Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (12)

W. Schänzer
H. Geyer
A. Gotzmann
U. Mareck
(Editors)

Sport und Buch Strauß, Köln, 2004

M. OKANO, M. SATO, M. UEKI:
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In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck (eds.) Recent advances in doping analysis (12). Sport und Buch Strauß, Köln (2004) 439-443

Masato OKANO, Mitsuhiko SATO, Makoto UEKI*)

Five year follow-up study on steroid profiles in individuals

-Stability and intra-individual variation of δ -values-

Doping Control Laboratory, Mitsubishi Kagaku Bio-Clinical Laboratories, Inc.
3-30-1 Shimura, Itabashi-ku, Tokyo 174-8555, JAPAN *) *Author for correspondence*

Introduction

The carbon isotope ratio of human steroids is considered to be stable as it depends solely on the origin of the carbon atom, and its acute change reflects the administration of a steroid, a source material or precursors. One possible influencing factor which may alter the δ -value is the carbon source from food. However, the long term stability of δ -value and the effects of different food customs is not well established yet. In this study, we refer to the long-term stability of the carbon isotope ratio of urinary steroids. The δ -parameters of non-vegetarians and strict vegetarians, are also compared.

Materials and Methods

Five volunteers including three males and two females were followed by longitudinal studies. Spot urines were also collected from 10 strict vegetarians in cooperation with the Sports Authority of India (SAI)'s doping control laboratory in New Delhi, and the samples were analyzed for steroid concentrations and δ -values in our Tokyo laboratory[1].

Results and Discussion

It has been observed that intra-individual variations of the concentration of androsterone(A) is as large as about 18 to 104 (CV)%, and the major influencing factors for the variations are sex and urine density. Ratios such as A/Etiocholanolone(E) and testosterone to epitestosterone(T/ET) were found to be more stable than steroid concentrations as the ratio compensates the variation sources. Due to a low concentration of androgens in the urine of females, however, variations of T/ET in females were larger than those in men (Table 1). On the other hand, the SD of δ - parameters of marker steroids in the same urine specimens was as small as 0.2 to 0.7 ‰ for A, E and 0.1 to 1.1 ‰ for 5 α - androstan-3 α ,17 β -diol(5 α A_2) and 5 β - androstan-3 α ,17 β -diol(5 β A_2). The δ -parameters were found to be essentially constant throughout the period(Table 2). Maximum values of the difference of δ -parameters from pregnanediol(P_2) were 1.27, 0.89, 0.50, 1.17 and 0.72 for DHEA, A, E, 5 α -A_2 and 5 β -A_2, respectively. On the other hand, the difference of average δ -values between Japanese non-vegetarians (n=108) and Indian vegetarians (n=10) was 0.3 to 1.5 ‰, and the difference was not significant. Major fuel of Indian vegetarians are vegetables which have low ^{13}C-content, however, the vegetarians also accept non-meat products from animal sources such as beverages from cow's milk and chicken eggs that may contain animal cholesterol. The results of our study unexpectedly suggested that for vegetarians, minor animal cholesterol is more predominant than carbon sources from vegetables. These results support the theory that isotope methodology allows a differentiation of steroid doping also among vegetarians. Long term inter-continental traveling was not the case for all the volunteers so the influence of inter-continental difference of foods was not considered in our studies.

Individual case considerations

Due to their special physiologic conditions, analytical results on two of the volunteers were further evaluated as follows. After a 1 year trial of reduction by case MU started in 2002, his height, weight, triglyceride, total cholesterol, HDL cholesterol, body fat%, AST, ALT changed from/to 169/169cm, 76/62kg, 165/62mg/dl, 206/173mg/dl, 45/58mg/dl, 24/14%, 68/20IU/l, 143/18IU/l respectively. Even though there were remarkable decreases of lipid fraction and change of liver function, the δ -value of his urinary steroids remained unchanged(Table1).

The T/ET ratio of case AR was 7.7 with an elevated testosterone concentration at a level of 164ng/ml, and his 5 α - and 5 β -A2 were also elevated such as 310 and 709 ng/ml respectively. However, elevated T/ET of this case was considered to be a natural as no significant difference of the δ -parameters from the other Indian volunteers was found.

Conclusion

No direct scientific evidence that demonstrates conversion of plant carbon sources to androgens in human body is available yet. The overall results showed that δ -parameters are fairly stable, and these of urinary steroids could hardly be altered by changing food, thus, athletes would need alternative sources of steroids or the precursors to establish manipulation.

