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7-hydroxylated derivatives of dehydroepiandrosterone as possibly related to menstrual mood change in healthy women

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Objective. Mood changes occur often in the luteal phase of menstrual cycle. Steroids modulating $GABA_A$ and NAMD receptors in the brain, namely allopregnanolone, were suggested as a factor of premenstrual syndrome. Another neurosteroid influencing the well-being is dehydroepiandrosterone. In the past decade it was shown by several authors that some dehydroepiandrosterone derivatives, especially those with 7-hydroxy- or 7-oxo group, exert a higher activity than dehydroepiandrosterone itself. It was also reasonable to see whether the levels of circulating 7-hydroxy-derivatives of dehydroepiandrosterone differ in the follicular and luteal phase of the menstrual cycle.

Methods. Steroids known to exert neuroprotective effects, namely 7α- and 7β-hydroxydehydroepiandrosterone, 5-androstene-3β,7α,17β-triol and 5-androstene-3β,7β,17β-triol, were determined in midfollicular and midluteal phase of the menstrual cycle of 22 healthy women with a regular menstruation cycle.

Results. Whereas the maternal steroids, dehydroepiandrosterone and androstene-3β,17β-diol showed no significant difference between the phases of menstrual cycle, the levels of their 7-hydroxylated metabolites were significantly lower in the luteal phase.

Conclusion. It is suggested that the observed decrease of 7-hydroxylated metabolites during the luteal phase may be a factor related to the etiopathogenesis of mood change and neurocognitive disturbances, which are known to be more accented in that particular phase of the menstrual cycle.

Key words: menstrual cycle, menstrual mood change, mealthy women, mehydroepiandrosterone, 7-hydroxydehydroepiandrosterone

In many women of reproductive age the well-being and health status is closely associated with the phase of menstrual cycle and with the secretion of ovarian steroids. Neurocognitive functioning and mood may be also be impaired in the luteal phase of the menstrual cycle due to associated changes in hypothalamic-pituitary-adrenal (HPA) axis function (Symonds et al. 2004). Not only adrenal steroid secretion, but also the effects expressed by steroids formed from adrenal androgens such as DHEA in peripheral organs including the brain may take part in this condition. Among the candidates responsible for this process are the 7-hydroxylated derivatives of dehydroepiandrosterone (DHEA), which have been shown as neuroactive steroids, (Akwa et al. 1992, 1993; Doostzadeh and Morfin 1996; Doostzadeh et al. 1997; Rose et al. 1997; Morfin and Starka 2001), immunomodulators (Morfin and Courtay 1994; Lafaye et al. 1999; Chmielewski et al. 2000; Morfin et al. 2000), and potentially regulators of cortisol-cortisone interconversion in the target tissues (Hennebert et al. 2009) or even as factors in development of Alzheimer´s disease (Attal-Khemis et al. 1998, Weill-Engerer et al 2003, Bicikova et al. 2004). The unique effect of 7-hydroxydehydroepiandrosterone or 7-keto-dehydroepiandrosterone to compete with cortisol or cortisone in the enzyme controlled interconversion by 11-hydroxysteroid de-

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Tab.1.

Free and conjugated dehydroepiandrosterone and its 7-hydroxylated derivatives in follicula and luteal phase of menstrual cycle in regulary menstruating women.

