Combined oral contraceptives containing estradiol valerate vs ethinylestradiol on coagulation : A randomized clinical trial

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ORIGINAL RESEARCH ARTICLE

Combined oral contraceptives containing estradiol valerate vs ethinylestradiol on coagulation: A randomized clinical trial

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Abstract

Introduction: Contraceptives containing ethinylestradiol (EE) induce changes in the coagulation system and are associated with a risk of venous thromboembolism. However, studies comparing the effects of combined oral contraceptives containing EE and low-potency estrogens (ie, estradiol [E_2] and estradiol valerate [EV]) on coagulation biomarkers are limited. This study represents secondary outcomes of a randomized trial comparing combined oral contraceptives containing EV + dienogest (DNG), EE + DNG, and DNG alone on selected coagulation biomarkers. We could compare the specific effects of the different estrogen components owing to the inclusion of preparations containing the same progestin.

Material and methods: We enrolled 59 healthy, 18- to 35-year-old, non-smoking women, of whom three discontinued. The participants were randomly allocated to 9 weeks of continuous treatment with EV 2 mg + DNG 2–3 mg ($n = 20$), EE 0.03 mg + DNG 2 mg ($n = 20$), or DNG 2 mg ($n = 19$). Blood samples were collected at baseline and after 9 weeks. We assessed coagulation in vitro by thrombin generation using the Calibrated Automated Thrombogram. Thrombin generation was evaluated by lag time, time to thrombin peak, thrombin peak, and endogenous thrombin potential in response to tissue factor (1 pM). In vivo coagulation assessment was based on levels of prothrombin fragment 1 + 2 (F1+ 2) (thrombin generation) and D-dimer (fibrin turnover). Clinical trial registration: NCT02352090.

Results: Lag time and time to thrombin peak remained unaltered after exposure to EV + DNG, whereas EE + DNG shortened both lag time (mean percentage change −24%, 95% confidence interval [CI] −32% to −15%; $p < 0.01$) and time to thrombin

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peak (−26%, 95% CI −37% to −16%; $p < 0.01$). EV + DNG induced lower thrombin peak and endogenous thrombin potential than EE + DNG (peak; +45%, 95% CI 22%–67% vs +147%,95% CI 96%–198%; $p < 0.01$, and endogenous thrombin potential; +26%, 95% CI 15%–38% vs +64%, 95% CI 51%–76%; $p < 0.01$). Median F1 + 2 levels remained unchanged with EV + DNG ($p = 0.22$) but increased within normal ranges with EE + DNG (from 152 pmol/L, 95% CI 127–206] pmol/L to 194 pmol/L, 95% CI 149–250 pmol/L, $p = 0.04$). The within-group change in D-dimer levels was not significant in any of the groups. DNG alone did not affect these biomarkers.

Conclusions: Both in vitro and in vivo thrombin generation was lower after exposure to EV + DNG compared with EE + DNG. The lower thrombin generation measures after treatment with EV + DNG indicate less enhancement of coagulation potential and suggest that EV may be favorable to EE as a component of combined oral contraceptives.

KEYWORDS
coagulation, combined oral contraception, dienogest, estradiol valerate, thrombin generation

1 | INTRODUCTION

The use of combined oral contraceptives (COCs) is a well-established risk factor for venous thromboembolism (VTE), increasing the risk two- to four-fold compared with non-use.[1] In COCs, the estrogen component, mainly ethinylestradiol (EE), induces a hypercoagulative state, and progestins seem to modify the overall effect depending on androgenic activity.[2] Also, the risk of VTE appears dose-dependent on EE and varies according to the progestin component.[3]

COCs containing low-potency estrogens, ie, estradiol (E_2) and estradiol valerate (EV), have been developed to reduce the effects on the coagulation system and lower the VTE risk. Indeed, the changes induced in the coagulation system by COCs containing E_2/EV appear comparable to or even lower than the changes induced by EE + levonorgestrel (LNG),[4–8] a second-generation COC acknowledged for carrying the lowest VTE risk.[1] Accordingly, prospective surveillance studies have reported similar VTE risk for E_2/EV containing COCs and EE + LNG.[9,10] Moreover, a recent epidemiological study from Finland revealed an unaltered risk of VTE associated with the use of E_2/EV-based COCs.[11] Nevertheless, COCs containing E_2/EV remain sparsely studied, and previous comparative studies of EE vs E_2/EV in COCs have mainly included preparations with different progestin types, making it difficult to evaluate the specific impact of the substitution of EE with E_2/EV.

