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U. MARECK-ENGELKE, U. FLENKER, W. SCHÄNZER:  
Stability of Steroid Profiles (6): The Influence of Oral Contraceptives on Steroid Profiles  
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## **Stability of Steroid Profiles (6): The Influence of Oral Contraceptives on Steroid Profiles**

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### **Abstract**

The present examination was performed to investigate the influence of oral contraceptives on steroid profile parameter of female volunteers.

Morning urine samples from one female menstrual cycle each with and without application of oral contraceptives from four female healthy volunteers were collected.

The urine samples were prepared according to the screening procedure of conjugated anabolic steroids and analyzed by GC/MS .

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

The stability of the most important ratios and excretion rates of endogenous steroids concerning judgement of steroid profiles in dope analyzes were investigated.

Urine samples collected during application of oral contraceptives were compared to the urines collected during withdrawal of oral contraceptives for each volunteer.

As the most important result of this study no significant change of the ratio A/E has been seen.

The ratio A/E has also been proved to be the most stable parameter in steroid profiles.

The ratio T/epiT shows a decrease due to an increase of the epitestosterone excretion during withdrawal oral contraceptives.

Due to the missing suppression from the gestogen-component of the oral contraceptive the excretion of pregnanediol shows a strong increase during the second part of the female menstrual cycle for volunteers taking no oral contraceptives.

## Introduction

The presented data is taken from basic studies about stability of steroid profiles.

The following samples have been investigated in former studies (2,3,4,5,6):

- Morning urine samples of one female menstrual cycle
- Morning urine samples collected 30 days for male volunteers
- Urine samples collected every two hours over 24 hours for male and female volunteers
- Morning urine samples collected over one year two times a month for female volunteers

The stability of important ratios and excretion rates of endogenous steroids concerning judgement of steroid profiles in dope analytic was investigated.

The most stable parameters of steroid profiles were A/E, Adiol/Bdiol and (for male volunteers) T/epiT. For female volunteers ratios with testosterone and epitestosterone show much more variation, because the concentrations of those steroids are near the detection limit and other endogenous substances frequently coelute with them.

## Experimental

### *Sample preparation (1)*

2 ml of urine and 20 µl of an internal standard mixture (17 $\alpha$ -methyltestosterone 50ppm, [2,2,4,4,<sup>2</sup>H<sub>4</sub>]-etiocholanolone 50ppm, [16,16,17,<sup>2</sup>H<sub>3</sub>]-testosterone 2ppm, [2,2,4,4,<sup>2</sup>H<sub>4</sub>]-11 $\beta$ -hydroxyandrosterone 14ppm) are added to a Amberlite XAD-2 column. The column (pasteur pipette, closed with glass pearl, bed height 2 cm) is washed with 2 ml of bidistilled water and the absorbed fraction is eluted with 2 ml of methanol. The methanolic eluate is evaporated to dryness and the residue is dissolved in 1 ml of 0.2 M sodium phosphate buffer pH 7.

To the buffer solution, 50 µl of beta-glucuronidase from E.coli is added and hydrolysis is performed for 1 h at 50°C. The buffered solution is alkalized with 250µl of 7% potassium carbonate solution to pH 9-10 and the steroids are extracted with 5 ml of tert.-butylmethylether on a mechanical shaker for 5 minutes. After centrifugation the ethereal layer is transferred and evaporated to dryness under vacuo.

### *Derivatisation*

The dry residue is derivatised with 100 µl of MSTFA/NH<sub>4</sub>I/ethanethiol 1000:2:3 (v:w:v) and heated for 15 min at 60°C.

3 µl of the solution are injected into the GC/MS.

### *GC/MS parameters*

GC/MS: HP 5890/HP 5971A (Hewlett Packard)

column: HP Ultra I (OV-1), 17m, 0.2mm i.d., 0.11 µm film thickness

carrier gas: 1ml helium at 180°C, split 1:10

temperature programm: 180°C, 3°C per min, 229°C, 40°C per min, 320°C

### *GC parameters*

GC: F45 Perkin Elmer

column: 2 m stainless steel 1/8" i.d. with 15% Carbowax 1500 on Chromosorb W-NAW 80-100 mesh

temperatures: oven 80°C, injector and detector 150°C

### *Volunteers and Experimental Protocol*

Four healthy female volunteers participated. Morning urines were collected for two female menstrual cycle each; one female menstrual cycle under the influence of oral contraceptives and one female menstrual cycle without the influence of oral contraceptives.

Table 1 shows the age of the volunteers during the urine collection time, the kind of applicated oral contraceptive, the total application time of the contraceptive and the time of withdrawl before collecting the second time morning urines.

Two different kind of oral contraceptives were applicated. A one phase oral contraceptive containing desogestrel and ethinylestradiol and two different three phase contraceptives containing levonorgestrel and ethinylestradiol.

## Results and Discussion

In former investigations the ratios A/E, Adiol/Bdiol and T/epiT (for male volunteers) were the most stable parameters.

In table 2 is shown the comparison of those ratios from urines, collected with and without application of oral contraceptives.

For all volunteers the ratio Adiol/Bdiol shows few variation.

The ratio A/E shows for all volunteers less variation for urines collected without oral contraceptives. Urines collected with application of oral contraceptives show an increase of the ratio A/E in the end of the female menstrual cycle (fig 1,2). The form of the curve is similar for all volunteers although it is not possible to proof this statistically, due to the small number of volunteers. For urines collected without application of oral contraceptives no course is obvious.

For all collected urines high variation is seen for the ratio T/epiT (tab 2) . The concentrations of testosterone and epitestosterone are near the detection limit and other endogenous substances frequently coelute with them.

The mean values of the ratio T/epiT are significantly lower for the urines collected without application of oral contraceptives (tab3, fig 1,3,4).

To explain this decrease of T/epiT-ratio it is necessary to investigate the excretion rates of the endogenous steroids as well.

Former studies (3,4,5,6) proved that some selected steroid ratios are more stable than excretion rates of endogenous steroid. In tab 4 is shown the stability (coefficient of variation) of excretion rates from some important endogenous steroids. Low variation is found for excretion of androsterone and etiocholanolone.

Excretion of testosterone shows much more intraindividual variation, as its concentration is near the detection limit as well as other endogenous substances frequently coelute with testosterone.

Epitestosterone and pregnanediol concentration in urines collected without application of oral contraceptives shows much higher variation than in urines collected under the influence of oral contraceptives (tab 4,5).

The excretion of epitestosterone shows a strong increase in the second part of the female menstrual cycle (fig 5,6) for all volunteers without application of oral contraceptives. The mean values of epitestosterone also increase when oral contraceptives are withdrawn to an individual formally taking no medication at all (fig 8).

Furthermore excretion rates (tab 5; fig 5,7) and mean values (fig 8) of pregnanediol rise in urines collected from volunteers taking no oral contraceptives.

These changes are due to the progesterone production of the corpus luteum with consecutive metabolism of progesterone to pregnanediol.

In the first part of the female menstrual cycle the follicle is stimulated by FSH. While growing the follicle produces estradiol. Due to a high level of estradiol the production of FSH is suppressed. After the ovulation the follicle transforms to a corpus luteum. The corpus luteum is stimulated by LH and produces progesterone which itself suppresses the LH-production via negative feed-back. With decreasing of the LH levels the progesterone-production stops and the menstruation starts.

