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The steroid metabolome in lamotrigine-treated women with epilepsy

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ABSTRACT

Background: Epilepsy in women may be associated with reproductive disorders and alterations in serum steroid levels. Some steroids can be induced by epilepsy and/or treatment with antiepileptic drugs; however, there are still limited data available concerning this effect on the levels of other neuroactive steroid metabolites such as 3a-hydroxy-5a/b-reduced androstanes.
Aim: To evaluate steroid alterations in women with epilepsy (WWE) on lamotrigine monotherapy.
Subjects and methods: Eleven WWE and 11 age-matched healthy women underwent blood sampling in both phases of their menstrual cycles (MCs). The steroid metabolome, which included 30 unconjugated steroids, 17 steroid polar conjugates, gonadotropins, and sex hormone-binding globulin (SHBG), was measured using gas chromatography–mass spectrometry (GC–MS) and radioimmunoassay (RIA).
Results: WWE had lower cortisol levels (status $p < 0.001$), but elevated levels of unconjugated 17-hydroxypregnenolone (status $p < 0.001$). Progesterone was higher in the follicular menstrual phase (FP) in WWE than in the controls (status × menstrual phase $p < 0.05$, Bonferroni multiple comparisons $p < 0.05$), whereas 17-hydroxyprogesterone was higher in WWE in both menstrual phases (status $p < 0.001$). The steroid conjugates were mostly elevated in WWE. The levels of 5α/β-reduced androstanes in WWE that were significantly higher than the controls were etiocholanolone (status $p < 0.001$), 5α-androstane-3α,17β-diol (status $p < 0.001$), and the 5α/β-reduced androstane polar conjugates (status $p < 0.001$).
Conclusions: WWE showed a trend toward higher circulating 3α-hydroxy-5α/β-reduced androstanes, increased activity of 17α-hydroxylase/17,20 lyase in the Δ^5-steroid metabolic pathway, and increased levels of the steroid polar conjugates.

1. Introduction

Epilepsy in women is connected with various reproductive disorders, such as hypogonadotropic hypogonadism, [1–4], lower fertility [5,6], abnormalities in luteotropin secretion [6–8], a higher frequency of polycystic ovary syndrome (PCOS) [2,4,9], a higher frequency of anovulatory cycles, and disturbances in the formation of ovarian steroids [6,8]. Some antiepileptic drugs (AED) may induce the development of PCOS.

In contrast to progesterone and estradiol, there are limited data available concerning the role of Δ^5 C19-steroids and the neuroactive C19 3α-hydroxy-5α/β-metabolites in women with epilepsy (WWE) on lamotrigine monotherapy. These steroids, however, could play a role in the pathogenesis of epilepsy. Reddy and colleagues [10] demonstrated in mice that testosterone-derived neurosteroid 5α-androstane-3α,17β-diol (A3α5α17β) has a powerful protective effect against seizures induced by $GABA_A$ receptor antagonists. The authors suggested that A3α5α17β could be an endogenous modulator of seizure susceptibility in men with epilepsy [10]. Anticonvulsant properties were also reported for androsterone and etiocholanolone [11]. Although these steroids are of lower potency, in the serum, they are present in relatively high amounts, particularly in the sulfated forms, and can reach even micromolar concentrations [12] in the human circulation. Although sulfated 3α-hydroxy-5α/β-metabolites are inactive, they might be locally hydrolyzed to active unconjugated substances,

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which can then operate as endogenous modulators of seizure susceptibility.

The goal of the present study was to find alterations in the steroid metabolome in WWE of fertile age who were treated with

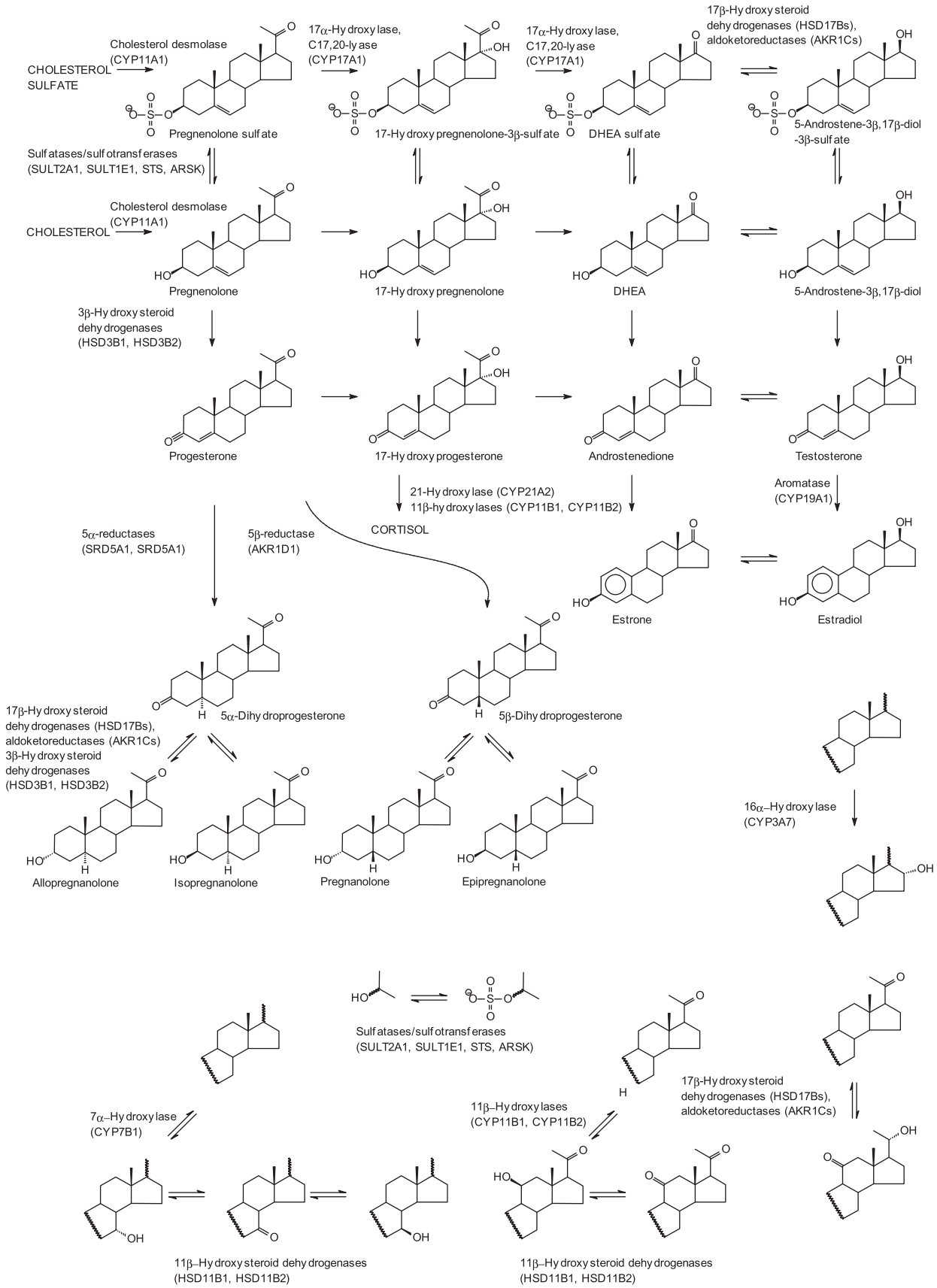

Fig. 1. Simplified scheme of steroid biosynthesis.

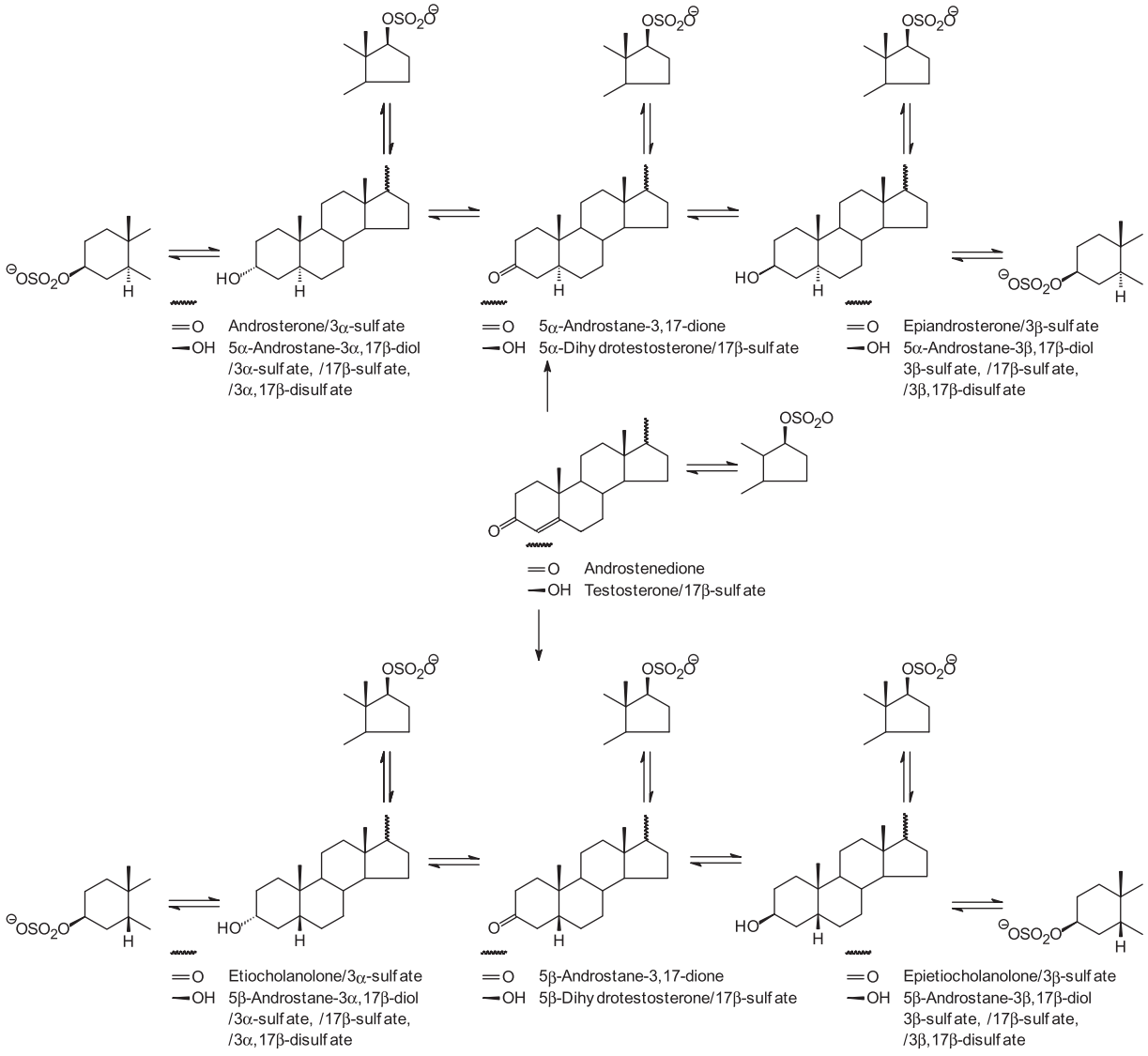

Fig. 2. Simplified scheme of the biosynthesis of 5α/β-reduced androstane metabolites.

lamotrigine. For a better illustration of the pathways involved, a scheme for the biosynthesis of common steroids and reduced progesterone metabolites is shown in Fig. 1, and Fig. 2 displays a scheme for the biosynthesis of reduced androstane metabolites. We selected WWE on lamotrigine monotherapy, as lamotrigine appeared to have a minimum effect on gonadal steroidogenesis [13–16]. In contrast to previous studies that included a greater number of patients and focused on particular steroids, we have selected a limited number of lamotrigine-treated WWE and a wider spectrum of analytes. The metabolites measured covered almost all of the steroid metabolome and included 30 unconjugated steroids, 17 steroid polar conjugates and related compounds, such as gonadotropins, and sex hormone-binding globulin (SHBG).

2. Methods

2.1. Subjects

The study was cross-sectional and included 11 women with epilepsy and 11 healthy control subjects. All of the subjects were followed in both the follicular menstrual phase (FP) and the luteal menstrual phase (LP). Women with catamenial epilepsy were excluded from the study. All patients were on a stable lamotrigine dosage and most of them had been seizure-free for more than one year. The patients were treated with lamotrigine monotherapy for 11–70 months, and for three of them, lamotrigine was their first antiepileptic drug. The study subjects did not use any drug known to interfere with steroid biosynthesis and metabolism and did not have any other endocrine disorder. All of the women had regular menstrual cycles (MCs), were non-smokers and did not consume more than one alcoholic beverage per week. After signing an informed consent form that was approved by the Ethics Committee of the Institute of Endocrinology, all of the women underwent blood sampling twice: once between the 1st and 5th and once between the 22nd and 24th day of the spontaneous MC. No patient was sampled within 7 days following their last seizure. For the evaluation of the analytes, 10 mL of blood was withdrawn on fasting in the morning. Blood samples were centrifuged and stored at −20 °C until they were analyzed.

2.2. Steroid analysis

Most of the steroids and their polar conjugates were measured using the previously described gas chromatography-mass spectrometry (GC-MS) method [17]. The 17-hydroxy-pregnenolone was measured by radioimmunoassay (RIA) as described in our

previous report [18], and conjugated 17-hydroxy-pregnenolone was measured using the RIA after hydrolysis, as described previously [17]. Estradiol was measured by an RIA kit from Orion, Finland (intra-assay CV = 4.4%, inter-assay CV = 4.6%) and 17-hydroxy-progesterone was assayed by a RIA kit from Immunotech, France (intra-assay CV = 5.2%, inter-assay CV = 6.5%). Cortisol was assayed using an RIA kit from Orion, Finland (intra-assay CV (coefficient of variation) = 3.8%, inter-assay CV = 4.4%.); LH levels were determined by an immunoradiometric assay (IRMA) kit from Immunotech, France (intra-assay CV = 3.7%, inter-assay CV = 4.3%); FSH was assayed by an IRMA kit from Immunotech, France (intra-assay CV = 2.6%, inter-assay CV = 4.5%); and SHBG was assayed by an IRMA kit from Orion, Finland (intra-assay CV = 6.1%, inter-assay CV = 7.9%).

