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METABOLIC PROFILE IN HUMAN URINE AFTER ORAL ADMINISTRATION OF 19-NORANDROSTENEDIOL AS A NUTRITIONAL SUPPLEMENT

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INTRODUCTION

19-Norandrostenediol is a prohormone of nandrolone. Both substances are included in the WADA´s List of Prohibited Classes of Substances [1]. Consumption of substances is determined by the presence of 19-norandrosterone (NA) with a concentration over 2 ng/mL in urine. Routine analytical procedures allow the determination of NA excreted free and conjugated with glucuronic acid, but amounts of nandrolone and 19-norandrostenediol metabolites are also excreted in the sulphate fraction.

The aim of this study is to determine the urine levels of 19-norandrostenediol and metabolites in the free (FF), glucuronide (GF) and sulphate (SF) fractions after oral administration of a nutritional supplement containing 19-nor-4-androstenediol.

EXPERIMENTAL

Clinical study

Two capsules of Norandrodiol Select 300 (Ergopharm, USA) containing 19-nor-4-androstenediol (theoretical content, 150 mg; measured content, 90.7±11.2 mg) were orally administered to 6 healthy male volunteers (protocol AEMPS 04-0012). Urine samples were collected before and at 0-2, 2-4, 4-8, 8-16, 16-24, 24-48, 48-72 and 72-96 h intervals following supplement administration.

Extraction procedure

Samples were extracted at pH 7 with t-butylmethyl ether (TBME) (FF). To obtain the GF, the remaining aqueous phase was extracted with Detectabuse cartridges and then subjected to enzymatic hydrolysis using β-glucuronidase from *E.coli* (55 ºC, 1 h). To obtain the SF, the aqueous phase was extracted with C_{18} cartridges and then subjected to solvolysis using a mixture of ethyl acetate: methanol: sulphuric acid (80:20:0.06) (55 ºC, 2 h). Extraction with TBME at pH 9 was performed after the hydrolyses steps. Derivatization of all fractions was made with MSTFA: NH_4I: 2-mercaptoethanol (60 ºC, 20 min).

Chromatographic conditions

Trimethylsilyl derivatives were analyzed by GC-MS. A dimethylpolysiloxane (17 m x 0.2 mm, 0.11 μm) column was used with helium as carrier gas with a flow rate of 0.8 mL min-1 (measured at 180 ºC). The temperature program started at 180°C, increased by 3°C/min up to 230°C, finally 40°C/min up to 310°C and held for 3 min. A 2 μL aliquot of sample was injected in the split mode (1:10). The injector and the detector were set at 280 ºC. Electron ionization (70 eV) and SIM acquisition modes (see table 1) were used.

Compound	Diagnostic ions	RT	RRT
Norandrosterone-d4 (ISTD.)	409, 424, 319	8.9	0.59
Norandrosterone (NA)	405, 420, 315	8.9	0.59
Norepietiocholanolone (ENE)	405, 420, 315	9.7	0.65
Noretiocholanolone (NE)	405, 420, 315	9.9	0.66
Norepiandrosterone (ENA)	405, 420, 315	10.2	0.69
Norandrostenediol (Nol)	420, 405, 330	11.1	0.74
Norandrostenedione (None)	416, 401, 311	11.9	0.79
Nandrolone (N)	418, 403, 194	12.3	0.82
Methyltestosterone (ISTD)	446, 301	14.9	1.00

Table 1**:** Diagnostic ions used for identification and quantification, retention times and relative retention times of the bis-O-TMS derivatives of the compounds in study.