Table 1 Intra-individual variation of steroid profiles (Units: ng/ml, mol/mol)

Volunteer	Date	pH	S.G.	A	E	A/E	T	ET	T/ET	DHT	5α-A2	5β-A2
	1999-7	6.8	1.019	1,453	863	1.7	21.7	8.5	2.56	1.38	39.7	35.2
	2000-11	7.1	1.016	1,142	899	1.3	24.1	9.9	2.42	0.62	39.0	95.0
M.U. (Male)	2001-6	6.1	1.021	1,413	1,154	1.2	24.7	8.5	2.90	1.56	52.8	125.3
	2002-2	6.8	1.022	1,399	1,357	1.0	41.4	21.7	1.91	3.77	52.9	128.0
	2003-5	5.3	1.030	963	1,263	0.8	29.2	11.8	2.47	1.15	17.8	93.8
Mean				1,274	1,107	1.2	28.3	12.1	2.45	1.70	40.4	95.4
SDn-1				213	219	0.3	7.8	5.5	0.35	1.21	14.3	37.4
CV(%)				16.7%	19.8%	28.4%	27.8%	45.7%	14.5%	71.2%	35.5%	39.2%
	1999-9	6.5	1.023	1,952	2,420	0.8	5.2	55.4	0.09	0.37	39.5	36.8
	2000-7	7.4	1.017	1,018	1,289	0.8	3.4	33.0	0.10	0.73	19.6	18.4
M.S.(Male)	2001-1	6.1	1.025	1,450	2,036	0.7	4.3	35.1	0.12	2.09	34.0	37.9
	2002-5	7.1	1.019	984	1,114	0.9	2.6	24.3	0.11	1.56	20.1	16.3
	2003-5	6.5	1.026	1,443	2,007	0.7	7.6	66.9	0.11	6.28	53.1	45.6
Mean				1,369	1,773	0.8	4.6	43.0	0.11	2.21	33.3	31.0
SDn-1				394.7	550.4	0.1	1.9	17.6	0.01	2.38	14.1	12.9
CV(%)				28.8%	31.0%	9.0%	41.4%	41.0%	9.8%	107.8%	42.3%	41.7%
	1999-8	6.8	1.017	697	823	0.8	2.5	10.1	0.25	1.97	22.2	22.6
	2000-12	5.8	1.018	1,466	1,868	0.8	5.5	20.5	0.27	2.98	49.3	47.5
M.O.(Male)	2001-6	6.8	1.020	1,275	1,329	1.0	4.1	14.1	0.29	4.45	48.2	47.1
	2002-2	5.3	1.023	2,271	2,325	1.0	5.2	15.8	0.33	6.71	81.3	65.8
	2003-5	5.3	1.027	1,837	2,317	0.8	5.4	20.4	0.27	5.71	91.9	87.5
Mean				1,509	1,732	0.9	4.5	16.2	0.28	4.36	58.6	54.1
SDn-1				593	652	0.1	1.3	4.4	0.03	1.93	28.0	24.2
CV(%)				39.3%	37.6%	10.4%	27.8%	27.2%	10.9%	44.3%	47.8%	44.7%
	1999-8	5.3	1.030	1,590	1,742	0.9	1.1	10.2	0.11	1.29	33.0	25.7
Y.T.(Female)	2001-6	6.8	1.022	1,609	1,364	1.2	0.9	19.1	0.05	0.77	33.8	19.0
	2002-5	6.5	1.025	1,442	1,388	1.0	1.0	6.5	0.15	0.96	28.3	19.9
	2003-5	6.5	1.020	818	765	1.1	0.8	3.3	0.23	0.33	17.5	7.6
Mean				1,365	1,315	1.1	1.0	9.8	0.14	0.84	28.2	18.0
SDn-1				372	405	0.1	0.2	6.9	0.08	0.40	7.5	7.6
CV(%)				27.3%	30.8%	10.4%	16.2%	70.1%	57.1%	47.7%	26.7%	42.0%
	1999-9	5.3	1.028	1,457	1,656	0.9	14.6	28.1	0.52	0.00	33.0	46.2
	2000-7	6.5	1.021	480	554	0.9	2.8	5.7	0.49	0.00	12.8	16.2
A.I. (Female)	2001-6	5.5	1.011	336	569	0.6	1.8	7.1	0.25	0.30	11.2	7.3
	2002-12	6.5	1.028	419	633	0.7	3.5	8.8	0.40	0.84	24.9	14.2
	2003-5	5.3	1.016	332	596	0.6	2.0	6.9	0.29	0.62	24.6	13.4
Mean				605	802	0.7	4.9	11.3	0.39	0.35	21.3	19.4
SDn-1				480	478	0.2	5.4	9.4	0.12	0.37	9.2	15.3
CV(%)				79.4%	59.7%	21.5%	110.1%	83.6%	29.8%	106.6%	43.0%	78.8%

