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Original Research Article

Effects of estradiol- and ethinylestradiol-based contraceptives on adrenal steroids: A randomized trial ☆,☆☆

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ABSTRACT

Objectives: Ethinylestradiol (EE)-based combined oral contraceptives (COC) affect adrenal function by altering steroid and corticosteroid-binding globulin (CBG) synthesis that may contribute to adverse effects related to these drugs. The effects of COCs containing natural estrogens remain unclear. We compared the effects of COCs containing estradiol valerate (EV) and EE on cortisol and other adrenal steroid hormones.
Study design: A spin-off study of a randomized, open-label trial. Fifty-nine healthy women were allocated to groups that engaged in the continuous use of EV+dienogest (DNG), EE+DNG, or DNG only for 9 weeks. We measured changes in adrenal steroids, CBG, and the free cortisol index (FCI).
Results: Treatment with EE+DNG increased total cortisol (mean increment 668 nmol/L, $p < 0.001$) and cortisone (10 nmol/L, $p = 0.001$) levels, whereas the change from the baseline was insignificant for the EV+DNG and DNG-only groups. Dehydroepiandrosterone sulfate decreased by 24% in the EE+DNG group but remained unchanged in the EV+DNG and DNG-only groups. Aldosterone and 17-hydroxyprogesterone levels did not differ between the groups. All preparations increased CBG, but the increase in the EE+DNG group (median increment 42 μg/mL, $p < 0.001$) was 9- and 49-fold higher than that in the EV+DNG and DNG-only groups, respectively. The FCI remained unchanged in all study groups, indicating that cortisol and CBG mainly increased in parallel, although some individuals demonstrated larger alterations in the cortisol–CBG balance.
Conclusion: In COCs, EV had a milder effect on circulating CBG and adrenal steroid levels than EE; however, further research is necessary to determine the long-term effects.
Trial Registration: ClinicalTrials.gov NCT02352090
Implications: EV-based COC had reduced effects on circulating CBG and adrenal steroids compared to EE, probably due to a lower hepatic impact. Whether the sensitization of the adrenals to ACTH varies according to COC contents and whether it relates to experienced side effects needs to be investigated. These results encourage further research and development of contraceptives containing natural estrogens.

1. Introduction

Millions of women worldwide use combined oral contraceptives (COCs), but studies on their effects on adrenal endocrine function are limited. The adrenal cortex produces glucocorticoids, mineralocorticoids, and androgens, which regulate, for instance, energy metabolism, salt–water homeostasis, inflammatory functions, and mood [1]. Steroid hormones in the circulation are mostly bound to carrier proteins, and only the unbound free fraction is considered biologically active. Cortisol, the most important glucocorticoid in humans, binds to corticosteroid-binding globulin (CBG). Both CBG and cortisol levels increase during COC use [2–6], as es-

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trogens modulate cortisol balance by stimulating hepatic CBG synthesis [1]. Increases in CBG levels during COC use are followed by concomitant increases in cortisol production, resulting in a new altered CBG–cortisol equilibrium [1]. COC use also increases adrenal responsiveness to adrenocorticotrophic hormone (ACTH) [7].

COC use also decreases circulating androgen levels by upregulating sex hormone binding globulin and inhibiting ovarian and adrenal androgen production [8]. Adrenal androgen production plays a role in hyperandrogenic conditions, such as polycystic ovary syndrome (PCOS), which is often managed using COCs [9,10]. Although the mechanism underlying adrenal suppression by COCs remains unclear, decreased ACTH and increased cortisol levels have been proposed as a candidate [8].

Most COCs contain ethinylestradiol (EE) combined with a progestin. EE is a highly potent estrogen with an up-to-600-fold effect on hepatic protein synthesis compared to estradiol (E2) [11]. EE-containing COCs also affect cortisol-related inflammatory cascades, glucose metabolism, and blood coagulation [12–17]. To avoid these unfavorable effects from EE, COCs containing natural estrogens, such as E2 (and its valerate, EV) and estetrol (E4), have been developed. However, due to their recent market introduction, the differences between natural estrogens and EE in COCs are still poorly understood. Previous studies have mainly compared E2/EV/E4 and EE in combination with different progestins. However, since the progestin component delivers its own effect and modulates the estrogens' effects, a meaningful comparison is made using preparations containing the same progestin [18]. Nevertheless, the impact of different E2/EV/E4 combinations on adrenal steroids and CBG seems to be less significant than that resulting from EE-based COCs [3,6,19].