		FOLLICULAR PHASE								LUTEAL PHASE								
Variable	Unit	n	mean	SD	median	Lower quartile	Upper quartile	Minimum	Maximum	n	mean	SD	median	Lower quartile	Upper quartile	Minimum	Maximum	p-value
Age	years	22	34,1	7,69	34,8	27,9	39,3	22,8	51	22	34,2	7,66	34,9	28	39,4	22,9	51,1	
DHEA	nmol/l	22	9,55	5,97	7,26	5,51	12,4	2,1	24,9	22	10,8	9,51	7,16	4,91	13,7	2,74	44,4	NS
Adiol	nmol/l	22	1,09	0,819	0,979	0,722	1,11	0,268	4,25	22	0,792	0,392	0,709	0,5	0,966	0,328	1,68	0.001
DHEA7α	nmol/l	22	1,81	0,872	1,51	1,26	2,21	0,7	4,76	22	1,21	0,573	1,02	0,84	1,54	0,537	2,95	0.0001
DHEA7β	nmol/l	22	0,787	0,924	0,529	0,371	0,797	0,134	4,62	22	0,403	0,212	0,361	0,237	0,49	0,161	0,869	0.0006
AT7α	nmol/l	22	0,194	0,144	0,172	0,0987	0,235	0,0344	0,679	22	0,138	0,101	0,108	0,0575	0,176	0,0335	0,457	0.004
AT7β	nmol/l	22	0,114	0,0625	0,108	0,0784	0,132	0,0284	0,289	22	0,0805	0,0464	0,0704	0,0415	0,0984	0,0258	0,193	0.0002
DHEAC	μmol/l	22	1705	739	1494	1200	2147	699	3082	21	854	315	786	666	1111	340	1570	0.0001
AdiolC	nmol/l	22	195	148	154	83,5	293	30,6	602	22	150	93	127	105	166	71,1	515	NS
DHEA7αC	nmol/l	22	8,55	7,44	5,87	2,94	13,3	1,25	27,9	21	0,992	0,499	0,83	0,644	1,19	0,469	2,34	0.0001
DHEA7βC	nmol/l	22	6,9	6,12	4,2	2,59	8,92	0,647	21,1	21	0,888	0,492	0,763	0,501	1,31	0,182	1,8	0.0001
AT7αC	nmol/l	22	0,977	1,13	0,438	0,184	1,41	0,0534	4,14	21	0,0634	0,0531	0,0511	0,0301	0,0782	0,00291	0,226	0.0001
AT7βC	nmol/l	22	0,98	1,07	0,485	0,255	1,38	0,0809	3,57	21	0,0909	0,0608	0,0754	0,0376	0,137	0,00605	0,201	0.0001

DHEA – dehydroepiandrosterone; Adiol – 5-androstene-3β,17β-diol; DHEA7α – 7α-hydroxy-dehydroepiandrosterone; DHEA7β – 7β-hydroxy-dehydroepiandrosterone; AT7α – 5-androstene-3β,7α,17β-triol; AT7β – 5-androstene-3β,7β,17β-triol; C - conjugate

hydrogenase and thus regulate the local concentration of cortisol became a basis of the recent top novelty in supplements for reducing body mass.

So far, any studies on the circulating 7-hydroxy derivatives of dehydroepiandrosterone in the menstrual cycle of normal healthy women were not published.

Patients and Methods

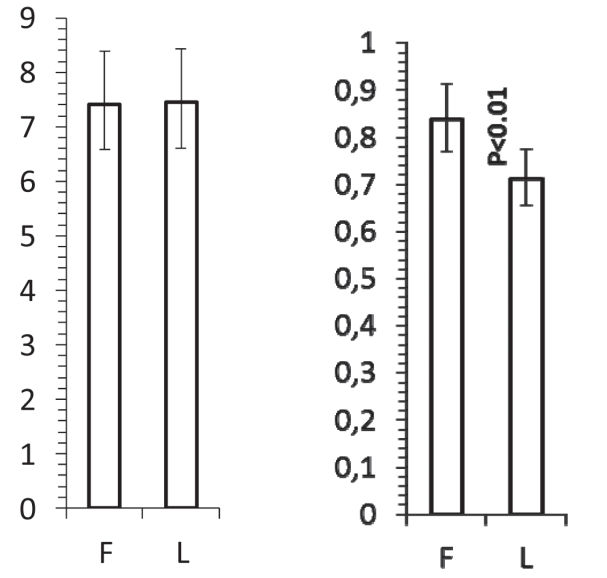
Patients. Twenty two healthy regularly menstruating women aged 34.1 ± 7.6 years (mean ± SD; median 34.8, lower quartile 27.9, upper quartile 39.4) not taking any hormonal contraception were included in the study after signing the informed consent. Peripheral blood sample (10 ml) was taken from the cubital vein in the midfollicular phase of the menstrual cycle (6-10th days after menstruation) and in the luteal phase (17-22nd day after menstruation). The phase of the cycle was checked by the determination of circulating gonadotropins, estradiol and progesterone. Serum was obtained after centrifugation for 5 min at 2000 g at 0 °C and stored at -20 °C until analyzed.

Steroids and chemicals. The steroids were obtained from Steraloids (Wilton, NH, USA) and the solvents of analytical grade for the extraction and HPLC were from Merck (Darmstadt, Germany). The derivatization agent Sylon BFT was purchased from Supelco (Bellefonte, PA, USA).

Instruments. The GC-MS system was supplied by Shimadzu (Kyoto, Japan). The GCMS-QP2010 Plus system consisted of a gas chromatograph equipped with automatic flow control, AOC-20s autosampler and a quadrupole electron-impact detector with an adjustable electron voltage of 10-195 V. A capillary column with a medium polarity RESTEK Rxi (diameter 0.25 mm, length 15 m, film thickness 0.1 μm) was used for analyses.

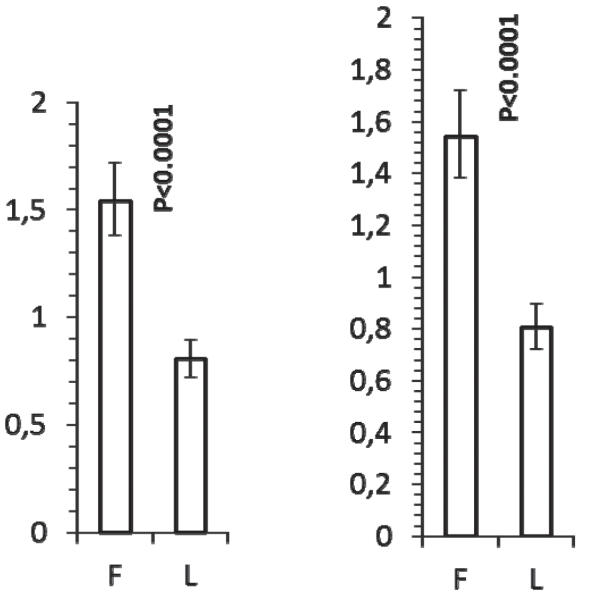
Steroid analysis. GC-MS method used was as published in detail elsewhere (Starka et al. 2009). Briefly, unconjugated steroids were extracted from 1 ml serum by diethyl-ether (3 ml), the extract was dried in the block heater at 37 °C. The lipids in dry residue of that extract were separated by partitioning between a mixture of methanol-water 4:1 (1 ml) and pentane (1 ml). The pentane phase was discarded and the polar phase was dried in the vacuum centrifuge at 60 °C (2 hours).

Steroid conjugates remaining in the polar residues after diethyl-ether extraction were analysed as follows: The polar residues were dried in the vacuum centrifuge at 37 °C (5 hours) and dry residues were hydrolyzed as



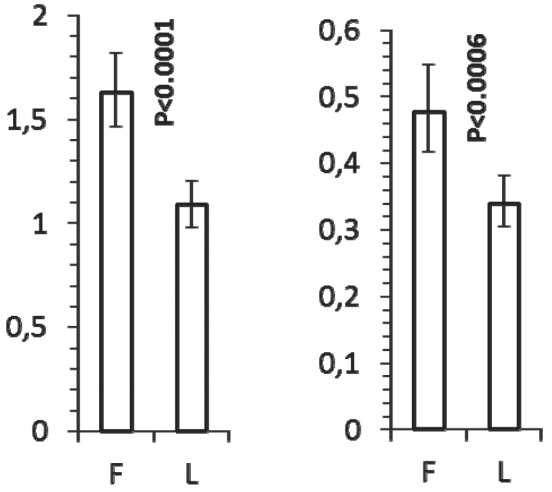
Free DHEA (μmol/l) **Free 5-androstene-3β,17β-diol (nmol/l)**

Fig. 1. Free dehydroepiandrosterone (DHEA) and free 5-androstene-3β,17β-diol in the follicular and luteal phase of the menstrual cycle.
F = follicular phase L = luteal phase



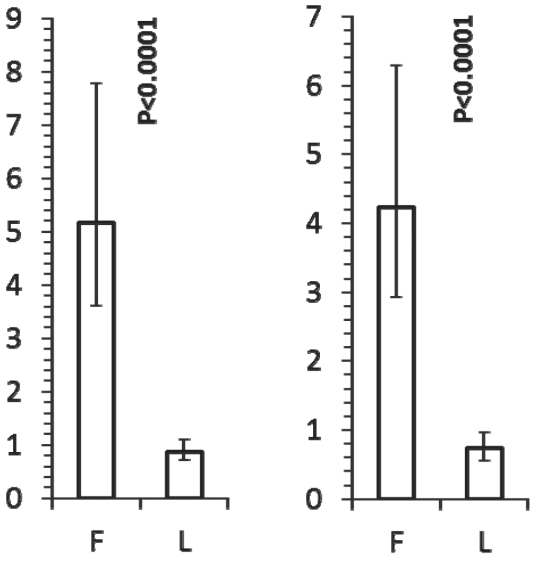
Conjugated DHEA (μmol/l) **Conjugated 5-androstene-3β,17β-diol (nmol/l)**