Table 2 Intra-individual variation of δ -parameters (Unit:‰)

		δ -value (S:sulfate, G:glucuronide, T:Total))							difference δ -value					
Volunteer	Date	$DHEA_{(S)}$	$A_{(T)}$	$E_{(T)}$	5α $A_{2(G)}$	5β $A_{2(G)}$	$P2_{(G)}$	$P3_{(G)}$	P2-DHEA	P2-A	P2-E	A-E	P2-5αA2	P2-5βA2
	1999-7	-18.5	-19.4	-18.5	-19.7	-20.0	-18.7	-19.6	-0.21	0.67	-0.29	-0.83	1.00	1.20
	2000-11	-19.0	-18.7	-18.8		-19.0	-19.2	-19.8	-0.24	-0.57	-0.38	0.17		-0.18
M.U. (Male)	2001-6	-18.6	-19.3	-18.3			-18.8		-0.19	0.48	-0.49	-0.84		
	2002-2	-18.7	-19.1	-18.5	-18.2	-19.2	-19.0	-20.2	-0.23	0.15	-0.46	-0.53	-0.77	0.23
	2003-5	-18.9	-18.7	-18.2	-19.6	-19.1	-18.9	-19.9	-0.05	-0.24	-0.72	-0.41	0.73	0.18
Mean		-18.7	-19.0	-18.5	-19.2	-19.3	-18.9	-19.9	-0.18	0.10	-0.47	-0.49	0.26	0.38
SDn-1		0.2	0.4	0.2	0.9	0.4	0.2	0.3						
	1999-9	-19.2	-19.6	-19.0	-18.9	-19.7	-20.0	-19.3	-0.80	-0.45	-0.99	-0.47	-1.15	-0.30
	2000-7	-19.2	-19.4	-19.0	-20.7	-19.1	-18.8	-18.1	0.47	0.62	0.27	-0.30	1.89	0.30
M.S. (Male)	2001-1	-19.2	-19.1	-18.4	-18.0	-18.8	-20.2	-18.8	-0.97	-1.04	-1.76	-0.62	-2.23	-1.40
	2002-5	-18.8	-19.9	-19.9	-19.7	-19.9	-19.9	-19.2	-1.14	0.00	0.06	0.05	-0.23	-0.04
	2003-5	-19.7	-19.7	-20.0	-18.4	-19.9	-20.1	-20.1	-0.31	-0.40	-0.03	0.32	-1.63	-0.14
Mean		-19.2	-19.5	-19.3	-19.1	-19.5	-19.8	-19.1	-0.55	-0.25	-0.49	-0.20	-0.67	-0.32
SDn-1		0.4	0.3	0.7	1.1	0.5	0.6	0.7						
	1999-8	-19.3	-21.7	-22.0		-18.9	-20.1	-19.5	-0.83	1.59	1.88	0.25		-1.26
	2000-12	-19.6	-21.4	-21.6	-19.8	-19.0	-19.6	-18.3	-0.04	1.84	2.03	0.17	0.18	-0.61
M.O. (Male)	2001-6	-20.5	-21.2	-20.8	-19.1	-18.5	-19.2	-18.1	1.34	1.97	1.65	-0.28	-0.12	-0.64
	2002-2	-19.7	-21.5	-20.7	-19.7	-19.7	-19.8	-18.6	-0.10	1.69	0.94	-0.65	-0.10	-0.08
	2003-5	-20.9	-21.5	-21.0	-19.7	-19.1	-19.9	-19.6	1.02	1.61	1.17	-0.38	-0.18	-0.76
Mean		-20.0	-21.5*)	-21.2*)	-19.6	-19.0	-19.7	-18.8	0.28	1.80**)	1.50**)	-0.18	-0.16	-0.67
SDn-1		0.7	0.2	0.5	0.3	0.4	0.3	0.7						
	1999-8	-19.9	-19.4	-20.0	-19.1	-19.6	-19.8	-19.6	0.16	-0.38	0.21	0.51	-0.69	-0.17
Y.T. (Female)	2001-6	-20.1	-19.5	-20.1		-19.9	-20.1	-20.4	0.00	-0.64	0.02	0.57		-0.21
	2002-5	-20.1	-18.8	-19.7	-18.8	-19.1	-20.7	-20.0	-0.62	-1.92	-1.02	0.78	-1.94	-1.55
	2003-5	-20.1	-20.0	-20.1	-19.2	-19.1	-20.1	-20.3	0.03	-0.13	0.02	0.13	-0.92	-0.95
Mean		-20.1	-19.4	-20.0	-19.0	-19.4	-20.2	-20.1	-0.11	-0.77	-0.19	-0.50	-1.17	-0.72
SDn-1		0.1	0.5	0.2	0.2	0.4	0.4	0.3						
	1999-9	-18.8	-19.7	-19.4	-19.2	-19.8	-19.9	-19.0	-1.02	-0.17	-0.46	-0.25	-0.61	-0.04
	2000-7	-19.2	-19.8	-19.5		-19.5	-20.1	-19.7	-0.91	-0.34	-0.59	-0.21		-0.61
A.I. (Female)	2001-6	-19.1	-18.7	-18.9	-19.6	-19.5	-20.3	-19.4	-1.23	-1.61	-1.39	0.19	-0.70	-0.78
	2002-12	-18.4	-18.9	-18.4	-19.2	-19.8	-20.1	-18.9	-1.72	-1.15	-1.64	-0.42	-0.91	-0.29
	2003-5	-18.7	-19.0	-19.1	-19.6	-19.6	-20.2	-19.2	-1.44	-1.19	-1.09	0.09	-0.56	-0.59
Mean		-18.8	-19.2	-19.1	-19.4	-19.6	-20.1	-19.2	-1.27	-0.89	-1.03	-0.12	-0.70	-0.46
SDn-1		0.3	0.5	0.4	0.2	0.1	0.2	0.3						