This spin-off study aimed to compare the effects of EE+DNG, EV+DNG, and DNG only on adrenal steroids and CBG. This work is part of a randomized trial comparing COCs containing EE and EV with the same progestin, primarily focusing on glucose metabolism [20].

2. Materials and methods

2.1. Study design

This study is a spin-off from a researcher-initiated randomized open-label trial conducted at the Helsinki and Oulu University Hospitals, Finland, between April 2015 and January 2018. The detailed study protocol has been described previously [17,20], and it was approved by the independent Ethics Committee of Helsinki University Central Hospital. The study was registered in the Clinical Trials database (NCT02352090; https://clinicaltrials.gov/) and the EU Clinical trials register (2014-001243-20; https://www.clinicaltrialsregister.eu). All subjects provided informed consent via a signed form. The sample size calculation was based on glucose metabolism, the trials' primary outcome [20].

2.2. Study subjects and intervention

Seventy-seven women volunteered for the study (Fig. 1). After the eligibility assessment, 59 healthy White women were enrolled. All women had regular menstrual cycles and a minimum wash-out period of 2 months from hormonal medication or 3 months from breastfeeding. Exclusion criteria were age $>$ 35 years, body mass index (BMI) $\geq$ 25 kg/m^2, blood pressure $\geq$ 140/90 mm Hg, smoking, alcohol or drug abuse, and abnormal findings in the standard 2-hour oral glucose tolerance test (OGTT) or in the gynecological ultrasound examination. The women had no contraindications for COC use.

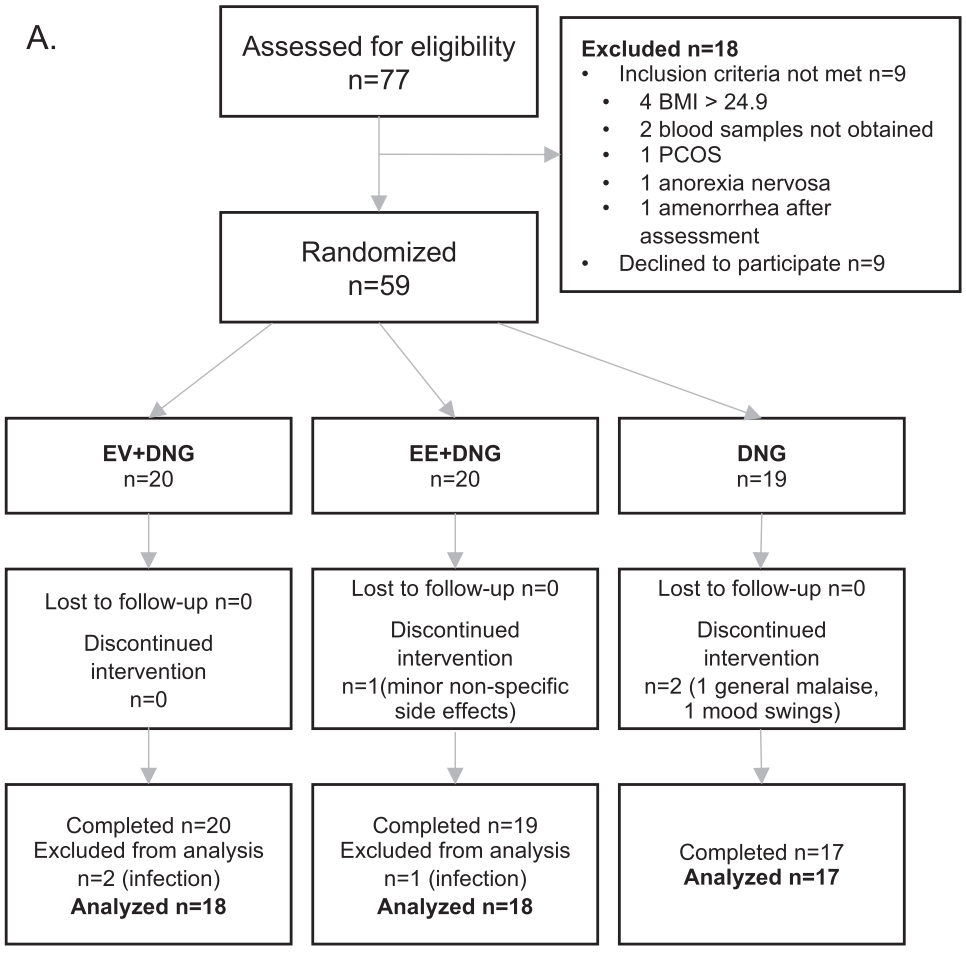
The women were randomized into groups that used either EV+DNG (Qlaira, Bayer AG, Germany), EE+DNG (Valette, Bayer AG, Germany), or DNG-only preparations (Visanne, Jenapharm, Bayer AG, Germany) for 9 weeks continuously. The original blister packs were modified to better match each other's hormonal contents (Fig. 1). The women were evaluated at baseline, during the first 5 days of the menstrual cycle, and during the fifth and ninth weeks of the study. After randomization, there was one dropout in the EE+DNG group due to minor nonspecific side effects and two dropouts in the DNG group due to general malaise and mood changes. Two women in the EV+DNG group and one woman in the EE+DNG group had a C-reactive protein value $>$ 10 mg/L at one appointment and were excluded from analyses as infection might have interfered with adrenal steroid levels.