Oral contraceptives often consist of two compounds. The first compound is an estradiol-derivative most often ethinylestradiol. It suppresses the FSH-production in the first part of the female menstrual cycle and hinders ovulation. The second compound is a progesterone-derivative.

Levonorgestrel or norethisterone is frequently used to both, the LH-production and the production of endogenous progesterone. In the second part of the female menstrual cycle the constant application of progesterone leads to a stable excretion of the main metabolite pregnanediol.

## **Conclusion**

Application of oral contraceptives can lead to an increase of the ratio T/epiT due to a suppression of the epitestosterone-excretion.

The ratios A/E, Adiol/Bdiol and the excretion of androsterone and testosterone are not influenced by oral contraceptives.

During application of oral contraceptives, the excretion of pregnanediol is a stable intraindividual parameter.

After withdrawal of oral contraceptives a strong increase can be observed in the second part of the female menstrual cycle (after ovulation) due to the missing suppression of LH by the gestogen component of the oral contraceptive.

For judging steroid profiles or endocrinological studies in female individuals one has to recognize, that changes in steroid profile parameters (especially T/epiT-ratio) can be due to application or withdrawal of oral contraceptives.

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Table 1: Influence of oral contraceptives on steroid profiles.

Morning urines collected two times for one female menstrual cycle.

(1 month with application of oral contraceptives and one month without)

(V1-V4 = volunteer 1-4).

	V1	V2	V3	V4
<b>with oral contraceptive</b>				
kind of oral contraceptive	3 phase	1 phase	3 phase	1 phase
age of volunteer	31	34	33	29
time taken o.c. before (years)	10	4	0.75	1
<b>without oral contraceptive</b>				
age of volunteer	34	36	36	27
pause (months)	12	4	29	3



Table 2: Influence of oral contraceptives on steroid profiles.

Morning urines collected from two female menstrual cycles each with and without application of oral contraceptives

Coefficient of variation (%) of some selected steroid concentration ratios (V1-V4 = volunteer 1-4).

O.C.: Oral Contraceptive

	A/E	T/epiT	Adiol/Bdiol
<b>V1</b>			
with O.C.	20	32	19
without O.C.	7	46	15
<b>V2</b>			
with O.C.	16	60	14
without O.C.	10	52	8
<b>V3</b>			
with O.C.	16	32	21
without O.C.	12	39	29
<b>V4</b>			
with O.C.	22	23	22
without O.C.	14	48	15

Table 3: Influence of oral contraceptives on steroid profiles.

Morning urines collected from two female menstrual cycles  
 each with and without application of oral contraceptives  
 Statistics of some selected steroid concentration ratios  
 (V1-V4 = volunteer 1-4).

+ : with Oral Contraceptive - : without Oral Contraceptive

min = minimum value                      max = maximum value

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

	V 1		V 2		V 3		V 4	
	+	-	+	-	+	-	+	-
<b>A/E</b>								
min	0.8	0.6	0.5	0.8	0.4	0.4	0.5	0.45
max	1.5	0.9	0.95	1.1	0.75	0.6	1.0	0.8
mean	1.1	0.75	0.75	0.9	0.6	0.5	0.7	0.6
st.dev.	0.23	0.05	0.1	0.09	0.09	0.06	0.2	0.09
c.v.(%)	20	7	16	10	16	12	22	14
<b>T/epiT</b>								
min	1.8	0.4	0.2	0.07	1.2	0.4	1.75	0.5
max	5.7	2.1	1.5	0.5	3.9	1.7	4.2	4
mean	3.4	1.1	0.6	0.2	1.8	0.9	2.4	1.5
st.dev.	1.1	0.5	0.4	0.1	0.6	0.3	0.6	0.7
c.v.(%)	32	46	60	52	32	39	23	48
<b>Adiol/Bdiol</b>								
min	2.3	1.6	1.3	0.9	7.1	3.2	1.9	1.6
max	4.2	2.9	2.1	1.3	15	12	4.9	2.6
mean	2.8	2.1	1.6	1.1	11	6.8	2.9	2.1
st.dev.	0.5	0.3	0.2	0.09	2.2	1.9	0.6	0.3
c.v.(%)	19	15	14	8	21	29	22	15

Table 4: Influence of oral contraceptives on steroid profiles.

Morning urines collected from two female menstrual cycles each with and without application of oral contraceptives

Coefficient of variation (%) of excretion rates

(V1-V4 = volunteer 1-4).

O.C.: Oral Contraceptive

	A	E	epiT	T	Pregnd
<b>V1</b>					
with O.C.	15	18	30	34	24
without O.C.	16	17	50	39	111
<b>V2</b>					
with O.C.	14	16	32	44	23
without O.C.	23	27	50	25	60
<b>V3</b>					
with O.C.	18	21	28	32	37
without O.C.	21	19	48	21	84
<b>V4</b>					
with O.C.	21	30	29	44	36
without O.C.	30	28	70	55	105

Table 5: Influence of oral contraceptives on steroid profiles.

Morning urines collected from two female menstrual cycles each with and without application of oral contraceptives