Measurement of in vitro thrombin generation can be used to evaluate both hypo- and hypercoagulative states,[12] and it appears to be a surrogate marker of VTE risk.[13,14] Thrombin generation reflects the overall balance of the coagulation system and EE-based COCs,[15,16] as well as other hypercoagulative states, such as pregnancy[17] and the use of postmenopausal hormone therapy,[18] are reported to increase thrombin generation.

Key message

Continuous exposure to the combined oral contraceptive containing ethinylestradiol + dienogest enhanced in vitro thrombin generation compared with estradiol valerate + dienogest, suggesting that the substitution of ethinylestradiol with estradiol valerate in combined oral contraceptives may beneficially lower coagulation potential.

We have recently completed a randomized trial comparing the metabolic effects of COCs containing EV + dienogest (DNG), EE + DNG, and DNG alone (included as an active control).[19–21] In the present study, to assess the impact of COCs on blood coagulation and explore possible differences between these preparations, we analyzed thrombin generation (with the Calibrated Automated Thrombogram, CAT)[12] under experimental conditions sensitive for detecting hypercoagulability induced by COC use.[22] We complemented this in vitro assay with specific biomarkers of in vivo coagulation activity—prothrombin fragment 1 + 2 (F1 + 2) and D-dimer, previously shown to be enhanced by the exposure to COCs containing EE.[23] Our purpose for including preparations with the same progestin was to enable a comparison of the specific effects of the estrogen components on the coagulation variables.

2 | MATERIAL AND METHODS

The present study reports secondary outcomes from our randomized clinical trial, previously described in detail[24] and conducted between April 2015 and January 2018 at Helsinki and Oulu University Hospitals.

Healthy women aged 18–35 years were invited to participate. Inclusion criteria were normal weight (body mass index 19–24.9 kg/m^2), regular menstrual cycle, normotension, and a 2-month washout from hormonal contraceptive use. Exclusion criteria were smoking, any chronic disease or regular medication, and any contraindication for combined hormonal contraceptive use, including known thrombophilia, previous VTE, or VTE in a first-degree relative.

The participants were randomly allocated in a 1:1:1 ratio into the three intervention groups: (1) EV 2 mg + DNG 2–3 mg (Qlaira®, Bayer AG, four-phasic regimen; days 1–2 EV 3 mg, days 3–7 EV 2 mg + DNG 2 mg, days 8–24 EV 2 mg + DNG 3 mg, days 25–26 EV 1 mg, days 27–28 placebo), (2) EE 0.03 mg + DNG 2 mg (Valette®, Jenapharm GmbH & Co. KG, days 1–21 EE 0.03 mg + DNG 2 mg, days 22–28 placebo), and (3) DNG 2 mg (Visanne®, Bayer AG, days 1–28 DNG 2 mg). We harmonized the preparations by eliminating the hormone-free interval (all placebo tablets) and removing the estrogen-only tablets (EV 3 mg and EV 1 mg) and one tablet containing EV 2 mg + DNG 2 mg from the EV + DNG preparation (leaving 21 tablets/blister pack) (Table 1). The treatment regimen was continuous for 63 days. Blood samples were collected at baseline, on menstrual cycle days 1–5 (the regimens were initiated the following day), and at 9 weeks (days 57–63).

Venous blood samples were collected into tubes containing 3.2% trisodium citrate (0.109 M, 0.1 vol). Platelet-poor plasma was prepared within 1 hour from blood collection by single centrifugation at 2500 **g** for 15 min at room temperature, split into aliquots, and stored at −70°C until analyzed. Plasma samples from 11 healthy, not age- or sex-matched individuals were used as controls for the thrombin generation assay.

Thrombin generation was measured in platelet-poor plasma using CAT (including all reagents; Thrombinoscope) as previously described.[12] The thrombin generation assay was performed in 96-well microtiter plates, where platelet-poor plasma (80 μL) was supplemented with either inner method Calibrator or tissue factor (TF) -containing reagent (20 μL). Reagents comprised TF (1 pM) and phospholipids (4 μM) in platelet-poor plasma (PPP Reagent Low). Thrombin generation was initiated by FluCA reagent mixture (20 μL). The analysis was carried out for a minimum of 60 minutes. The variables derived from the assay were: lag time (corresponding to clotting time); time to thrombin peak (time to highest thrombin concentration); thrombin peak (highest thrombin concentration); and endogenous thrombin potential (ETP; area under the curve). Our laboratory is standardized for the CAT,[25] but because of the technical challenges of the routine, we deviated from the protocol by centrifuging the samples only once. Analyses were performed at the Coagulation Disorders Research Unit in Biomedicum, Helsinki.

The F1 + 2 was analyzed by enzyme-linked immunosorbent assay (Enzygnost® F1 + 2, Siemens Healthcare Diagnostics Products GmbH). The manufacturer's reference range (5th to 95th centiles) was 69–229 pmol/L. D-dimer was measured using an immune-turbidimetric assay (STA®-Liatest® D-Di Plus, Diagnostica Stago) with the STA-R Max® analyzer (Diagnostica Stago). The assay's detection limit is 0.27 mg/L, and the working range is 0.27–4.00 mg/L. These analyses were performed at The Finnish Red Cross Blood Service, Department of Hemostasis.

2.1 | Statistical analyses

The sample size calculation was estimated by our study's primary outcome measure, a change in the whole-body insulin sensitivity index (the Matsuda Index), reported separately.[24] Accordingly, to reach a power of 0.8 at the significance level of 0.05, we had to enroll 48 participants. To compensate for possible loss to follow up, we intended to enroll 60 women.

The intra-individual changes from baseline were calculated for all variables, and data are summarized as mean (with standard deviation [SD] or 95% confidence interval [CI]) or median (with range [min–max]) and mean percentage (%) changes from baseline (with 95% CI). Means were also calculated for each time point during the thrombin generation assay (every 20 seconds) captured as thrombograms (Figure 2). Lag time, time to thrombin peak, and F1 + 2 were log-transformed before the analysis because of their skewed distribution (verified by Shapiro-Wilks test). The mean differences from baseline (the within-group change) of the thrombin generation variables were analyzed using the paired samples *t* tests. Furthermore, we used the Kruskal-Wallis test to compare EE + DNG, EV + DNG, and DNG-alone groups and Dunn's test for post hoc paired comparisons. Paired samples *t* test was used to analyze the within-groups change in F1 + 2, one-way analysis of variance for the between-groups comparison, and Tukey's *post hoc* test for paired comparisons. The corresponding tests for the change in D-dimer levels were Wilcoxon signed ranks tests, Kruskal-Wallis test, and Dunn test. Bonferroni corrections were used to adjust for multiple comparisons.

TABLE 1 Hormonal contents of the study preparations

| Study group | Estrogen | | Progestin | |
	Type and dose	Days	Dose	Days
EV + DNG	Estradiol valerate 2 mg	1–21	Dienogest 2 mg	1–5
			Dienogest 3 mg	6–21
EE + DNG	Ethinylestradiol 0.03 mg	1–21	Dienogest 2 mg	1–21
DNG			Dienogest 2 mg	1–21

Note: Preparations were used continuously for 63 days (3 × 21 days).
Abbreviations: DNG, dienogest, EE, ethinylestradiol; EV, estradiol valerate.

Statistical analyses were performed with SPSS Statistics 27 (IBM) and GraphPad Prism 9.0 for Mac iOS (GraphPad).

2.2 | Ethics statement

The study protocol was approved by the Independent Ethics committees of Helsinki and Oulu University Hospitals (reference number 150/13/03/03/2014) on April 30, 2014. The study was prospectively registered at clinicaltrials.gov on January 27, 2015 (NCT 02352090). The registration is available at https://clinicaltrials.gov/ct2/show/NCT02352090. The initial participant enrollment took place in April 2015. All women who enrolled signed an informed consent form.

