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Endogenous Steroid Profiles of Urine Samples Obtained from the 10th Chinese National Winter Games

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Introduction

The 10th Chinese National Winter Games took place in Heilongjiang Province on Jan. 5 to 17, 2003. 366 urine samples in competition and pre-competition were tested. Among them were 194 urine samples for males and 172 urine samples for females. Normal procedure IV for total fraction was implemented to estimate the concentrations of androsterone, etiocholanolone, 5α-androstanediol, 5β-androstane- diol, testosterone, epitestosterone, 11β-OH-androsterone and 11β-OH- etiocho- lanolone. The ratios of the 4 pairs of endogenous steroids were presented and discussed using the statistics for normal distribution.

The normal ranges of these steroids and their ratios were statistically analyzed using the non-parametric method [1].

Some of publications dealt with the endogenous steroid profiles [2-5] to investigate the normal ranges for athletes. It is well know about the difference of T/E ratio between Caucasian and Chinese athletes [6]. This paper is focused on the statistics of endogenous steroid profiles of the Chinese athletes in winter sports.

Experimental

5 ml urine was centrifuged with 2500 rpm for 5 min, 50μl of internal standard solution (methyltestosterone 50ng/μl) were added to the urine, then the urine sample was applied to C-18 column, the column was washed with 5 ml purified water and eluted with 2 ml methanol, the methanol phase was evaporated to dryness with rotation machine, the residue was reconsituted in 1 ml phosphate buffer (pH 6.8) and 100μl of β-glucuronidase (5000 unit, E.Coli. from Sigma), then the buffed mixture was incubated at 55$°$C for 3 hours. 100 mg of solid buffer (pH 8.8-8.9) was added to the incubated solution, then the solution was extracted with 5 ml t-butyl methyl ether for 10 min, the ether phase was separated from the solution and evaporated to dryness under

nitrogen, the residue was derivatized with $50\mu l$ of MSTFA/TMSI/dithioerythriol (1000:3:1) at 70 °C for 30 min. $1\mu l$ of the derivatized solution was injected into the GC/MSD.

HP5973 GC/MSD with HP 6890 gas chromatography was used. An Agilent HP 1 column (17 m x 0.2 mm I.D. x 0.11 μm film thickness) was used with helium as the carrier gas (0.9 ml/min at 180°C, flow constant mode). The injector was set at 280°C. Split mode was used with a ratio of 1:5. The oven temperature program was 180 °C–3.3 °C/min → 231 °C–30°C/min–310 °C (2.0 min). The column head pressure was about 75 kPa. Electron energy was 70 eV. The multiplier voltage was 200 V higher than the result of auto tuning. The purge vent was set at 2 ml/min and the split vent 10 ml/min.

Results and discussion

1) The data of endogenous steroids were calculated using the concentrations of mixed standards. The concentrations of the mixed standard solution are: androsterone 1000ng/ml, etiocholanolone 1000ng/ml, 5α-androstanediol 100ng/ml, 5β-androstanediol 100ng/ml, 11β-OH-androsterone 200ng/ml, 11β-OH-etiochola- nolone 100ng/ml, testosterone 100ng/ml, epitestosterone 50ng/ml.

2) The data was not corrected for specific gravity (SG).

$1.004 \leqslant S.G._{\cdot male} \leqslant 1.030$, $1.002 \leqslant S.G._{\cdot female} \leqslant 1.031$.

3) The histograms and non-parametric method results of endogenous steroids and its ratios are shown in Figure 1 to Figure 12 and Table 1 to Table 2.

References:

1) H.E.Solberg, Approved Recommendation (1987) on the Theory of Reference Values, J. Chem. Clin. Biochem. 1987; Vol.25:645-656.