Statistics of some selected excretion rates

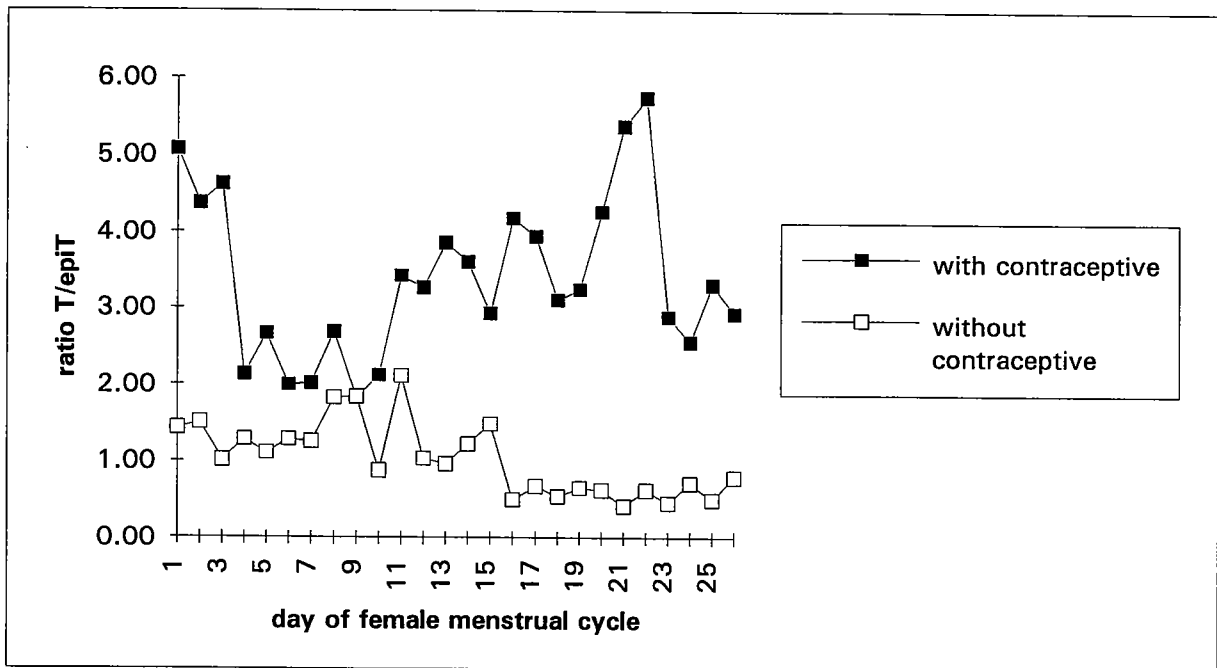
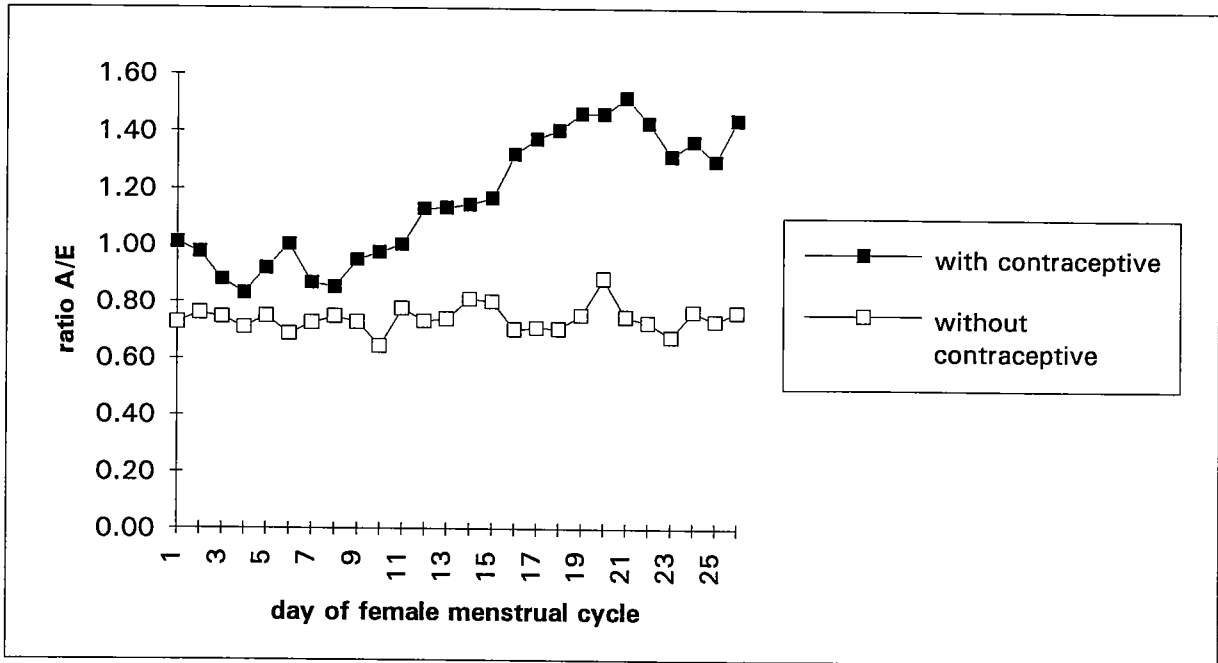
(V1-V4 = volunteer 1-4).

+ : with Oral Contraceptive - : without Oral Contraceptive

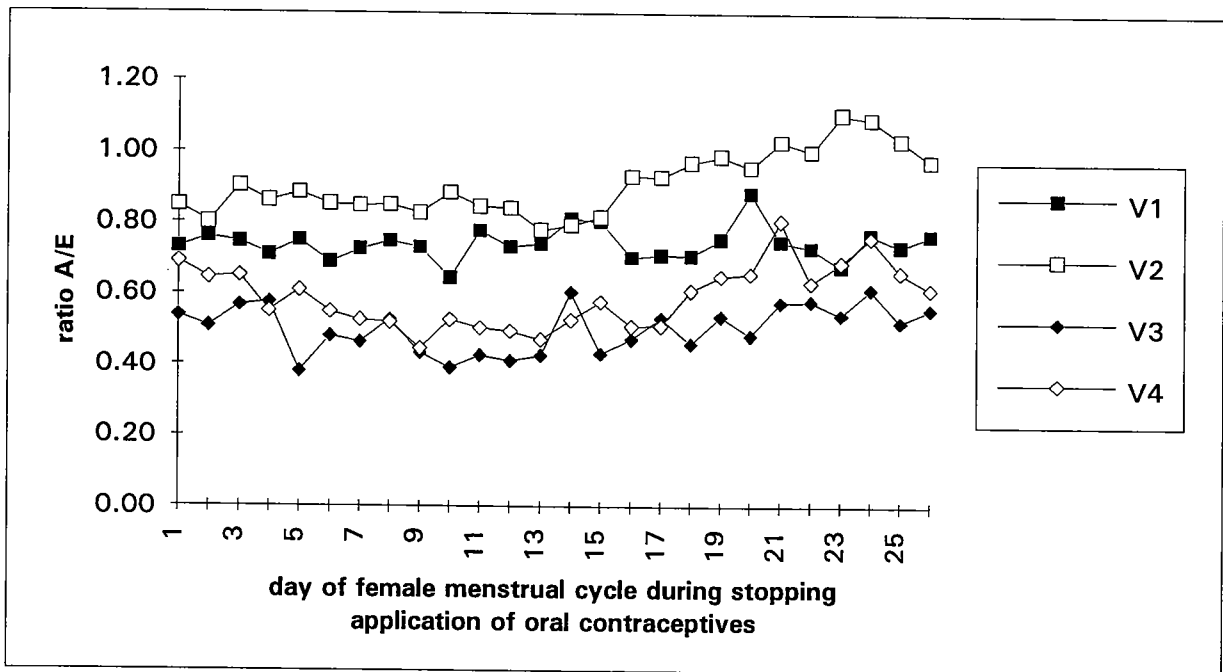
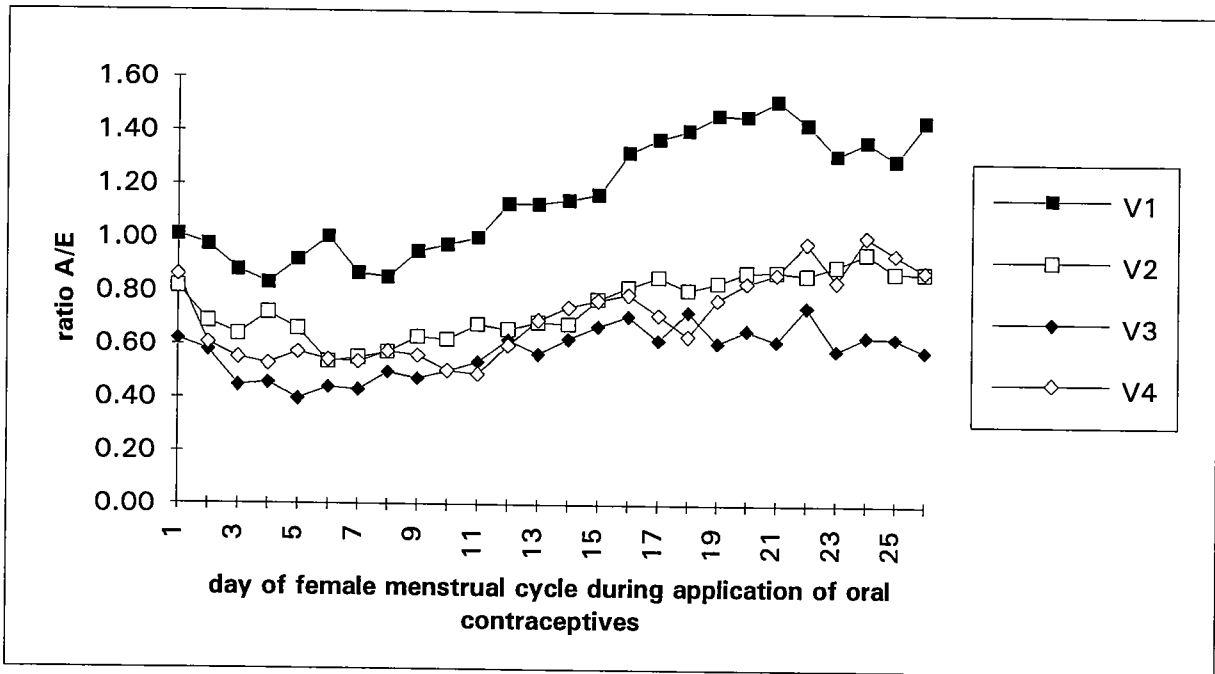
min = minimum value                      max = maximum value