3 | RESULTS

We assessed 77 volunteer women (all white) for eligibility, of whom 59 were randomized (Figure 1). After study completion, the samples were screened for activated protein C (APC) -resistance (Sta®-Staclot®-APC-R, Diagnostica Stago), and two cases of Factor V Leiden mutation were confirmed by DNA testing. Of the 59 women enrolled, 56 completed the study; three discontinued because of minor side effects ($n = 1$ mood changes, $n = 1$ general malaise, $n = 1$ nonspecific minor side-effects), and the two carriers of Factor V Leiden mutation were excluded ($n = 2$) (Figure 1). Two samples for CAT and two others for F1+2 and D-dimer were discarded because

of technical difficulties. The final numbers of participants analyzed for CAT were EV+DNG $n = 18$, EE+DNG $n = 18$, DNG $n = 16$, and for F1+2/D-dimer were EV+DNG $n = 17$, EE+DNG $n = 18$, DNG $n = 17$. Baseline clinical characteristics were comparable (Table 2).

Baseline thrombin generation data did not differ between the groups (Table 3) and were comparable to the measures from the 11 controls (lag time $p = 0.82$, time to thrombin peak $p = 26$, thrombin peak $p = 0.50$, ETP $p = 0.97$). The comparative CAT results are shown in Figure 2. In the EV+DNG group, the mean lag time and time to thrombin peak did not change, whereas the mean thrombin peak increased (mean % change from baseline) by +45% (95% CI 22%–67%; $p < 0.01$) and ETP by +26% (95% CI 15%–38%; $p < 0.01$). In the EE+DNG group, lag time and time to thrombin peak shortened by −24% (95% CI −32% to −15%; $p < 0.01$) and −26% (95% CI −37% to −16%; $p < 0.01$), whereas thrombin peak and ETP increased more than in the EV+DNG group, by +147% (95% CI 96%–198%; $p < 0.01$) and +64% (95% CI 51%–76%; $p < 0.01$), respectively. In contrast, all variables remained unchanged in the DNG group. The comparison between EV+DNG and EE+DNG was significant for all variables (lag time $p = 0.02$, time to thrombin peak $p = 0.01$, thrombin peak $p < 0.01$, and ETP $p < 0.01$) (Figure 2). Similarly, the comparisons between EE+DNG and DNG-alone groups were significant (all variables, $p < 0.01$), whereas the comparisons between EV+DNG and DNG-alone groups were not.

The mean change in levels of F1+2 in the EV+DNG (−6%, 95% CI −19% to 8%]; $p = 0.22$) and DNG-alone groups (−9%, 95% CI −20% to 4; $p = 0.08$) were non-significant. In contrast, F1+2 increased in
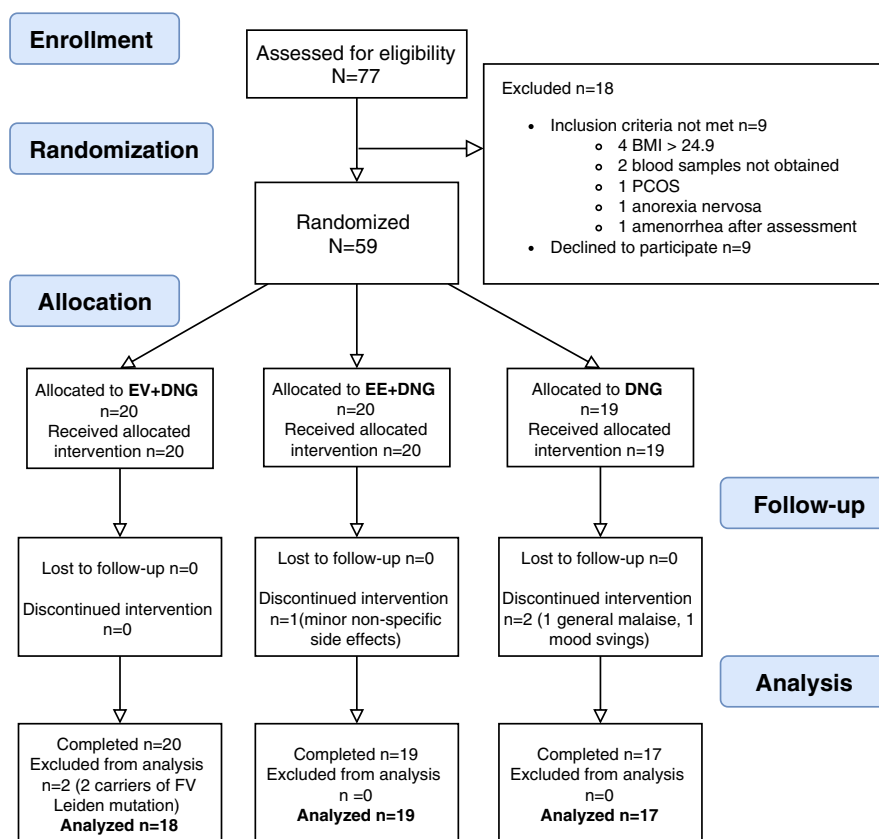
FIGURE 1 Flowchart of our 9-week randomized study comparing the effects of estradiol valerate+dienogest (EV+DNG), ethinylestradiol + dienogest (EE+DNG), and dienogest alone (DNG) on coagulation biomarkers.

