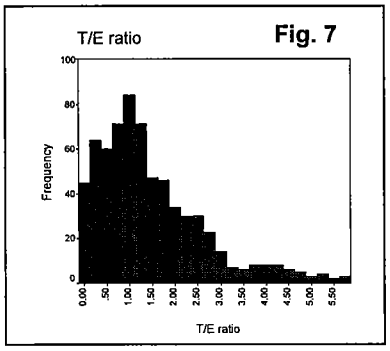


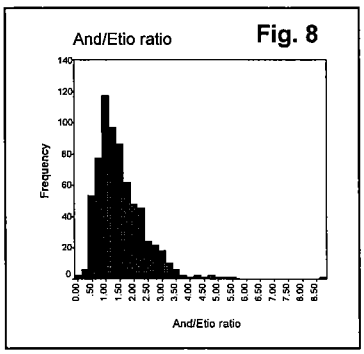


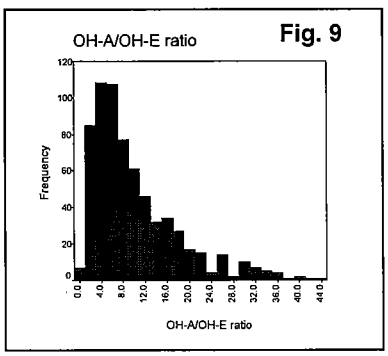












Figures 10 to 18: Endogenous Steroids distribution for females: