

Dehydroepiandrosterone (DHEA) Serum Levels Sufficiently Predict Cerebrospinal Fluid Levels of DHEA and Estradiol (E2) in Women at Term Pregnancy

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Research

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Abstract

Background and objectives:

Neuroactive steroids such as dehydroepiandrosterone (DHEA), estradiol (E2), and progesterone (P4) are associated with structural and functional changes in the central nervous system (CNS). Measurement of steroid levels in the CNS compartments, though restricted in accessibility, is essential for clinical evaluation. Consequently, there is only limited human data on the correlation and equilibrium for steroid levels between peripheral and central compartments. While some neuroactive steroids including DHEA and E2 have been reported to convey excitatory and proconvulsant properties, the opposite was demonstrated for progesterone. We aimed to elucidate the correlation between peripheral and central DHEA, E2, and P4 levels in women at term pregnancy.

Subjects and Methods:

CSF and serum samples of 27 healthy pregnant women (22-39 years) at term pregnancy were collected simultaneously under combined spinal and epidural anesthesia and used for DHEA, E2, and P4 ELISA.

Results:

All three neuroactive steroids were detected at markedly lower levels in CSF compared to their corresponding serum concentrations (decrease, mean \pm SD, 97.66 ± 0.83 %).

We found a strong correlation for DHEA between its serum and the corresponding CSF levels ($r=0.65$, $p=0.003$). While a significant but weak correlation ($r=0.39$, $p=0.046$) was detected for P4, serum and CSF levels of E2 ($r=0.31$, $p=0.12$) appeared not to correlate in the investigated cohort. DHEA serum concentration correlated significantly with both E2 ($r=0.58$, $p=0.0016$) and P4 in CSF ($r=0.39$, $p=0.046$). A strong correlation between the steroids DHEA and E2 measured in CSF was found ($r=0.65$, $p=0.0002$), while no substantial correlation between DHEA and P4 was evident in this compartment ($r=0.34$, $p=0.085$).

Conclusions:

Peripheral DHEA levels might serve as a valid predictor for central nervous levels of the neuroactive steroids DHEA and E2 in pregnant women.

1. Background

The neuroactive steroids dehydroepiandrosterone (DHEA), estradiol (E2), and progesterone (P4) have been found to modulate neural activity to a variable degree exerting excitatory and inhibitory effects in neural tissue (1-4). These properties are mediated by nuclear, mitochondrial or cell-membrane-bound steroid receptors, which occur in varying densities and subtypes throughout the CNS (5-7). The aforementioned receptors of neuroactive steroids can act as transcription factors via classical nuclear

steroid receptors as well as non-classical signaling pathways including modulation of synaptic and extrasynaptic neurotransmitters such as gamma-aminobutyric acid (GABA) A, N-methyl-D-aspartic acid (NMDA), glycine, serotonin nicotinic acetylcholine, and oxytocin receptors (8, 9).

Besides hypothalamic-pituitary-ovarian cycle control, menopausal climacteric complaints, cognitive performance and psychosocial behavior, neuroactive steroids seem to be relevant in several neurodegenerative diseases (10). Furthermore, regular cyclic fluctuations of hormone levels are associated with CNS symptoms such as premenstrual dysphoric syndrome (PMDS) or catamenial epilepsy patterns (11). However, there is an ongoing debate on their pathophysiology with contradictory clinical observations and research results (4). There is no strong scientific basis for the assumption, that estradiol is proconvulsant, nonetheless estradiol has potent neuroexcitatory effects under certain circumstances and without a doubt, estradiol influences seizure susceptibility in women with epilepsy (12-16). In contrast, there is sufficient evidence demonstrating anticonvulsant properties of progesterone and its metabolite allopregnanolone (17, 18).

Neuroactive steroids originate both from peripheral glands and de novo synthesis in the CNS in neurons and glial cells (19, 20). The steroidal hormone transport across the blood brain barrier varies according to the specific hormone (21) however the knowledge of precise mechanisms and distributional equilibrium between peripheral and central compartments is limited (22).