2.3. Steroid hormone measurements

Blood samples were collected at baseline and at the fifth and ninth weeks of treatment to measure the levels of adrenal steroids (progesterone, 17-hydroxyprogesterone [17-OHP], dehydroepiandrosterone sulfate [DHEAS], aldosterone, cortisol, and cortisone). Fasting samples were collected between 07:00 and 10:00 AM after 15 minutes of resting while subjects were sitting. Serum (300 µL) was used for analysis with liquid chromatography–tandem mass spectrometry (LC–MS/MS). Serum proteins were precipitated with acetonitrile, and the supernatant was subjected to liquid–liquid extraction with ethylacetate–heptane on a Hamilton STAR pipetting robot (Bonaduz, Switzerland). An Acquity UPLC system (Waters, Milford, MA) was used to chromatographically separate the steroids on a C-18 column (50 $\times$ 2.1 mm, 1.7 mm particle size), which was developed by gradient elution using water and methanol containing ammonium hydroxide as mobile phases. The UPLC system was connected to a Waters Xevo TQ-S tandem mass spectrometer equipped with an electrospray ionization source, and the steroids were detected in the multiple reaction monitoring mode. Two product ions were monitored for each compound to check for interference. Analytical sensitivity and precision were determined as the lower limit of detection and total coefficient of variation for intermediate concentrations, respectively, for progesterone (0.21 nmol/L and 10.3%), 17-OHP (0.021 nmol/L and 4.4%), DHEAS (0.021 µmol/L and 10.4%), cortisol (0.59 nmol/L and 4.0%), cortisone (0.17 nmol/L and 4.2%), and aldosterone (13 pmol/L and 7.5%). Accuracies were in the range 95% to 109%.

2.4. CBG ELISA

CBG was measured at baseline and at 9 weeks of treatment using sandwich enzyme immunoassay (Cat. No. RD192234200R, BioVendor, Brno, Czech Republic) according to the manufacturer's instructions. The detection limit of the assay was 0.1 ng/mL. Briefly, samples were first incubated in microplate wells precoated with polyclonal anti-human CBG antibodies. This was followed by biotin-labeled monoclonal anti-human CBG antibodies, streptavidin-horseradish peroxidase conjugate, and substrate solution (tetramethylbenzidine). The reaction was stopped, and absorbance was measured at a 450 nm wavelength and a 650 nm reference wavelength. To mitigate optical interference, 650 nm absorbance was deducted from the 450 nm measurement before analysis. A standard curve was constructed, and the concentrations were interpolated with GraphPad Prism 9 for macOS. The inter-assay coefficient of variation (CV) was 11.5%, and the intra-assay CV 7.0%.



B.

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
EV+DNG	EV 2mg + DNG 2 mg					EV 2mg + DNG 3mg															
EE+DNG	EE 0.03 mg + DNG 2mg																				
DNG	DNG 2mg																				

Fig. 1. A. Flowchart of the study. B. Hormonal contents of the study preparations. Each preparation was used for nine consecutive weeks without hormone-free intervals. DNG, dienogest; EE, ethinylestradiol; EV, estradiol valerate.

2.5. Statistical analysis

The hierarchical linear mixed model was used to analyze the repeated measurements of progesterone, 17-OHP, DHEAS, aldosterone, cortisol, and cortisone. Progesterone levels were primarily analyzed and reported in our previous publication [21]. Given that progesterone plays a role in adrenal steroid synthesis, the progesterone data are re-presented as part of the synthesis cascade (Fig. 2). Distributions of progesterone and 17-OHP residuals were skewed; therefore, these variables were logarithmically transformed. Concentrations above the upper limit of quantification (ULQ) were replaced with the ULQ value (7 samples of DHEAS $>$ 10 µmol/L and 1 sample of cortisol $>$ 1500 nmol/L). Concentrations that were below the lower limit of quantification (LLQ) were replaced with LLQ (2 samples of aldosterone $<$ 13 pmol/L and 61 samples of progesterone $<$ 0.21 nmol/L). Two women were excluded from the progesterone analysis due to major outliers at baseline.