2) M.Ueki et al., Nagano Strategy against Natural Hormone Doping (1998) in: Schänzer,W., Geyer,H., Gotzmann,A., Mareck-Engelke,U.(eds.) Recent Advanced in Doping Analysis (6) Sport und Buch Strauß, Köln, pp 145-146

3) C.Schweizer et al., Profile Variation through Puberty in Young Adolescent Girls, ibid, pp 205-222

4) X. de La. Torre et al., Steroid Profile and Sports – A Cluster Analysis fo Samples from Barcelona'92 Olympic Games (1996) in: Schänzer,W., Geyer,H., Gotzmann,A., Mareck-Engelke,U.(eds.) Recent Advanced in Doping Analysis (4) Sport und Buch Strauß, Köln, pp 59-70

5) U.Mareck-Engelke et al., The Interpretation of Female Steroid Profile (1997) in: Schänzer,W., Geyer,H., Gotzmann,A., Mareck-Engelke,U.(eds.) Recent Advanced in Doping Analysis (5) Sport und Buch Strauß, Köln, pp 51-70

6) X. de la Torre et al., Testosterone Detection in Different Ethnic Group (1996) in: Schänzer,W., Geyer,H., Gotzmann,A., Mareck-Engelke,U.(eds.) Recent Advanced in Doping Analysis (4) Sport und Buch Strauß, Köln, pp.59-70 pp 71-90
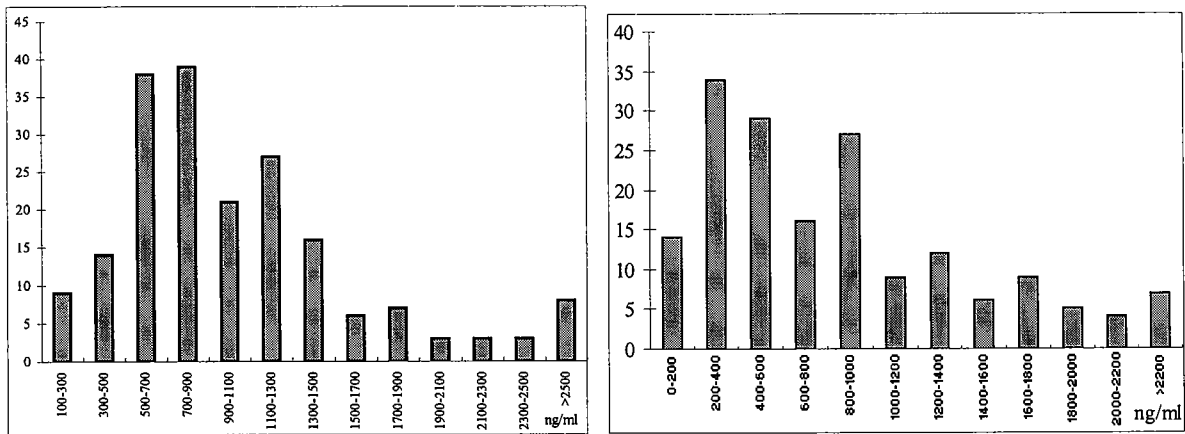
Figure 1: Histogram of androsterone (left: male, right: female)
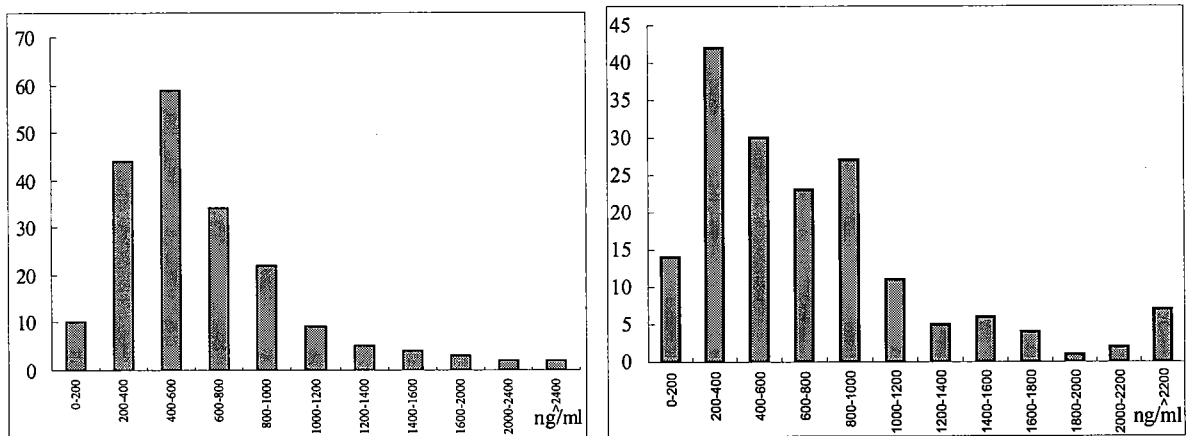

