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RECENT ADVANCES IN DOPING ANALYSIS (3)

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U. Mareck-Engelke, U. Flenker and M. Donike:
Stability of Steroid Profiles (5): The Annual Rhythm of Urinary Ratios and Excretion Rates of Endogenous Steroids in Female and its Menstrual Dependency
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Stability of Steroid Profiles (5): The Annual Rhythm of Urinary Ratios and Excretion Rates of Endogenous Steroids in Female and its Menstrual Dependency

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The stability of ratios and excretion rates of endogenous steroids in female urine was investigated. Five female (age: x= 30 ± 5.5 years) volunteers participated. Urine samples were collected two times a month (at the beginning and in the middle of the female menstrual cycle; n=24) over one year.

The urines were prepared according to the screening procedure of conjugated anabolic steroids and analysed by GC/MS (1).

The following steroid glucuronides were measured and quantified: androsterone (A), etiocholanolone (E), testosterone (T), epitestosterone (epiT), 11ß-OH-androsterone (OHA), 11ß-OH-etiocholanolone (OHE), 5α-androstan-3α,17ß-diol (Adiol) , 5ß-androstan-3α,17ß-diol (Bdiol), pregnandiol (Pregnd) and tetrahydrocortisol (THF).

The results of selected steroid concentration ratios, their excretion rates and statistical evaluations are shown in Tables 1- 6 and Figures 1- 5 .

Conclusions

There is no annual rhythm recognized for excretion rates (fig 1,2) or ratios (fig 3) of the examined endogenous steroids. Also, the excretion rates of the endogenous steroids show strong intraindividual and interindividual variation (tab 1,4).

The most stable steroid profile parameters for women over the whole year are the ratios A/E and Adiol/Bdiol (tab 1,2). Ratios of T and epiT show much more variation. The concentrations of testosterone and epitestosterone are near the detection limit and other endogenous substances frequently coelute with them (tab 1,2).
The ratios A/Adiol, A/Bdiol, E/Adiol and E/Bdiol show coefficient of variations less than 30 percent (tab 1,2).

Similar to the former studies (4,5) differences between excretion rates of all quantified steroids (max and min) and their corresponding mean values were calculated. The aim is borderline definitions of the subject-based reference ranges for persons under resting conditions within one year.
The calculated values for all steroids from all volunteers are presented in tab 5 and 6.
The borderlines (L_0) of the subject-based reference range for androsterone, etiocholanolone, testosterone, epitestosterone, 5α-androstan-3α,17ß-diol and 5ß-androstan-3α,17ß-diol are similar to the 24 hour ranges.
The definition may be (for n=24): L_0 = mean ± 3.0 * standard deviation
The values for 11ß-OH-androsterone, 11ß-OH-etiocholanolone, pregnandiol and tetrahydrocortisol are different to the 24 hour range. They are situated in a wider range characterised as: " mean ± 4.4 * standard deviation"
These data are based on 24 data points per person and steroid.

Excretion rates and ratios of the endogenous steroids from the first and 14th day of the female menstrual cycle were compared interindividually and evaluated statistically (Wilcox-Matched-Pair-Test). The result shows that there is no dependency between excretion of the investigated endogenous steroids and day of the female menstrual cycle (fig 4).

One of the volunteers (V5) stopped application of the contraceptive for 3 months during the collection period. The excretion rates of the endogenous steroids raised, the highest being pregnandiol (fig 5). Further studies to investigate this point are already underway.

References

(1) Donike M., Geyer H., Gotzmann A., Kraft M., Mandel F., Nolteernsting E., Opfermann G., Sigmung G., Schänzer W. and Zimmermann J.: Dope Analysis
In: Official Proceedings of the International Athletic Foundation World Symposium on Doping in Sport. P.Bellotti, G.Benzi, A.Ljungquist (Hrsg.) IAAF Florence (1988) 53-87.

(2) Mareck-Engelke U., Geyer H. and Donike M.: Stability of steroid profiles
in: M.Donike, H.Geyer, A.Gotzmann, U.Mareck-Engelke, S.Rauth (Eds.) 10th Cologne workshop on dope analysis 7th to 12th June 1992, Sport und Buch Strauß, Köln (1993) 87-89.

(3) Mareck-Engelke U., Geyer H. and Donike M.: Stability of steroid profiles (2) :
Excretion rates from morning urines.
in: M.Donike, H.Geyer, A.Gotzmann, U.Mareck-Engelke, S.Rauth (Eds.) Recent advances in doping analysis, Sport und Buch Strauß, Köln (1994) 85-89.

(4) Mareck-Engelke U., Geyer H. and Donike M.: Stability of steroid profiles (3) :
The Circadian Rhythm of Urinary Ratios and Excretion Rates of Endogenous Steroids in Male.
in: M.Donike, H.Geyer, A.Gotzmann, U.Mareck-Engelke (Eds.) Recent advances in doping analysis (2) Sport und Buch Strauß, Köln (1995) 121-133.

