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## **Steroid profile. Differences inside the Latin-American population of athletes**

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### **Abstract**

The factors influence the urinary steroid profile applied to control doping has been studied from different point of view. The most knowing factors are age, sex, consumption of some drugs, ethanol, and circadian rhythm, between others. More recently, the gene polymorphism UGT2B17, UGT2B7 and UGT2B15 have been described and therefore differences between Caucasians, Asians and Africans respect to excreted steroids in urine are well known. The aim of this investigation was to observe differences in concentrations of some parameters of steroid profile in urine between two populations of athletes concern the same geographic area. The concentration of (T), epitestosterone (epiT), androsterone (A), etiocholanolone (Et), 3 $\alpha$ ,5 $\beta$ - and 3 $\alpha$ ,5 $\alpha$ -androstane diols, as well as T/epiT, A/Et and 3 $\alpha$ ,5 $\beta$ /3 $\alpha$ ,5 $\alpha$ - ratios were compared in both sexes for Cubans and Mexicans athletes. Statistical analysis was carried out applying test U Mann Whitney ( $p < 0.05$ ). The results showed that all parameters, except 3 $\alpha$ ,5 $\alpha$ -androstane diol, exhibited higher values in Cubans athletes population with regard to Mexicans athletes population in both sexes. This result responds to the ethnic composition of these two populations. Cuba is considered an afro-creole country (51 % Africans descendants and 11 % Africans) meantime; Mexico is considered a *mestizo* country because an important percent is derived from emigrants mix and only 0.5 % is Africans descendants.

### **Introduction**

Researches related to differences in the urinary excretion of steroids in different ethnic populations due to gene polymorphism of UGT2 family have been already described [1,2]. Caucasian, Asian and African are the most studied populations, but areas where the miscegenation is more complex are outside of the analysis so far. That is the case of Latin America. The countries of this area present different degree of mixes depending of the migrations that begun more than 25,000 years ago [3-5].

**Aim:** Observe and describe the conduct of the concentrations of testosterone, epitestosterone, androsterone, etiocholanolone, 3 $\alpha$ ,5 $\alpha$ -androstane diol, 3 $\alpha$ ,5 $\beta$ -androstane diol and testosterone to epitestosterone, androsterone to etiocholanolone and 3 $\alpha$ ,5 $\alpha$ - to 3 $\alpha$ ,5 $\beta$ -androstane diol ratios in populations of athletes Cubans and Mexicans, which are populations "apparently" similar.

### **Experimental**

Urine samples were collected in and out of competition and were analyzed at the WADA accredited Antidoping Laboratory of Havana and National Laboratory for Prevention and Doping Control, Mexico. In order to guarantee a reliable result, the *z-scores* obtained in WADA EQAS 2012 for both laboratories were compared. No significant differences were observed so it was considered that both data can be compared.

The assays applied in both laboratories are validated, accredited and inside the scope following the stated in ISL (version 7.0) and ISO/IEC 17025.

Extraction: Trimethylsilyl derivatives of the analytes were analyzed after hydrolysis with  $\beta$ -glucuronidase (*E. coli*) and liquid-liquid extraction with *tert*-butylmethyleter at pH 9.5 of 2.5 mL of urine.

The values of endogenous steroid profile were obtained from 878 Cuban athletes (613 males and 265 females) and 828 Mexican athletes (553 males and 275 females).

Following analytes were evaluated:

- Concentrations (ng/mL): testosterone, epitestosterone, androsterone, etiocholanolone,  $3\alpha,5\alpha$ -androstenediol and  $3\alpha,5\beta$ -androstenediol.
- Ratios: testosterone to epitestosterone, androsterone to etiocholanolone and  $3\alpha,5\alpha$ - to  $3\alpha,5\beta$ -androstenediol.

Before the evaluation of the data, concentrations were corrected for specific gravity (1,020). Statistical evaluation was carried out using software MedCal (version 11.3.0.0). The differences were evaluated by Kruskal-Wallis test ( $p < 0.05$ ) for non-parametric data. Table 1 shows the ethnic composition of Cuban and Mexican populations.

## Results and Discussion

Male Athletes: After the statistical comparison applying Kruskal-Wallis test, significant differences was found for concentrations of T, epiT, A and  $3\alpha,5\beta$ -androstenediol. Graphics plotting relative distribution *versus* concentration (Figure 1) show that around the higher concentrations predominate the Cuban over the Mexican athletes. Concentrations of etiocholanolone and  $3\alpha,5\alpha$ -androstenediol exhibited no significant differences between both populations.