The free cortisol index (FCI) was calculated by dividing cortisol by the CBG. Wilcoxon's test was used for analyses since the distributions of CBG and the FCI were skewed, and CBG was measured only twice. The intraindividual change from baseline was calculated, and the Kruskal–Wallis test was used for the between-groups comparison. IBM SPSS Statistics 27 was used for the statistical analysis.

3. Results

3.1. Study groups

The groups were comparable in terms of age, BMI, waist–hip ratio, blood pressure, and metabolic measurements (Table 1). After dropouts and exclusions due to high C-reactive protein levels, 18 women in the EV+DNG group, 18 in the EE+DNG group, and 17 in the DNG-only group remained for analysis.

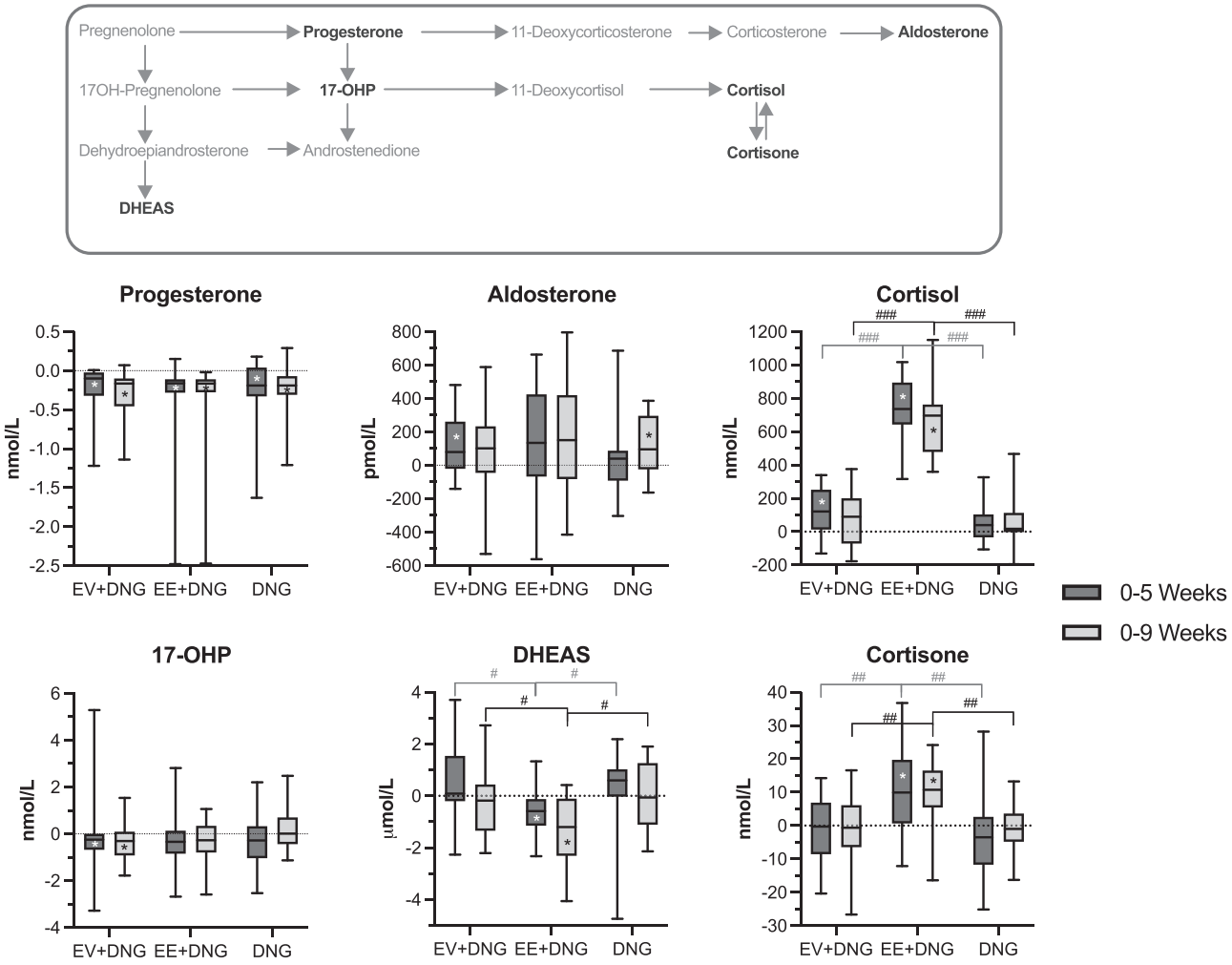


Fig. 2. Alterations in adrenal steroids during the trial with the steroid synthesis pathway. EE, ethinylestradiol; EV, estradiol valerate; DNG, dienogest; DHEAS, Dehydroepiandrosterone sulfate; 17-OHP, 17-Hydroxyprogesterone. *Significant change within the group; # $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$.