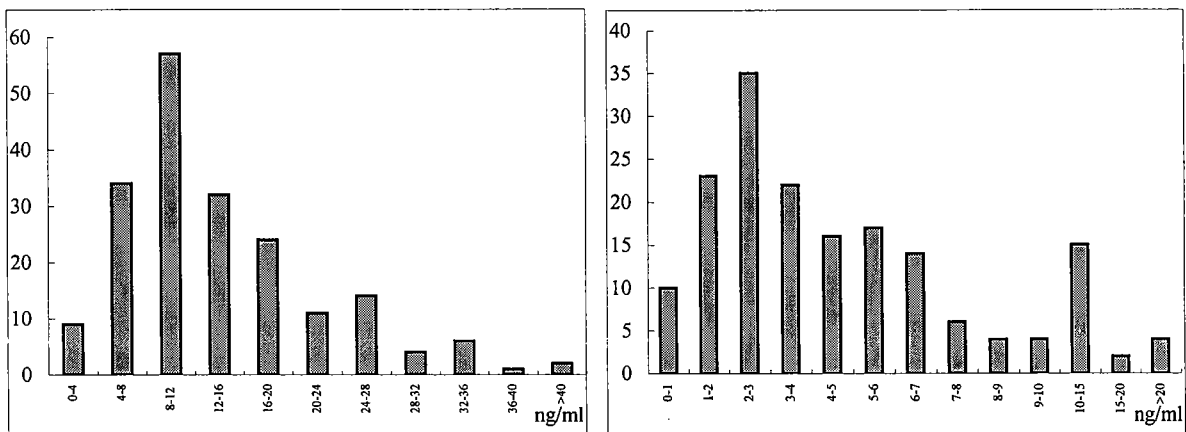Figure 2: Histogram of etiocholanolone (left: male, right: female)

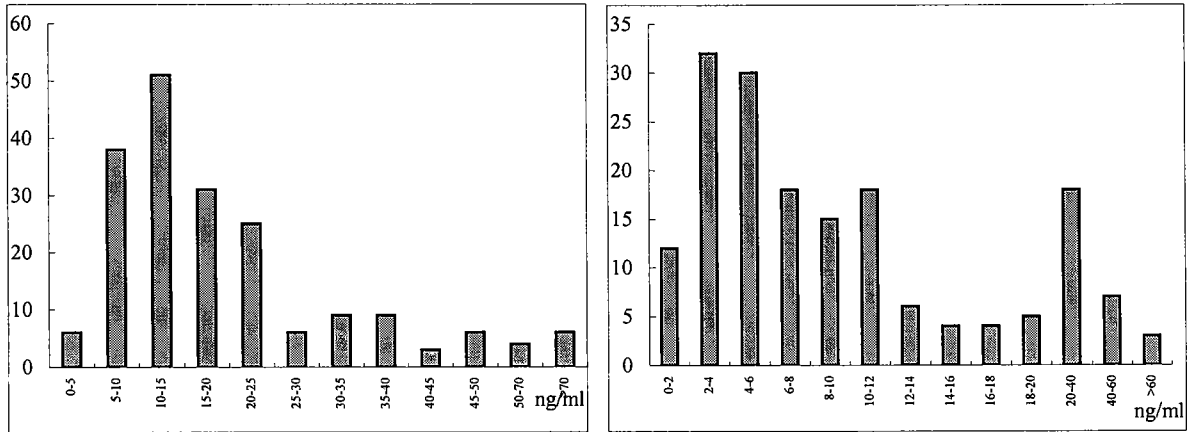Figure 3: Histogram of 5α-androstanediol (left: male, right: female)