(5) Mareck-Engelke U., Geyer H. and Donike M.: Stability of steroid profiles (4) :
The Circadian Rhythm of Urinary Ratios and Excretion Rates of Endogenous Steroids in Female and its Menstrual Dependency.
in: M.Donike, H.Geyer, A.Gotzmann, U.Mareck-Engelke (Eds.) Recent advances in doping analysis (2) Sport und Buch Strauß, Köln (1995) 135-155.

Table 1: Stability of steroid profiles (female urine).

Urine collected at the beginning and in the middle of the female menstrual cycle.
(Total collection time: 1 year)
Coefficient of variation (%) of some selected steroid concentration ratios
(V1-V5 = volunteer 1-5).

	V1	V2	V3	V4	V5
A/E	12	9	9	26	18
T/epiT	15	51	25	31	30
A/T	14	37	19	23	18
A/epiT	15	43	35	36	23
Adiol/Bdiol	17	11	19	16	22
A/Adiol	16	11	17	21	18
A/Bdiol	18	13	30	21	30
E/Adiol	14	14	18	24	20
E/Bdiol	16	9	30	20	22
OHA/OHE	70	35	72	44	39
A/THF	22	23	27	38	30

Table 2: Stability of steroid profiles (female urine).

Urine collected at the beginning and in the middle of the female menstrual cycle.

(Total collection time: 1 year)

Statistics of some selected steroid concentration ratios

min = minimum value max = maximum value

st.de. = standard deviation c.v. = coefficient of variation (%)

V1-V5 = volunteer 1-5

	V1	V2	V3	V4	V5
A/E					
min	0.76	0.71	0.48	0.52	0.46
max	1.27	0.94	0.69	1.39	0.85
mean	1.05	0.83	0.59	0.84	0.61
st.dev.	0.12	0.07	0.05	0.22	0.11
c.v. (%)	12	9	9	26	18

	V1	V2	V3	V4	V5
T/epiT					
min	1.53	0.06	0.49	1.09	0.80
max	3.45	0.35	1.34	5.11	2.19
mean	2.32	0.17	0.96	2.94	1.23
st.dev.	0.35	0.09	0.24	0.90	0.37
c.v. (%)	15	51	25	31	30

	V1	V2	V3	V4	V5
Adiol/Bdiol					
min	1.55	1.06	2.22	1.50	3.55
max	3.25	1.59	4.53	3.00	8.09
mean	2.37	1.27	3.02	2.23	5.63
st.dev.	0.41	0.14	0.59	0.36	1.23
c.v. (%)	17	11	19	16	22

Table 3: Stability of steroid profiles (female urine).

Urine collected at the beginning and in the middle of the female menstrual cycle.

(Total collection time: 1 year)

Coefficient of variation (%) of excretion rates

V1-V5 = volunteer 1-5

	V1	V2	V3	V4	V5
A	43	23	25	27	25
E	47	18	25	41	24
epiT	45	62	43	70	27
T	46	33	27	40	35
Adiol	45	16	43	47	45
Bdiol	63	35	44	51	38
OHA	48	21	29	35	28
OHE	54	19	36	35	33
Pregnd	51	62	45	121	29
THF	53	18	39	57	45

Table 4: Stability of steroid profiles (female urine).

Urine collected at the beginning and in the middle of the female menstrual cycle.

(Total collection time: 1 year)

Statistics of some selected excretion rates

min = minimum value max = maximum value

st.de. = standard deviation c.v. = coefficient of variation (%)

V1-V5 = volunteer 1-5

		V1	V2	V3	V4	V5
A (µg/h)	min	13	65	13	10	51
	max	91	171	59	45	134
	mean	45	107	38	29	86
	st.dev.	20	24	9	8	21
	c.v. (%)	43	23	25	27	25
E(µg/h)	min	10	89	22	12	75
	max	93	182	96	74	230
	mean	44	129	65	38	142
	st.dev.	21	24	16	16	34
	c.v. (%)	47	18	25	41	24
epiT (µg/h)	min	0.01	0.23	0.09	0.02	0.11
	max	0.12	1.46	0.75	0.20	0.30
	mean	0.06	0.61	0.33	0.07	0.21
	st.dev.	0.03	0.38	0.14	0.05	0.06
	c.v. (%)	45	62	43	70	27
T (µg/h)	min	0.04	0.03	0.11	0.08	0.16
	max	0.31	0.14	0.48	0.36	0.49
	mean	0.14	0.08	0.29	0.17	0.25
	st.dev.	0.07	0.03	0.08	0.07	0.09
	c.v. (%)	46	33	27	40	35

Table 5: Stability of steroid profiles (female urine).