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

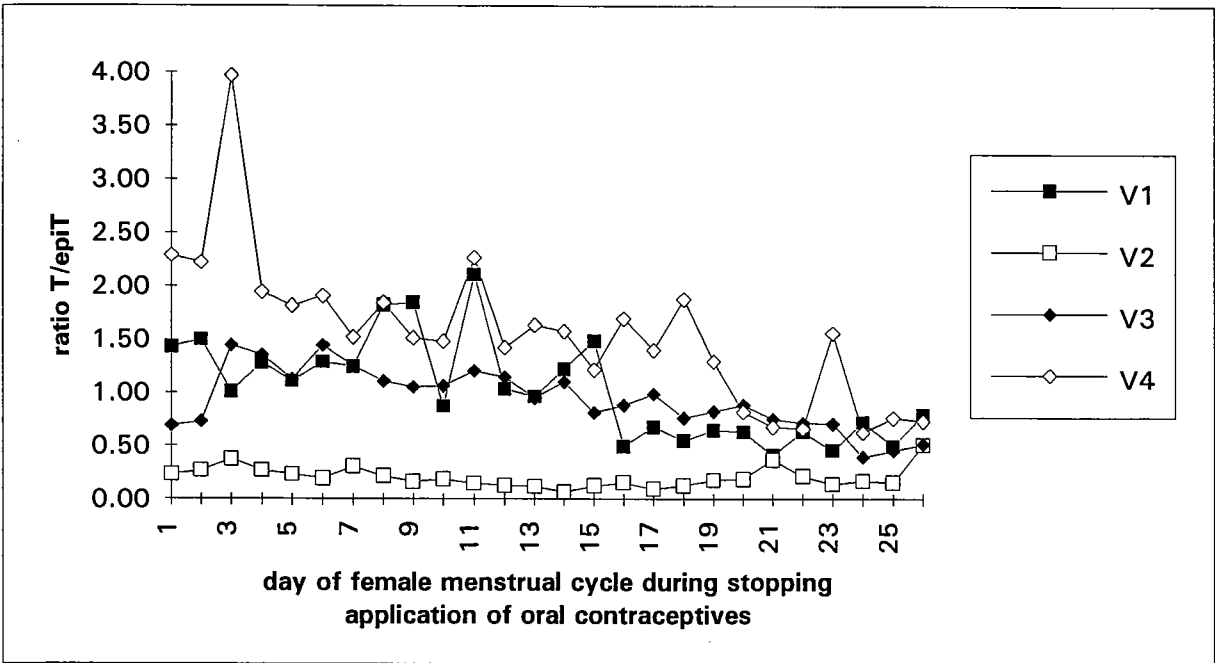
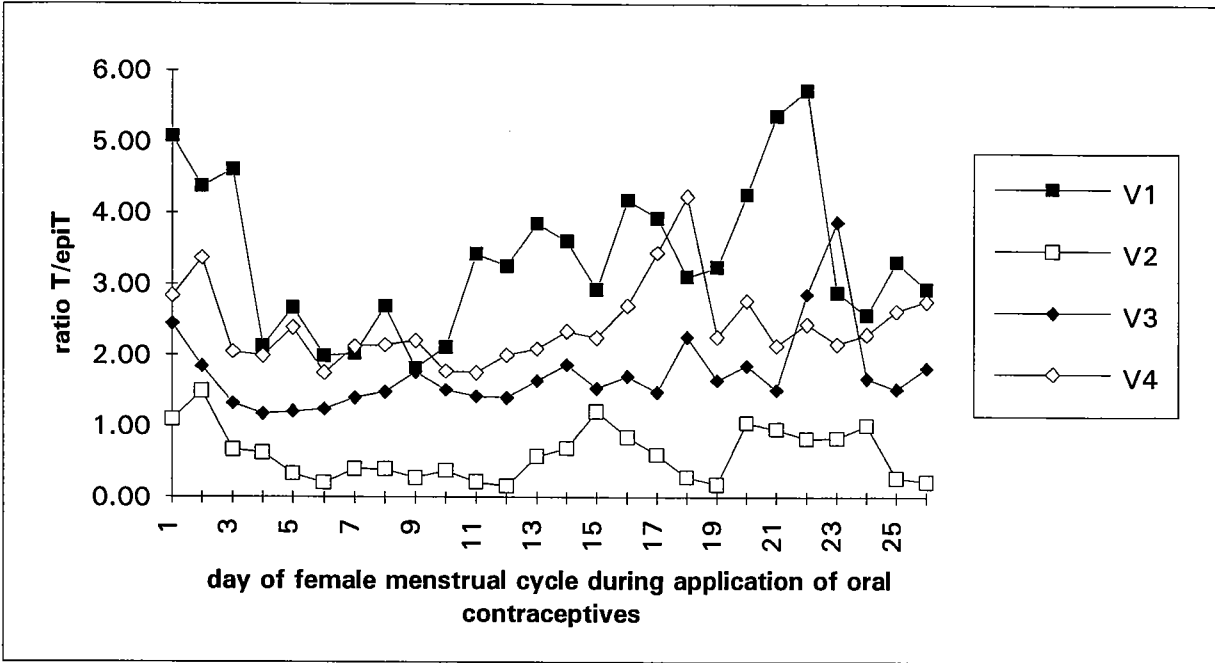
	V 1		V 2		V 3		V 4	
	+	-	+	-	+	-	+	-
<b>A (µg/h)</b>								
min	43	42	54	55	22	22	23	22
max	73	92	96	131	48	53	50	62
mean	56	66	71	92	37	35	35	39
st.dev.	8.2	10	10	21	7	7	7	12
c.v.(%)	15	16	14	23	18	21	21	30
<b>T (µg/h)</b>								
min	0.13	0.18	0.02	0.05	0.19	0.16	0.11	0.15
max	0.42	0.70	0.14	0.14	0.64	0.37	0.54	1.07
mean	0.20	0.28	0.08	0.08	0.32	0.25	0.18	0.34
st.dev.	0.07	0.11	0.04	0.02	0.1	0.05	0.08	0.19
c.v.(%)	34	39	44	25	32	21	44	55
<b>epiT (µg/h)</b>								
min	0.03	0.14	0.07	0.14	0.08	0.12	0.05	0.07
max	0.1	0.59	0.26	1.12	0.3	0.74	0.13	0.79
mean	0.06	0.29	0.15	0.42	0.19	0.34	0.08	0.29
st.dev.	0.02	0.15	0.05	0.21	0.05	0.16	0.02	0.20
c.v.(%)	30	50	32	50	28	48	29	70
<b>Pregd (µg/h)</b>								
min	5.3	6	13	14	10	11	5	7
max	13	214	30	99	36	125	18	117
mean	8.8	45	23	41	21	37	10	33
st.dev.	2.1	50	5	25	8	31	3	34
c.v.(%)	24	111	23	60	37	84	36	105