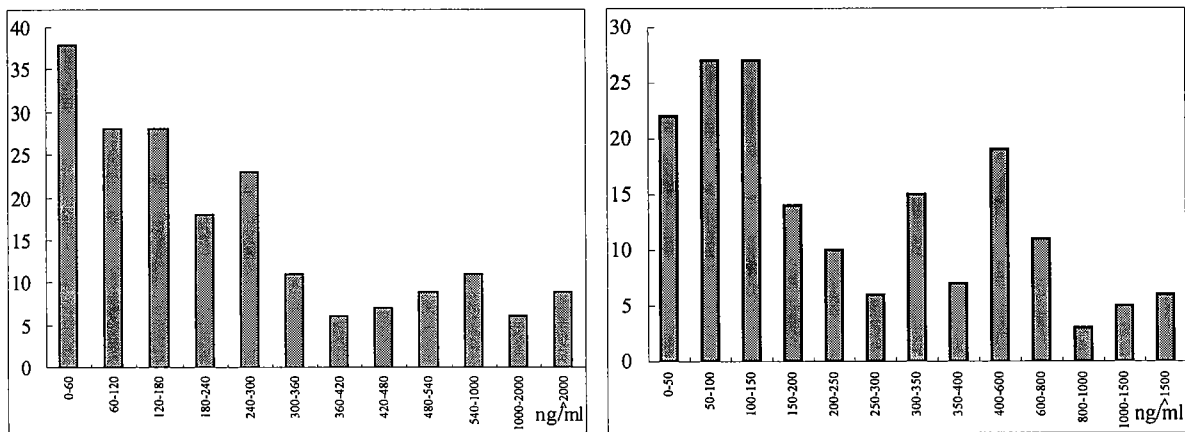Figure 4: Histogram of 5β- androstanediol (left: male, right: female)

Figure 5: Histogram of 11β-OH-androsterone (left: male, right: female)
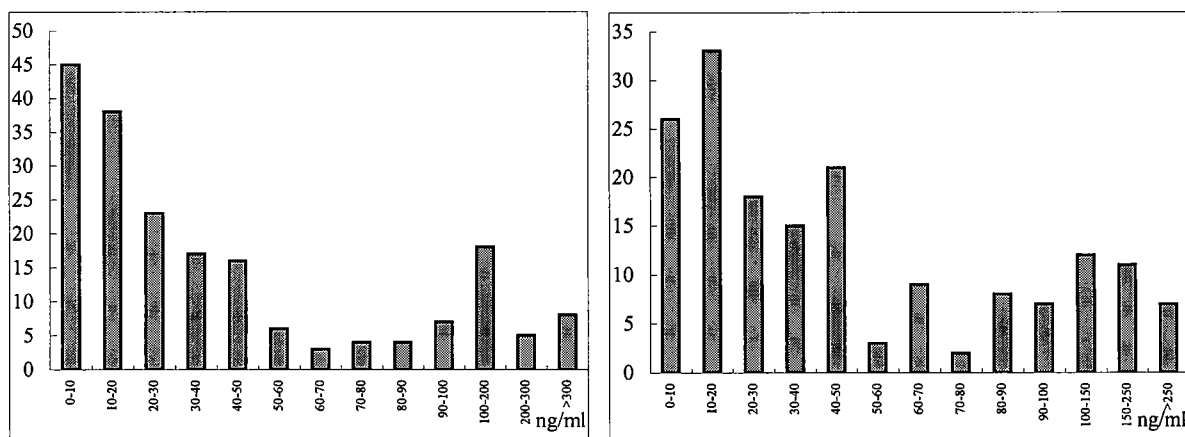