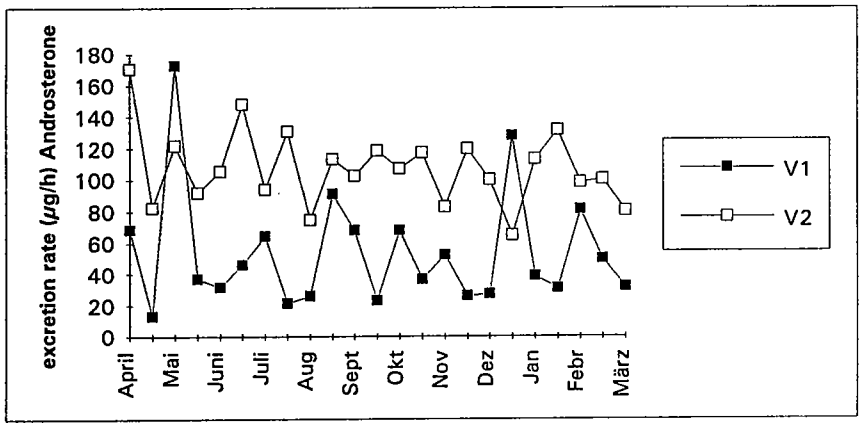
Urine collected at the beginning and in the middle of the female menstrual cycle.
(Total collection time: 1 year) V1-V5 = volunteer 1-5
Calculation factor (t) for the upper boarderline (u_0) of the subject-based reference range following the formula: u_0 = mean + t * st.dev.

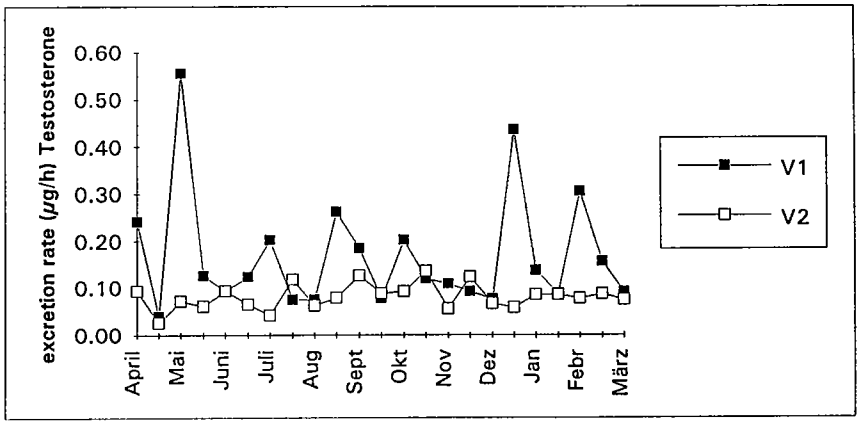
	V1	V2	V3	V4	V5
A	2.3	2.7	2.3	2	2.3
E	2.3	2.2	1.9	2.3	2.6
epiT	2	2.2	3.0	2.6	1.5
T	2.4	2.0	2.4	2.7	2.7
Adiol	2.8	2.2	2.6	2.4	3.4
Bdiol	2.0	2.8	1.8	2.4	3.2
11OHA	3.1	1.7	2.6	3.1	2.6
11OHE	3.0	2.1	2.0	2.5	2.3
Pregnd	3.3	3.0	3.1	4.4	2.4
THF	3.6	2.2	2.5	3.0	3.4
mean	2.6	2.3	2.4	2.7	2.6

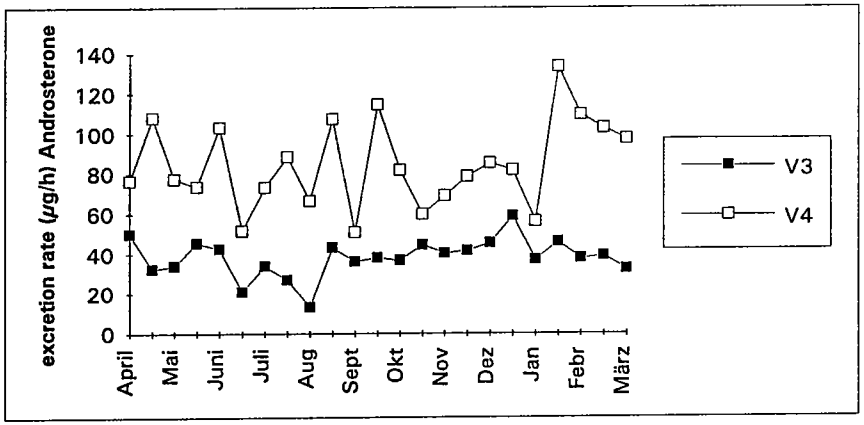
Table 6: Stability of steroid profiles (female urine).

Urine collected at the beginning and in the middle of the female menstrual cycle.
(Total collection time: 1 year) V1-V5 = volunteer 1-5
Calculation factor (t) for the lower boarderline (l_0) of the subject-based reference range following the formula: l_0 = mean + t * st.dev.

