ESTRADIOL VALERATE VS. ETHINYLESTRADIOL IN COMBINED ORAL CONTRACEPTIVES: EFFECTS ON THE PITUITARY-OVARIAN AXIS

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Funding

This work was supported by The Swedish Cultural Foundation in Finland (AH); Helsinki University Hospital research funds (JST, OH, AH, KL); The Sigrid Jusélius Foundation (JST, TP); The Finnish Medical Association (AH, TP, MHK); The Academy of Finland (JST, TP); University of Oulu Graduate School (MHK) and The Emil Aaltonen Foundation (MHK).

Disclosure statement

TP has received honoraria for lectures and consultation, and research grants from Exeltis, Gedeon Richter, MDS, Merck, Pfizer, Roche, and Mithra Pharmaceuticals. OH occasionally serves on advisory boards for Bayer AG and Gedeon Richter and has designed and lectured at educational events for these companies. The other authors have nothing to disclose.

ABSTRACT

Context: There are limited studies comparing the effects of combined oral contraceptives (COCs) containing natural estrogens and synthetic ethinylestradiol (EE) on reproductive hormones.

Objective: To compare estradiol valerate (EV)+dienogest (DNG), EE+DNG, and DNG alone (an active control) on levels of follicle stimulating hormone (FSH), luteinizing hormone, Anti-Müllerian hormone (AMH), ovarian steroids, sex hormone binding globulin (SHBG), and the Free Androgen Index (FAI).

Design: Spin-off study from a randomized trial.

Setting: Outpatient setting at Helsinki and Oulu University Hospitals, Finland.

Participants: 59 healthy, 18–35-year-old ovulatory women were enrolled. Three women discontinued. The groups were comparable as regards age and body mass index.

Interventions: EV 2mg+DNG 2–3mg (n=20), EE 0.03mg+DNG 2mg (n=20) and DNG 2mg (n=19) were used continuously for nine weeks. Blood samples were drawn at baseline, and at 5 and 9 weeks.

Main Outcome Measures: EV+DNG suppressed FSH by -27% (-51:-3) (median [95%CI]) vs. EE+DNG, -64% (-78: -51), *P*=0.04, but AMH levels decreased similarly by -9% (-18: -0.1) vs. -13% (-28:0.2), P=0.38, respectively. EV+DNG increased SHBG levels by 56% (30:82) and EE+DNG by 385% (313:423), *P*<0.001. Total testosterone (T) decreased by 16% (-27: -5) in the EV+DNG group but it did not decrease in the EE+DNG group, whereas the FAI decreased by -39% (-54: -25) vs. -72% (-78: -67), *P*<0.001. DNG alone did not induce changes in any of these parameters.

Conclusions: Compared with EE+DNG, treatment with EV+DNG resulted in milder pituitary downregulation and reduced induction of hepatic SHBG synthesis—potentially carrying more beneficial health effects.

Keywords: Anti-Müllerian hormone, combined oral contraception, dienogest, estradiol valerate, free androgen index, sex hormone binding globulin

INTRODUCTION

Combined oral contraceptives (COCs) inhibit ovulation by disrupting the hypothalamic-pituitary-ovarian axis, mainly through suppression of pituitary gonadotropin secretion (1). This results in arrested follicular development and suppression of ovarian activity, which is reflected in decreased circulating levels of estradiol (E2) and its metabolites, testosterone (T), androstenedione (A4) (2), progesterone (P4), and Anti-Müllerian Hormone (AMH) (3). Additionally, COCs upregulate hepatic synthesis of estrogen-sensitive proteins, such as sex hormone binding globulin (SHBG) (4), thereby increasing the binding capacity for testosterone (T) and decreasing the fraction of biologically active free T in the circulation (2). In addition to being an indicator of the impact of COC use on hepatic metabolism, SHBG is also a suggested marker of the risk of venous thromboembolism (VTE) in COC users (5).

COCs use is associated with an increased risk of VTE and arterial thrombosis (6,7) as well as metabolic changes such as altered glucose tolerance, increased inflammation and changes in the lipid profile (8,9). While reductions in initially high doses of ethinylestradiol (EE) during COC development have improved their safety (10,11), further improvement has been sought by replacing (highly potent) EE with low-potency natural estrogens. Two formulations containing either bioidentical estradiol (E2) or its ester, estradiol valerate (EV) (12,13), have previously been market-introduced and recently, a new formulation containing fetal-origin estetrol (E4) (14) was authorized by the European Medicines Agency.