RESULTS AND DISCUSSION

Acceptable validation parameters were obtained and no significant contribution of glucuronides in the SF was verified. After administration of Nol, the main metabolites detected were NA and NE in GF and SF. Epimers of both NA and NE, ENA and ENE respectively, were also detected in SF (**Table 2** and **3).** NA, NE and ENA were detected in SF of all volunteers at all collected times, however ENE was not detected in the first samples after administration (**Figure 1**). Few hours after administration the main metabolite was NA glucuronide and in the last sample (4 days after administration) the main metabolite turns to be NA sulphate, followed by NE glucuronide **(Figure 2).** As shown in **Table 3,** after 96 h almost half of the dose was excreted and main metabolites were still found in urine.

Free fraction

Hours	NA (ng mL^{-1})					NE (ng mL^{-1})					N (ng mL^{-1})					Nol (ng mL^{-1})					None (ng mL^{-1})				
	n^a	mean	±	sd	range	n^a	mean	±	sd	range	n^a	mean	±	sd	range	n^a	mean	±	sd	range	n^a	mean	±	sd	range
0		0,0	±	0,0			0,0	±	0,0			0,0	±	0,0			0,0	±	0,0			0,0	±	0,0	
2	6	3,4	±	2,9	0,6-8,5	6	14,5	±	15,7	1,7-44,1	6	1,8	±	1,1	0,6-3,7	6	10,0	±	6,2	3,8-20,8	6	79,1	±	54,8	13,3-153,5
4	6	9,8	±	11,7	2,1-32,1	6	28,2	±	36,6	4,0-97,2	6	2,6	±	2,7	0,8-7,9	6	21,6	±	17,6	3,6-54,8	6	188,0	±	176,6	57,3-489,8
8	6	3,8	±	3,1	1,4-9,8	6	6,6	±	3,7	3,-12,8	6	1,0	±	0,6	0,6-2,2	6	6,3	±	3,4	1,9-11,2	6	72,7	±	86,7	30,5-249,3
16	6	3,7	±	2,1	0,9-6,7	6	14,4	±	16,5	2,8-46,9	6	0,7	±	0,3	0,4-1,2	6	4,2	±	2,5	0,9-7,1	6	37,9	±	21,2	5,8-55,8
24	6	4,1	±	3,9	0,6-11,7	6	31,8	±	32,2	2,6-69,8	6	0,6	±	0,2	0,3-0,9	6	1,8	±	1,2	0,5-4,0	6	26,0	±	20,9	2,7-65,5
48	6	1,1	±	0,6	0,3-2,1	6	13,2	±	11,5	3,6-29,4	6	0,4	±	0,1	0,3-0,5	6	0,6	±	0,4	0,3-1,2	6	4,2	±	3,2	1,3-8,8
72	6	0,4	±	0,3	0,2-1,1	6	3,8	±	5,6	0,5-15,1	6	0,3	±	0,04	0,3-0,4	5	0,3	±	0,1	0,2-0,4	6	1,2	±	0,4	0,5-1,8
96	5	0,4	±	0,2	0,2-1,7	6	2,5	±	3,4	0,2-8,3	6	0,3	±	0,1	0,3-0,4	5	0,3	±	0,2	0,2-0,6	6	0,9	±	0,5	0,3-1,6