	V1	V2	V3	V4	V5
A	1.6	1.8	2.8	2.4	1.7
E	1.6	1.7	2.7	1.6	2.0
epiT	1.7	1.0	1.7	1.0	1.7
T	1.4	1.7	2.2	1.3	1.0
Adiol	1.4	2.0	1.6	1.7	1.1
Bdiol	1.4	1.8	1.8	1.3	1.3
11OHA	1.5	1.7	2.3	2.3	1.7
11OHE	1.2	1.7	1.7	2.0	1.9
Pregnd	1.5	1.1	1.6	0.5	1.8
THF	1.5	1.7	2.1	1.3	1.4
mean	1.5	1.6	2.1	1.5	1.6







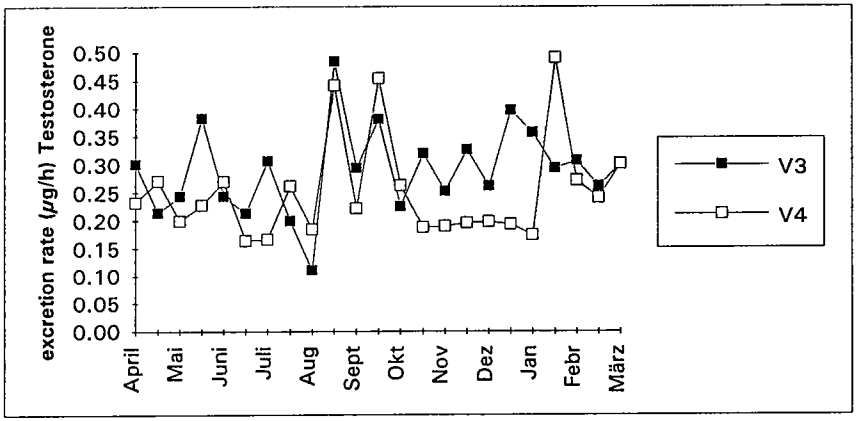
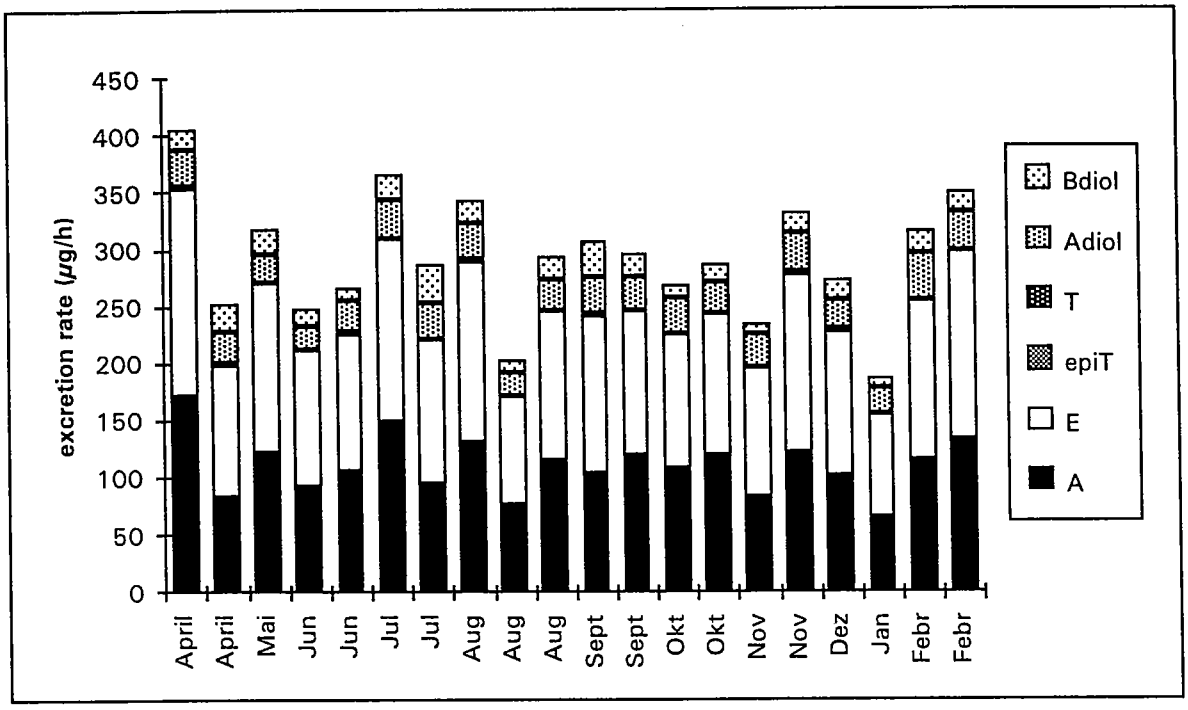
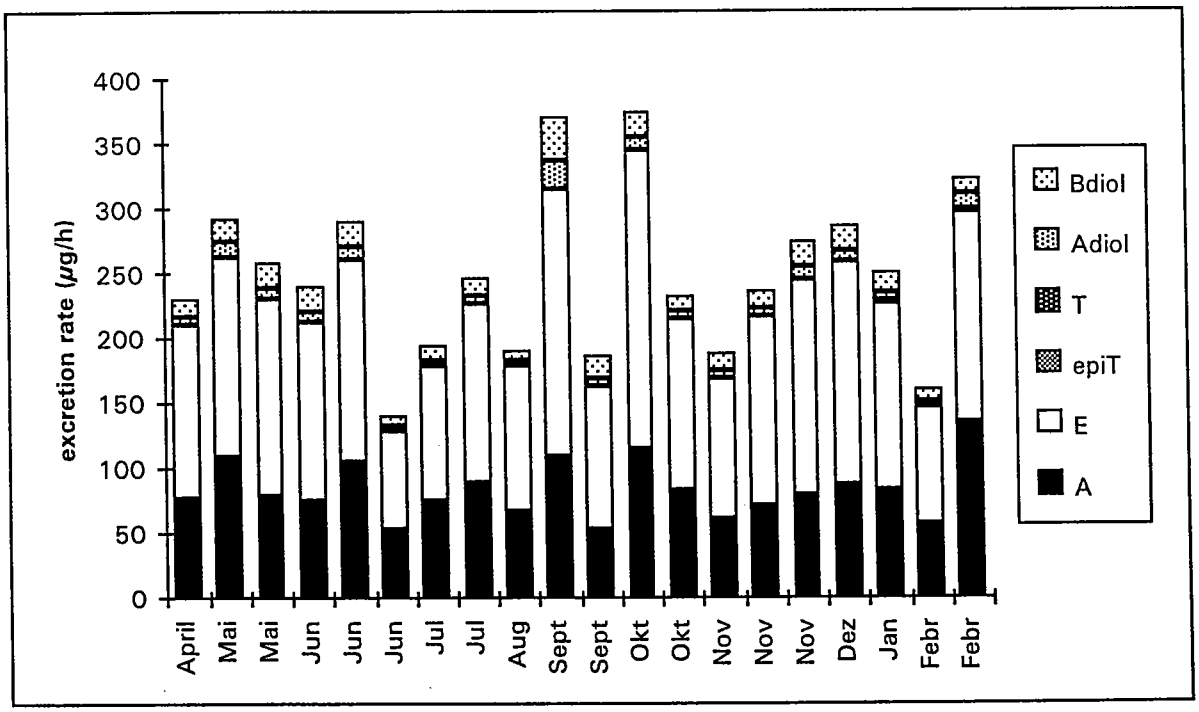