COCs containing E2/EV have high contraceptive efficacy (15,16) and have been found to induce less pronounced endocrine and metabolic effects than those containing EE (17,18). However, clinical evidence on their effects on reproductive hormones remains limited. COCs containing EE suppress

androgen synthesis in both ovaries (A4 and T synthesis) and adrenals, in addition to lowering free T levels by increasing SHBG synthesis (2). This is beneficial in managing hyperandrogenism (acne, hirsutism) in women but could also affect libido, which is thought to, at least partially, be driven by androgens (19). How E2/EV containing COCs affect female androgen status remains sparsely studied and in most available studies, E2/EV containing COCs have been compared with preparations containing EE combined with various progestins. This makes pinpointing the specific effects of the estrogen challenging as progestins also modulate at least the hepatic effects of COCs (20). In addition, AMH, which is increasingly being used as a tool for estimating remaining fertility potential (21), decreases during the use of COCs containing EE (3), but whether E2/EV-based COCs also decrease AMH levels is not well known.

The objective of this study was to compare the effects of a nine-week continuous regimen of COCs containing either EV+dienogest (DNG) or EE+DNG, and DNG alone (as an active control group) on the suppression of pituitary (FSH, LH) and ovarian activity (P4, E2 and its metabolites, and AMH), induction of hepatic metabolism (SHBG), and androgen suppression (T, A4 and the free androgen index [FAI]). Owing to the lower potency of EV, we anticipated that EV+DNG would have less marked effects on all variables compared with EE+DNG, while DNG alone was expected to have only a modest effect on most reproductive hormones as previously shown (22)

MATERIAL AND METHODS

This study is a spin-off from our randomized clinical trial focusing on glucose tolerance (23). Independent Ethics committees of Helsinki and Oulu University Hospitals approved the study protocol, and the study was registered at clinicaltrials.gov (NCT 02352090). The study was conducted between April 2014 and December 2016 at Helsinki and Oulu University Hospitals, in Finland.

PARTICIPANTS

The inclusion criteria were a two-month washout period after hormonal contraceptive use, age 18–35 years, normal weight (body mass index [BMI] 19-24.9 m^2/kg), non-smokers, no use of regular prescription medication, regular menstrual cycles (21–35 days), and no contraindications for combined hormonal contraceptive use (24). Before randomization, a gynecological examination including vaginal ultrasonography was performed to exclude polycystic ovarian morphology, since polycystic ovarian syndrome (PCOS) was also an exclusion criterion of the study.

INTERVENTION

The intervention treatments contained 1) EV 2mg + DNG 2–3mg, 2) EE 0.03mg + DNG 2mg, and 3) DNG 2mg in a continuous regimen for nine weeks (63 days). We used commercial preparations (Qlaira®, Bayer AG: fourphasic regimen; day 1–2 EV 3mg, day 3–7 EV 2mg + DNG 2mg, day 8–24 EV 2mg + DNG 3mg, day 25–26 EV 1mg, day 27–28 placebo; Valette®, Jenapharm, day 1–21 EE 0.03mg + DNG 2mg, day 22–28 placebo; Visanne®, Bayer AG, day 1–28 DNG 2mg), but removed all placebo tablets (EE+DNG and DNG alone) and the tablets containing only EV and one tablet containing EV 2mg + DNG 2mg from the EV+DNG packages to harmonize the hormonal content of the preparations and to eliminate the hormone free interval. After omitting the hormone free interval, all packages contained 21 tablets of active hormone, taken continuously for 63 days. The participants were randomly allocated to one of the three treatment groups: 1) EV+DNG, 2) EE+DNG, and 3) DNG alone. In the EV + DNG group, the DNG dose was 3mg for 16/21 days and 2mg for 5/21 days. Baseline blood samples were drawn after a 12h fast on menstrual cycle day 1–5 and at the

fifth (days 28–35) and ninth (days 47–63) weeks of treatment. The preparations were initiated after baseline blood sampling (day 2–6).

SAMPLE HANDLING AND LABORATORY ASSAYS

Serum samples were prepared by a single 10-min centrifugation at 2000 g and stored at -70°C until analysis.

Serum concentrations of FSH and SHBG were analyzed using chemiluminescent microparticle immunoassays, ARCHI FSH (Abbott Cat# 7K75-25, RRID:AB_2813910) (analytical sensitivity 0.05 IU/L, total coefficient of variation [CV%] 3.2–4.6%) and ARCHI SHBG (Abbott Cat# 8K26, RRID:AB_2895255 (analytical sensitivity 0.02 nmol/L, total CV% 5.6–9.54%) (Abbot Architect i2000SR analyzer, Abbot Diagnostics). Levels of LH were analyzed by chemiluminescent immunoassays using Atellica IM LH (Siemens Cat# 01756298, RRID:AB_2895592) (analytical sensitivity 0.07 IU/L, intra-assay CV% 2.1–2.4%) (Siemens Healthcare Diagnostics) and AMH was quantified by Elecsys AMH Plus immunoassays (Roche Cat# 06331076, RRID:AB_2895131) (analytical sensitivity 0.010 ng/mL, intra-assay CV% 1–2.6%) (Roche Diagnostics). The above analyses were performed at Huslab (Helsinki, Finland). The Free Androgen Index (FAI) was used to estimate bioavailable free T levels and was calculated as follows: FAI=(T/SHBG) *100)(25).