Unit : $\delta^{13}C$(‰) *)overlapped peak(A(S)-20.2±0.7 , E(S)-20.4±0.6) **) overlapped peak (P2-A(S) 0.50 , P2-E(S)0.70)

Table 3 δ-parameters for Indian vegetarians (Unit: ‰)

			δ-value (S:sulfate, G:glucuronide, T:Total))							difference δ-value				
Volunteer	pH	S.G.	$DHEA_{(S)}$	$A_{(T)}$	$E_{(T)}$	$5\alpha A_{2(G)}$	$5\beta A_{2(G)}$	$P2_{(G)}$	$P3_{(G)}$	P2-DHEA	P2-A	P2-E	A-E	P2-5αA2
A.B. (Female)	7.7	1.007	-21.7	-20.9	-21.7								-0.83	
P.Y. (Female)	5.8	1.011	-19.7	-19.5	-19.5	-18.0	-18.1	-19.6	-18.3	0.07	-0.09	-0.17	-0.83	-1.64
M.D. (Female)	5.5	1.010	-20.1	-19.7	-19.2	-18.1	-18.2	-19.9	-19.1	0.19	-0.27	-0.70	-0.83	-1.80
J.G. (Female)	5.3	1.012	-22.4	-21.3	-21.5	-20.3	-20.0	-20.9	-20.0	1.43	0.38	0.55	-0.83	-0.64
T.K. (Female)	5.8	1.009	-19.2	-19.7	-19.8	-17.2	-17.9	-19.0	-18.4	0.16	0.66	0.79	-0.83	-1.83
S.J. (Female)	5.5	1.025	-20.6	-19.8	-20.1	-20.9	-20.4	-20.6	-20.1	-0.08	-0.85	-0.58	-0.83	0.27
J.S. (Male)	8.3	1.028	-20.4	-19.2	-18.7	-18.5	-18.5	-19.5	-19.3	0.91	-0.24	-0.76	-0.83	-0.99
S(Male)	5.5	1.012	-21.7	-20.9	-20.6	-19.8	-20.2	-20.4		1.31	0.57	0.23	-0.83	-0.51
A.R. (Male)	8.3	1.032	-21.4	-20.0	-19.7	-20.3	-19.4	-20.5	-20.9	0.91	-0.48	-0.76	-0.83	-0.21
J.G. (Male)	7.1	1.004		-19.8	-19.6	-17.2	-17.1	-19.1	-18.1		0.67	0.49	-0.83	-1.97
Mean			-20.8	-20.1	-20.0	-18.9	-18.9	-20.0	-19.3					
SDn-1			1.1	0.7	1.0	1.4	1.2	0.7	1.0					
Reference(Japanese) n=108			-20.5±2.8	-20.9±1.4	-21.5±2.2	-20.4±3.3	-20.4±3.3	-20.6±3.1		-0.1±2.6	-1.3±2.0	0.3±2.9	0.8±0.9	-0.4±1.4

Unit : $\delta^{13}C$(‰)

Reference

1) M.Ueki, M.Okano, *Rapid Commun. Mass Spectrom.*,13,2237-2243(1999)