Fig. 2. Conjugated dehydroepiandrosterone (DHEA) and 5-androstene-3β,17β-diol in the follicular and luteal phase of the menstrual cycle.
F = follicular phase L = luteal phase



Free 7α-hydroxy-DHEA (nmol/l) **Free 7β-hydroxy-DHEA (nmol/l)**

Fig. 3. Free 7α-hydroxdehydroepiandrosterone (7α-hydroxy-DHEA) and 7β-hydroxdehydroepiandrosterone (7β-hydroxy-DHEA) in the follicular and luteal phase of the menstrual cycle.
F = follicular phase L = luteal phase



Conjugated 7α-hydroxy-DHEA (nmol/l) **Conjugated 7β-hydroxy-DHEA (nmol/l)**

Fig.4. Conjugated 7α- and 7β-hydroxydehydroepiandrostero ne in the follicular and luteal phase of the menstrual cycle.
F = follicular phase L = luteal phase

described elsewhere (Dehenin et al. 1996). Hydrolyzed samples were again dried in the vacuum centrifuge at 37 °C (5 hours). The dried residues were reconstituted with 1 ml of chromatographic water and further processed in the same way as free steroids. In contrast to the preparation of free steroids sample, the dry residue after the second derivatization step was dissolved in 200 µl isooctane instead of 20 µl isooctane.

Dry residue from the polar phase was derivatized first with methoxylamine-hydrochloride solution in pyridine (2 %) on oxo-groups (60 °C, 1 hour). The mixture after the first derivatization was dried in the flow of nitrogen and dry residue was treated with Sylon B (99 % of bis(trimethylsilyl)-trifluoroacetamide and 1 % of trimethylchlorosilane) forming trimethylsilyl derivatives on hydroxy-groups (TMS-MOX derivatives) (90 °C, 1 hour). Finally, the mixture after the second derivatization step was dried in nitrogen flow, while the dry residue was dissolved in 20 µl isooctane and 1 µl of the solution was used for GC-MS analysis.

Prior to further processing, original samples were spiked with 17α-estradiol (as an internal standard) to attain a concentration of 1 ng/ml and 10 ng/ml, respectively, and that standard was recorded at effective masses m/z = 231, 285 and 416. The addition of internal standard to body fluid before sample preparation (free steroids) and to polar phase after diethyl-ether extraction (conjugated steroids) assured that the losses during the sample processing were not critical for steroid quantification.

Electron-impact ionization was used for the analyses. Electron voltage was set up to 70 V and emission current to 160 µA. The temperature of ion source and interface were maintained at 260 °C and 310 °C, respectively. Analyses were carried out with a constant linear velocity of carrier gas (He), which was maintained at 60 cm/s. The septum purge flow was set up to 3 ml/min. Samples were injected using on-column injection mode, detector voltage being set to 1.4 kV

Temperature and pressure gradients for GC-MS analysis of steroids after derivatization and the retention times of the steroids.

To utilize the biological material effectively, individual samples were applied in three independent courses, in each case employing a part of the steroids under investigation. The selection of steroids measured within the individual courses, the temperature and pressure gradients as well as the effective masses used for measurement in selected ion monitoring (SIM) mode were all optimized to attain minimum limit of detection (LOD) at sufficient selectivity. The temperatures and pressure gradients for the detection of steroids were

described elsewhere (Starka et al. 2009). The effective masses used for quantification were: for dehydroepiandrosterone m/z (Da) 268, for 7-hydroxy-DHEA m/z 387 and for androstene-triols m/z 437. Retention times of chromatographic peaks, sequence number of injection for steroid groups and gradients that were used for quantification of individual steroids are the same as published recently (Starka et al. 2009). In all cases, the mixtures of authentic standards were processed by the same way as samples and were specific for each of independent courses as mentioned above. The standards were injected in three different amounts for each steroid (10, 100 and 1000 pg).

For evaluation of linearity, increasing volumes of mixtures of pooled maternal serum with water for chromatography (300+700, 400+600, 500+500, 600+400, 700+300, 800+200, 900+100 and 1000+0 ml) were assayed. Two-parameter linear regression was used for the evaluation of relationships between peak areas and serum volume.

Statistical evaluation. Wilcoxon's robust paired test was used for the evaluation of the results.