Figure 6: Histogram of 11β-OH-etiochonalone (left: male, right: female)
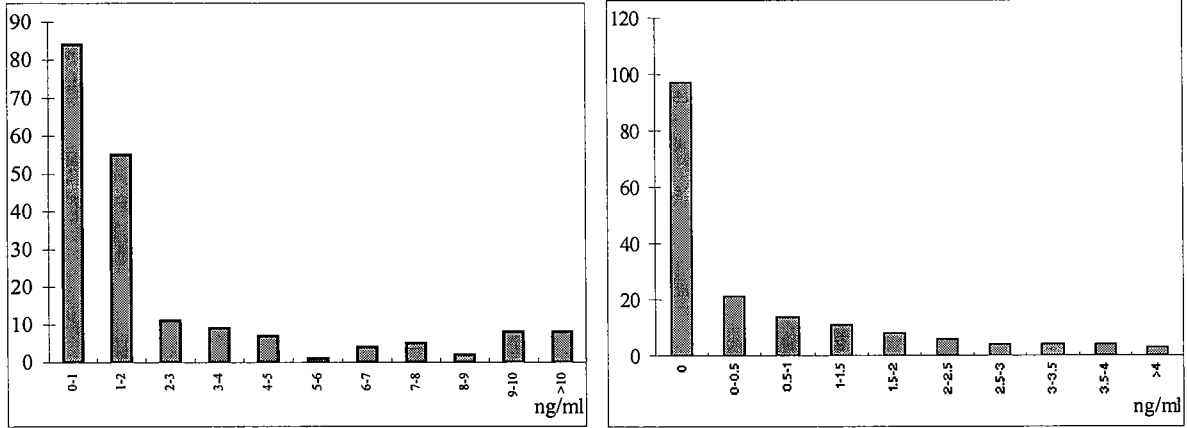
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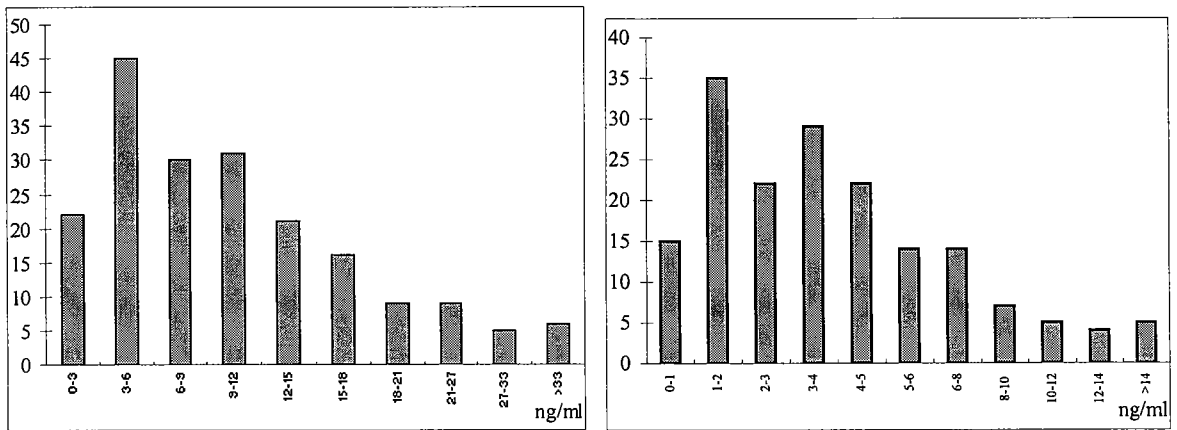Figure 7: Histogram of testosterone (left: male, right: female)

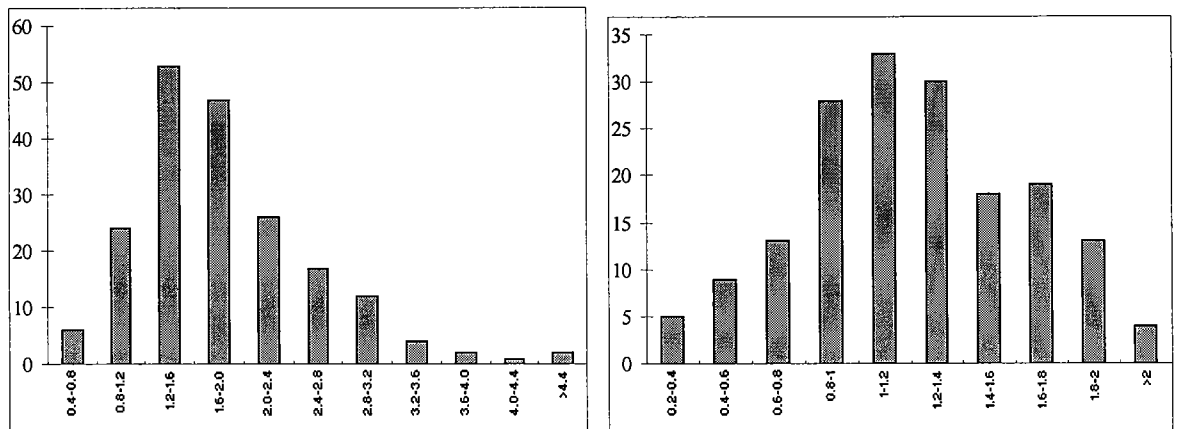Figure 8: Histogram of epitestosterone   (left: male, right: female)