Table 1
Baseline demographics of the women participating in the study

	EV+DNG		EE+DNG		DNG		
	Mean	(SD)	Mean	(SD)	Mean	(SD)	*p*-value[a]
Number of subjects	18		18		17		
Age, y	24.28	(3.75)	25.89	(3.92)	24.00	(3.86)	0.296
BMI, kg/m2	22.42	(1.63)	23.02	(1.95)	21.87	(1.94)	0.197
WHR	0.76	(0.04)	0.78	(0.05)	0.78	(0.04)	0.425
Systolic BP, mm Hg	118.00	(7.19)	118.22	(8.72)	111.94	(9.73)	0.060
Diastolic BP, mm Hg	74.44	(7.09)	73.44	(7.54)	72.53	(7.38)	0.743
Fasting Glucose, mmol/L	5.18	(0.44)	5.08	(0.31)	4.93	(0.35)	0.139
Total Cholesterol, mmol/L	3.96	(0.68)	4.18	(0.57)	4.07	(0.45)	0.504
HDL, mmol/L	1.64	(0.36)	1.76	(0.37)	1.62	(0.30)	0.430
LDL, mmol/L	2.16	(0.63)	2.23	(0.50)	2.39	(0.55)	0.465
Triglycerides, mmol/L	0.66	(0.24)	0.70	(0.17)	0.65	(0.17)	0.740
HbA1c, mmol/mol	33.11	(2.52)	32.94	(2.24)	32.00	(2.37)	0.341
hs-CRP, mg/L	0.62	(0.51)	0.95	(0.86)	0.65	(0.57)	0.282

BMI, body mass index; BP, blood pressure; DNG, dienogest; EE, estradiol valerate; EV, estradiol valerate; hs-CRP, high sensitivity C-reactive protein; WHR, waist-hip ratio.
[a] Between-the-groups comparison with Anova.

3.2. Adrenal steroids

The steroid levels at each time point are shown in Table 2, and the changes during the study period are shown in Figure 2. During the EV+DNG treatment, 17-OHP decreased slightly, but the difference was not significant between the groups. DHEAS decreased during EE+DNG treatment (median [95% confidence interval, CI] -1.28 µmol/L [-1.92 to -0.64]) but did not change during EV+DNG and DNG-only use. Aldosterone increased from baseline to 9 weeks in the DNG-only group (mean 109.76 pmol/L [25.49–194.03]) but did not differ between the study groups. Cortisol levels increased during EE+DNG treatment (668.00 nmol/L [563.87–772.14]), whereas no significant change was seen in the EV+DNG (71.83 nmol/L [-2.37 to 146.03]) nor DNG-only (58.81 nmol/L [-

Table 2
Measurements of adrenal steroid hormones and corticosteroid-binding globulin