fig 1: excretion rates of endogenous steroids in female urine
urine collected at the beginning and in the middle of the female menstrual cycle (total collection time: 1 year)
V1 - V4 = volunteer 1 - 4

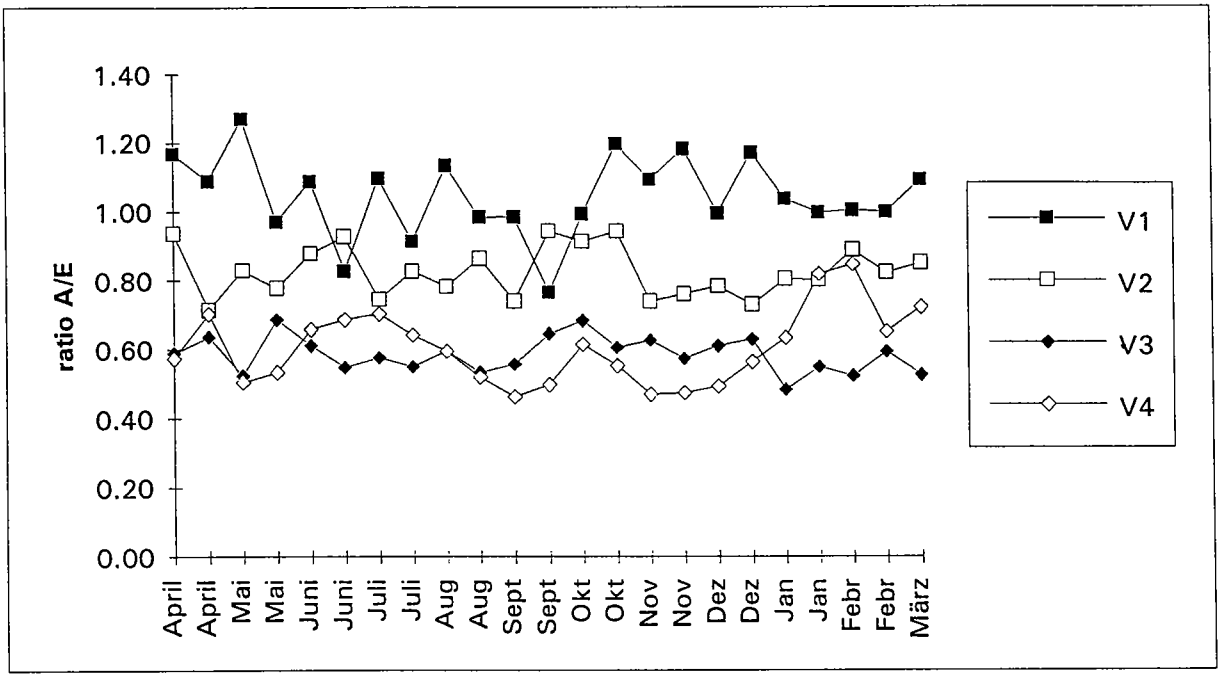


volunteer 2



volunteer 4

fig 2: excretion rates of endogenous steroids in female urine
urine collected over 1 year
(at the beginning and in the middle of the female menstrual cycle)