Given the current uncertainties, an examination of steroid fluctuation and distribution between central and peripheral compartments in clinical populations of interest would be a sensible direction for further studies. The access to CNS tissue for steroid measurement from individuals without CNS pathology is limited for ethical reasons. Within certain diagnostic or therapeutic clinical procedures including combined spinal-epidural anesthesia cerebrospinal fluid (CSF) is available and can be utilized for analyses. The measurement of neuroactive hormone concentrations in CSF is an approximation to endocrine tissue conditions in the brain in an in vivo observation without tissue damage.

We aimed to investigate a putative correlation of the neuroactive steroid hormones between serum and CSF as well as whether the peripheral hormones might serve as predictor for the respective and the other steroid hormones in CSF. In this study, we provide evidence that peripheral DHEA shows a strong correlation with central nervous hormone levels of DHEA and E2 and thus could be a suitable predictor of CSF hormone concentrations.

2. Subjects And Methods

This prospective study was approved by the local institutional ethics committee of the Medical Faculty, RWTH Aachen University, Aachen, Germany in accordance to the Declaration Helsinki Declaration on Ethical Principles for Medical Research Involving Human Subjects and the Guideline for Good Clinical Practice (EK 201/14). All participants signed informed consent. A total of 27 healthy pregnant women in the 38-40th gestational week, who presented themselves for a planned cesarian section between 2014

and 2015 were included. CSF and blood samples were collected simultaneously during combined spinal epidural anesthesia for elective cesarian section. After epidural puncture liquor was rinsed to verify correct intrathecal needle position. 1 ml of clear CSF without macroscopic blood contamination was then collected and stored in an Eppendorf tube in -80° for further analysis. Blood samples were centrifuged, and serum and cerebrospinal fluid were assayed for DHEA, estradiol and progesterone in the same laboratory using an electrochemiluminescence immunoassay (ECLIA, Cobas Roche Diagnostics, Mannheim, Germany).

2.1 Statistics

Data analysis and visualization was performed using GraphPad Prism (version 8.4.3, San Diego, CA, USA). Bivariate correlation of quantitative data was assessed by nonparametric Spearman's rank correlation. A correlation coefficient of $(\rho = r)$ 0.00 – 0.19 indicates a very weak correlation, 0.20 – 0.39 a weak correlation, 0.40 – 0.59 a moderate correlation, 0.60 – 0.79 a strong correlation and 0.8 – 1.00 a very strong correlation. A p-value of < 0.05 was considered significant.

3. Results

Serum DHEA levels significantly correlate with DHEA and E2 levels in CSF

To elucidate a potential correlation between serum and CSF levels of the neuroactive steroids dehydroepiandrosterone (DHEA), progesterone (P4) and estradiol (E2) at term pregnancy, we included a total of 27 pregnant women (mean age: 30.5, range: 22-39 years) with elective cesarean section in the 38-40th gestational week in this study (Table 1). In all subjects, both CSF and serum samples were collected simultaneously under combined spinal and epidural anesthesia.

We observed a 98.83% (SD: ± 0.80), decrease to a mean DHEA level in CSF of 0.18 ng/ml (range: 0 - 0.47 ng/ml) (Fig. 1A) from a mean serum DHEA level of 15.38 ng/ml (range: 4.14 - 37.33 ng/ml). A similar pattern of serum and CSF levels was evident for both E2 and P4. Estrogen was found at a mean serum level of 13.9 ng/mL (range: 2.2 – 43.6 ng/mL) with a 97.15% (SD: $\pm 2.2\%$) reduction in CSF (mean: 281.44 ng/mL, range: 123 - 490 ng/mL) (Fig. 1B). Also P4 was reduced by approximately 97.01 % (SD: ± 1.66) in the CSF (mean: 2.96 ng/ml, range: 0.41-5.55 ng/ml) compared to the corresponding serum levels (mean: 114.04 ng/ml, range: 25 - 375 ng/ml) of pregnant women (Fig. 1C). Although all three steroids displayed similar relative decreases between serum and CSF levels, a Spearman rank correlation analysis between serum and CSF revealed a significant strong correlation only for DHEA ($r=0.65$, $p=0.003$) (Fig. 1D). While a significant but weak correlation ($r=0.39$, $p=0.046$) was detected for P4, only serum and CSF levels of E2 ($r=0.31$, $p=0.12$) appeared not to correlate in the investigated cohort (Fig. 1E/F).

Next, we evaluated if peripheral and central DHEA levels correlated with central E2 and P4 levels and if DHEA could be a suitable predictor.

DHEA serum concentration correlated significantly with both central nervous E2 ($r=0.58$, $p=0.0016$) and P4 in CSF ($r=0.39$, $p=0.046$) (Fig. 2A/C). The correlation of peripheral DHEA levels with central E2 was stronger than with P4. The latter also held true for the correlation of central DHEA levels with central E2 and P4 levels. While a significant and strong correlation between the steroids DHEA and E2 measured in CSF was found ($r=0.65$, $p=0.0002$), no significant correlation between DHEA and P4 was evident in this compartment ($r=0.34$, $p=0.085$) (Fig. 2B/D).

In summary, our data strongly suggest that peripheral DHEA levels may serve as a good predictor for central nervous levels of the neuroactive steroids DHEA (strong correlation) and E2 (moderate correlation) in pregnant women. For P4 the intercompartment correlation was weak in the tested cohort, similar to the measurements between peripheral DHEA and central P4.

4. Discussion

The correlations of serum to CSF concentrations of the measured steroid hormones (DHEA, E2, P4) vary according to the specific steroid in our cohort of 27 women at term pregnancy. Our results for pregnant women are consistent with the repeated observation in non-pregnant subjects, that steroid concentrations broadly differ at least by one to two orders of magnitude between the serum to CNS compartments (23, 24). We found the strongest inter-compartment correlation for DHEA. On the contrary, correlations for estradiol were rather weak, and for progesterone weak but significant. Interestingly central E2 concentration correlated best to serum DHEA (moderate correlation) and to central DHEA (strong correlation).

To our knowledge this is the first study observing CSF to serum concentrations of neuroactive steroid hormones in women at term pregnancy. CSF and serum concentrations were measured from simultaneously collected samples. Robust correlation effects could be shown over a wide range of the hormone serum concentrations that are typically found in pregnancy. We choose a set of steroids with neuroactive potential that are most available in clinical routine analysis. A second and more important reason for measuring estradiol and progesterone is that these are the two most discussed neuroactive steroids in seizure susceptibility in women with epilepsy (4).

Pregnancy is a distinct physiological condition that might include changes in blood-brain-barrier function as well as central and peripheral steroid metabolism compared to non-pregnant subjects. Therefore the results may not be valuable for non-pregnant women and men. Our study population of 27 subjects was small, though comparatively large regarding published populations in this field. Statistical evidence base is limited due to a small sample size. ELISA were not constructed for hormone analysis in CSF (25) Mass spectrometry might be superior for precise steroid detection in CSF, but was not available for this study.

The transformation from cholesterol to progesterone or DHEA and further from DHEA via the downstream androgens androstendione and testosterone towards their aromatization to the estrogens estron and estradiol (E2) are major human steroidogenic pathways (26). The main serum availability of these steroids in premenopausal women derives from peripheral glands (ovaries, adrenals) and in case of

pregnancy from the fetoplacental unit. Extraordinarily high circulating E2 concentrations during pregnancy result from placental aromatase activity. The placental steroid production exceeds physiologic concentrations in non-pregnant women by several orders of magnitude. Estradiol serum concentrations rise 100 to 500-fold during pregnancy, compared to normal cycle conditions (27).

Steroid hormones cross the blood brain barrier to a certain extent, but the intercompartment equilibrium between serum, CSF and neural tissue is unclear (10). In line with our results, *in vivo* data of patients, though scarce in number, suggest that the steroidal environment in CNS versus intravascular compartments differs by a magnitude of 10 to 100 with lower concentrations in the CNS. Animal models support these findings (10, 28). CNS tissue concentrations are not necessarily reflected in CSF (23). Most stable correlations were found for DHEA in CSF and temporal brain tissue (29). The study of intra-tissue steroid hormone formation and metabolism (intracrinology) reveals the expression of required enzymes for steroid hormone synthesis in topical variance in the brain tissue, although there are conflicting data concerning the complete steroid biosynthetic pathways in human CNS (30). The balance between intra tissue *de novo* synthesis to systemic delivery of hormone precursors or ready for function steroid effectors has not yet been disentangled.

In conclusion of these findings and our data central E2 seems to originate mainly from local synthesis based on local or peripheral DHEA supply as a precursor hormone to estrogen after aromatization. In this regard peripheral DHEA can be interpreted as a predictor of central E2, whereas our data suggest at least a partial peripheral to central P4 transport. A DHEA to P4 ratio from serum hormone concentrations would allow a prediction of the E2 to P4 ratio in a central compartment.

Since estradiol is a potentially proconvulsive neuroactive steroid, and progesterone has a tendency to have a depressant effect, a proportional increase of estradiol compared to progesterone concentration in the CSF in the course of pregnancy would result in an excitatory neurosteroidal environment due to overweighing neuroactive estradiol effects.

In most women with epilepsy (WWE) the status quo of seizure frequency appears to remain controlled during pregnancy. But studies report an increase in seizure frequency for 20-50 percent of WWE during pregnancy (31-34). A reliable predictor has not been identified yet. In an attempt to exclude anti-epileptic drugs (AED) serum concentration dependent effects such as accelerated metabolism in pregnancy or non-compliance to medication intake Vajda et al. still found a significant rise in seizure frequency in a Australian pregnancy register based case series of women with medically untreated epilepsy during pregnancy (35).

Neuroactive hormone balance signatures are not systematically examined or reflected as predictors of seizure frequency worsening in pregnant WWE. A pregnancy is an exceptional endocrine situation with excessive hormone synthesis and highly variable serum concentrations compared to normal cyclic conditions. The clinically observed increase of seizures in patients without AED may partly be influenced by rising estradiol to progesterone ratios in the CNS. The predictive potential of peripheral DHEA

concentrations as a surrogate marker for central E2 concentrations might identify patients at risk for seizure e.g. from epilepsy or preeclampsia due to endocrine CNS modulation.

Therefore, clinical observation with clear concomitant measurement of neuroactive hormonal distribution is required. Here, we offer a clinical tool (peripheral DHEA/P4 ratio to calculate CSF E2/P4 ratio) that allows predictive insight of neuroactive steroid concentrations in a CNS compartment from the easily accessible peripheral serum compartment.

5. Conclusions

Central E2 comes from local metabolization rather than via blood brain barrier transport at term pregnancy and serum DHEA seems to predicts central levels of E2 and DHEA. Peripheral DHEA / P4 ratio may serve as a prognostic factor for pregnant women at risk for seizures.

Declarations

Ethics Approval and Consent to Participate

This prrospective observational cohort analysis was approved by the local institutional ethics committee of the Medical Faculty, RWTH Aachen University, Aachen, Germany (EK 201/14) in accordance to the Declaration of Helsinki and the Guideline for Good Clinical Practice. All participants signed informed consent.

Consent for publication

This study does not include images or other personal or clinical details of participants that compromise anonymity. With their consent to participate, all patients have also signed a written consent to publish their data anonymously.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request

Competing interests

The authors declare no competing interests.

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Authors' contributions

Conceptualization: PH, BR; methodology: PH, BR; investigation/experiments:PH, BR; validation: PH, BR; formal analysis: PH, JN, SH, BR; visualization: PH, BR; resources: JN, BR; original draft preparation: PH, BR; review and editing: PH, JN, SH, BR; supervision: BR;. All authors have read and approved the manuscript

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Not Applicable

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Tables

Due to technical limitations, Table 1 is only available as a download in the Supplemental Files section.