	Week	EV+DNG ($N = 18$) Mean/Median (SD/IQR)		*p*-value	EE+DNG ($N = 18$) Mean/Median (SD/IQR)		*p*-value	DNG ($N = 17$) Mean/Median (SD/IQR)		*p*-value
17-OHP	0	1.82	[1.38–2.68]	0.044[a]	1.28	[1.11–1.75]	0.130[a]	1.76	[1.08–2.05]	0.110[a]
nmol/L	5	1.58	[0.83–2.24]	0.028[b]	1.05	[0.66–1.35]		1.23	[0.95–1.53]	
	9	1.78	[0.70–2.38]	0.033[b]	1.05	[0.52–1.43]		1.64	[1.30–2.20]	
DHEAS µmol/L	0	5.34	(2.67)	0.036[a]	4.87	(1.98)	0.002[a]	4.86	(1.79)	0.548[a]
	5	5.79	(2.69)	0.166[b]	4.28	(2.07)	0.014[b]	5.24	(1.95)	
	9	5.09	(2.57)	0.468[b]	3.59	(1.59)	0.001[b]	4.89	(1.44)	
Aldosterone pmol/L	0	289.28	(154.00)	0.030[a]	267.92	(221.80)	0.154[a]	236.43	(168.50)	0.033[a]
	5	414.19	(154.18)	0.009[b]	421.23	(223.09)		271.80	(225.89)	0.514[b]
	9	380.19	(245.97)	0.157[b]	414.14	(252.48)		346.19	(191.28)	0.014[b]
Cortisol nmol/L	0	540.08	(91.71)	0.006[a]	495.95	(139.42)	<0.001[a]	493.21	(165.31)	0.182[a]
	5	663.15	(173.37)	0.002[b]	1231.82	(157.31)	<0.001[b]	537.41	(134.6)	
	9	611.92	(165.70)	0.057[b]	1163.95	(198.3)	<0.001[b]	552.02	(191.81)	
Cortisone nmol/L	0	69.40	(7.87)	0.838[a]	62.45	(9.50)	<0.001[a]	63.84	(7.54)	0.653[a]
	5	68.51	(10.69)		73.33	(15.34)	0.001[b]	61.01	(12.08)	
	9	67.85	(10.26)		72.47	(8.64)	0.001[b]	62.68	(7.82)	
CBG µg/mL	0	22.04	[17.92–25.03]		25.42	[21.84–28.53]		20.82	[19.28–24.76]	
	9	27.16	[22.12–30.93]	<0.001[b]	67.33	[58.85–75.62]	<0.001[b]	24.09	[19.72–26.77]	0.049[b]
FCI µmol/g	0	24.02	[21.64–27.96]		19.47	[15.00–23.31]		21.34	[16.41–26.50]	
	9	22.50	[18.18–26.10]	0.231[b]	17.46	[13.94–20.33]	0.145[b]	23.32	[15.64–29.00]	0.723[b]

CBG, Corticosteroid-binding globulin; DHEAS, dehydroepiandrosterone sulfate; DNG, dienogest; EV, estradiol valerate; EE, estradiol valerate; FCI, free cortisol index; 17OHP, 17-hydroksiprogesterone.
Adrenal steroid hormone and binding protein measurements during the trial. Data is presented as median [interquartile range, IQR] or mean (standard deviation, SD). Concentrations outside the range of measurement were replaced with the limit value (DHEAS >10 µmol/L, cortisol >1500 nmol/L, and aldosterone <13 pmol/L).
[a] within-the-group comparison, change during the trial.
[b] pairwise analysis, compared to baseline.

27.48 to 145.1]) groups. Cortisone levels changed with a similar pattern to its precursor, cortisol.

3.3. CBG and FCI

CBG increased in all study groups, but the increase in the EE+DNG group was nine-fold higher than in the EV+DNG group, and 49-fold higher than in the DNG-only group (Fig. 3). Even though both cortisol and CBG levels changed, the FCI remained unchanged in all three groups. This indicates that CBG and cortisol mostly increased proportionately; however, some individuals showed wider deviations in cortisol–CBG balance (Fig. 3).

4. Discussion

To our knowledge, this is the first comparison of COCs containing EE and EV combined with the same progestin on the effects on adrenal steroids. We found that EE+DNG increased CBG and cortisol levels, resulting in a new modified CBG–cortisol equilibrium. Furthermore, DHEAS decreased with the EE-containing COC. The EV+DNG and DNG-only treatments had only limited effects on adrenal steroids.

In the present study, EE+DNG increased cortisol and CBG levels, which is a well-known effect of EE [2,4,5,11]. In contrast, natural estrogens seemed to have a milder effect on cortisol and CBG levels. In a study comparing EV+DNG and EE+levonorgestrel (LNG), CBG levels increased in both groups; however, the increase was less significant with EV+DNG treatment [19]. Another study comparing E2+nomegestrol acetate (NOMAC) and EE+LNG reported a greater increase in total cortisol and CBG in the EE+LNG group [3]. Furthermore, the combination of drospirenone (DRSP) and the most recent natural estrogen on the market, E4, had a milder effect on cortisol and CBG than EE+DRSP [6]. Taken together, it appears that natural estrogens in COCs appear to exert less of an impact on both CBG and cortisol concentrations than EE. We have demonstrated the induction of hepatic protein synthesis also in our previous studies as greater increases in SHBG and prothrombin levels during EE+DNG use compared with EV+DNG or DNG-only use [21,22].