Glucuronide fraction

Hours	NA (ng mL^{-1})					NE (ng mL^{-1})					N (ng mL^{-1})					Nol (ng mL^{-1})				
	n^a	mean	±	sd	range	n^a	mean	±	sd	range	n^a	mean	±	sd	range	n^a	mean	±	sd	range
0		0,0	±	0,0			0,0	±	0,0			0,0	±	0,0			0,0	±	0,0	
2	6	13942,0	±	9784,2	2242,2-28562,2	6	6898,8	±	6933,1	1663,7-18124,5	6	487,2	±	776,3	6,3-2009,5	6	31,7	±	27,6	9,9-84,0
4	6	25671,2	±	18773,9	9711,6-60936,3	6	8677,7	±	8456,8	2934,6-25128,2	6	451,1	±	665,4	9,4-1764,0	6	40,9	±	35,5	19,0-113,0
8	6	11225,4	±	4555,3	5817,5-17814,3	6	4031,6	±	3275,4	1051,7-9673,2	6	173,3	±	204,3	2,8-553,8	6	22,1	±	14,0	9,3-47,3
16	6	10707,6	±	5283,2	2347,6-18551,2	6	10479,8	±	12983,1	3786,8-36632,9	5	110,7	±	133,8	1,5-282,5	6	23,4	±	16,2	5,1-44,1
24	6	5664,4	±	3822,6	997,1-11706,5	6	11536,0	±	9806,4	494,8-29977,9	5	32,0	±	26,2	2,3-60,5	6	10,0	±	7,3	2,4-21,9
48	6	1156,2	±	409,1	575,8-1733,2	6	4513,3	±	2823,3	1512,9-9456,2	4	13,9	±	7,6	4,8-23,2	6	3,0	±	1,9	0,9-6,2
72	6	217,4	±	266,2	29,4-639,9	6	797,6	±	1041,8	55,8-2666,3	4	1,8	±	1,41	0,7-3,6	5	14,4	±	30,4	0,4-68,9
96	6	146,7	±	200,6	6,0-437,9	6	412,8	±	627,0	4,3-1545,3	4	1,4	±	0,9	0,6-2,3	5	6,6	±	13,3	0,4-30,4

Sulphate fraction

Hours	NA (ng mL^{-1})					NE (ng mL^{-1})					ENA (ng mL^{-1})					ENE (ng mL^{-1})				
	n^a	mean	±	sd	range	n^a	mean	±	sd	range	n^a	mean	±	sd	range	n^a	mean	±	sd	range
0		0,0	±	0,0			0,0	±	0,0			0,0	±	0,0			0,0	±	0,0	
2	6	6499,8	±	5097,2	275,5-12821,8	6	2596,8	±	2236,3	247,3-5440,2	6	7189,9	±	6199,8	506,9-16906,3		0,0	±	0,0	
4	6	22888,7	±	20075,6	4168,2-50793,8	6	5690,9	±	6167,2	495,1-16509,9	6	17394,0	±	17181,6	3270,2-40229,5		0,0	±	0,0	
8	6	14905,0	±	8082,2	5276,3-25880,7	6	2325,7	±	1527,1	569,2-4399,2	6	6976,9	±	4325,2	3095,4-14313,4	1	51,1	±		
16	6	16589,0	±	6769,5	9624,7-26966,3	6	4084,6	±	4323,8	415,5-12308,9	6	4418,8	±	2127,8	1528,9-6995,6	3	136,8	±	161,1	24,9-321,4
24	6	12450,2	±	10288,8	1965,4-32320,2	6	4861,4	±	5821,7	551,1-14251,0	6	2506,3	±	2223,9	1088,7-6802,2	6	197,5	±	218,5	56,5-598,1
48	6	3197,3	±	1987,7	1785,2-6718,0	6	1967,3	±	1771,9	133,9-4448,6	6	412,3	±	267,3	149,1-880,9	6	122,9	±	101,2	36,5-306,7
72	6	695,7	±	447,5	271,5-1520,5	6	345,9	±	348,2	25,4-775,1	6	67,0	±	51,19	17,4-159,6	6	35,4	±	28,9	5,7-78,2
96	6	460,9	±	469,0	77,6-1305,5	6	169,3	±	207,6	5,8-493,0	6	47,1	±	56,6	3,9-154,5	6	24,5	±	24,1	2,3-54,7

Table 2: Concentrations detected for each metabolite in each fraction (n^a: number of volunteers in which the metabolite was detected).