Figure 9: Histogram of androsterone/etiocholanolone   (left: male, right: female)
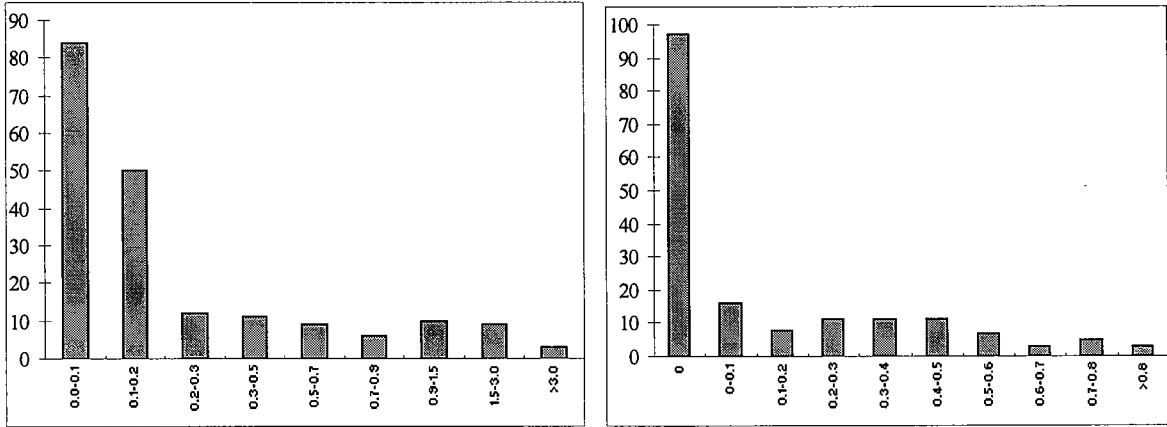
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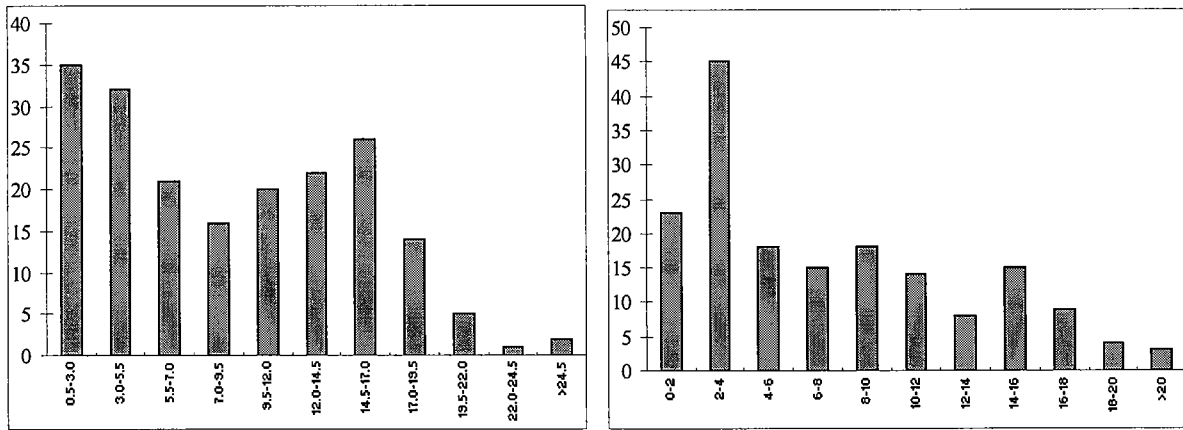Figure 10: Histogram of T/epi-T (left: male, right: female)