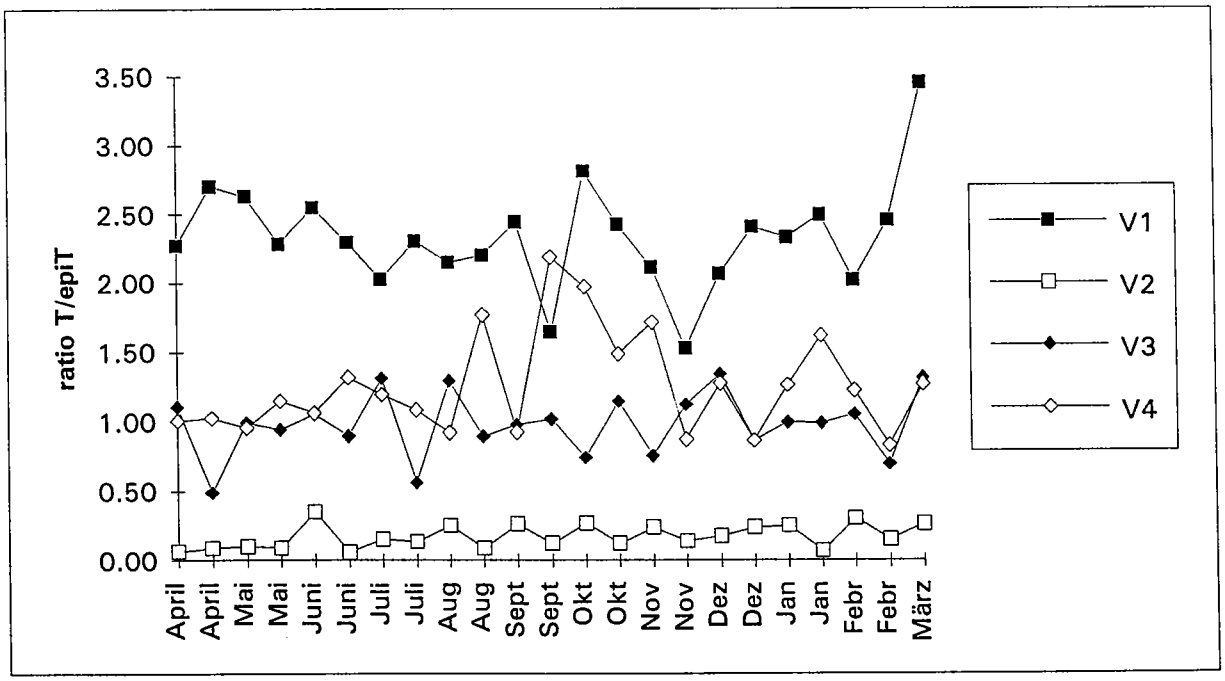
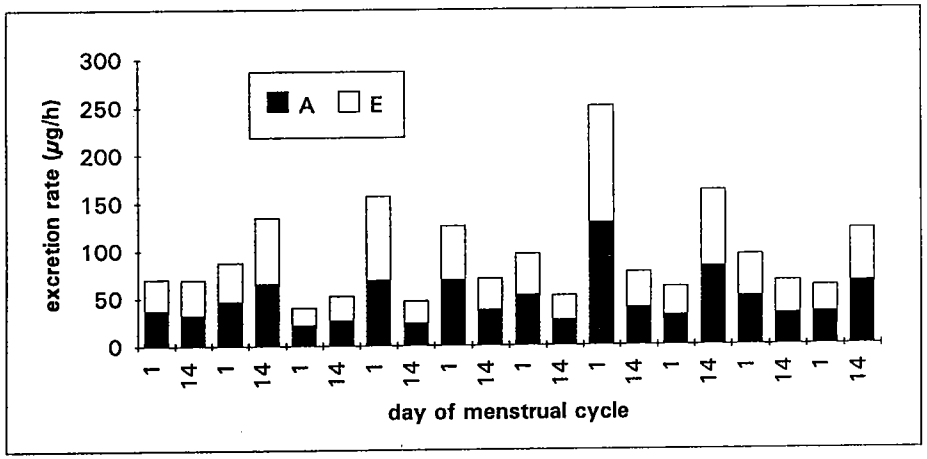
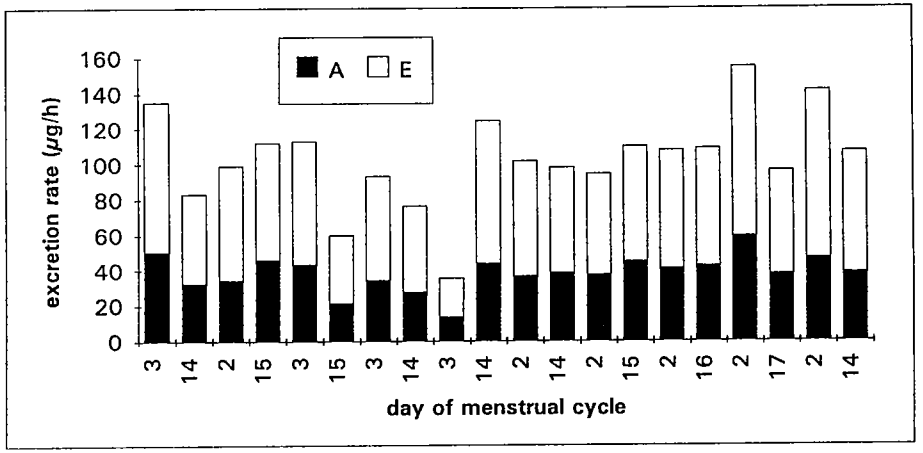