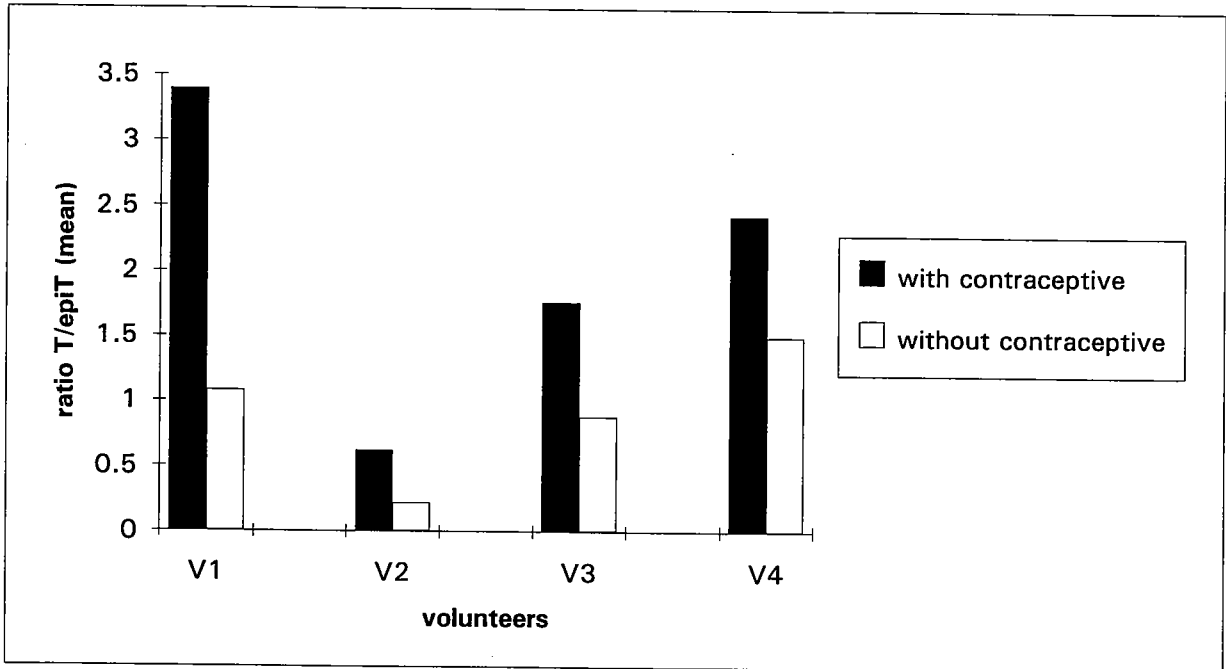
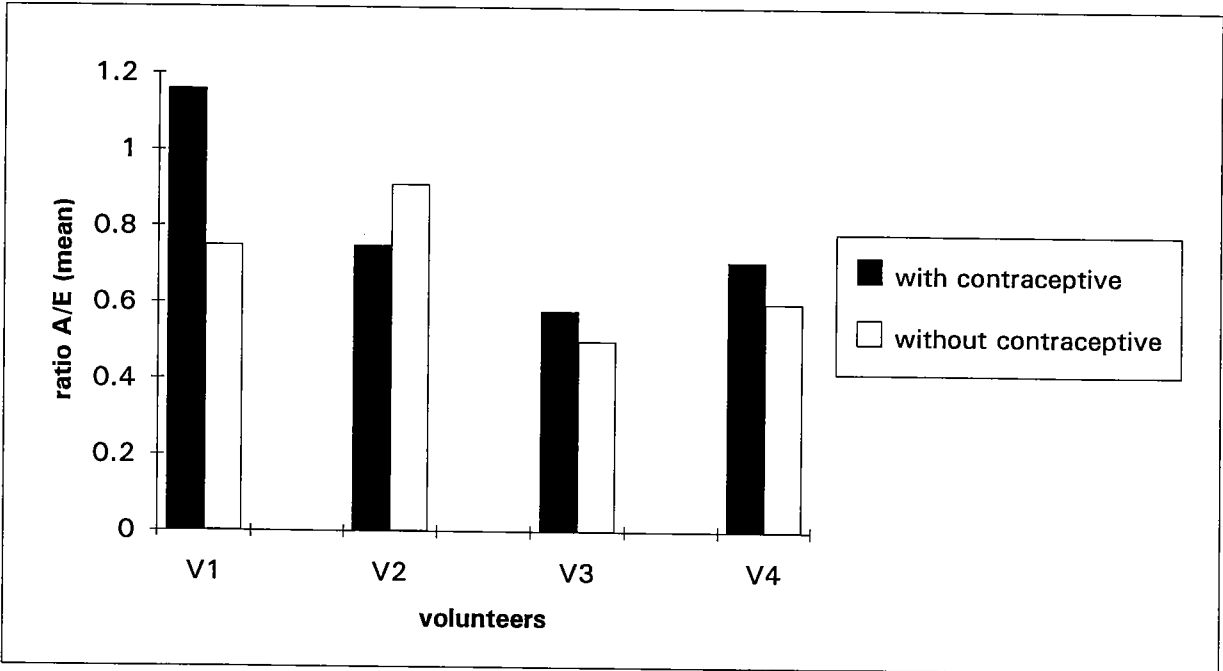
**Fig 1: Influence of oral contraceptives on female steroid profiles.**  
**Comparison of the ratios A/E and T/epiT over the course of one female menstrual cycle each with and without oral contraceptive. volunteer 1**



**Fig 2: Influence of oral contraceptives on female steroid profiles. Comparison of the ratio A/E over the course of one female menstrual cycle each with and without oral contraceptive. volunteer 1-4**