2.3. Statistical data analysis

A comparison between the groups of WWE and the controls was carried out using an ANOVA model consisting of the between-subject factor Status (WWE vs. untreated healthy controls); Subject factor (separating the inter-individual variability); within-subject factor Phase of the Menstrual Cycle (follicular phase vs. luteal phase) and the Status × Phase of the Menstrual Cycle interaction (evaluating the difference between trends in the follicular and luteal phases).

Least significance multiple comparisons followed the ANOVA. Due to the skewed distribution and heteroscedasticity (non-constant variance) in most variables, a power transformation to data symmetry and homoscedasticity was used before ANOVA [19]. The homogeneity of the transformed data and symmetry in the distribution of residuals were tested using a residual analysis as described elsewhere [20]. The main factors, their interaction, and multiple comparison tests were considered significant for $p < 0.05$.

3. Results

The group of eleven patients was composed of three women suffering from focal epilepsy, seven women suffering from idiopathic generalized epilepsy, and one unclassified woman. Epilepsy onset occurred between 10 and 31 years of age and lasted from 2 to 18 years. The clinical characteristics of the study participants are summarized in Table 1.

Table 1
Clinical characteristics of the study participants.

Variables	Controls	WWE[a] on lamotrigine
Number of subjects	11	11
Age (years)	28 (26; 31)[b]	28 (24; 31)
Range (years)	25–35	24–34
BMI (kg/m^2)	22.1 (19.8; 25.7)	22.5 (20.3; 25.2)
Range (kg/m^2)	18.1–26.9	18.8–26.3
Duration of menstrual cycle (days)	28 (27; 29)	30 (29; 31)
Range (days)	26–30	24–38
Type of epilepsy	–	focal (n = 4) generalized (n = 7)
Duration of epilepsy (years)	–	11.5 (3; 13)
Range (years)	–	2-18
Duration of current drug therapy (years)	–	1.95 (1.08; 5.00)
Range (years)	–	0.917-5.93
Drug dose (mg/day)	–	250 (150; 300)
Range (mg/day)	–	150-400
Serum drug concentration, μmol/L	–	7.32 (5.52; 15.2)

[a] Women with epilepsy.
[b] Median with quartiles.

The comparison between the group of lamotrigine-treated WWE and the controls for unconjugated steroids is illustrated in Table 2, and Table 3 shows the data for steroid polar conjugates, gonadotropins, and SHBG. Progesterone was higher in the FP in WWE than in the controls (status × MC $p < 0.01$, Bonferroni multiple comparisons CF < PF, $p < 0.05$), whereas 17-hydroxyprogesterone was higher in WWE in both the FP and the LP (status $p < 0.001$). Unconjugated 20α-dihydropregnenolone levels in WWE were higher in both the FP and the LP (status $p < 0.001$). The 5α-pregnanolone isomers allopregnanolone and isopregnanolone did not differ between WWE and the controls, whereas the 5β-epimers pregnanolone and epipregnanolone trended toward higher levels in WWE compared to the controls (status $p < 0.05$ and $p < 0.01$, for pregnanolone and epipregnanolone, respectively). Pregnenolone in the LP was lower in WWE than in the controls (status × MC $p < 0.05$, Bonferroni multiple comparisons CL>PL, $p < 0.05$). In both the FP and the LP, the WWE had elevated levels of conjugated Δ^5 steroids (conjugates of pregnenolone, 17-hydroxypregnenolone, DHEA, and androstenediol) (status $p < 0.001$). WWE also showed elevated levels of the polar conjugates of allopregnanolone (status $p < 0.01$), isopregnanolone (status $p < 0.001$), pregnanolone (status $p < 0.001$), 5α-pregnane-3β,20α-diol (status p<0.001), and 20α-dihydropregnenolone (status $p < 0.001$), in both phases of the MC.

In both phases of the MC, the levels of testosterone (status $p < 0.001$), etiocholanolone (status $p < 0.001$), androsterone (status $p < 0.05$), androstenedione (status $p < 0.001$), and 5α-androstane-3α,17β-diol (status $p < 0.001$), as well as the concentrations of all conjugated 5α/β androstanes (status $p < 0.001$), were higher in WWE than in the controls. Estradiol in the FP was higher in the controls than in WWE (status × MC $p < 0.05$, Bonferroni multiple comparisons CF > PF $p < 0.05$). WWE showed lower cortisol levels than the controls regardless of the phase of MC (status $p < 0.001$).

4. Discussion

This study presents the most comprehensive analysis of serum steroid levels, both gonadal and adrenal, in patients with epilepsy, and it is the first study to look at both conjugated and unconjugated levels of steroids in epilepsy patients. However, we are aware that our study is limited by the absence of a group of untreated WWE. Although oral contraceptives do accelerate lamotrigine catabolism [21,22], and lamotrigine may increase FSH and LH concentrations (having no effect on progesterone production) [23], studies evaluating the effect of lamotrigine on the serum levels of endogenous steroids in WWE reported that this drug did not cause any significant hormonal change in major androgen levels [14,16,24]. *In vitro* data also did not support a significant influence of lamotrigine on steroid synthesis and production. In a yeast cell system, therapeutic concentrations of lamotrigine had no significant effect on the activities of expressed human steroid 17α-hydroxylase/17,20 lyase (CYP17A1) or type 2 3β-hydroxysteroid dehydrogenase (HSD3B2) [25]. The aforementioned data, which indicated that the lamotrigine effect on steroidogenesis is negligible, allow us to speculate that the alterations in steroid levels in our group of lamotrigine-treated WWE compared with healthy controls are related to epilepsy status *per se*. However, most of the steroids/conjugates analyzed in this study have not been previously evaluated in patients with epilepsy.

In both the FP and the LP, WWE had elevated levels of conjugated Δ^5 steroids (conjugates of pregnenolone, 17-hydroxypregnenolone, DHEA, androstenediol, and 20α-dihydropregnenolone). The levels of testosterone were higher in the FP in WWE when compared with the controls. The levels of etiocholanolone and A3α5α17β were higher in both phases of the MC in WWE than in the controls. The levels of the other free C19 steroids did not differ

Table 2
Levels of unconjugated steroids (nmol/L) for controls and lamotrigine-treated women with epilepsy in fertile age; the results are shown as medians with quartiles.