Progesterone (P4), androstenedione (A4), testosterone (T), estradiol (E2), estrone (E1), and estrone-3-sulfate (E1S), were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Core Facility for Metabolomics, University of Bergen, Norway). 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, USA) was used to chromatographically separate the steroids on a C-18 column (50 x 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 MRM mode. Two product ions were monitored for each compound to check for interference. Analytical sensitivity and precision were determined as lower limit of quantification (LLQ) and total coefficient of variation (CV) for intermediate concentrations, respectively: 17β-estradiol (3.6 pmol/L and 5.0%), estrone (2.1 pmol/L and 3.6%), estrone 3-sulfate (0.24 nmol/L and 4.8%), progesterone (0.21 nmol/L and 10.3%), testosterone (0.11 nmol/L and 3.2%), androstenedione (0.02 nmol/L and 5.4%). Accuracies were in the range 95–109%.

STATISTICAL ANALYSIS

Data are presented as means (with SDs) or medians (with interquartile ranges [IQRs]) depending on their distribution (verified by the Shapiro–Wilk test). The treatment effect is summarized as mean or median percentage (%) change from baseline, with confidence intervals (95%CIs). A significance level of $P<0.05$ was considered statistically significant. We log-transformed skewed data before analysis and replaced values under the quantification limit with the value of the quantification limit. The treatment effect within and between the groups was analyzed using repeated-measures ANOVA with one within (time) and one between factor (group) and Tukey's *post hoc* test. P4 data was analyzed using the Wilcoxon's signed ranks test since it was not normally distributed even after logarithmic transformation. The Kruskal-Wallis test and Dunn's *post hoc* tests were used for comparisons across the groups. Variables analyzed in samples collected only at two different time points (baseline and nine weeks [FSH, LH, AMH]) were analyzed using the paired samples T-test or Wilcoxon's signed-rank test, and one-way ANOVA or the Kruskal-Wallis test to analyze the between-group effect including Tukey's or Dunn's *post hoc* tests. The *post hoc* tests were adjusted for multiple

comparisons (Bonferroni). IBM SPSS Statistics 27 and Graph Pad Prism 9.2 for macOS were used for the analyses.

RESULTS

Seventy-seven women (all White) volunteered and were screened, of which 59 were eligible and 56 completed the study, leaving the final groups as follows: 1) EV+DNG n=20 2) EE+DNG n=19, and 3) DNG n=17 (Figure 1; flowchart). In the DNG alone group, one LH sample (baseline) could not be analyzed owing to technical problems. The groups were comparable regarding age, BMI (Table 1) and hormonal baseline values (Table 2). Serum concentrations of the variables at five and nine weeks of treatment, and comparisons between the groups are shown in Figure 2 and Table 2.

GONADOTROPINS AND AMH

Serum levels of FSH (median percentage change from baseline [%, 95%CI]) decreased in the EV+DNG group by -27% (-51: -3) vs. -64% (-78: -51) in the EE+DNG group, *P*=0.001. A similar decrease in LH levels was detected in both COC groups; treatment with EV+DNG decreased LH by -67% (-78: -32) and EE+DNG treatment by -77% (-97: -69), (*P*=0.58). Treatment with DNG alone did not alter FSH levels, while LH levels increased by 39% (0:78, *P*=0.05). At nine weeks one woman in the DNG-alone group had a significantly higher LH level (14.7 IU/L) than the other participants (<7.2 IU/L) in that group. Omitting this subject from analyses did not affect the significance of the comparisons between the groups (Table 2); however, the change from baseline within the DNG alone group became non-significant (*P*=0.09).

Serum levels of AMH decreased over the nine-week treatment period in both the EV+DNG and EE+DNG groups by -9.2% (-18: -0.2, *P*=0.04) vs. -13.0% (-28:0.2, *P*=0.01), *P*=0.38, but remained unchanged in the DNG alone group (0.7% [-10:12]).

OVARIAN STEROIDS

As expected, serum levels of estradiol (E2), estrone (E1) and estrone sulfate (E1S) increased significantly following the intake of EV+DNG; E2 levels increased by 161% (50:227), E1 by 1341% (620:1700) and E1S by 1890% (974:2267). In contrast, EE suppressed levels of E2, E1 and E1S by -92% (-93: -83), -30% (-44:10), and -68(-81: -53), respectively. Treatment with DNG alone resulted in a slight increase in E2 levels (the change from five to nine weeks, *P*=0.02), and in E1 and E1S levels, by +72% (-13:283), 43% (0:100) and 47% (1:137), respectively. We calculated the E1:E2 ratios as a means of assessing compliance to the EV+DNG treatment, since the E1:E2 ratio increases following oral intake of EV/E2 owing to the extensive metabolism of E2 to E1 in the intestinal mucosa during absorption and during hepatic first-pass (26). At baseline all groups had physiological E1:E2 ratios close to 1 (median [IQR]); EV+DNG 1.24 (0.88:1.64), EE+DNG 1.27 (0.94:1.99) and DNG alone 1.03 (0.81:1.47). At nine weeks the corresponding ratios were 7.0 (5.06:9.47, *P*<0.001), reflecting oral intake of E2/EV in the EV+DNG group; 10.4 (7.92:13.62, *P*>0.001), reflecting relatively more profound suppression of E2 than E1 in the EE+DNG group, even though both E2 and E1 were clearly decreased; and 1.13 (0.58:1.66, *P*=0.53), reflecting a sustained physiological E1:E2 ratio in the DNG alone group.

All women (56/56) had comparable low levels of P4 (< 1.6 nmol/L) consistent with anovulation at five and nine weeks of treatment. At five weeks, 60% (12/20) and at nine weeks, 50% (10/20) of the women in the EV+DNG group had concentrations of P4 under the lower limit of quantification

(<0.21nmol/L). The corresponding values in the EE+DNG group were 47% (9/19) and 60% (12/19), and in the DNG-alone group, 53% (9/17) and 47% (8/17). P4 levels decreased similarly across the groups over the treatment period (*P*=0.74) and no pregnancies occurred in any of the groups.

Serum androstenedione (A4) levels (mean % change [95%CI]) declined similarly over nine weeks in both the EV+DNG and EE+DNG groups, by -22% (-34:11) and -14% (-28: -0.6) respectively. Serum levels of T (median % [95%CI]) decreased from baseline during treatment with EV+DNG by -16% (-27: -5), whereas a tendency towards increased T levels was observed in the EE+DNG group (the change from baseline to five weeks *P*=0.05); at nine weeks T levels had increased by 24% (5:44). However, the increase did not reach statistical significance (P=0.07). Treatment with DNG alone did not induce changes in T or A4 levels (-5% [-20:8] and 3% [-15:20], respectively).

SHBG AND FAI

Serum concentrations of SHBG increased in both COC groups over the nine-week treatment period, but the change was more pronounced in the EE+DNG group, as SHBG levels increased by 386% (313:423) vs. 56% (30:82) in the EV+DNG group (*P*<0.001). Correspondingly, the FAI decreased by -72% (-78: -67) vs. -39% (-54: -25), (*P*<0.001). SHBG levels and the FAI remained unchanged in the DNG-alone group.

DISCUSSION

In this study we showed that EV+DNG had a minor impact on most of the hormonal variables compared with EE+DNG, while the effects of DNG alone were mostly neutral. Our key findings include the less pronounced suppression of FSH and only moderately increased levels of SHBG.

Consequently the FAI was reduced in both COC groups; however, following treatment with EV+DNG the FAI remained higher. Furthermore, treatment with EV+DNG and EE+DNG resulted in comparable decreases in serum AMH levels.

Combined contraceptives containing natural estrogens have been found to have good contraceptive efficacy, as they suppress gonadotropin secretion and inhibit ovulation (27,28). This was also demonstrated in our study, where both COC regimens similarly decreased LH levels. However, treatment with EV+DNG resulted in less pronounced suppression of FSH, and possibly a lesser degree of follicular arrest than EE+DNG, yet with effective ovulation inhibition, as reflected in low or undetectable P4 levels and decreased serum concentrations of T, A4, and AMH. Conversely, resulting from the oral intake of EV, levels of E2 and its metabolites (E1 and E1S) were elevated. The potential role for the approximately tenfold increment in E1 (+1341%) and E1S (+1890%) levels compared to E2 (+161%) is to serve as a pool of estrogens that can be converted back to E2, thus increasing the biological half-life of orally administered E2 (29). Whether or not some endogenous E2 production persists cannot be determined, but it is possible owing to the submaximal suppression of FSH. As expected, EE+DNG treatment resulted in efficient pituitary downregulation, reflected in low FSH and LH levels, and undetectable P4 levels. Efficient follicular arrest was reflected in decreased levels of E2 (and E1, E1S), A4 and AMH. In contrast to previous studies (2), in the EE+DNG-group, there was a tendency towards increased total T levels, which is unlikely clinically significant and may reflect lower T clearance resulting from the concomitant nearly fourfold increase in SHBG levels (+386%). As T levels were measured using a state-of-the-art method (LC-MS/MS) (30), we do not suspect that analytical difficulties could explain these results.

One of the major findings was the higher FAI values in the EV+DNG group than in the EE+DNG group (change from baseline; -39% vs. -72%) as a result of the weaker effect of EV on SHBG synthesis. Whether or not the smaller reduction in FAI values observed with EV+DNG has clinical implications, i.e., results in less effective treatment of hyperandrogenic conditions, such as hirsutism and acne, remains to be elucidated in future studies. However, reassuring preliminary data have been published, suggesting improvement of acne and insulin resistance in hyperandrogenic women with PCOS treated with EV+DNG (31,32). These findings suggest clinical efficacy of EV+DNG in hyperandrogenism despite only moderately increased SHBG levels (56% vs. 386% in the EE+DNG group). Also, the direct antiandrogenic effect of DNG on peripheral androgen receptors (33) may contribute to the resolution of the symptoms. The modest suppression of androgens with EV+DNG treatment could also potentially be beneficial, since androgens have been implied to affect libido in women (19). In fact, improved sexual function has been observed during the use of COCs containing EV/E2 (34–36). However, whether or not the reduction in circulating androgens during COC use affects sexual function remains controversial (37).

Treatment with DNG alone resulted in anovulatory P4 levels, and unchanged FSH and androgen levels, which align with the concept that DNG (≥2mg/d) efficiently inhibits ovulation (38) regardless of having limited antigonadotrophic effect (39). Furthermore, LH and E2 levels increased moderately. The increase in LH has not previously been reported during DNG treatment (22) and probably reflects our limited sample size (one outlier). The increment in E2 levels (within early follicular range) is in line with a previous study (22), and possibly indicates increased granulosa-cell mass and persisting follicular development during DNG use. There was, however, large intraindividual differences in E2 levels, reflected in wide confidence intervals. Similarly increased E2 levels has been shown with drospirenone alone, while use of desogestrel alone has resulted in even

higher E2 levels (40). Like other progestins (41), treatment with DNG alone did not increase SHBG levels nor alter FAI values.

Previous studies have shown AMH levels to be 20–50% lower in women using EE-based COC than non-users (3,42–46). The reduction of circulating AMH during COC use is most likely explained by decreased FSH levels and follicle development, and consequently reduced granulosa-cell mass, mainly in the small antral stage (3). We found that AMH levels declined by 9% in the EV+DNG group and 13% in the EE+DNG group, which was somewhat less than previously observed. This could be explained by the relatively short follow-up period, or the specific estrogen-progesterone combinations studied, as it is unknown whether the decrease in AMH continues over time or whether it is formulation-specific. Furthermore, we found that treatment with DNG alone did not alter AMH levels, which is in line with a previous report (47) but contrasts others showing that monotherapy with progestins (drospirenone and norethisterone) is associated with 15-30% lower AMH levels compared with non-use (43,46). Nevertheless, the clinical usefulness of AMH as a marker of ovarian reserve (48) seems to be limited among all women using COCs regardless of the estrogen type. The effects of DNG alone need further exploration in studies with longer follow-up, because there may be clinical interest in using AMH as a marker of ovarian reserve in women treated with DNG alone for endometriosis.

Estrogens upregulate the synthesis of many hepatic proteins, of which SHBG has received the most attention. The synthesis of SHBG increases dose-dependently with EE administration (49) and decreases according to the androgenicity of the progestin component (50), and has accordingly been suggested to be a marker of total COC estrogenicity (41). SHBG has also been suggested as a surrogate marker of the risk of VTE associated with the use of hormonal contraception (51), even

though it is not directly related to any hemostatic processes. The mechanism underlying the VTE risk associated with COC use is not fully understood, but a plausible mechanism includes COC-induced activated protein C (APC) resistance (52). The degree of APC resistance (a suggested surrogate marker for VTE risk (53)), induced by different COCs has, in turn, been correlated with SHBG levels (54–56). Agreeing with the results of previous studies on COCs containing natural estrogens (41,57–61), we found that treatment with EV+DNG only modestly increased SHBG levels (+56%), whereas EE+DNG resulted in a major increment (+386%). The increase in SHBG in the EV+DNG group is comparable to that induced by EE+levonorgestrel (LNG) (~50%) (41), a second-generation COC acknowledged to carry a low VTE risk (62). The results of two large post-marketing surveillance studies also indicated a comparable VTE risk as regards E2/EV containing COCs and EE+LNG (63,64). A recently published extension of the active surveillance on the EV+DNG preparation showed an even lower VTE risk for EV+DNG compared with EE+LNG (65). Since E2/EV based COCs have been shown to have comparable contraceptive efficacy to EE based COCs (16,66), the lower impact on hepatic metabolism and emerging evidence of a low VTE risk indicate a favorable risk-benefit ratio for E2/EV containing COCs.

The strengths of this study include the direct comparison of COCs differing only in their estrogen component and the inclusion of a DNG-alone group as control. The DNG dose was higher for 51 out of 63 days in the EV+DNG group than in the other groups (3 vs. 2 mg), which could have had an impact on our results. However, antiandrogenic progestins, such as DNG, are not expected to antagonize the hepatic effects of estrogens. Moreover, DNG does not bind to SHBG, interfering with the binding of T and E2, nor does it have any significant steroid-receptor affinity for other than the progesterone receptor (33). The study could not be blinded because DNG on its own lacks a contraceptive indication. Adherence to the study protocol was considered good, owing to low anovulatory P4 levels in all women. Furthermore, all subjects in the EE+DNG group had profoundly

suppressed E2 levels, indicative of COC use, and in the EV+DNG group, exogenous EV use was apparent by the altered E1:E2 ratio.

Limitations of this study include the short follow-up time and the relatively low number of participants. However, nine weeks of continuous combined contraceptive use seems to be long enough to show alterations in metabolic parameters (67), and for instance, effects on blood coagulation emerge even more rapidly, already after the first week of COC use (68). Since the sample size was based on power calculations for the primary endpoint of the main study (23), the risk of type II statistical error must be considered for the present analyses. The sample size may be too small to detect differences between the EV+DNG and DNG alone groups. The analyses were, in addition, conservative owing to adjustments for multiple comparisons.

In conclusion, EV+DNG seems to exert less pronounced pituitary downregulation and lower hepatic impact than EE+DNG. Moreover, even though the FAI values clearly decreased after treatment with EV+DNG (-39 %), the FAI remained higher than after treatment with EE+DNG (-72%), which may be of clinical significance. E2/EV containing COCs may not be as efficient in managing hyperandrogenism but could have less effect on libido. Furthermore, based on our results, data on AMH as a marker of the ovarian reserve should be interpreted with caution not only in users of COCs containing EE, but also E2/EV. Further research carried out to assess long-term clinical consequences such as VTE risk, efficacy in hyperandrogenic conditions, and the effect on libido and metabolism are needed to establish the potential benefits of COCs containing natural estrogens.

DATA AVAILABILITY STATEMENT

Restrictions apply to the availability of some or all data generated or analyzed during this study to preserve patient confidentiality. The corresponding author will, on request, detail the restrictions and any conditions under which access to some data may be provided.

ACKNOWLEDGEMENTS

Research nurses Pirjo Ikonen and Elina Huikari are thanked for their invaluable assistance during this study.

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FIGURE LEGENDS

Figure 1. Flowchart

Flowchart of our nine-week, randomized trial comparing endocrine effects of continuous treatment with estradiol valerate+dienogest (EV+DNG), ethinylestradiol+dienogest (EE+DNG) and dienogest alone (DNG)

Figure 2. Hormonal parameters at baseline and after five and nine weeks of continuous treatment

Hormone serum concentrations (mean or median [95%CI]) in the three treatment groups, EV+DNG, EE+DNG and DNG alone at baseline before treatment (black bars), and at five (dark gray bars) and nine weeks (light gray bars) of treatment. Panel A) Follicle stimulating hormone (FSH) (mean [SD], B) Luteinizing hormone (LH) (median [95%CI]) C) Anti-Müllerian hormone (AMH) (median [95%CI]), D) Sex hormone binding globulin (SHBG) (mean [95%CI]), E) total testosterone (T), (median [96%C]), F) Free Androgen Index (FAI)(median [95%CI]), G) Estradiol (E2) (median [95%CI]), H) Androstenedione (A4) (median[95%CI])

*Change from baseline $P < 0.05$

**Change from baseline $P < 0.001$

*** Change from baseline $P = 0.05$

**** the change from five to nine weeks was significant P=0.02

[a] Within this group, one subject had a significantly higher LH value (14.8 IU/L) than the other subjects (<7.2 IU/L). Omitting this subject from the analyzes yielded a non-significant result, the change from baseline P=0.09, but it did not affect the comparisons between the groups.

Baseline values were comparable across the groups for all variables ($P > 0.05$)

TABLES

TABLE 1. Clinical characteristics

	EV+DNG	EE+DNG	DNG	*P-value*
n	20	19	17	
Age (years)	24.1 (3.6)	25.8 (3.8)	24.0 (3.9)	0.24
Weight (kg)	61.4 (5.8)	63.3 (4.5)	58.0 (7.1)	0.03
Body mass index (kg/m^2)	22.4 (1.6)	23.1 (1.9)	21.9 (1.9)	0.13

Clinical characteristics of the women participating in this nine-week randomized clinical trial (mean [SD]) analyzed using ANOVA

TABLE 2. Change from baseline to nine weeks and comparisons between the treatment groups

VARIABLE	BASELINE	5 WEEKS	9 WEEKS	EV+DNG vs. EE+DNG	EV+DNG vs. DNG	EE+DNG vs. DNG
	mean/median (SD/IQR)			*P*-value		
FSH IU/L						
EV+DNG	5.2 (3.8–7.2)		**3.2 (2.0–5.2) ***	**0.04**	0.29	**<0.001**
EE+DNG	5.5 (4.6–6.8)		**1.6 (0.9–2.8) ****			
DNG	5.0 (3.8–6.5)		4.6 (2.4–6.2)			
LH IU/L						
EV+DNG	5.2 (3.6–6.4)		**2.0 (0.8–4.4) ****	0.58	**0.002**	**<0.001**
EE+DNG	4.3 (3.1–5.6)		**0.7 (0.1–1.6) ****			
DNG	3.7 (2.9–5.3)		**5.7 (3.4–6.7) * [a]**			
E2 pmol/L						
EV+DNG	95.6 (59.9–143.1)	198.9 (128.5–241.3)	**197.3 (148–287.3) ***	**<0.001**	0.18	**<0.001**
EE+DNG	82.6 (63.8–123.9]	9.4 (5.2–13.5)	**8.2 (5.0–11.3) ****			
DNG	87.7 (68.5–135.7)	100.9 (59.4–174.4)	**131.4 (79.0–544.5) ******			
E1 pmol/L						
EV+DNG	129.5 (59.4)	**1155 (705.0) ****	**1580 (774.0) ****	**<0.001**	0.88	**<0.001**
EE+DNG	118.3 (61.0)	85.31 (39.3)	85.99 (31.9)			
DNG	118.0 (42.8)	121.3 (54.7)	183.2 (89.7)			
E1-S nmol/L						
EV+DNG	1.6 (0.8–2.3)	**35.3 (11.9–40.0) ****	**40.0 (23.4–40.0) ****	**<0.001**	**<0.001**	**<0.001**
EE+DNG	1.4 (0.9–2.9)	**0.4 (0.2–1.0) ****	**0.4 (0.2–0.8) ****			

DNG	1.8 (1.3–2.7)	1.7 (1.0–3.7)	2.4 (1.5–5.7)			
P4 nmol/L						
EV+DNG	0.46 (0.31–0.71)	**0.21 0.21–0.34) ****	**0.22 (0.21–0.26) ****	>0.99	>0.99	>0.99
EE+DNG	0.41 (0.35–0.52)	**0.22 (0.21–0.27) ***	**0.21 (0.21–0.25) ****			
DNG	0.42 (0.38–0.95)	**0.21 (0.21–0.31) ***	**0.27 (0.21–0.53) ***			
Total T nmol/L						
EV+DNG	1.1 (0.9–1.4)	1.09 (0.86–1.44)	**1.0 (0.8–1.3) ***	0.72	0.41	0.12
EE+DNG	0.93 (0.78–1.2)	**1.25 (0.96–1.58) *****	1.3 (1.0–1.6)			
DNG	0.93 (0.83–1.2)	0.87 (0.74–1.15)	0.97 (0.7–1.6)			
A4 nmol/L						
EV+DNG	6.2 (2.3)	5.0 (2.0)	**4.7 (1.9) ***	0.31	0.76	0.75
EE+DNG	5.2 (2.3)	4.4 (1.0)	**4.1 (1.2) ***			
DNG	5.3 (2.3)	4.6 (1.6)	5.1 (2.0)			
AMH ng/mL						
EV+DNG	3.55 (2.90–6.86)		**3.30 (2.76–5.05) ***	0.38	0.36	**0.03**
EE+DNG	3.23 (2.27–4.74)		**2.86 (1.58–4.30) ***			
DNG	2.81 (2.12–3.85)		2.67 (2.06–4.03)			
SHBG nmol/L						
EV+DNG	79.5 (32.7)	112.3 (43.7)	**117.7 (46.2) ***	**<0.001**	0.29	**<0.001**
EE+DNG	69.2 (22.0)	285.9 (67.8)	**306.1 (60.9) ****			
DNG	84.6 (30.7)	84.6 (56.8)	78.4 (53.6)			
FAI						
EV+DNG	1.6	1.1	**0.8**	**<0.001**	0.54	**<0.001**

	(1.2-1.9)	(0.7–1.4)	**(0.6-1.2) ****
EE+DNG	1.6	0.4	**0.4**
	(1.0–1.8)	(0.3–0.6)	**(0.3–0.5) ****
DNG	1.1	1.2	1.3
	(1.0–1.6)	(0.9–1.7)	(1.0–2.1)

***** treatment effect, the change from baseline $P < 0.05$, ** $P < 0.001$, ***$P=0.05$, **** the change between five- and nine weeks, $P=0.02$

[a] Within this group, one subject had a significantly higher LH value (14.8 IU/L) than the other subjects (<7.2 IU/L). Omitting this subject from the analyzes yielded a non-significant result, the change from baseline $P=0.09$, but it did not affect the comparisons between the groups.