Figures

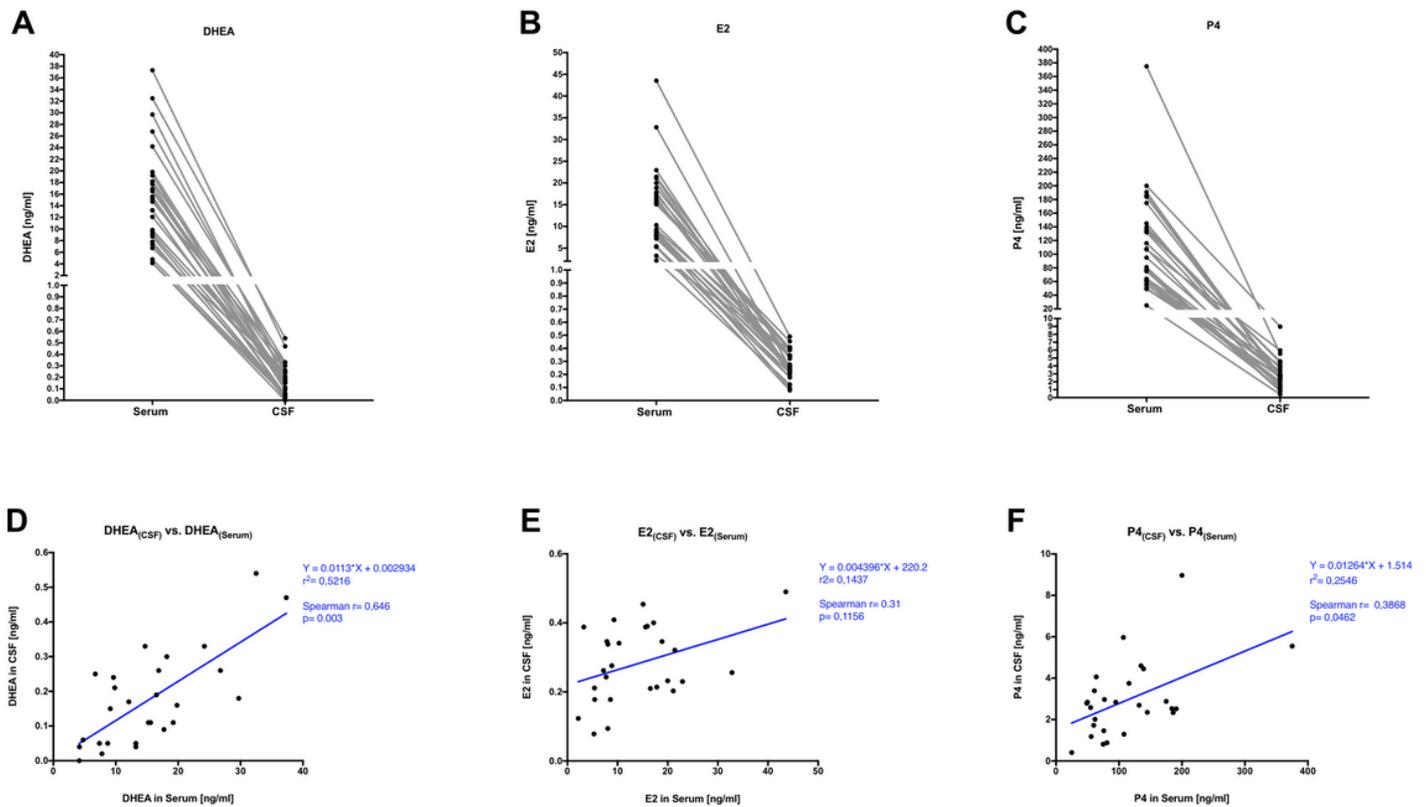


Figure 1

Associations between serum and CSF levels of neuroactive steroids in pregnant women. Serum and CSF of 27 women at term-pregnancy was sampled during cesarian section. A) Dehydroepiandrosterone (DHEA) concentrations, B) Estradiol (E2) and C) Progesterone (P4) in serum and CSF per subject is given respectively. Spearman rank correlation analysis between serum and CSF revealed in D) a significant strong correlation for DHEA ($r=0.65$, $p=0.003$) in E) a non-significant correlation for E2 ($r=0.31$, $p=0.12$) and in F) a weak correlation for P4 ($r=0.39$, $p=0.046$).