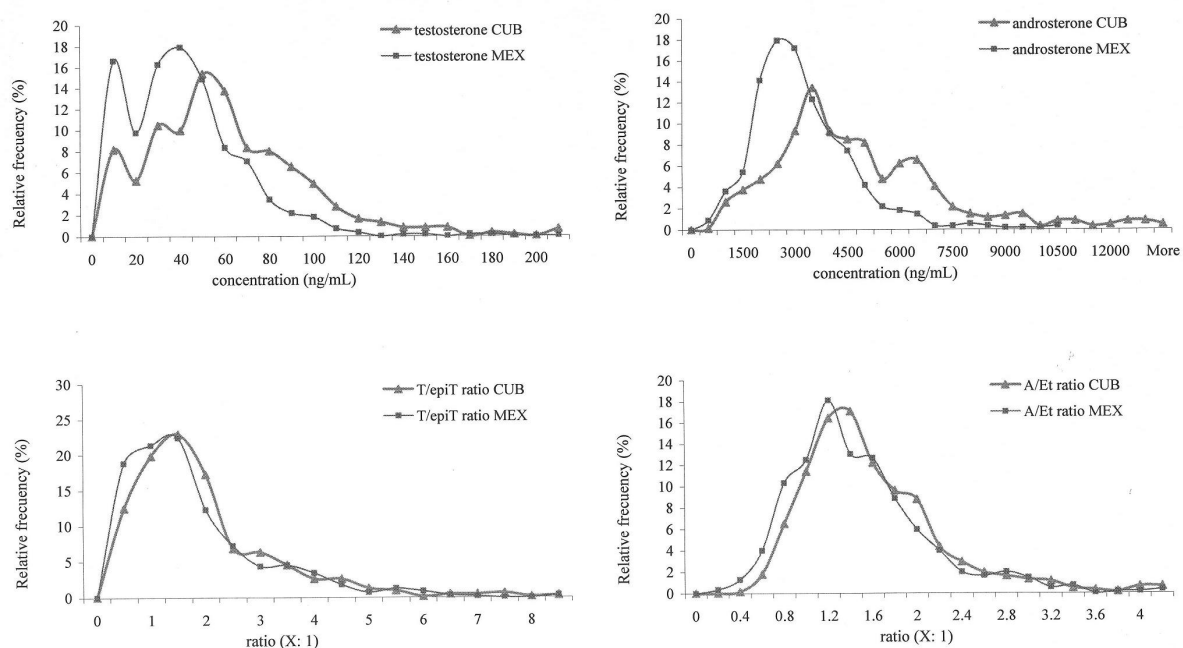


Figure 1. Relative frequency *versus* concentrations in male population corresponding to cuban and mexican athletes.

Ratios T/epiT and A/Et showed no significant differences, but the ratio  $3\alpha,5\alpha$ -/ $3\alpha,5\beta$ -androstenediols displayed differences caused obviously, by the differences found in concentrations of  $3\alpha,5\beta$ - only. In this case the lower values were observed in Cuban athletes.

Female Athletes: Females showed exactly the same behavior than male population. Concentrations of T, epiT, A and  $3\alpha,5\beta$ -androstenediol and ratio  $3\alpha,5\alpha$ -/ $3\alpha,5\beta$ - showed significant differences between Mexican and Cuban athletes. Over the higher concentrations were observed the prevalence of Cuban data (Figure 2).