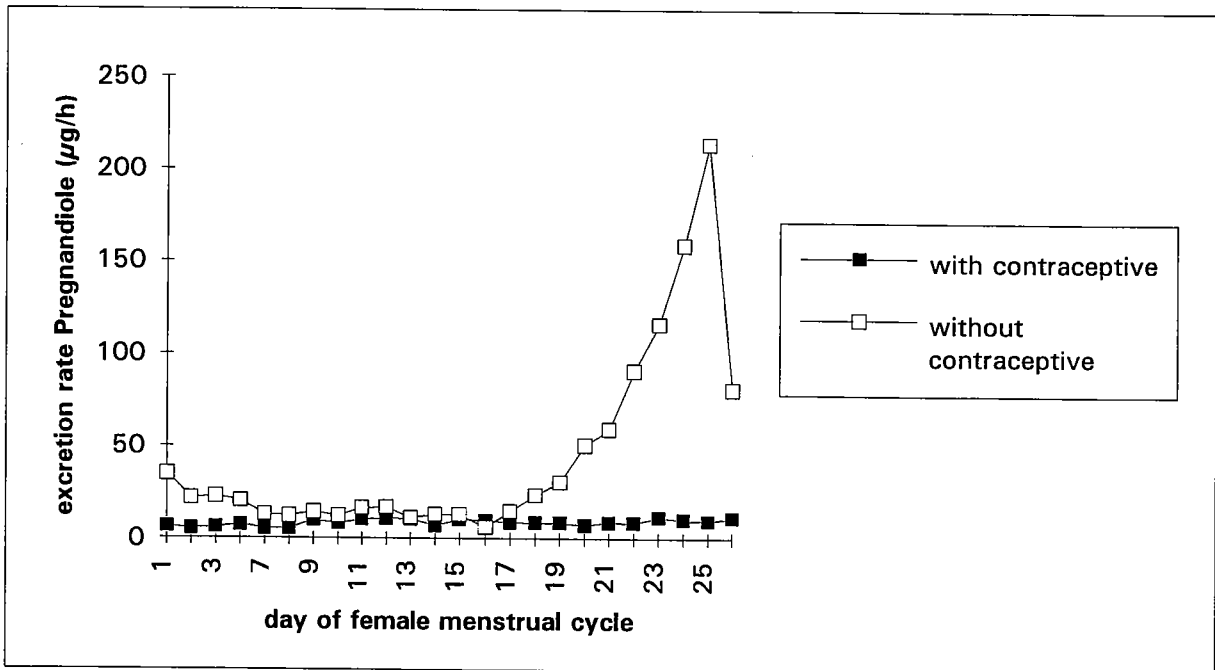
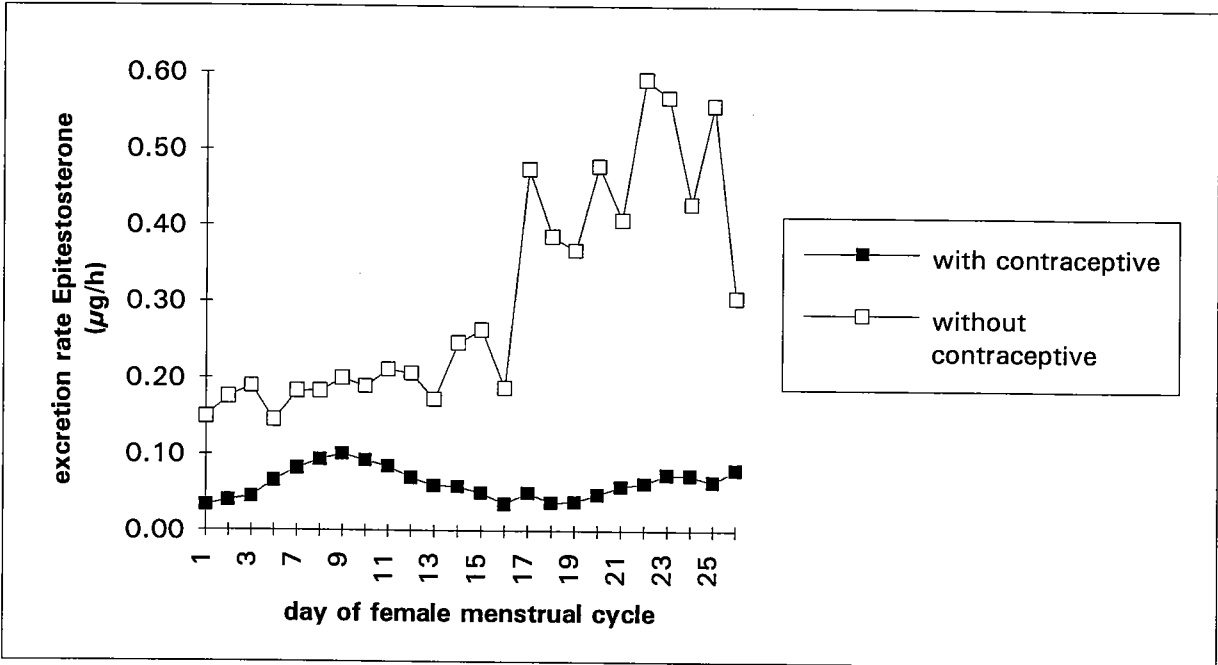


**Fig 3: Influence of oral contraceptives on female steroid profiles. Comparison of the ratioT/epiT over the course of one female menstrual cycle each with and without oral contraceptive. volunteer 1-4**

