Jain S, Lal R, Garg T, Jamal H, Goswami M, Nimker V, Soni A, Beotra A, Kaur T.

Comparative study of endogenous steroid profile of Indian athletes with other Commonwealth Games 2010 athletes

National Dope Testing Laboratory, New Delhi, India

1.0 Introduction

The evaluation of endogenous steroid profile has been successfully used as an additional tool in doping control and therefore, the reference ranges of endogenous steroids have been established. Since these are obtained from the samples collected in Western countries, extrapolating data to different ethnic populations appears inefficient. The objective of this study is to compare the endogenous steroid profile and the concentration ratios of Indian athletes with athletes from Commonwealth Games (CWG), 2010.

2.0 Material and methods

Reference standards of endogenous steroids were procured from Sigma-Aldrich, USA, Roche or National Measurement Institute, Australia. C-18 cartridges were procured from 3 M Empore, Varion. CWG 2010 involved participation of 6,081 athletes from 71 Commonwealth nations and various ethnic groups such as Indian, Asian, European and Pacific Basin nationalities. Urine samples which were negative for the doping agent and showed no bacterial degradation was included in the present study (Indian athletes: 2654 male and 1567 female and CWG athletes: 808 male and 562 female). Routine screening procedure IV consisting of automated solid phase clean up followed by derivatization and instrumental analysis on GC-MS was implemented for androgenic anabolic steroids (AAS) to determine the concentration of androsterone (A), etiocholanone (E), testosterone (T), epitestosterone (EPIT), 5α- androstanediol (5A), 5β- androstanediol (5B) and dehydroepiandrosterone (DHEA)[1.] Deuterated compounds such as d4 androsterone glucuronide (500 ng/ml), d5 Etiocholanone (300 ng/ml), d3 5α- androstanediol (30 ng/ml), d5 5β- androstanediol (60 ng/ml), d3 testosterone (40 ng/ml) and d3- epitestosterone (10 ng/ml) were used as internal standards. Instrumental analysis was done on GC-MSD (Model:Agilent 5973 and 5975). Statistical evaluation was performed using non-parametric statistics in the STATA software for Windows.

3.0 Results and Discussion

The concentration of endogenous steroids in sample was calculated using the calibration mixture containing standards prepared at concentration of A -3000 ng/ml, E- 3000 ng/ml, T- 45 ng/ml, EPIT- 45 ng/ml, 5A- 90ng/ml, 5B -240ng/ml and DHEA- 500ng/ml and was injected at the beginning of each batch. Samples with specific gravity higher than 1.020 were corrected and those below 1.007 were eliminated to avoid large correction factors. The histograms and non parametric method results of endogenous steroids and its ratios are shown in Figure 1 and 2.

The 95% central reference interval estimated for the concentrations of EPIT, T, A, E and for the ratios of A/E and T/EPIT in Indian athletes are found to be within the acceptable range suggested by WADA in the TD EAAS2009[2] (Table 1). The urinary levels of all steroids show variation between Indian and CWG athletes probably because CWG consists of mixed population from 71 different countries which is in line with the reported literature that fluctuations in steroid profile parameters are attributed to inter-individual effects (such as gender, genotype, ethnic descent) and intra-individual factors (such as diet, age, exercise, drugs, etc.)[3].

As shown in Table 1 there is no difference in the ratios of A/E, T/EPIT and 5A/5B among the two groups (Indian and CWG) and also between males and females. This could be because these ratios are not influenced by factors such as exercise, severe physical endurance performance, menstrual cycle, circadian or annual rhythms[3].

The urinary ratios of A/E and T/E in male and female of Indian population (For males A/E=3.3; T/EPIT=3.3 and for females A/E=2.4; T/EPIT= 1.8) are lower than the Caucasian population (For males A/E=3.6, T/EPIT=4.3 and for females A/E=2.7; T/EPIT=2.7)[4] which may be due to higher incidence of deletion of UGT2B17 gene in Asian population leading to lower excretion of testosterone[5]. The ratio of 5A/5B in Indian males and females are higher (> 2.5) as compared to Caucasian males and females (< 1.7) which could be attributed to the higher activity of 5-alpha reductase in Asian population.

In order to make data interpretation more meaningful, the reference ranges of Indian population and CWG 2010 players was compared after log transformation and using the 1-way ANOVA test followed by Bonferroni's multiple comparison test. The values obtained were significantly different for all 7 natural steroids and 3 ratios except A/E ratio in females and T/EPIT ratio in males at P<0.001.

