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Delta values of endogenous steroids in Indian population: Establishing Reference Ranges

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Introduction

The development of gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) has allowed high precision measurements of isotopic composition in each compound ⁽¹⁾. India's ethnic and regional complexity sets it apart from the other countries as its population is polygenetic and an amalgamation of various races and cultures. Geographically, India is mainly divided into four regions i.e. Northern, Southern, Eastern and Western and the diet pattern of these regions show marked variation. The aim of the present study was to investigate the variability in $\delta^{13}\text{C}$ values of endogenous steroids in different regions of India, thereby establishing reference ranges.

Materials and Methods

Reference Population: The urine samples were collected from 143 male and 89 female (14-30 years of age) volunteers from different regions of India out of which 91 samples were from Eastern India, 76 samples from Northern India and remaining 65 from Southern India.

Quality Control samples: Testosterone undecanoate (40 mg) was administered orally to a healthy male volunteer and urine sample collected from 0-72 hours. The study was reviewed and approved by ethical committee of NDTL, India. The excretion study samples were pooled and kept in small aliquots at -20 °C. A single aliquot was processed and injected with each batch as positive Quality Control Sample.

Sample preparation: The samples were prepared using enzymatic hydrolysis and solid phase extraction by C18 cartridges ⁽²⁾ and analysed after acetylation on GC (HP 6890) connected with Isoprime IRMS. The $\delta^{13}\text{C}$ values of the following endogenous steroids were measured: Etiocholanolone (Etio), Androsterone (Andro) and 11-keto Etiocholanone (11 keto etio-used as endogenous reference compound (ERC)). All the steroids were measured as acetylated derivatives

Results and Discussion

The criteria used to differentiate between the natural or synthetic endogenous steroids as per WADA Technical document – TD2004EAAS⁽³⁾ was as follows:-

The differences between the $\delta^{13}\text{C}$ of Andro and/or Etio from $\delta^{13}\text{C}$ of ERC must be greater than 3 ‰ and/or $\delta^{13}\text{C}$ of Andro and/or $\delta^{13}\text{C}$ Etio is below -28 ‰ based on underivatized steroids.

Summary of $\delta^{13}\text{C}$ values of endogenous steroids: Etio, Andro and ERC and differences obtained in urine samples collected from different regions is shown in Table-1 & 2. The mean values of $\delta^{13}\text{C}$ endogenous steroids from all the three regions were comparable ($p < 0.05$).

Endogenous Steroid	Region wise delta values (uncorrected)				
		North	East	South	Combined
Etiocholanolone	Mean	-24.26±0.93	-24.91± 0.88	-24.99 ± 0.96	-24.72 ± 0.92
Androsterone	Mean	-24.27±0.91	-24.62 ± 0.90	-24.92 ± 0.92	-24.58 ±0.91
11-Ketoetiocholanone	Mean	-23.35± 1.36	-24.27 ± 1.51	-23.69 ± 1.02	-23.77 ± 1.3

Table-1: Summary of $\delta^{13}\text{C}$ values of endogenous steroids : Etio, Andro and ERC

Table-2: Summary of difference obtained from different regions of India

Difference	Region wise) Delta values in ‰				
		North	East	South	Combined
Etiocholanolone – 11-Ketoetio	Mean	0.91	0.64	1.30	0.95
Androsterone – 11-Ketoetio	Mean	0.92	0.35	1.23	0.81

The difference of $\delta^{13}\text{C}$ values between endogenous steroids and ERC for all the regions was less than 3 delta units; the cutoff value set up by WADA for a positive sample (Table-2) . The diet pattern of southern region was mainly rice/vegetarian/non vegetarian and eastern region rice eater/non vegetarian whereas; northern region had mix of wheat/rice, vegetarian/non vegetarian. Hence the variation in diet did not contribute any significant change in $\Delta^{13}\text{C}$ values of endogenous steroids which is in conformity with the findings of Piper et al⁽⁴⁾.

Table-3: Summary of result obtained from each country

Country	Number	Mean Etio	Mean Andro	Mean 11-keto
Australia	59	-23.0 SD 0.97	-22.5 SD 1.16	-21.1 SD 1.29
New Zealand	108	-23.3 SD 1.07	-23.0 SD 1.16	-20.9 SD 1.21
China	157	-22.5 SD 1.29	-21.6 SD 1.53	-20.1 SD 1.59
Kenya	127	-20.5 SD 1.53	-20.3 SD 1.38	-18.6 SD 1.81
India	180	-22.68 SD 0.84	-22.53 SD 0.83	-21.63 SD 1.18

The mean $\delta^{13}\text{C}$ values (corrected for acetylation) for natural endogenous steroids in Indian population are comparable with other Asian – Oceania countries⁽⁵⁾ and distributions are close to normal (Table-3).

