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Stability of the alternative steroid profile and stereodomic model: Part II -Evaluation of female steroid profiles: influences of contraceptives and pregnancy

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Abstract

In the past years, the detection of misuse with endogenous steroids has evolved. New metabolites that improve the detection of misuse with endogenous steroids have been introduced with extended steroid profiling. Also the developments of an adaptive Bayesian model for systematic longitudinal follow-up of steroid profile parameters and a steroidomic model that recognizes abnormal steroid profiles have shown to be able to boost the detection sensitivity of modern steroids profiling. Until now, these new markers and technologies have not been evaluated yet with the steroid profiles of females. The stability of female steroid profiles was reinvestigated with regards to the minor steroid metabolites, the steroidomic model and adaptive model from the steroid passport. In addition, the influence of oral contraceptives and pregnancy was studied using these technologies.

Introduction

Throughout the last decades, steroid profiling has been studied and became an established tool to screen for misuse with endogenous steroids. Recently, several biomarkers including minor hydroxylated steroids were introduced to increase the steroid profiling specificity [1]. The combination of these additional metabolites and the traditional markers with the longitudinal model as used in the biological passport and a discriminative algorithm resulted in a very doping-sensitive steroidomic model [2]. This model facilitates the interpretation of all monitored biomarkers by reducing them to a single abnormal steroid profile score. These new strategies however were elaborated using male steroid profiles. As the endocrine system of females differs from those of men, these new markers should be investigated onto female steroid profiles.

During the menstrual cycle the female endocrine system shows very large intra-individual variances of progestogens and estrogens, the female hormones which are in close relation with those of their androgenic steroid profile. There is close interaction of the female hormones with secretagogues follicle stimulating hormone (FSH) and luteinizing hormone (LH) that might influence androgen levels and its metabolites. A normal menstrual cycle starts with the follicular phase where the FSH induced maturing of a follicle takes place. This follicle produces estradiol, which in turn suppresses FSH. A sufficient concentration of estradiol triggers an acute production of LH in pituitary, inducing the ovulation. After ovulation and during the luteal phase, the follicle becomes a corpus luteum which is stimulated by LH. The corpus luteum produces progesterone, which inhibits LH production by feedback. Progesterone concentrations drop inducing the menstruation.

The close relation of the involved secretagogues LH and FSH and progesterone as androgen precursor is shown in Figure 1. Female endocrine fluctuation causes certain alterations in the steroid profile as we monitor. Often pregnanediol (PD), a progesterone metabolite is also monitored as an endogenous reference compound for IRMS. Often, these natural infradian rhythms are suppressed by hormonal birth control pills. These oral contraceptives usually consist of a combination of estrogen, usually ethinyl estradiol and progesterone derivatives in different concentrations depending on the type of contraceptive pill. These synthetic analogs inhibit the production of the secretagogues LH and FSH and prevent maturing of the follicle. Other large changes in the female endocrine system occur during an emerging pregnancy where human chorionic gonadotropine (hCG) is released by the syncytiotrophoblast which forms the placenta in later stages of the pregnancy.

Hence, this study contributes to the information and experience needed to correctly interpret these new markers and steroidomic model as well as traditional steroid profile markers in female athletes with a focus on the influence of the menstrual cycle, hormonal contraceptives and pregnancy.





Figure 1: Evolution of the main hormones during a menstrual cycle

Experimental

Study design contraceptives:

Six healthy young females (25-30 years) participated in a controlled trial where they were asked to provide morning urines on a daily basis during two menstrual cycles, one with oral contraceptive (OC) and one without using oral contraceptives (Table1). Between collection periods was a lag time of 1 month in order to let the endocrine system get used to the new situation. Each volunteer provided at least 2 times 28 urines, 387 steroid profiles were assessed in total. All volunteers used single phased OCs i.e. OC pills containing a fixed concentration of conceptive hormones. The six females used their own brand of OC pill. Five of them used the common combination pill without OC administration in the fourth week. F6 used a mini pill where this so called 'stop week' was absent. Hence, a continuous administration of the progestogen analog occurs in total absence of any estrogen analog.

Volunteer							Administration					
	Age	Brand	Estrogen	amount	Progestagen	amount	Туре	during weeks	Generation	Phase		
۷1	28	Yasmin	Ethinylestradiol	0,03mg	Drospirenon	3mg	combination	1-3	4	1		
V2	25	Desorelle 30	Ethinylestradiol	0,15mg	desogestrel	0,03mg	combination	1-3	3	1		
V3	30	Nora 30	Ethinylestradiol	0,03mg	levonorgestrel	0,15mg	combination	1-3	2	1		
V4	26	Nora 30	Ethinylestradiol	0,03mg	levonorgestrel	0,15mg	combination	1-3	2	1		
V5	26	deso 20	Ethinylestradiol	0,15mg	desogestrel	0,020mg	combination	1-3	3	1		
V6	26	Cerazette	2		desogestrel	0,075mg	mini pil	1-4		1		

Table 1: Information on the volunteers participating in the trial using single phase oral contraceptives.