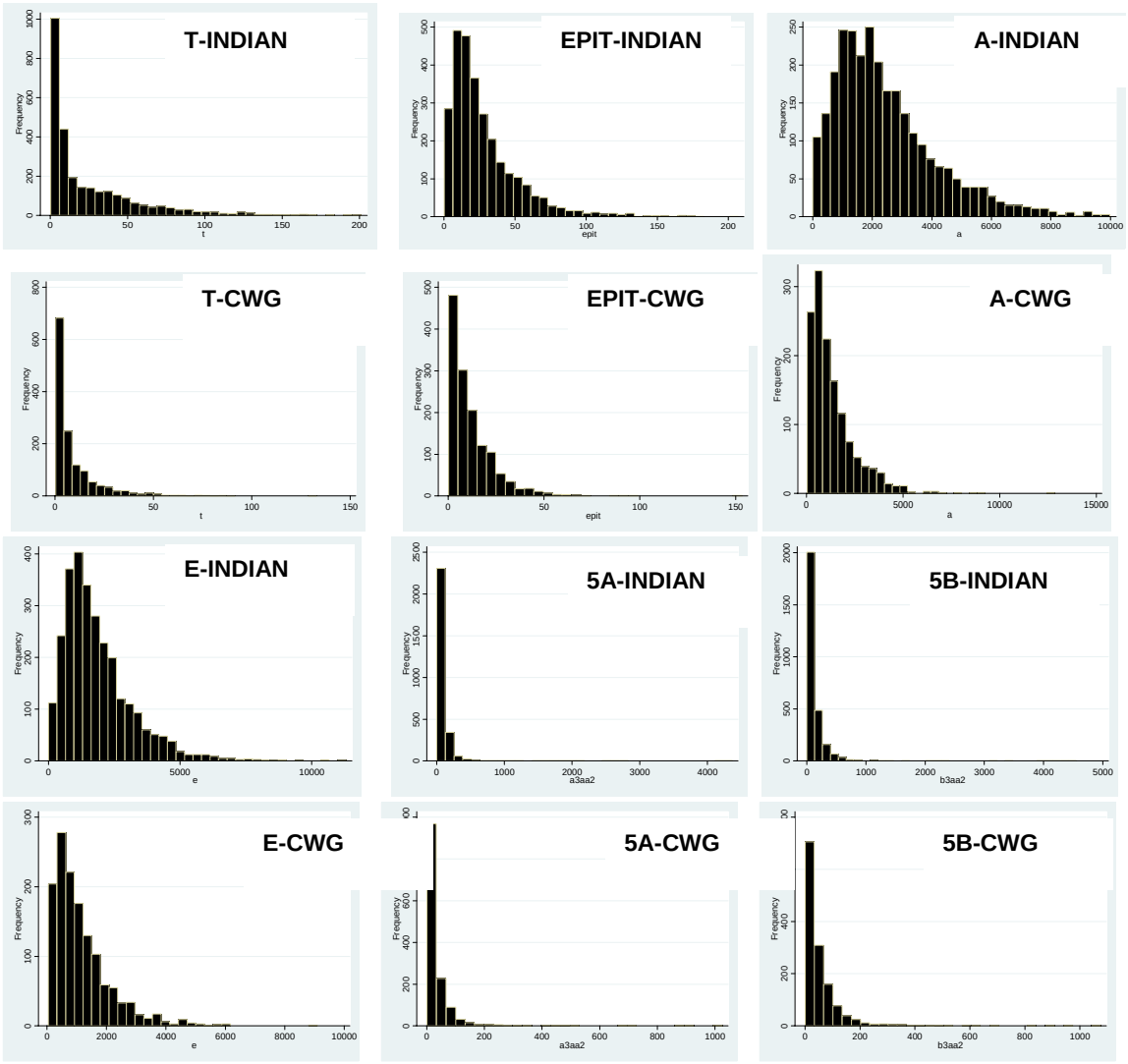

Figure 1: Endogenous steroid concentration (ng/ml) distribution for Indian athletes and those who participated in Commonwealth Games (CWG) 2010