Variable	EV + DNG	EE + DNG	DNG	p^a
Women, n	20	19	17	
Age (y), mean (SD)	24.1 (3.6)	25.8 (3.8)	24.0 (3.9)	0.24
Weight (kg), mean (SD)	61.4 (5.8)	63.3 (4.5)	58.0 (7.1)	0.03
BMI (kg/m², mean (SD)	22.4 (1.6)	23.1 (1.9)	21.9 (1.9)	0.13
WHR), mean (SD)	0.76 (0.04)	0.79 (0.05)	0.78 (0.03)	0.16

Abbreviations: BMI, body mass index; DNG, dienogest; EE, ethinylestradiol; EV, estradiol valerate; SD, standard deviation; WHR, waist-to-hip ratio.

^{a}Analyzed by one-way analysis of variance.

the EE + DNG group by +24% (95% CI 4%–44%; $p = 0.04$) (Table 3). The EV + DNG vs EE + DNG and EE + DNG vs DNG comparisons were significant ($p = 0.04$ and $p = 0.03$, respectively), but the comparison between EV + DNG and DNG alone was not.

Most D-dimer values were at or below the detection limit (73% at baseline and 75% at 9 weeks), so we arbitrarily replaced all low values below 0.27 mg/L with 0.27 mg/L. From baseline, D-dimer levels changed by +2% (95% CI −14% to 19%) in the EV + DNG group, +13% (95% CI 3%–28%) in the EE + DNG group and 2% (95% CI −4% to 0.4%) in the DNG alone group. The within-group differences from baseline were nonsignificant (Table 3). However, the difference across the groups was significant ($p = 0.03$), as well as the post hoc comparison EV + DNG vs EE + DNG ($p = 0.04$) (adjusted for multiplicity).

4 | DISCUSSION

In this study, we found that all thrombin generation variables differed substantially between the EV + DNG and EE + DNG groups. We demonstrated enhanced thrombin generation response in plasma triggered by TF in the EE + DNG group compared with the EV + DNG group. Furthermore, treatment with EE + DNG increased F1 + 2 compatible with enhanced thrombin generation in vivo. Additionally, treatment with DNG alone did not affect the coagulation biomarkers, aligning with studies on progestin-only contraceptives, and showing no relevant effects on blood coagulation or increased VTE risk.[26,27]

VTE is a multifactorial disease to which various environmental and genetic risk factors contribute. The enhancement of thrombin generation during COC use reflects increased coagulation potential without indicating immediate VTE risk. VTE is a rare event in young, healthy women that seldom occurs unprovoked, even during COC use. The enhanced coagulation potential during COC use could, however, indicate a lowered threshold for thrombosis under transient conditions, such as trauma, surgery, and prolonged immobilization, or further increase the VTE risk associated with inherent risk factors, such as advanced age,[28] obesity,[29] and genetic thrombophilia.[30]

Our results indicate that the estrogen component contributes to thrombin generation and show that EV-induced coagulation potential is lower than that of EE at the present contraceptive doses.