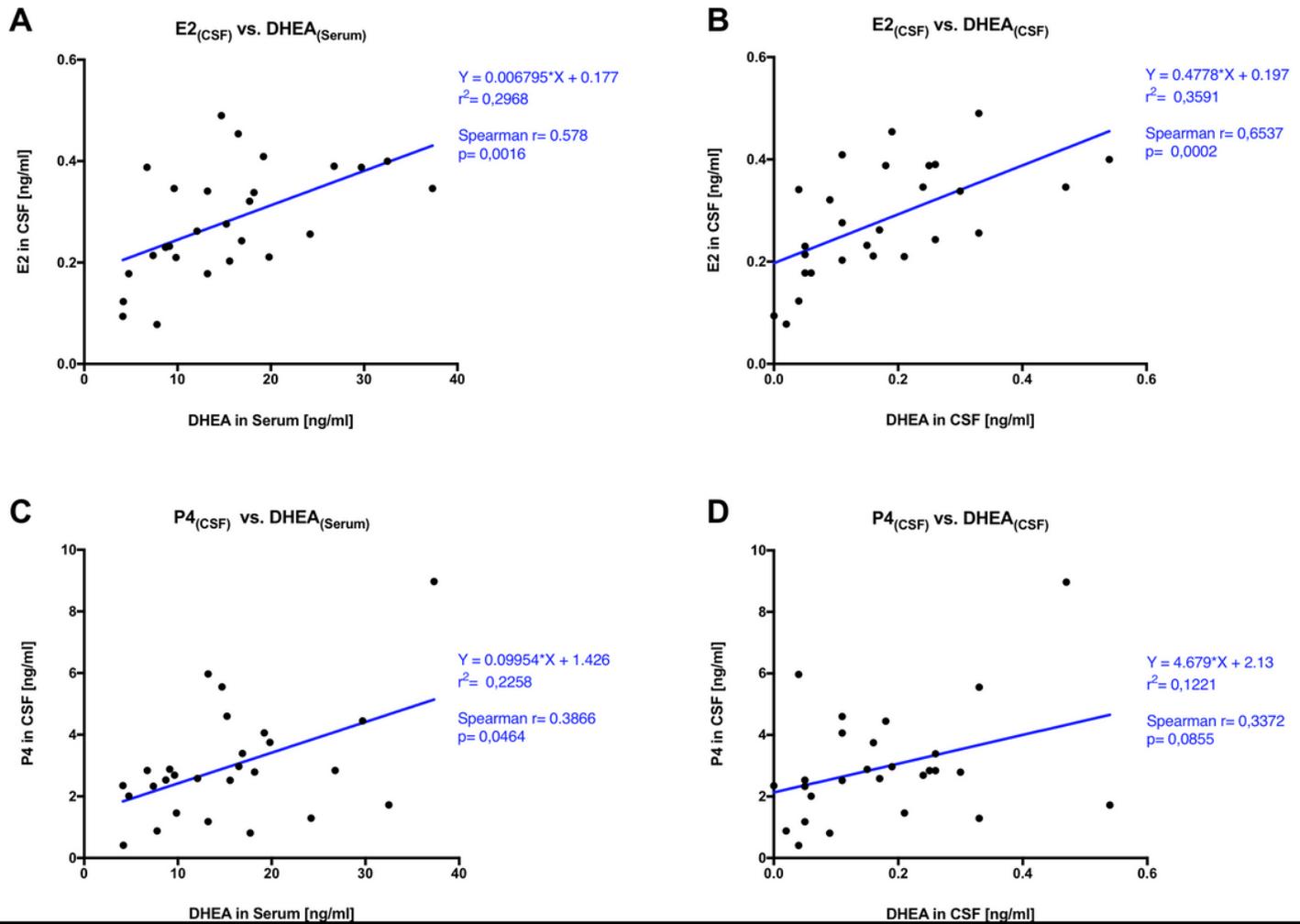


Figure 2

DHEA serum levels significantly correlate with CSF levels of E2 and P4 in pregnant women DHEA levels in serum ($r = 0.58$, $p=0.0016$) (A) and in CSF ($r=0.65$, $p=0.0002$) (B) display a significant correlation with central E2. (C) DHEA levels in serum show a significant but weak correlation with P4 levels in CSF ($r=0.39$, $p=0.046$), while DHEA in CSF seems not to correlate with central P4 levels ($r=0.34$, $p=0.085$) (D).

Supplementary Files

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