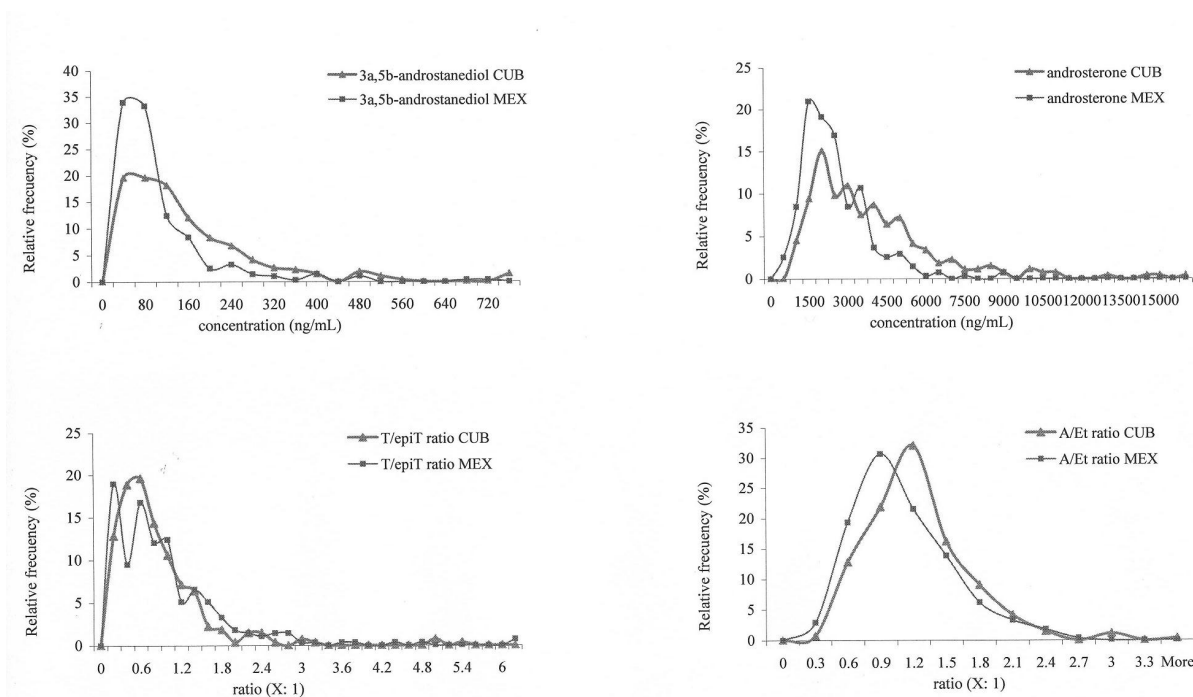


Figure 2. Relative frequency versus concentrations in female populations corresponding to cuban and mexican athletes.

It was not possible set up a pattern to support the existence of possible differences on metabolism (i.e. 5 $\beta$ -path favored over 5 $\alpha$ - or vice versa). The evaluation of other analytes included into the testosterone metabolic cascade could give more information related to the differences observed in the present work.

Looking closer the ethnic composition of this two population (Table 1), it could be observed that Cuban one is influenced by Africans genes (11%) more than Mexican population (0.5%). Besides, the Asian influence originated by the miscegenation with native Americans, is higher in Mexicans (14% and 0.5% direct descendent from Asians) compare to Cuban population (1.0 %). These factors are associated with the already studied genetic polymorphism of the family UGT2B and its conduct on Africans and Asians populations.

Certainly, endogenous steroid profile still has new observations points. Biological passport definitively resolve this problem but is a fact that the analyst that evaluate day after day the steroid profile face a challenge at the moment to asseverate a sample as "negative" or "suspected".

Origin of the studied sample		% of the population	
		Cuba	Mexico
Amerindians	Descendent of Asians and Americans	0.0	14.0
Creoles	Descendent of Europeans; mainly the Spanish	37.0	15.0
Afro-americans	Descendent of Africans and Americans	51.0	70.0*
Africans	Descendent of Africans	11.0	0.5
Asians	Descendent of Asians	1.0	0.5

(\*) referred only mestizos: descendants of different races parents, i.e. Africans and Europeans, Africans and Amerindians

Table 1. Ethnic composition of Cuba and Mexico populations [5].

## Conclusions

Comparison of Cuban and Mexican male and female populations showed statistical differences ( $p < 0.05$ ) for concentrations of T, epiT, A and  $3\alpha,5\beta$ -androstenediol and  $3\alpha,5\alpha$ -/ $3\alpha,5\beta$ -ratio. The highest concentrations of androgens were focused in Cubans which are more influenced by African genes than Mexicans. Even when the behavior of the z-scores makes the data comparable, it can not be excluded the logical differences by the fact that the method was applied in two different labs. Some questions about the profile behavior are still without a definitive and unique response. It is known the differences derived of the ethnic origin, but the search for one or more variables that do not be affected by this issue is enforced in order to set some good sense to the initial steroid profile evaluation.

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