This is in line with the concept of estrogen potency, meaning that larger doses of E_2 than EE are needed for the same clinical effect. However, the dose-equivalence is tissue-specific and depends on the end points. EE is a highly potent estrogen with high bioavailability and long half-life, whereas the low potency estrogens, such as EV (an ester of naturally occurring E_2) have low bioavailability and short half-life.[31] The higher potency of EE is exemplified by the hepatic effects; EE exerts a 500- to 600-fold upregulation of certain proteins compared with E_2/EV.[32] Our results could offer a biological basis for the results of recent prospective epidemiological studies, where E_2/EV-containing COCs carry similar or even lower VTE risk than EE + LNG, which carries low VTE risk.[9,10] In the EE + LNG preparation, the androgenic LNG counteracts some of EE's hepatic effects, which could explain the low impact on the coagulation system.[33]

In addition to increased in vitro thrombin generation, exposure to EE + DNG activated in vivo thrombin generation compared with EV + DNG. Levels of F1 + 2 increased (within normal range) in the EE + DNG group, whereas in the EV + DNG group, F1 + 2 remained unaltered, in line with previous work.[8] Excessive fibrin formation and degradation could be excluded in all study groups by low D-dimer levels without significant within-group effects. Whether the increase in F1 + 2 levels seen during use of EE-based COC is clinically significant is unclear because the values usually remain within normal ranges.

EE-containing COCs induce both pro- and anticoagulant factors as well as fibrinolytic activity,[34] culminating in increased thrombin generation (Figure 3), as shown here and previously under various assay conditions. In previous comparative studies, E_2/EV-containing COCs have influenced several blood coagulation variables less than EE + LNG, including fibrinogen, antithrombin, protein S, and APC.[4–8] As the changes induced in the coagulation system by COCs manifest as an acquired APC resistance,[2,16,35] previous studies on COCs containing different estrogens have dealt with thrombin generation probing the APC pathway (ie, the ETP-based activated Protein C resistance test). Thrombin generation has also been triggered with a higher TF concentration (5 pM), which also renders the thrombin generation assays more sensitive for the APC pathway. Most, but not all,[6] of these studies, have shown lower thrombin generation after exposure to COCs containing E_2/EV than EE + LNG.[4,5,7,8]

TABLE 3 Effects of EV+DNG, EE+DNG, and DNG alone on thrombin generation, prothrombin fragment F1+2 and D-dimer

Variable	Group	n	Reference Mean (SD)	Baseline[a] Mean (SD)	9 Weeks Mean (SD)	% Change Mean	95% CI	p[b]	p[c]
Lag time[d] (min)	EV+DNG	18	5.6 (1.1)	5.68 (1.87)	5.29 (1.32)	−3.2%	−13.1 to 6.7	0.30	<0.01
	EE+DNG	18		5.80 (1.81)	4.28 (1.10)	−23.5%	−31.8 to −15.1	<0.01	
	DNG	16		6.14 (1.84)	6.19 (1.47)	3.4%	−6.6 to 13.5	0.67	
Time to thrombin peak[d] (min)	EV+DNG	18	10.2 (1.5)	11.4 (2.9)	10.5 (2.6)	−6.2%	−13.7 to 1.2	0.80	<0.01
	EE+DNG	18		11.5 (2.6)	8.3 (2.3)	−26.2%	−36.6 to −16.1	<0.01	
	DNG	16		12.3 (2.7)	12.0 (2.6)	−0.8%	−10.7 to 12.3	0.76	
Thrombin peak[d] (nM)	EV+DNG	18	126 (37.5)	110.6 (45.1)	155.2 (71.7)	44.8%	22.4 to 67.2	<0.01	<0.01
	EE+DNG	18		115.1 (45.8)	260.0 (90.9)	147.1%	95.9 to 198.4	<0.01	
	DNG	16		99.5 (32.6)	105.7 (39.7)	7.6%	−12.1 to 27.3	0.71	
ETP[d] (nM*min)	EV+DNG	18	1038 (249.3)	1023 (253.7)	1262 (290.8)	26.4%	14.5 to 38.2	<0.01	<0.01
	EE+DNG	18		1060 (185.9)	1704 (205.3)	63.9%	51.4 to 76.3	<0.01	
	DNG	16		1025 (157.6)	1098 (187.9)	7.1%	−3.2 to 17.4	0.23	
			Normal values	Median (range)	Median (range)				
Prothrombin F1+2 (pmol/L)	EV+DNG	17	69–229 pmol/L	156.2 (80.5–246.9)	133.7 (80.0–260.5)	−5.5%	−19.4 to 8.4	0.22	0.02
	EE+DNG	18		151.7 (97.9–313.7)	193.5 (125.4–274.5)	24.1%	4.2 to 43.9	0.04	
	DNG	17		140.3 (83.1–230.5)	125.6 (87.9–205.4)	−8.5%	−20.4 to 3.5	0.08	
D-dimer (mg/L)	EV+DNG	17	<50 mg/L	0.28 (0.27–0.48)	0.27 (0.27–0.57)	+2.4%	−14.0 to 18.7	0.42	0.03
	EE+DNG	18		0.27 (0.27–0.74)	0.27 (0.27–0.77)	+12.6%	−3.3 to 28.4	0.11	
	DNG	17		0.27 (0.27–0.31)	0.27 (0.27–0.27)	−1.6%	−3.6 to 0.4	0.25	

