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Preliminary results on the carbon isotope ratios of endogenous steroids in urine collected from Asian countries

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Preliminary results on the carbon isotope ratios of endogenous steroids in urine collected from Asian countries

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Introduction

Gas Chromatography Combustion Isotope Ratio Mass Spectrometry (GC/C/IRMS) is a technique to detect and confirm the abuse of endogenous anabolic steroids. The administered steroids are chemically identical to that produced in the body, but the ratio of ^{13}C to ^{12}C of the synthetic products may be different due to the source of carbon. Carbon isotope ratio is expressed in term of $\delta^{13}C$ values [(1)], with unit in per mil. Variation in the reference range of the $\delta^{13}C$ value of the endogenous steroids is due to the effect of diet [(2)].

This work reports the variability in the $\delta^{13}C$ values of Asian athletes, who took part in the 1st Asian Indoor Games held in Bangkok, Thailand, 12 – 19 November 2005.

Experimental

Sample Preparation

Urine samples (5 ml) were applied onto C-18 cartridge (Sep-Pak®), eluted with methanol and then dried. The residues were reconstituted in 1.0 ml buffer (pH 7.0) and free-form steroids in the aliquots extracted with *t*-butyl methyl ether. After addition of the ISTD (5α-androstan-3β-ol), the aqueous fractions were hydrolyzed by β-glucuronidase (*E. coli*) for 1 hr at 55°C. After extraction by n-pentane, the aliquots were evaporated to dryness, acetylated by acetic anhydride in the presence of pyridine, re-evaporated to dryness, and reconstituted in cyclohexane (50 μl).

The acetylated aliquots (2 μl) were injected for GC/C/IRMS analysis and another 2 μl aliquots were injected for GC/MS analysis in order to identify the steroids.

Instrumentation

GC/C/IRMS analyses were performed on an HP 6890 GC connected to Micromass Isoprime IRMS. The GC was equipped with CP-Sil 24 CB column (Chrompack, 30 m x 0.25 mm i.d. x 0.25 μm). Helium was the carrier gas at constant flow of 1.5 ml/min. The GC temperature program was initial at 160°C, then 20°C/min to 270°C, 2°C/min to 290°C, 5°C/min to 300°C and held for 8 min. GC/MS analyses were performed on an Agilent 6890N GC / 5973N MSD.

Results and Discussion

The $\delta^{13}C$ values of the following endogenous steroids were measured: Etiocholanolone (Etio), Androsterone (Andro), 5β-androstan-3α,17β-diol (5β-diol), 5α-androstan-3α,17β-diol (5α-diol), 11-Ketoetiocholanolone (11-Keto), Pregnandiol (P2) and Pregnantriol (P3). 11-ketoetiocholanolone is the endogenous reference compound (ERC) for androsterone and etiocholanolone (one OH-group) and pregnandiol is ERC for 5β-androstandiol and 5α-androstandiol (two OH-groups). All the $\delta^{13}C$ values were corrected for the derivatization using the equation of D.M. Johnes. [3]

The results obtained from the 1st Asian Indoor Games are shown in Table 1 and 2 and Figures 1 – 6.

Table 1. Summary of results obtained from the 1st Asian Indoor Games

	Delta value (‰) (n = 189)						
	Etio	Andro	5β-diol	5α-diol	11-Keto	P2	P3
MEAN	-21.04	-21.44	-22.04	-23.75	-23.23	-22.45	-21.00
SD	1.09	0.96	1.43	1.02	1.15	1.21	1.14

Table 2. Summary of the difference obtained from the 1st Asian Indoor Games

	Difference ($\delta^{13}_{steroid} - \delta^{13}_{11\text{-}Keto}$)		Difference ($\delta^{13}_{steroid} - \delta^{13}_{P2}$)	
	Etio	Andro	5β-diol	5α-diol
MEAN	1.06	2.19	0.41	-1.30

From Figure 1 and 2, the mode values of $\delta^{13}C$ value for etiocholanolone, androsterone, 11-ketoetiocholanolone, 5β-androstandiol, 5α-androstandiol and pregnandiol are -21 ‰, -21 ‰, -23 ‰, -22 ‰, -24 ‰ and -22 ‰, respectively. All $\delta^{13}C$ value measured (n = 189) are less than -28.0 ‰ [2, 4], the cut-off value for a possible positive sample.

The difference of $\delta^{13}C$ value between endogenous steroid and ERC (Table 2) is less than 3 delta unit [2, 4], the cut-off value for a possible positive sample.

All samples, from the 1st Asian Indoor Games are negative according to the WADA directive[4], whereby all criteria must be met for a sample to be declared positive.

Conclusions

In the 1st Asian Indoor Games Bangkok 2005, there were 45 participating nations. The data are representative of the $\delta^{13}C$ values for athletes from Asia.