Study design pregnancy:

Weekly, urines of three pregnant women were collected from the 5th week in pregnancy until delivery. Blanc urines from before the pregnancy of two volunteers were available. Two volunteers delivered a girl in the 40th week. Volunteer 3 gave birth to a boy in the 39th week. More details on the protocol and ethical approval can be found elsewhere [3].

Steroid Profile markers:

The monitored steroid profile markers include hydroxylated steroid metabolites to focus on the steroid ratio testosterone/epitestosterone (T/E), androsterone/etiocholanolone (Andro/Etio), 5α -androstane- 3α ,17 β -diol/5 β -androstane- 3α ,17 β -diol/ 5α -Adiol), dihydrotestosterone (DHT)/E, dehydroepiandrosterone (DHEA)/E, DHT/5 β -Adiol, 7 β -OH-dehydroepiandrosterone (1 6α -OH-DHEA)/E, 1 6α -OH-dehydroepiandrosterone (1 6α -OH-DHEA)/E, 6 α -OH-androstenedione (6 α -OH-Adion)/1 6α -OH-DHEA, 4-OH-androstenedione (4-OH-Adion)/1 6α -OH-androstenedione (1 6α -OH-Adion). Behaviour of the abnormal steroid profile score (ASPS, [2]) was investigated in females.

Results and Discussion

Oral Contraceptives (OC)

Most obvious changes in the menstrual cycle without OC changes were raised concentrations of PD and E (Figure 2). We observed a large peak in the fourth week for PD which could rise up to 80 times the basal levels ($\mu = 1321 \pm 820$ ng/mL) and significantly elevated E concentrations (paired T-test, p>>0.05) from the follicular phase (average E conc: 11.3 ± 4.9 ng/mL) towards the luteal phase (average E conc: 14.4 ± 7.3 ng/mL). Inter-individual differences of E-excretion between follicular and luteal phase were 4.5% whereas maximal E increases were observed up to 10 times the basal concentrations. Application of OC induced a suppression of the PD peak and elevated E concentration in the luteal phase.

A decrease of 25% less variation is observed for E concentrations in females taking OC whereas the PD peak fully disappears as no corpus luteum to produce progesterone.



Figure 2: The upper graphs show the PD and E excretion profiles observed in two female volunteers that were not using OCs and thus the influence of the menstrual cycle. The lower graphs indicate the effect of the use of OC for a given female.



In the normal menstrual cycle, the T/E ratio shows a drop during the luteal phase as the E excretion is suppressed [4] resulting in a mean CV of 47% was found with a intra-individual maximum of 58% (Table 2). During the luteal phase, the T/E ratio cycle drops 21% during normal menstrual which can cause atypical values in the ABP (Figure 3).

Steroid profiles with the application of OC are not featured by luteal drop causing the mean T/E to rise with 44%. The maximal intra-individual difference observed between OC and nOC was found to be up to 120% in a female that was not used to taking OC before participating in this study. The mean CV decreases with 10% to a mean of 35% when OC are applied.

Period	Contracep tion	Stat	T/E	T/Andro	Andro / Etio	5α/β- A diol	DHT/5β- Adiol	DHT/E	DHEA/ E	16αOH- DHEA/ E	7β-OH- DHEA <i>I</i> E	4-OH- A dion/ 16α-OH- A dion	6α-OH- Adion/ 16α-OH- DHEA
Week1-4	nOC	mean	0,71	0,0043	0,69	0,22	0,02	0,23	5,40	2,03	2,62	0,73	0,05
Week1-4	nOC	CV	0,46	0,31	0,17	0,35	0,47	0,66	0,44	0,48	0,61	0,49	0,28
Week1-4	ос	mean	1,26	0,0040	0,78	0,19	0,015	0,35	11,79	3,48	5,28	0,73	0,07
Week1-4	oc	CV	0,35	0,41	0,17	0,81	0,65	0,65	0,27	0,29	0,33	0,51	0,29
Difference (%)	nOC-OC	mean	44,0	-6,7	10,8	-13,8	-22,6	33,7	54,2	41,8	50,3	-0,2	24,7
Difference (%)	nOC-OC	CV	-10,5	9,5	0,6	46,4	17,4	-0,4	-16,7	-19,4	-28,2	1,8	1,5
Week1-3	oc	mean	1,27	0,0043	0,65	0,19	0,01	0,29	12,61	3,66	5,70	0,69	0,07
Week1-3	oc	CV	0,42	0,48	0,17	0,45	0,74	0,77	0,28	0,34	0,30	0,36	0,32
Week4	oc	mean	1,63	0,0049	0,77	0,25	0,015	0,36	14,54	4,26	6,86	0,97	0,06
Week4	oc	CV	0,44	0,66	0,14	0,70	0,56	0,57	0,21	0,19	0,28	0,45	0,24
Difference (%)	oc	mean	22,4	12,6	16,2	23,7	8,5	19,9	13,3	14,0	16,8	28,3	-7,1
Difference (%)	oc	CV	2,5	18,7	-3,1	24,8	-17,2	-19,9	-6,9	-15,1	-2,4	8,8	-8,1
Week1-2	nOC	mean	0,87	0,0044	0,69	0,20	0,02	0,28	6,46	2,27	3,32	0,64	0,05
Week1-2	nOC	CV	0,29	0,26	0,12	0,31	0,54	0,61	0,30	0,29	0,44	0,28	0,25
Week 3-4	nOC	mean	0,62	0,0043	0,70	0,16	0,018	0,20	4,97	1,83	2,40	0,74	0,05
Week3-4	nOC	CV	0,50	0,33	0,18	0,33	0,41	0,68	0,52	0,55	0,67	0,37	0,25
Difference (%)	nOC	mean	-39,0	-1,8	1,2	-25,0	-0,4	-43,8	-30,0	-24,4	-38,5	13,8	-3,3
Difference (%)	nOC	CV	21,7	6,9	6,3	2,4	-13,2	7,2	22,6	25,6	23,0	8,9	0.4