The FCI correlates with serum free cortisol levels and describes the balance of active cortisol in the body [23]. Consistent with the results of a study comparing E4+DRSP and EE+DRSP [6], the FCI remained unchanged in all study groups. Although most women maintained this FCI-equilibrium, some individuals in all groups showed greater variation from the baseline in the FCI at 9 weeks (Fig. 3). Whether deviations in cortisol balance can explain some of the individual variations in side effects, such as severe mood swings, general malaise, and deteriorated glucose tolerance, should be further explored. However, as side effects are usually most pronounced during the initial months of contraceptive use and resolve with time [24,25], this could hypothetically be related to the re-obtained cortisol equilibrium. Unfortunately, we could not correlate the side effects with the CBG–cortisol response, as our study was not designed for this purpose. Furthermore, a study investigating the responsiveness to exogenous ACTH demonstrated an increased adrenal response in COC users compared with non-users [7]. Thus, it appears that COCs influence adrenal hormones also independently of increasing basal cortisol and CBG levels. It remains to be investigated whether the altered adrenal responsiveness impacts stress tolerance or affects the long-term health of COC users and whether EE and EV in COCs alter this response differently.

In contrast to increases in CBG and cortisol, we found that DHEAS levels decreased by 24% in the EE+DNG group, whereas DHEAS remained unchanged in the EV+DNG and DNG-only groups. Our results are consistent with earlier studies showing that both EE+LNG and EE+DRSP decrease DHEAS levels more than E2+NOMAC [3] and E4+DRSP [6]. Indeed, the increased activity of the cortisol pathway during COC use most likely resulted in the deceleration of the DHEAS synthesis pathway. Even though increased ACTH is required for achieving the new CBG–cortisol equilibrium at early stage [1], high cortisol and reduced release of ACTH could decrease adrenal androgen synthesis in the long term [8]. Given all this, the use of EE-containing COC could benefit especially women with congenital adrenal hyperplasia or some women with PCOS who present with increased adrenal androgen levels [9,10].

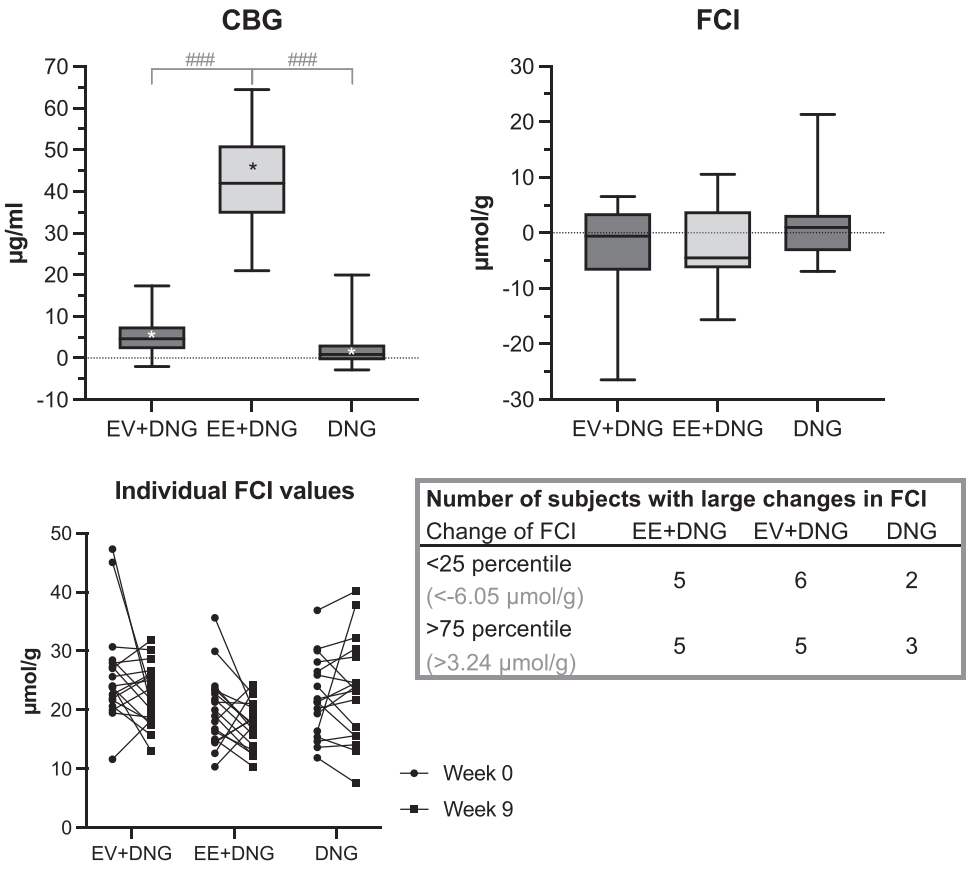


Fig 3. Changes in corticosteroid-binding globulin (CBG) and the free cortisol index (FCI) during the trial. Even though CBG increased during the use of EE+DNG, the FCI did not change in any of the treatment groups. However, a few subjects in all groups showed notable changes in the FCI. CBG, corticosteroid-binding globulin; DNG, dienogest; EE, ethinylestradiol; EV, estradiol valerate; FCI, free cortisol index; PCTL, percentile. *Significant change within the group; ### p-value < 0.001.