Substance	Follicular phase		Luteal phase		Menstrual cycle (MC)	Status	Status × MC	Bonferroni multiple comparisons ($p < 0.05$)
	Controls (CF)	Patients (PF)	Controls (CL)	Patients (PL)				
Pregnenolone	1.5 (1.2; 2.7)	1.5 (1.1; 2.5)	2.7 (2.1; 4.8)	2.5 (1.2; 3.4)	$p < 0.05$	$p < 0.05$	$p < 0.05$	CF < CL, CL > PL
17-Hydroxypregnenolone	4.2 (1.6; 7.8)	14 (8.4; 20)	4.9 (2.9; 9.2)	11 (4.4; 14)	NS	$p < 0.05$	NS	CF < PF
Dehydroepiandrosterone	10 (6.1; 15)	14 (10; 19)	12 (7.8; 18)	12 (8.5; 26)	NS	p<0.05	NS	
Androstenediol	1.1 (0.9; 1.5)	1.1 (1; 1.2)	0.9 (0.68; 1.2)	1.5 (0.82; 1.7)	NS	NS	NS	
Progesterone	0.49 (0.45; 0.53)	1.6 (0.7; 2)	22 (16; 31)	14 (8.2; 19)	$p < 0.001$	NS	$p < 0.01$	CF < CL, PF < PL
17-Hydroxyprogesterone	1.7 (1.3; 2.6)	2.5 (1.9; 3.4)	8.6 (6.1; 10)	6.1 (4.5; 9)	$p < 0.001$	$p < 0.05$	NS	CF < CL, PF < PL
Androstenedione	3.9 (3.3; 4.5)	4.9 (3.6; 5.5)	4.9 (3.4; 6.6)	6.7 (4.1; 8)	$p < 0.01$	$p < 0.001$	NS	CF < PF, CL < PL
Testosterone	0.93 (0.75; 1.1)	2.2 (1.5; 2.5)	1.2 (0.91; 1.3)	1.8 (0.94; 2.2)	NS	$p < 0.001$	$p < 0.05$	CF < PF
Estradiol	0.18 (0.12; 0.22)	0.1 (0.086; 0.12)	0.36 (0.31; 0.64)	0.48 (0.31; 0.58)	$p < 0.001$	NS	$p < 0.05$	CF < CL, PF < PL, CF>PF,
5α-Dihydroprogesterone	0.56 (0.52; 0.63)	0.26 (0.11; 0.34)	0.92 (0.8; 1.2)	0.46 (0.28; 0.68)	p<0.001	p<0.001	NS	CF<CL, CF>PF, CL>PL
Allopregnanolone	0.17 (0.13; 0.22)	0.16 (0.14; 0.27)	0.85 (0.53; 1)	0.55 (0.41; 1.1)	p<0.001	NS	NS	CF < CL,
Isopregnanolone	0.14 (0.094; 0.2)	0.22 (0.15; 0.28)	0.47 (0.37; 0.8)	0.37 (0.21; 0.61)	$p < 0.001$	NS	$p < 0.01$	CF < CL,
Pregnanolone	0.065 (0.054; 0.092)	0.13 (0.11; 0.19)	0.23 (0.16; 0.39)	0.22 (0.18; 0.44)	$p < 0.001$	$p < 0.05$	NS	CF < CL, CF < PF,
Epipregnanolone	0.025 (0.022; 0.033)	0.05 (0.022; 0.1)	0.045 (0.026; 0.082)	0.091 (0.076; 0.11)	NS	$p < 0.01$	NS	
20α-Dihydropregnenolone	0.71 (0.65; 1.1)	1.4 (1.1; 2.1)	0.98 (0.7; 1.4)	1.4 (1.1; 2.4)	NS	$p < 0.001$	NS	CF < PF
20α-Dihydroprogesterone	0.64 (0.48; 0.81)	0.64 (0.58; 0.91)	5.5 (4.6; 6.9)	2.9 (1.1; 5.7)	$p < 0.001$	NS	$p < 0.05$	CF < CL, PF < PL
20α-Hydroxy-5α-pregnane-3-one	0.33 (0.22; 0.46)	0.47 (0.3; 0.97)	2.3 (2; 3.4)	1.2 (0.99; 1.8)	$p < 0.001$	NS	$p < 0.001$	CF < CL, PF < PL, CL > PL
5α-Dihydrotestosterone	0.19 (0.094; 0.42)	0.4 (0.33; 0.45)	0.39 (0.26; 0.76)	0.38 (0.22; 0.52)	NS	NS	NS	
Androsterone	0.47 (0.3; 0.57)	0.47 (0.38; 0.59)	0.5 (0.33; 0.62)	0.67 (0.42; 0.74)	NS	$p < 0.05$	NS	
Epiandrosterone	0.59 (0.35; 0.75)	0.62 (0.44; 0.79)	0.56 (0.43; 0.84)	0.68 (0.41; 1.2)	NS	NS	NS	
Etiocholanolone	0.26 (0.2; 0.33)	1 (0.47; 2.4)	0.16 (0.15; 0.26)	0.47 (0.26; 1.6)	NS	$p < 0.001$	NS	CF < PF, CL < PL
Epietiocholanolone	0.014 (0.01; 0.02)	0.018 (0.011; 0.032)	0.012 (0.0091; 0.015)	0.02 (0.0054; 0.038)	NS	NS	NS	
5α-Androstane-3α,17β-diol	0.051 (0.034; 0.064)	0.14 (0.091; 0.26)	0.032 (0.029; 0.058)	0.13 (0.13; 0.22)	NS	$p < 0.001$	NS	CF < PF, CL < PL
7α-Hydroxydehydroepiandrosterone	2.1 (1.6; 2.4)	1.4 (1.2; 2)	1.6 (1.2; 1.7)	1.4 (1.2; 2.1)	$p < 0.05$	NS	NS	
7β-Hydroxydehydroepiandrosterone	0.62 (0.51; 0.79)	0.55 (0.45; 1.2)	0.48 (0.37; 0.68)	0.5 (0.39; 0.91)	$p < 0.01$	NS	NS	
5-Androstene-3β,7α,17β-triol	0.23 (0.17; 0.32)	0.12 (0.067; 0.14)	0.18 (0.13; 0.2)	0.1 (0.089; 0.15)	NS	$p < 0.001$	NS	CF > PF, CL > PL
5-Androstene-3β,7β17β-triol	0.12 (0.11; 0.19)	0.11 (0.041; 0.13)	0.095 (0.07; 0.14)	0.092 (0.067; 0.12)	NS	NS	NS	
Cortisol	409 (337; 453)	246 (214; 285)	433 (330; 516)	208 (152; 366)	NS	$p < 0.001$	NS	CL > PL

Status × MC = Status – MC interaction (evaluating difference between trends in the follicular and lueal phases); CF = controls in the follicular phase, PF = patients in the follicular phase, CL = controls in the luteal phase, PL = patients in the luteal phase; only significant least significant difference (LSD) multiple comparisons were shown; the multiple comparisons were considered as significant for $p < 0.05$.