fig 3: ratios of endogenous steroids in female urine
urine collected at the beginning and in the middle
of the female menstrual cycle (total collection time: 1 year)
V1 - V4 = volunteer 1 - 4



volunteer 1



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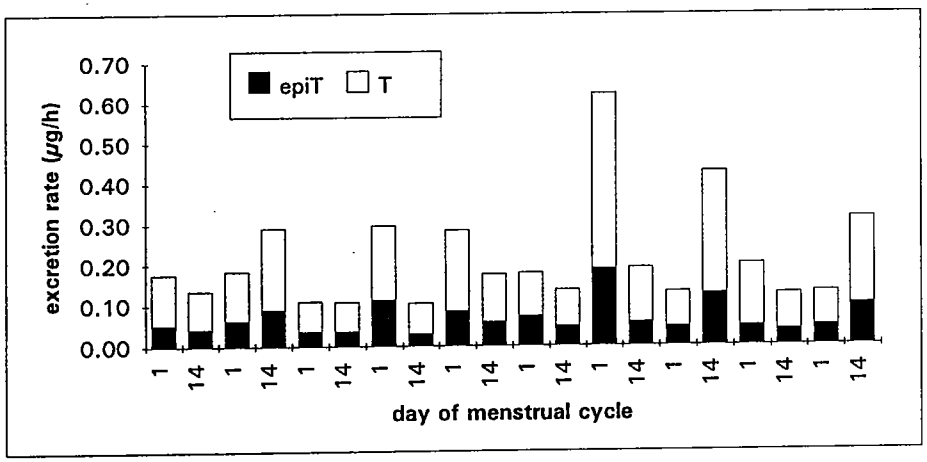