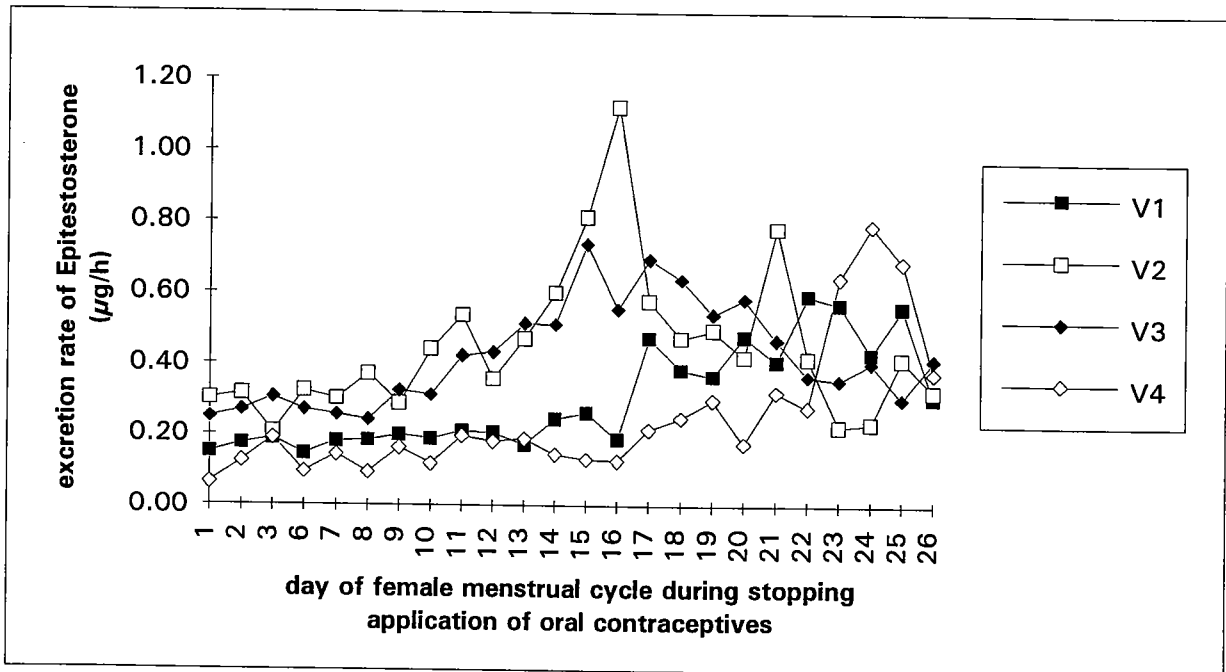
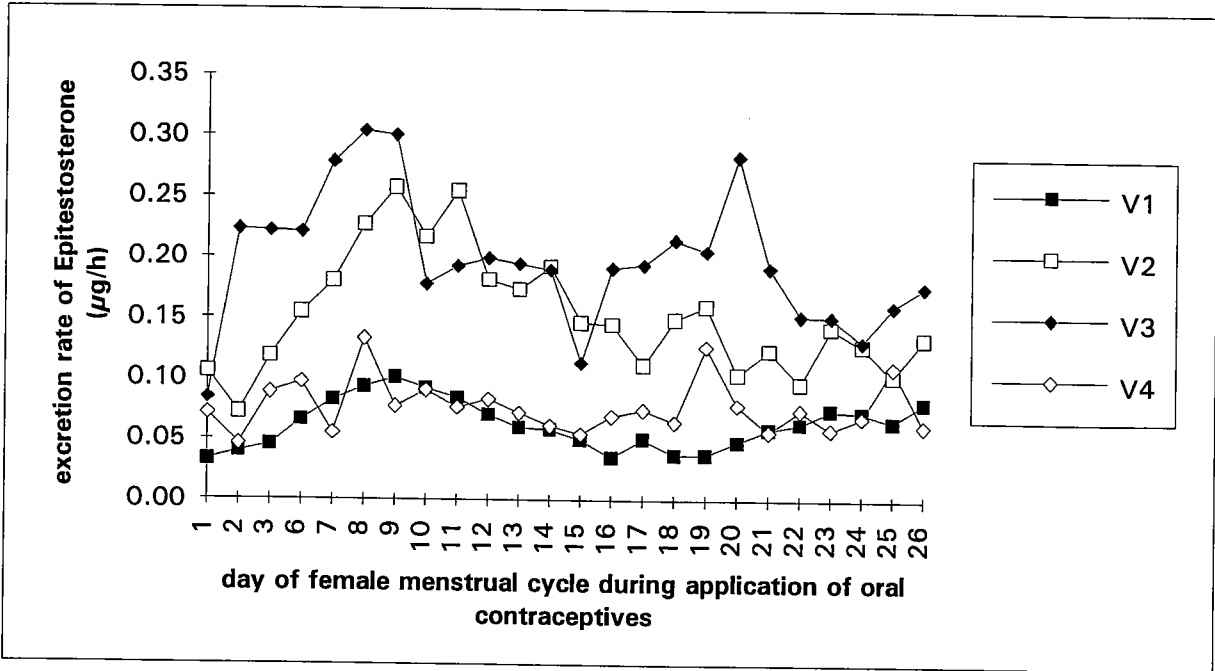


**Fig 4: Influence of oral contraceptives on female steroid profiles.**  
**Differences between mean values of the ratios A/E and T/epiT**  
**over the course of one female menstrual cycle each with and**  
**without oral contraceptive.**  
**volunteer 1-4**

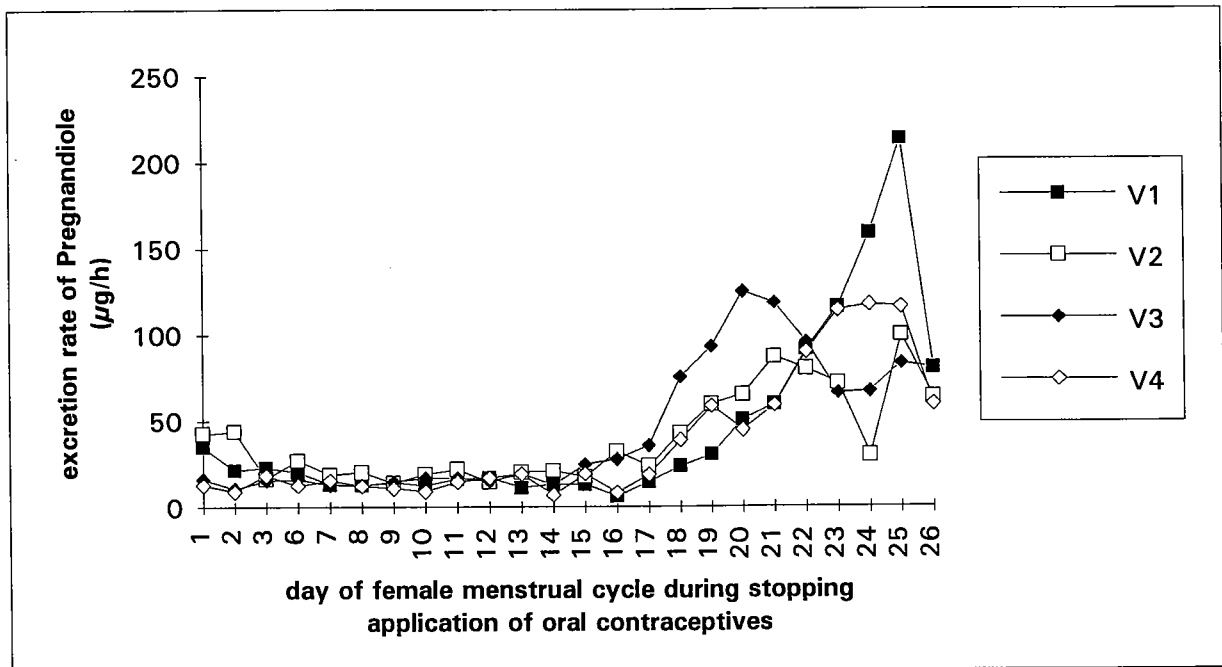
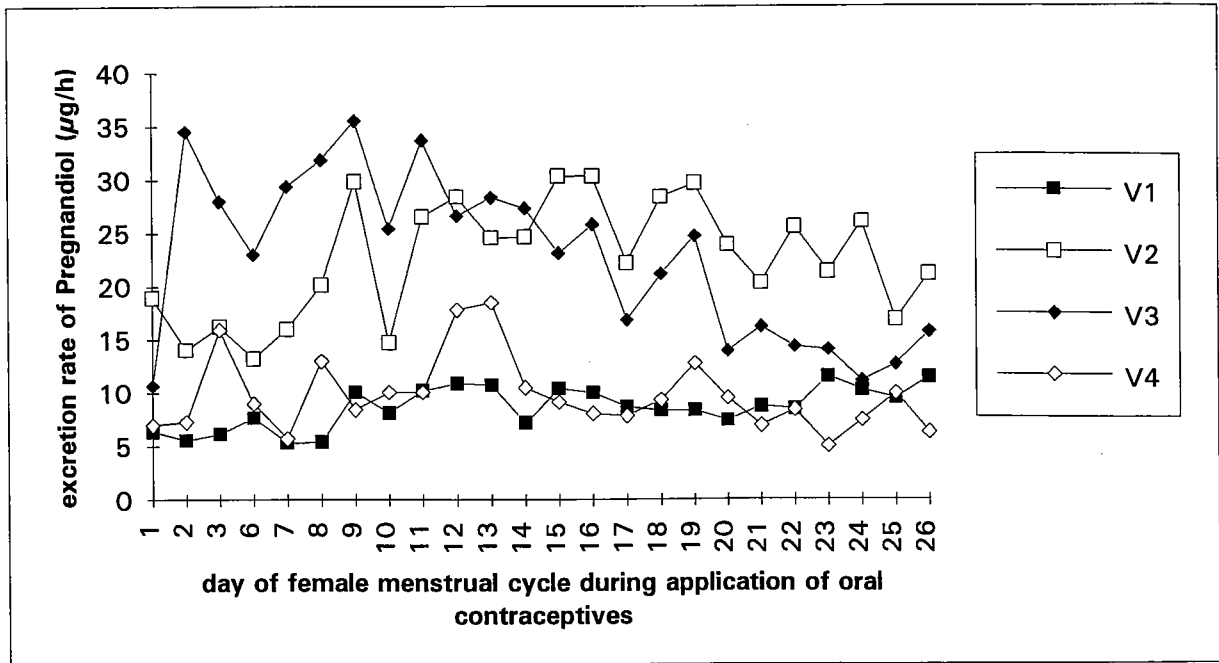