Figure 11: The histogram of 11β-OH-androsterone/11β-OH-etiocholanolone
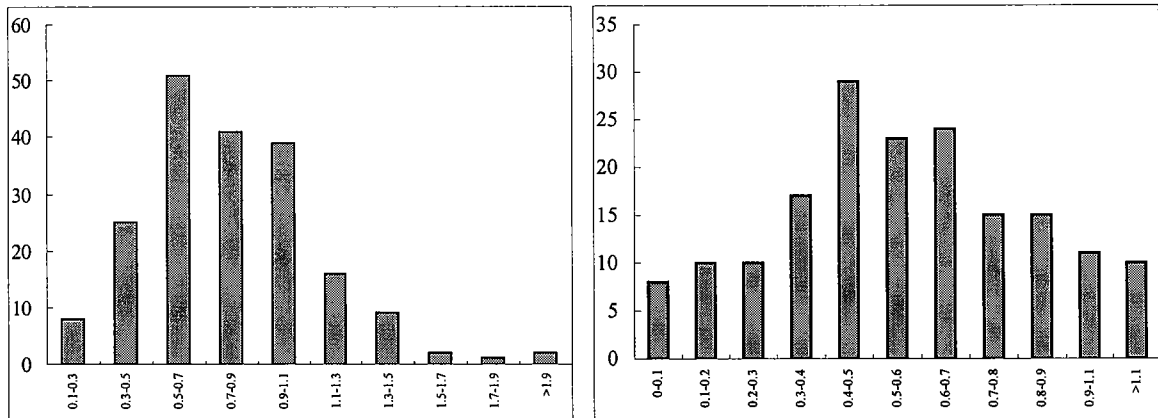(left : male, right : female)

Figure 12: The histogram of 5α-androstanediol/5β-androstanediol
(left : male, right : female)

Table1: non-parametric method results of male

	2.5% (ng/ml)	90% confide (ng/ml)		97.5% (ng/ml)	90% confide (ng/ml)	
androsterone	245	184	311	3686	2485	4749
etiocholanolone	127	94	192	1928	1460	3076
5α-androstanediol	3.0	2.7	4.1	34.6	31.8	40.0
5β-androstanediol	4.4	2.6	5.4	70.9	51.7	136.1
11β-OH-androsterone	13.6	9.4	21.7	3158.0	1816.6	4304.9
11β-OH-etiocholanolone	1.6	1.4	2.1	443.3	285.8	837.1
testosterone	0.1	0.0	0.3	12.5	9.9	16.6
epitestosterone	1.2	0.8	2.2	34.6	28.6	39.4
androsterone/etiocholanolone	0.77	0.67	0.93	3.78	3.12	4.41
5α-Androstanediol/5β-Androstanediol	0.26	0.12	0.34	1.58	1.36	2.00
11β-OH-androsterone/11β-OH-etiocholanolone	0.92	0.72	1.24	21.13	19.11	25.43
T/epi-T	0.03	0.00	0.04	2.54	1.89	3.30

Table 2: non-parametric method results of female

	2.5% (ng/ml)	90% confide (ng/ml)		97.5% (ng/ml)	90% confide (ng/ml)	
androsterone	69	8	156	3033	2176	3891
etiocholanolone	85	9	138	2594	2116	6341
5α-androstanediol	0.5	0.1	0.8	20.5	12.8	29.9
5β-androstanediol	1.3	0.2	1.8	57.2	41.7	217.9
11β-OH-androsterone	12.4	1.8	23.5	2369.6	1103.9	3287.4
11β-OH-etiocholanolone	2.1	0.5	3.8	333.6	218.2	3005.0
testosterone	0.0	0.0	0.0	4.0	3.4	8.5
epitestosterone	0.5	0.2	0.7	18.3	12.0	29.0
androsterone/etiocholanolone	0.37	0.30	0.48	2.08	1.89	3.27
5α-Androstanediol/5β-Androstanediol	0.08	0.07	0.10	1.22	1.12	1.44
11β-OH-androsterone/11β-OH-etiocholanolone	0.48	0.25	1.13	18.70	17.67	21.28
T/epi-T	0.00	0.00	0.00	0.79	0.65	1.90