Table 3
Levels of steroid polar conjugates (nmol/L), gonadotropins (U/L), and sex hormone binding globulin (nmol/L) for controls and lamotrigine-treated women with epilepsy in fertile age; the results are shown as medians with quartiles.

Variable	Follicular phase		Luteal phase		Menstrual cycle (MC)	Status	Status × MC	Bonferroni multiple comparisons ($p < 0.05$)
	Controls (CF)	Patients (PF)	Controls (CL)	Patients (PL)				
Conjugated pregnenolone	56 (38; 88)	140 (99; 240)	80 (43; 110)	130 (70; 270)	NS	$p < 0.001$	NS	CF < PF, CL < PL
Conjugated 17-hydroxypregnenolone	7 (5.9; 13)	39 (29; 51)	6.6 (4.9; 8.2)	39 (35; 42)	NS	$p < 0.001$	NS	CF < PF, CL < PL
Conjugated dehydroepiandrosterone	1514 (1369; 2015)	2687 (2475; 4219)	957 (821; 1120)	3264 (1511; 5081)	$p < 0.01$	$p < 0.001$	$p < 0.05$	CF > CL, CF < PF, CL < PL
Conjugated androstenediol	100 (79; 210)	550 (330; 770)	130 (110; 150)	460 (290; 980)	NS	$p < 0.001$	NS	CF < PF, CL < PL
Conjugated allopregnanolone	4.6 (3.4; 7.7)	10 (8.1; 18)	25 (21; 31)	54 (17; 69)	$p < 0.001$	$p < 0.01$	NS	CF < CL, PF < PL, CF < PF,
Conjugated isopregnanolone	4.7 (3.2; 7)	13 (10; 22)	15 (9; 20)	46 (11; 58)	$p < 0.001$	$p < 0.001$	NS	CF < CL, CF < PF, CL < PL
Conjugated pregnanolone	7.9 (5.4; 12)	21 (15; 33)	21 (17; 26)	58 (24; 79)	$p < 0.001$	$p < 0.001$	NS	CF < CL, PF < PL, CF < PF, CL < PL
Conjugated epipregnanolone	2.1 (1.3; 2.8)	2.3 (1.7; 3.1)	3.8 (2.3; 4.6)	5.3 (2.8; 8.7)	$p < 0.01$	NS	NS	
Conjugated 20α-dihydropregnenolone	176 (147; 292)	345 (317; 407)	172 (144; 273)	394 (242; 475)	NS	$p < 0.001$	NS	CF < PF, CL < PL
Conjugated 5β-pregnane-3α,20α-diol	40 (32; 66)	42 (30; 52)	120 (100; 180)	96 (67; 270)	$p < 0.001$	NS	NS	CF < CL
Conjugated 5α-pregnane-3β,20α-diol	14 (12; 19)	140 (97; 170)	84 (66; 100)	150 (77; 210)	$p < 0.001$	$p < 0.001$	$p < 0.01$	CF < CL, CF < PF, CL < PL
Conjugated androsterone	788 (552; 1180)	1725 (1004; 3187)	346 (263; 457)	1480 (830; 3340)	$p < 0.01$	$p < 0.001$	$p < 0.01$	CF > CL, CF < PF, CL < PL
Conjugated epiandrosterone	313 (212; 352)	559 (397; 686)	163 (116; 216)	483 (314; 745)	$p < 0.001$	$p < 0.001$	$p < 0.05$	CF > CL, CF < PF, CL < PL
Conjugated etiocholanolone	46 (33; 91)	200 (81; 290)	25 (22; 34)	98 (58; 310)	$p < 0.001$	$p < 0.001$	$p < 0.01$	CF > CL, CF < PF, CL < PL
Conjugated epietiocholanolone	11 (6.9; 22)	29 (15; 70)	6.1 (5.1; 8.2)	25 (16; 88)	NS	$p < 0.001$	$p < 0.01$	CF > CL, CF < PF, CL < PL
Conjugated 5α-androstane-3α,17β-diol	3.4 (2.1; 5.1)	36 (33; 40)	4.4 (2.8; 5.6)	30 (22; 56)	NS	$p < 0.001$	NS	CF < PF, CL < PL
Conjugated 5α-androstane-3β,17β-diol	12 (6.9; 16)	38 (33; 40)	11 (8.6; 13)	47 (24; 63)	NS	$p < 0.001$	NS	CF < PF, CL < PL
Lutropin (LH)	4.3 (2.4; 7.3)	4.9 (3.2; 12)	7.9 (5; 10)	5.4 (3.5; 14)	NS	NS	NS	
Follitropin (FSH)	7.9 (3.8; 8.8)	6.6 (5.9; 8.8)	4.2 (2.4; 5.3)	3.8 (2.6; 4.5)	$p < 0.05$	NS	NS	CF > CL, PF > PL,
Sex hormone binding globulin (SHBG)	67 (56; 140)	47 (43; 56)	66 (46; 100)	50 (42; 62)	NS	$p < 0.05$	NS	CF > PF, CL > PL