Table 2: A summary of all the statistics of the monitored ratios observed in 6 females with and without contraceptives



Figure 3: The T/E profiles with and without oral contraceptives (OC) of one female are plotted together in the left graph. T/E passports with adaptive thresholds are given in the right graphs.



In a normal menstrual cycle, Andro/Etio shows a small CV of 17%. When OC are taken, for Andro/Etio we also [4] observed an increasing trend towards the end of the cycle with a 6% to maximal 12% increase of the mean in the stop week. In volunteer 6, which used the mini pill, this trend was not observed but a 38% higher Andro/Etio ratio was observed when OC were taken due to higher Andro excretion. This difference was not observed for the $5\alpha/\beta$ -Adiol ratio. In general, $5\alpha/\beta$ -Adiol is unaffected by the neither menstrual cycle nor OC as no significant changes or trends could be found.

Similar to T/E a declining trend was observed for DHT/E, DHEA/E, 7 β -OH-DHEA/E and 16 α -OH-DHEA/E due to elevated E in the luteal phase lowering the inter-individuals mean 30%-44%. The influence of OC on these markers returns increases the average with 34%-55% due to a gradually increasing trend towards the stop week, where 13%-20% higher means were found than in the three weeks with estrogens and progestogens. The volunteer taking the mini pill did not show this altered profile. Despite the observed trend while on OC, the CV in these markers reduces with 10-20%, even 30% for 7 β -OH-DHEA/E. The other monitored markers (T/Andro, DHT/5 β -Adiol, 4-OH-Adion/16 α -OH-Adion and 6 α -OH-Adion/16 α -OH-DHEA) showed no difference between follicular and luteal phase. No significant changes were observed for these markers with the application of OC's. The calculated ASPS values follows similar trends after application of OCs but none exceeds the threshold of 0.79 [2].

Pregnancy

Pregnancy causes hCG levels to rise in the first three months of the pregnancy. hCG is known to promote T production in the testes leading to altered steroid profile markers [5]. This increase was only observed in one volunteer in the first semester of the pregnancy. A decreasing trend however could be observed for the androgens whereas the same was true for E (Figure 4).

These altered excretion profiles result in a clear decrease up to 54% of the T/E ratio over the course of the pregnancy with a clear nadir in the third trimester. The CV's did not exceed 60% and hence T/E did not exceed the biological passports thresholds. As the Andro/Etio remains unaltered, the $5\alpha/\beta$ -Adiol ratio showed up to 70% lowered values towards the delivery. Early pregnancy however did no significantly change Andro/Etio and $5\alpha/\beta$ -Adiol values. A steady decrease towards delivery was observed for DHEA/E, DHT/E, 7β -OH-DHEA, 6α -OH-Adion/16 α -OH-DHEA and 4α -OH-Adion/16 α -OH-Adion were the difference in mean values in the between first and third trimester could reach 80%. In contrast, 16 α -OH-DHEA/E showed a completely different behavior. Elevated values between 80% and 600% were reached near delivery compared to basal levels. This elevation is attributed to fetal liver and adrenal gland production of 16 α -OH-DHEA as a precursor for estriol formation in the placenta. Women carrying a baby girl demonstrated at least a double increase of 16 α -OH-DHEA/E compared to those who delivered a boy.

The steroidomic model based on six traditional steroid metabolites showed no significant changes whereas if it was extended with the minor metabolites a significant increase was found with an apex near the delivery. This apex did not exceed the threshold of 0.79 [2].



Figure 4: T and E excretion profiles during pregnancy

Conclusions

Female E and PD levels in the luteal phase are highly suppressed by oral contraceptives. T/E ratio become more stable with OC whereas Andro/Etio slightly increases. The ratios of DHEA (metabolites) over E increase midcycle while using OC. The same trends are observed for the ASPS but none exceeds the proposed threshold.

During pregnancy the T/E ratio can decrease more than 50% whereas Andro/Etio remains a stable parameter. $5\alpha/\beta$ -Adiol has shown a decrease up on 70% which might cross the threshold calculated in the biological passport. 16α -OH-DHEA/E increases due to fetal liver production whereas all other ratios present a declining trend towards delivery. ASPS is hence also altered but does not exceed the threshold of 0.79.

References

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Acknowledgements

The world anti-doping agency (WADA) is gratefully acknowledged for its financial support.