A4, androstenedione; AMH, anti-Müllerian hormone; E1, estrone; E1-S, estrone sulfate; E2, estradiol; DNG, dienogest; EE, ethinylestradiol; EV, estradiol valerate; FAI, free androgen index; P4, progesterone

Figure 1 Flow chart

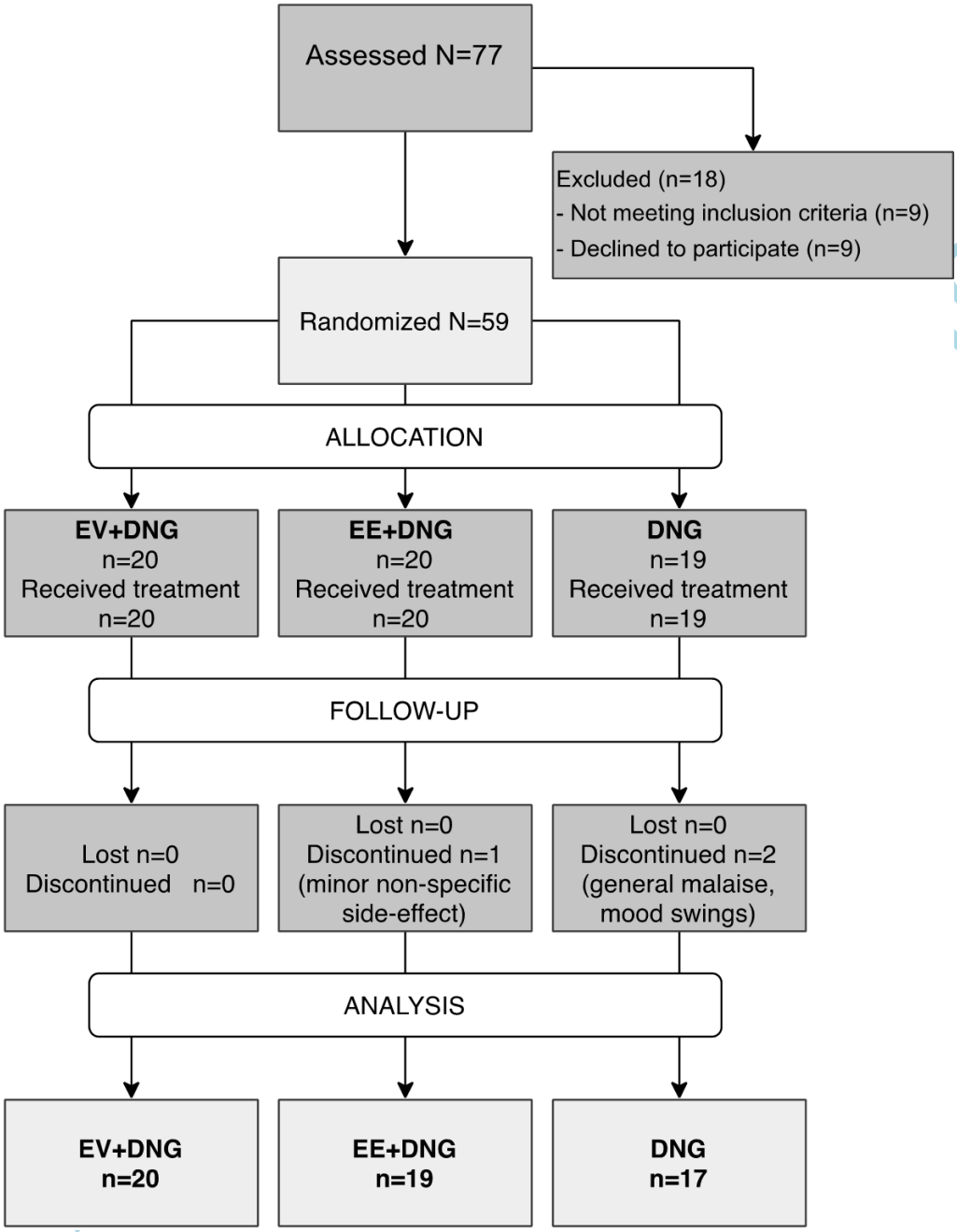

Figure 2 Hormonal parameters at baseline and after five and nine weeks of continuous treatment