References

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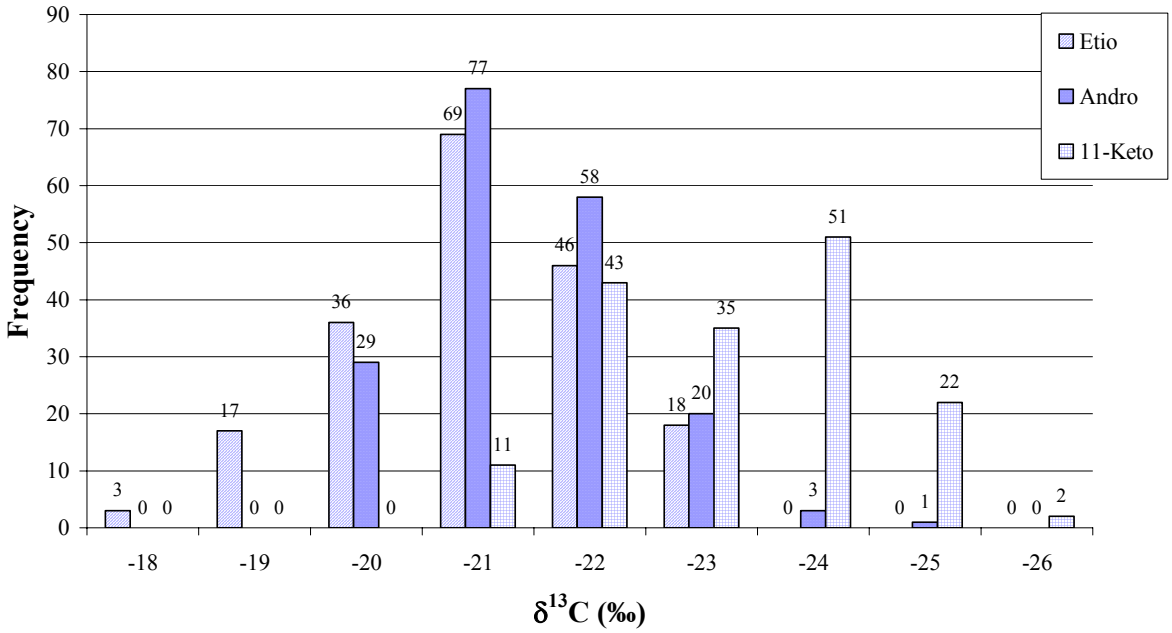

Figure 1. The distribution of $\delta^{13}C$ values of etiocholanolone, androsterone and 11-ketoetiocholanolone.

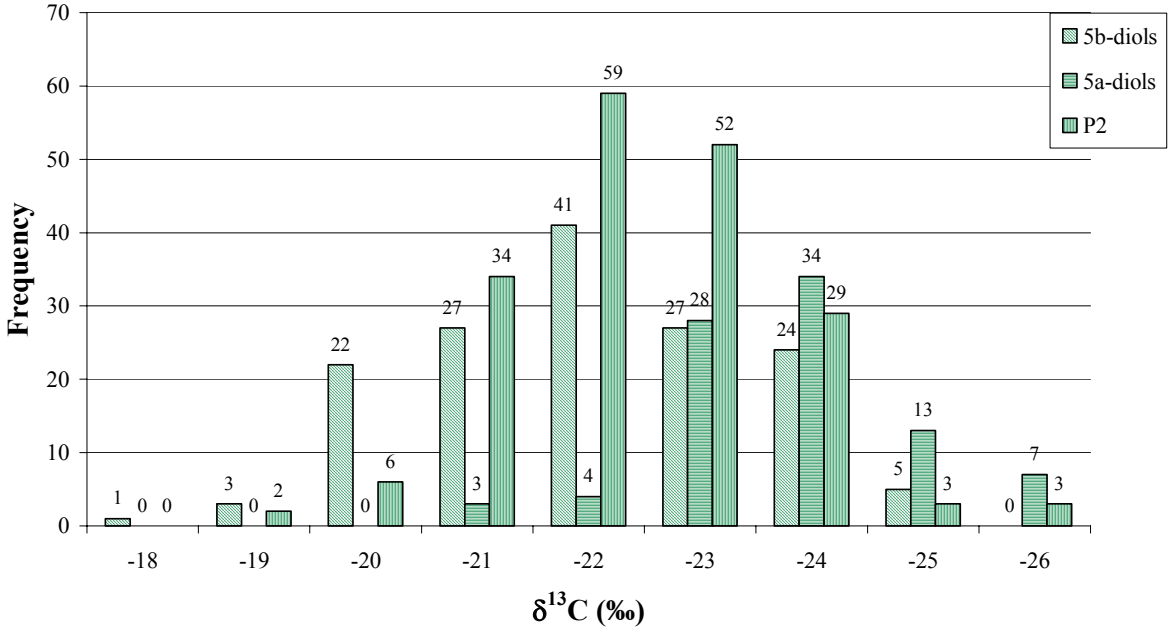

Figure 2. The distribution of $\delta^{13}C$ values of 5β-androstandiol, 5α-androstandiol and pregnandiol.