Results

The concentrations of DHEA, 5-androstene-3β,17β-diol and their 7α- and 7β-hydroxymetabolites, free and conjugated, in midfollicular and midluteal phase of menstrual cycle are shown in Table 1, which shows mean and median values of C_{19} steroids and significance of the differences between both cycle phases. Fig.1- 5 show mean ± SD values for the individual steroid under study in follicular and luteal phase. Concentrations of all circulating C_{19} steroid were within the normal range, as compared to those of 7-hydroxyderivatives and previously reported by Lapcik et al. (1998, 1999). In contrast to significant increase of progesterone and its metabolites as well as that of estradiol in the luteal phase, the androgens either decreased or did not change. The secretion of free dehydroepiandrosterone does not differ in both phases of the menstrual cycle; however, the conjugated form is circulating in the luteal phase in significantly lower concentration than at the beginning of the cycle. A significant decrease of free and conjugated 7-hydroxylated derivatives of DHEA (7α- and 7β-hydroxy-DHEA, 5-androstene-3β,7α,17β-triol and 5-androstene-3β,7β,17β-triol) was observed in the luteal phase, the difference between the values in follicular and luteal phase being more pronounced for free steroids than for their conjugates.

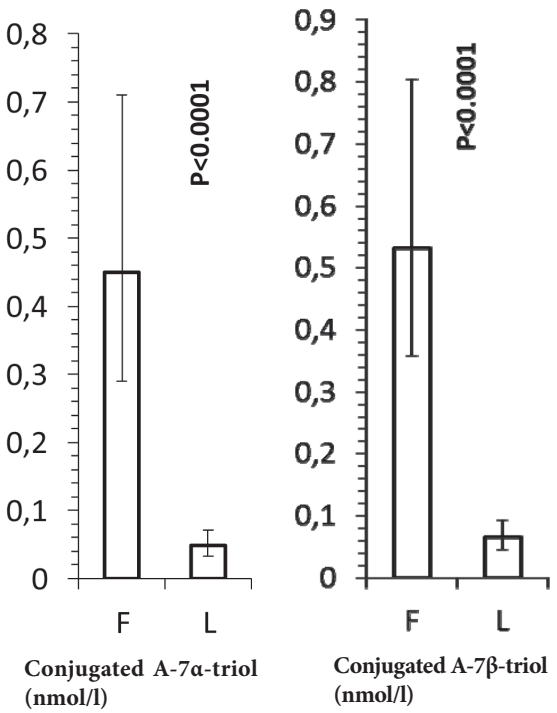


Fig. 5. Conjugated 5-androstene-3β,7α,17β-triol (A-7α-triol) and 5-androstene-3β,7β,17β-triol (A-7β-triol) in the follicular and luteal phase of the menstrual cycle.
F = follicular phase L = luteal phase

Discussion

Dehydroepiandrosterone is one of the most abundant steroids with multiple important functions including the influence on neuronal development and neuroprotection (Li and Bigelow 2010). Actually, the mechanism of neuroprotective function of dehydroepiandrosterone is not yet fully understood and, currently, there are two major hypotheses. Firstly, as an endogenous excitatory neurosteroid, dehydroepiandrosterone directly modulates $GABA_A$ and NMDA receptors, although any convincing evidence on the direct inhibition of $GABA_A$ receptor or the stimulation of NMDA receptor still is not available. Secondly, dehydroepiandrosterone may be metabolized to active metabolites in various tissues (Starka and Kutova 1962, Starka et al. 1962) including the human or rat brain, as already shown by several authors (Akwa et al. 1992; Rose et al 2001; Kim et al. 2004; Steckelbroeck et al. 2002; Weill-Engerer et al. 2003; Li and Bigelow 2010) and this should be responsible for either some or all of the neuroprotective effects. The occurrence of most abundant metabolites of dehydroepiandrosterone in the brain, 7α- and 7β-hydroxy-

dehydroepiandrosterone, was reported by Morfin and Starka (2001).

At the cellular level, irreversible 7α-hydroxylation of 3β-hydroxy-5-ene steroids produces derivatives, which exert anti-glucocorticoid, immunity-promoting, and protective activities. In the brain, as „neuroprotective steroids" and as immunity promoters, 7-hydroxysteroids could contribute to the panels of cellular protection and defense.

Among many women, not only their mood, but also their sexual function and sexual desire are varying according to the phase of their menstrual cycle (Salonia et al. 2006). Relative to the follicular phase, verbal fluency was impaired in the luteal phase and reaction times speeded on a continuous performance task, without affecting overall accuracy. 'Hedonic' scores on the UWIST-MACL scale were decreased in the luteal phase (Symonds et al. 2004).

The putative biological difference in the level of 7-hydroxylated metabolites in the phases of menstrual cycle may be important for our understanding of the etiopathogenesis of menstrual related mood change and neurocognitive disturbances.

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