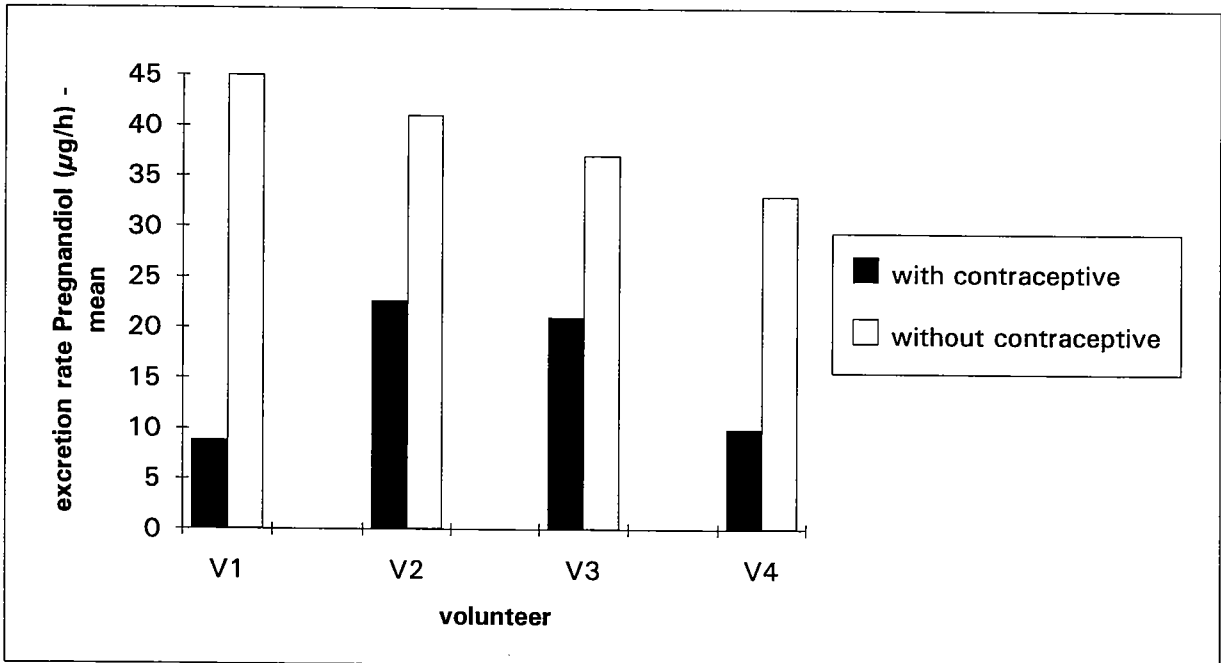
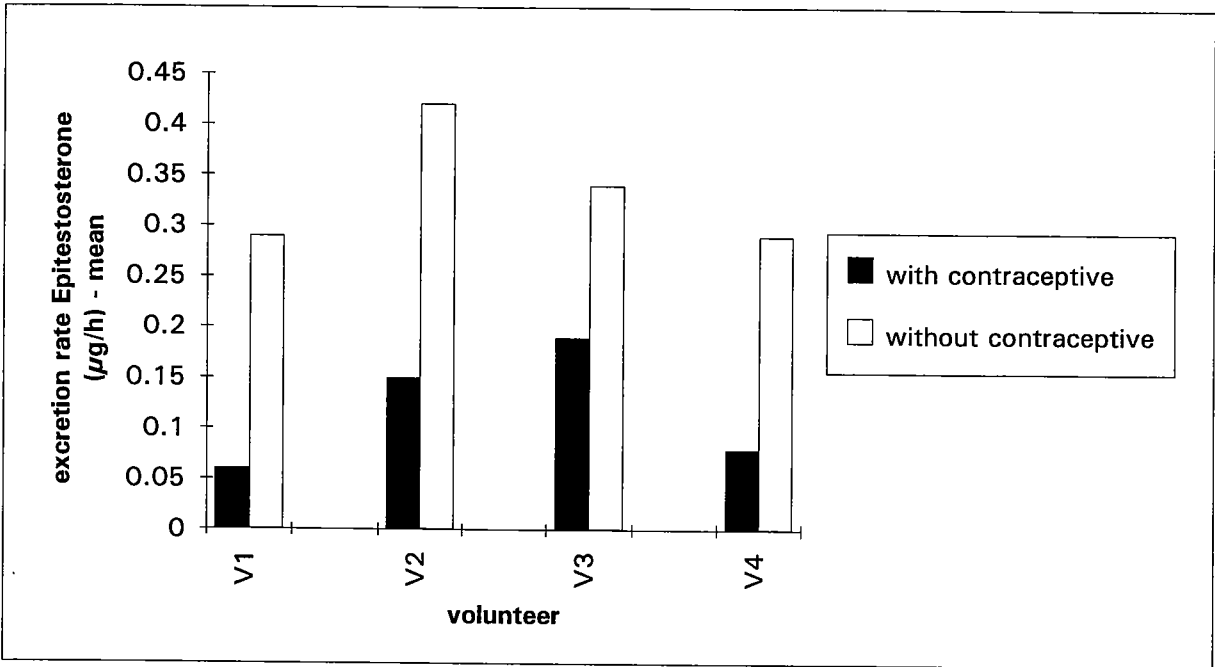
**Fig 5: Influence of oral contraceptives on female steroid profiles. Comparison of the excretion from Epitestosterone and Pregnanediol over the course of one female menstrual cycle each with and without oral contraceptive. volunteer 1**



**Fig 6: Influence of oral contraceptives on female steroid profiles.**  
**Comparison of the excretion from Epitestosterone**  
**over the course of one female menstrual cycle**  
**each with and without oral contraceptive.**  
**volunteer 1-4**



**Fig 7: Influence of oral contraceptives on female steroid profiles. Comparison of the excretion from Pregnanediol over the course of one female menstrual cycle each with and without oral contraceptive. volunteer 1-4**



**Fig 8: Influence of oral contraceptives on female steroid profiles.**  
**Differences between mean values of the excretion rates from Epitestosterone and Pregnanadiole over the course of one female menstrual cycle each with and without oral contraceptive.**  
**volunteer 1-4**