Status × MC = Status – MC interaction (evaluating difference between trends in the follicular and lueal phases); CF = controls in the follicular phase, PF = patients in the follicular phase, CL = controls in the luteal phase, PL = patients in the luteal phase; only significant least significant difference (LSD) multiple comparisons were shown; the multiple comparisons were considered as significant for $p < 0.05$.

between the groups. However, all conjugated 5α/β androstane levels were higher in WWE than in the controls in both phases of the MC. The 3α-hydroxy-5α/β-reduced-androstanes are potent antiseizure agents. It has been demonstrated that the systemic administration of A3α5α17β in female rats reduces kainic acid-induced seizures [26]. Men with epilepsy treated with AED excrete diminished quantities of androsterone and etiocholanolone compared with age-matched control subjects [27]. However, to date, there are no reports on the levels of 5α/β-reduced androstane steroids in WWE. We had anticipated that androstane levels in WWE would be decreased, as was found in males [27]. However, the consistently increased levels of both free and conjugated C19 steroids surprised us. It is tempting to speculate that the observed increase in androstane levels might be a neuroprotective mechanism that partially counteracts the detrimental effects of epilepsy.

We observed reduced estradiol levels of borderline significance in the FP of WWE compared with the controls, but no difference was found in the LP. This is in agreement with the data of Murialdo et al. [28], but conflict with the data of Galimberti et al., who described reduced estradiol levels in WWE [29] even in the LP. However, in those studies, most of the women were treated by enzyme-inducing drugs, such as carbamazepine (CBZ) and phenytoin, or by valproate. In contrast to those studies, women treated by monotherapy with non-enzyme inducing drugs only showed a small difference compared with the control group [29]. Our estradiol data are also in accordance with those of Svalheim et al. [16], who reported no difference in estradiol levels in the FP between patients on lamotrigine therapy and controls [16].

Increased progesterone concentrations were found in the FP of WWE. The levels of 5α-dihydroprogesterone were consistently lower in both phases of the MC in WWE, whereas pregnanolone isomers in the FP were higher in WWE than in the controls. However, the unconjugated allopregnanolone showed no difference regardless of the MC phase. Our progesterone data in the FP differed from the results of previous studies. Progesterone levels in the FP in WWE treated with various enzyme inducing drugs were lower with CBZ treatment than in the controls [30,31]. The lower progesterone levels during the FP could be related to the different methodologies. Most studies use chemiluminescence or IRMA to quantify these steroids, which are inferior compared with GC–MS.

A progesterone deficiency in the LP of WWE may be associated with a lack of neuroinhibiting metabolites (3α-PI), which may correlate with a higher frequency of epileptic seizures. Several studies have indicated a connection between catamenial epilepsy and disturbances in the biosynthesis of progesterone and its reduced metabolites [32–34]. A number of studies in WWE have evaluated the role of estradiol and progesterone balance [8,28,29,31] in the pathogenesis of catamenial epilepsy, and progesterone and its derivatives have also been suggested as treatments for anticonvulsant therapy [35–37]. Our progesterone data in the LP were in agreement with results showing no significant change in the LP [29], but they differed from other results showing lower progesterone levels in WWE than in controls [31].

Concerning the unconjugated allopregnanolone, our data are in accordance with the results of other authors who described no difference in the allopregnanolone levels in the interictal period between children with epilepsy and controls [38] or between women with temporal lobe epilepsy and healthy controls [39].

WWE showed suppressed cortisol levels. This result, together with increased levels of C19 steroids, pointed to the increased activity of the *zona reticularis* and suppressed cortisol production in the *zona fasciculata*. Our data are in contrast to others who have described either an increase [40,41] or no change [42] in serum cortisol compared with healthy controls. Although we did not measure adrenocorticotropic hormone (ACTH) or corticoliberin (CRH) levels, we hypothesize that our results could be a consequence of

a deficient production of ACTH and the increased influence of CRH in our patients. This imbalance may lead to a preferential production of androgens. The data of Motta and colleagues [43] that described lower ACTH in epileptics than in the controls support this idea. CRH acts as an adrenal secretagogue, as revealed by Ibanez et al. [44], who showed that after CRH infusion, levels of serum dehydroepiandrosterone sulfate increased, DHEA doubled, and androstenedione tripled.

In conclusion, the main finding of our study is that female patients suffering from generalized or focal epilepsy on lamotrigine therapy have increased levels of free and conjugated androstane metabolites, but unaltered levels of allopregnanolone and pregnanolone.

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