Table 3
Changes in previously reported measurements related to adrenal steroids

	EV+DNG		EE+DNG		DNG		
	Mean	(95,0% CI)	Mean	(95,0% CI)	Mean	(95,0% CI)	p-value[a]
Weight (kg)	-0.49	(-0.95 to -0.03)	-0.08	(-0.81 to 0.65)	-0.57	(-1.09 to -0.05)	0.41
Systolic BP, mm Hg	-2.89	(-6.46 to 0.68)	-3.33	(-8.15 to 1.49)	-3.41	(-6.85 to 0.02)	0.98
Diastolic BP, mm Hg	-1.56	(-4.53 to 1.41)	-0.72	(-4.81 to 3.36)	-2.53	(-5.01 to -0.05)	0.72
Fasting glucose, mmol/L	-0.08	(-0.23 to 0.08)	0.03	(-0.12 to 0.19)	0.11	(-0.1 to 0.31)	0.28
HbA1c, mmol/mol	-0.56	(-2.07 to 0.96)	-0.22	(-1.41 to 0.97)	-0.88	(-2.38 to 0.62)	0.79

BP, blood pressure; DNG, dienogest; EV, estradiol valerate; EE, estradiol valerate; HbA1c, hemoglobin A1c; OGTT, oral glucose tolerance test.
Changes in measurements related to adrenal steroids, that were reported in our previous papers [17,20]. Values presented here were calculated based on the subjects included to this study: subjects with C-reactive protein >10 mg/L at any appointment were excluded from the analysis.
[a] Group comparison with Anova.

Aldosterone, the main mineralocorticoid in humans, increases the reabsorption of Na^+ in the kidneys and regulates extracellular fluid volume [1]. We found that aldosterone levels remained unaltered in both the EE+DNG and EV+DNG groups. This aligns with a previous study that compared EE+DNG with EE+LNG, the results of which showed no consistent alterations in aldosterone levels [26]. However, we found individual variation to be high and a significant increase in the DNG-only group. The type of progestin seems to be a more important factor than the type of estrogen in determining the effects of COC on aldosterone; in a recent study, both E4+DRSP and EE+DRSP increased aldosterone, whereas EE+LNG decreased aldosterone [6]. This finding relates to the antimineralocorticoid effect of DRSP, which is a spironolactone-derived progestin [27].

We have previously reported several endpoints from this trial relating to the physiological functions of adrenal steroid hormones (Table 3). We found that blood pressure, body weight [17] and glucose tolerance [20] remained unchanged in all study groups during the trial. Since glucocorticoids and mineralocorticoids regulate glucose metabolism, blood pressure, and salt-water balance, the findings indicate that elevated CBG indeed balances the increase in cortisol levels during EE-based COC use. Whether individuals with poor CBG balance experience detrimental effects of EE-based COC use, in the long run, remains to be investigated.

This study has several strengths. Randomization of the groups was successful, as reflected in comparable baseline characteristics. Additionally, the dropout rate (three women) was low. Moreover, all preparations contained the same progestin, which allowed

for a meaningful comparison of the two estrogens and DNG only. Furthermore, the use of LC–MS/MS technology allowed for high-quality steroid hormone data. A limitation of our study is the 9-week follow-up, which is too short to draw conclusions regarding possible long-term effects. Moreover, the possibility of a type II statistical error must be considered, as the sample size calculation was not based on the endpoints of this study. However, the differences between the groups were significant for many of the endpoints, and the results were consistent with previous studies, indicating a sufficient sample size.

In conclusion, EV in a COC had less of an impact on DHEAS, cortisol, and CBG levels compared to EE. Even though the FCI remained mainly stable with both combinations, women could benefit from less significant changes in CBG and cortisol levels. Until recently, EE dose and progestin type has been the main factors in COC choice. However, with accumulating data showing the milder metabolic impact of natural estrogens in COC, the estrogen type should also be considered. Although more research regarding the long-term effects of COCs on adrenal steroid hormones is warranted, this pilot study emphasized the neutral effect of EV in favor of EE, encouraging further research and development of COCs containing natural estrogens.

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