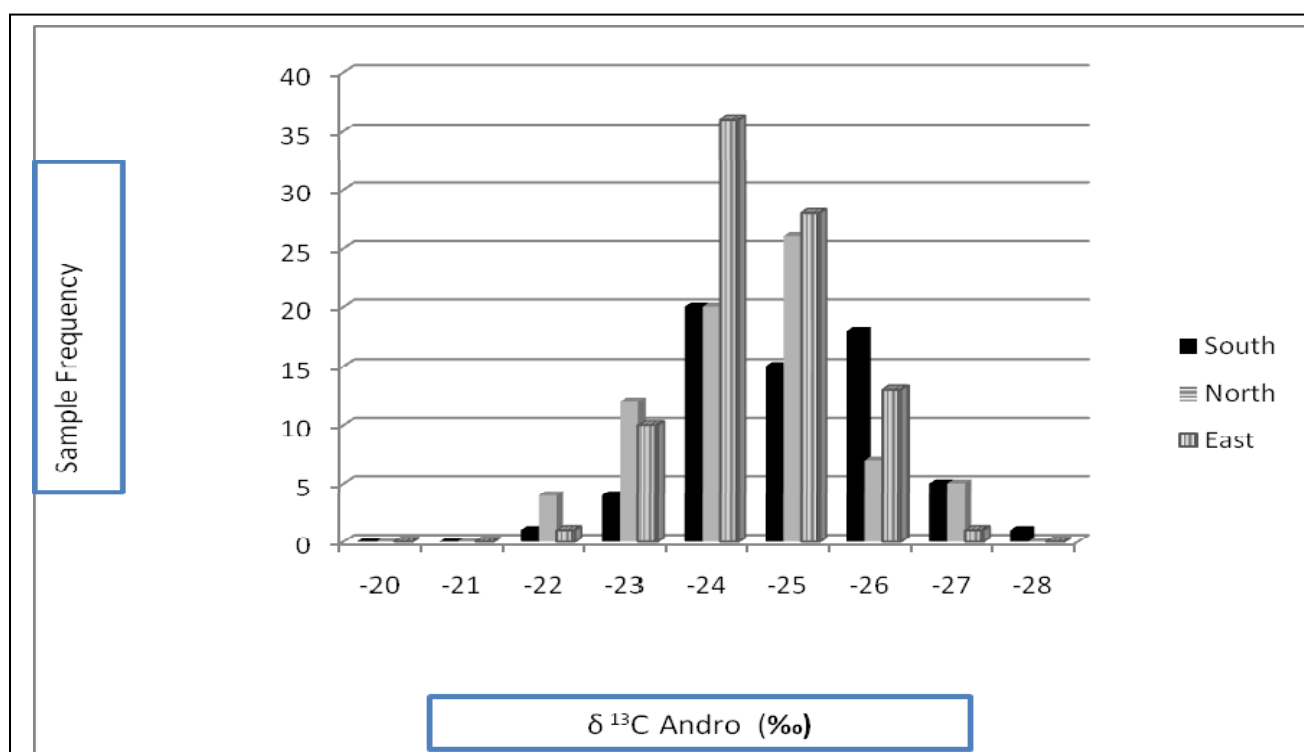
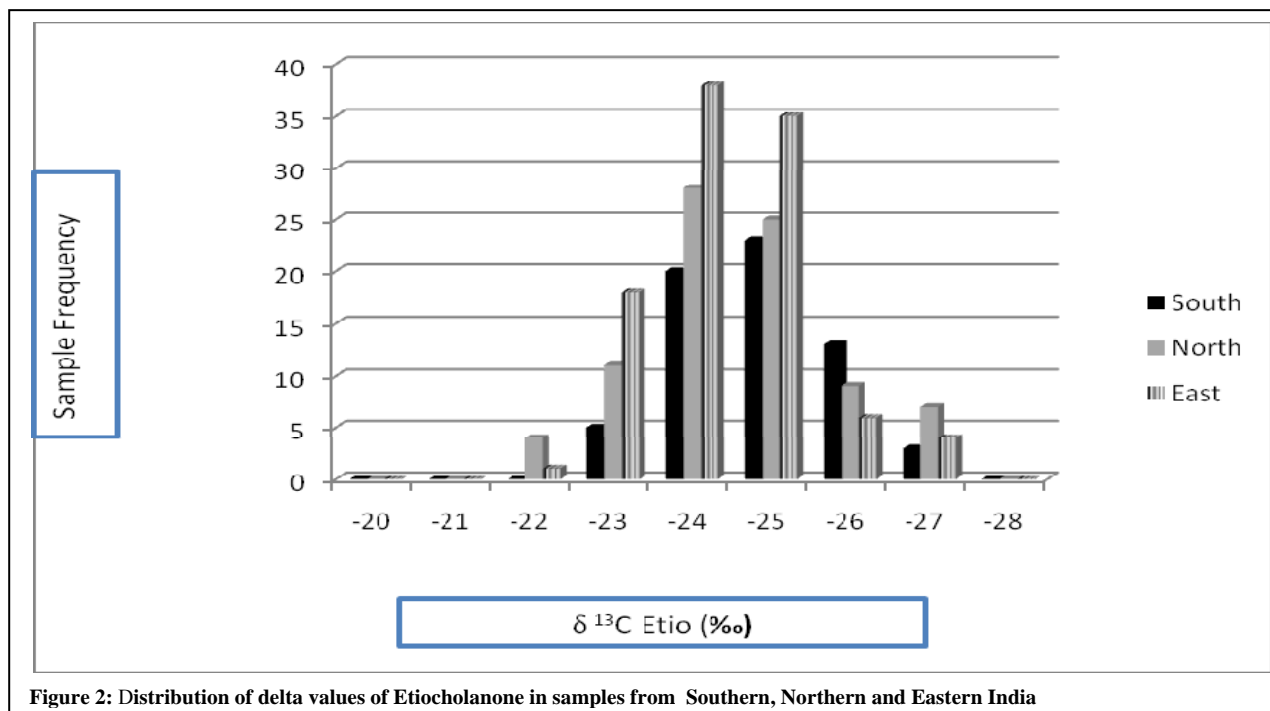


Figure 1; Distribution of delta values of Androsterone in samples from Southern, Northern and Eastern India



Though statistically, there was no significant effect of diet on $\delta^{13}\text{C}$ values of endogenous steroids but sample distribution of southern India samples showed slightly more depleted values than the other regions of India (Fig 1 & 2). Hence, the present study showed no significant difference between $\delta^{13}\text{C}$ values of endogenous steroids of eastern, southern and northern India. Further work is in progress to have more number of samples from western part of the country.

Reference:

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