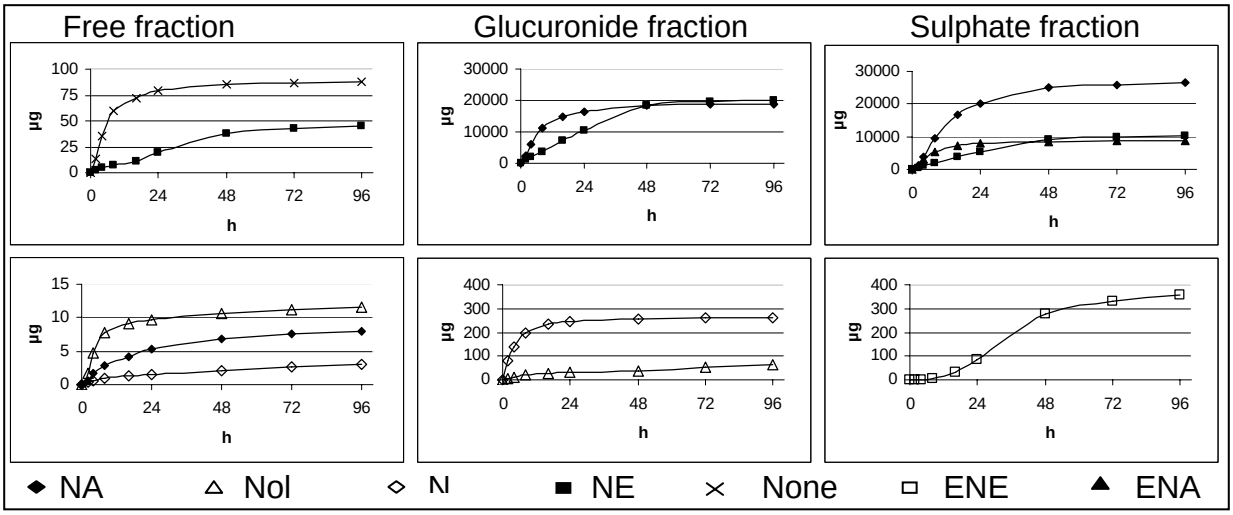

Figure 1: Mean cumulative excreted amounts for each metabolite in the different fractions.

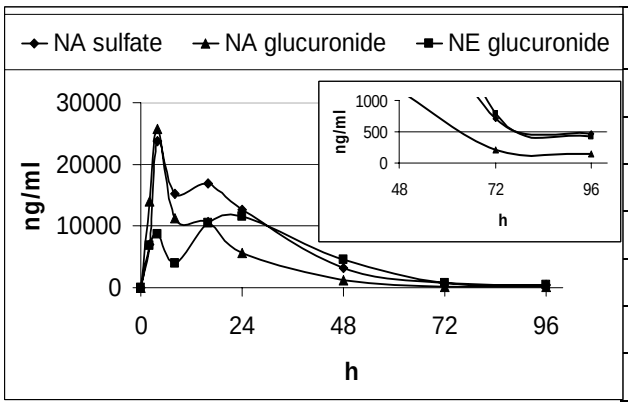

Figure 2: Mean concentration of main metabolites.

	FF Mean (SD)	GF Mean (SD)	SF Mean (SD)	Total Mean (SD)
NA	0,005 (0,001)	10,4 (4,0)	14,3 (4,1)	24,6 (6,7)
NE	0,025 (0,010)	11,0 (6,2)	5,0 (4,6)	16,0 (9,7)
ENA	-	-	4,8 (1,5)	4,8 (1,5)
ENE	-	-	0,2 (0,1)	0,2 (0,1)
N	0,002 (0,001)	0,1 (0,1)	-	0,1 (0,1)
None	0,049 (0,018)	-	-	0,05 (0,02)
Nol	0,006 (0,003)	0,03 (0,03)	-	0,04 (0,03)

Table 3: Percentage of the dose excreted of each metabolite in each fraction (n=6).

CONCLUSIONS

- The metabolic profile in urine after oral administration of Nol is presented.
- Relative abundance of NA and NE in the glucuronide and sulphate fractions fluctuates with time; in accordance with other authors [2]. NA sulphate is the main metabolite few days after administration.
- Detection of ENE has not been reported in previous studies [3, 4].

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