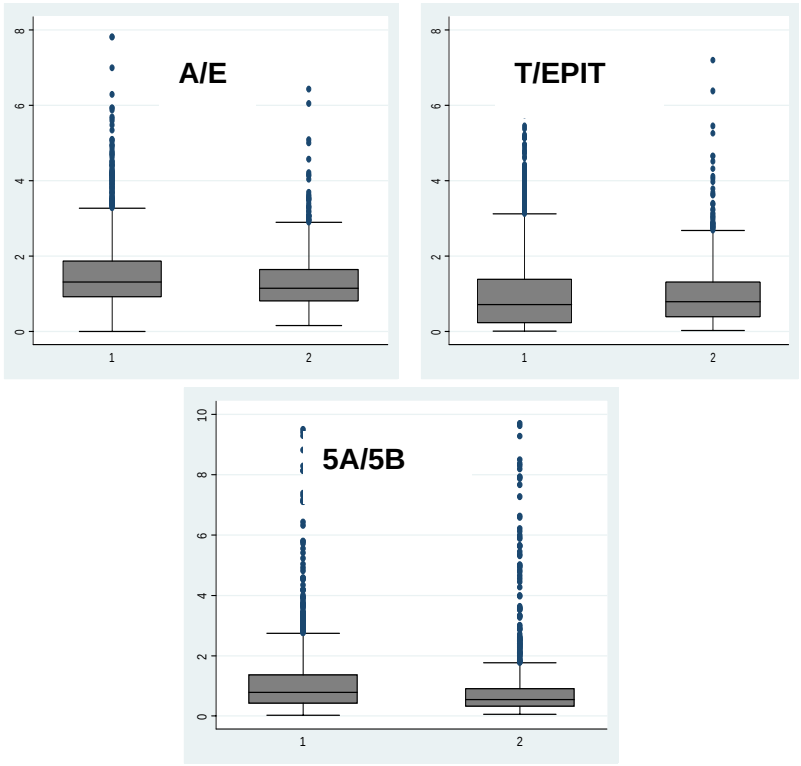

Figure 2: Box plot of medians and 95% reference ranges of two sample groups viz. 1-Indian athletes population; 2- Common wealth Games (CWG) athletes, 2010

Table 1: Comparison of the upper limits of 95% central reference value for endogenous parameters among Indian athletes, CWG athletes and WADA reference values of urinary concentrations (ng/ml) and steroid ratios

Population		Concentration of endogenous steroids (ng/ml)							Ratios		
		EPIT	T	A	E	5A	5B	DHEA	A/E	T/EPIT	5A/5B
WADA's threshold[2]		200	200	10000	10000	200	200	100	4	4	2
Indian	Combined	76	90	5893	4454	225	407	91	3.1	3.0	2.5
	Male (n=2654)	86	104	6181	4227	265	442	89	3.3	3.3	2.6
	Female (n=1567)	44	33	5429	4878	122	322	93.4	2.4	1.8	2.5
CWG	Combined	34	35	3839	3089	116	182	71	2.5	2.30	2.40
	Male (n=808)	42	48	3975	2924	152	233	73	2.8	2.40	2.50
	Female (n=562)	18	9	3436.0	3315.0	53	108.0	70	2.1	2.1	2.20

4.0 References

1) Jain S, Garg T, Lal R, Jamal H, Goswami M, Beotra A. Automated solid phase extraction method for rapid screening of anabolic steroids and few other doping agents on GC-MSD. Presented at 43rd Annual Conference of Indian Pharmacological Society, from 13th -16th December 2010 in Hyderabad, India

2) WADA Laboratory Committee, Endogenous anabolic Androgenic Steroids-testing, Reporting and Interpretative Guidance, World Anti-Doping Agency: Montreal, 2009, WADA Technical Document –TD2009EAAS (Draft version)

3) Mareck U, Geyer H, Opfermann G, Thevis M, Schanzer W. Factors affecting the steroid profile in doping control analysis. *J. Mass Spectrom* **43**, 2008, 877-91

4) Van Renterghem P, Van Eenoo P, Geyer H, Schanzer W, Delbeke FT. (2009) Alternative steroid profiling: Reference ranges for urinary endogenous steroids in a Caucasian population of athletes. In: Schänzer W, Geyer H, Gotzmann A, Mareck U, (eds.) *Recent Advances in Doping Analysis (17)*, Köln, pp 81-90.

5) Strahm E, Sottas P.E, Schweizer C, Saugy M, Dvorak J, Saudan C, (2009) Steroid profiles of professional soccer players: an international comparative study *Br J Sports Med* **43**, 1126-30