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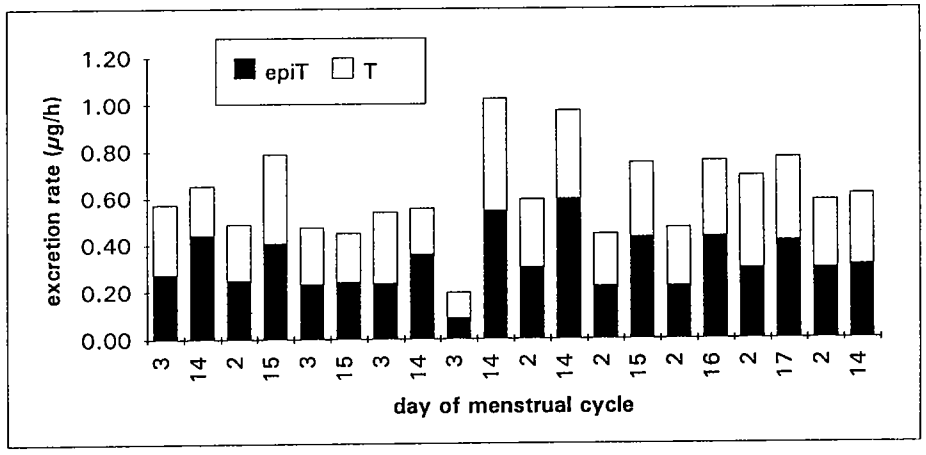
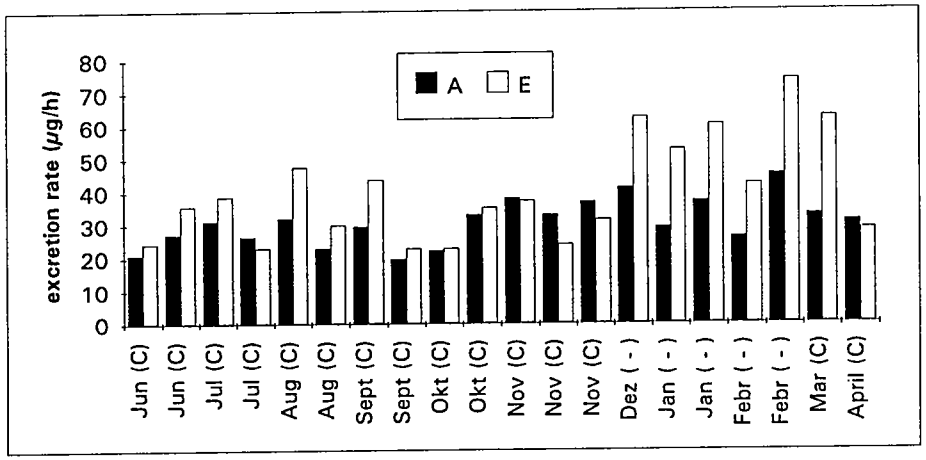
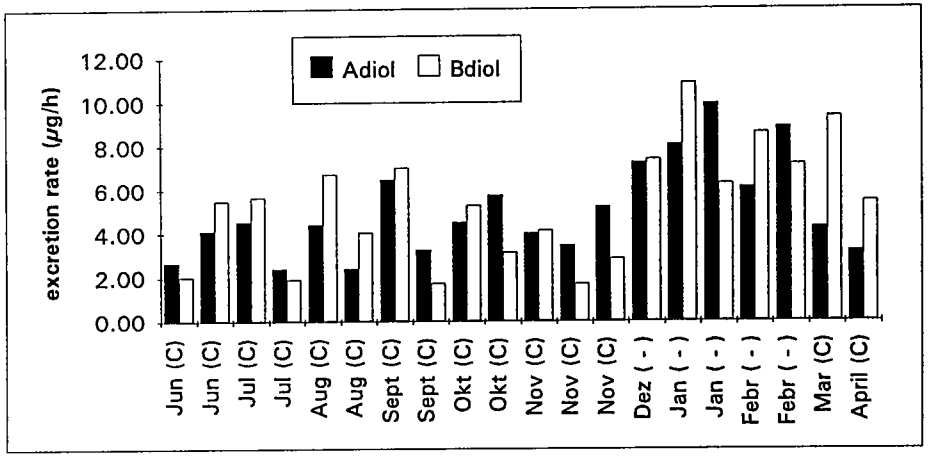
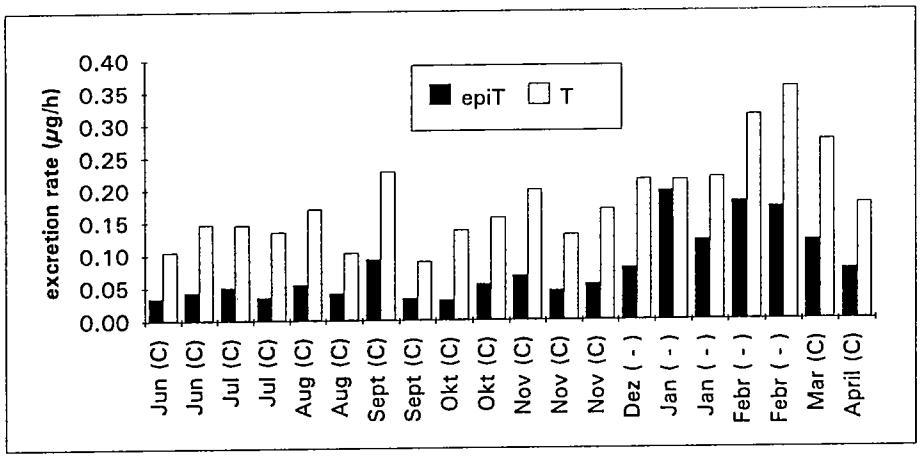


volunteer 3

fig 4: comparison of excretion rates of endogenous steroids in female urine
urine collected at the beginning and in the middle of the female menstrual cycle (total collection time: 1 year)







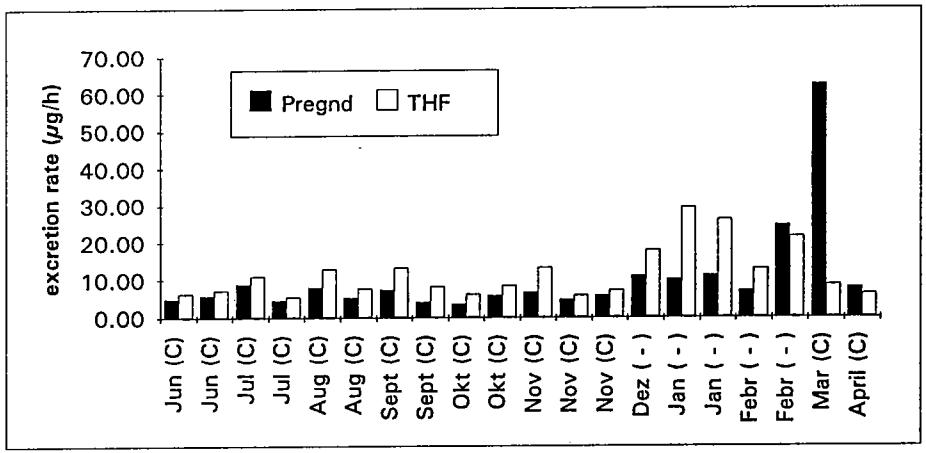


fig 5: excretion rates of endogenous steroids in female urine
volunteer 5
the application of the contraceptive (C) was stopped for 3 months (Dez-Febr)