Note: The thrombin generation reference values are from 11 healthy, not age- or sex-matched individuals. Baseline values did not differ between study groups and controls (lag time $p = 0.82$, time to thrombin peak $p = 0.26$, thrombin peak $p = 0.50$, ETP $p = 0.97$).CAT variables were analyzed with paired samples *t* test and Kruskal-Wallis test, prothrombin fragment F1+2 with paired samples *t* test and one-way analysis of variance, and D-dimer with Wilcoxon signed ranks test and Kruskal-Wallis test. Statistically significant *p*-values ($p < 0.05$) are bolded.

Abbreviations: CAT, Calibrated Automated Thrombogram; DNG, dienogest; EE, ethinylestradiol; ETP, endogenous thrombin potential; EV, estradiol valerate; F1+2, prothrombin fragment 1+2; SD, standard deviation.

[a]Baseline values were comparable ($p = 0.63$ to $p = −0.86$), except for D-dimer (EV+DNG vs DNG $p = 0.02$).

[b]*p*-value for the within-group change from baseline to 9 weeks (within-group treatment effect).

[c]*p*-value for the between-groups change from baseline to 9 weeks.

[d]Variables derived from the CAT.

To the best of our knowledge, the specific experimental conditions of the present study have not previously been reported in a study comparing different estrogens as components of COC. Overall, thrombin generation reflects the balance of pro- and anticoagulant interactions upstream of thrombin beyond end point markers, with the main determinants at low TF being fibrinogen, Factor VII, antithrombin, and tissue factor pathway inhibitor (TFPI). In general, low TF highlights the intrinsic coagulation pathway, whereas higher TF (5 pM) engages the extrinsic pathway.[22] We chose the low TF concentration (1 pM) to further explore the effects of the different COCs on thrombin generation, not only for its sensitivity for COC use,[22] but also because the anticoagulant activity of the TFPI/protein S system is revealed at low TF concentrations.[22] This is important because the mechanism of the acquired APC resistance associated with COC use seems to relate to impaired functions of the natural anticoagulants TFPI/Protein S.[36]

Strengths of our study include its randomized design and the inclusion of DNG alone as an active control. All preparations contained the same progestin, thus allowing the assessment of the specific effects of the different estrogens. The DNG dose was higher (3 vs 2 mg) in the EV+DNG group than in the other two groups for most of the study duration (51/63 days), but this should only have a limited impact because DNG as an antiandrogenic progestin is not expected to antagonize the hepatic effects of estrogens. Because DNG alone lacks indication for contraceptive use, our study had to be unblinded. Although the follow up was short, 9 weeks is long enough to demonstrate effects on blood coagulation. For instance, thrombin generation measures are already increased after the first week of COC use.[15] However, conclusions on the potential long-term effects cannot be drawn.

Regarding the validity of our CAT results, it is known that factors such as race, age, sex, and the specific assay conditions applied contribute to large variation in thrombin generation measures.

PANEL A
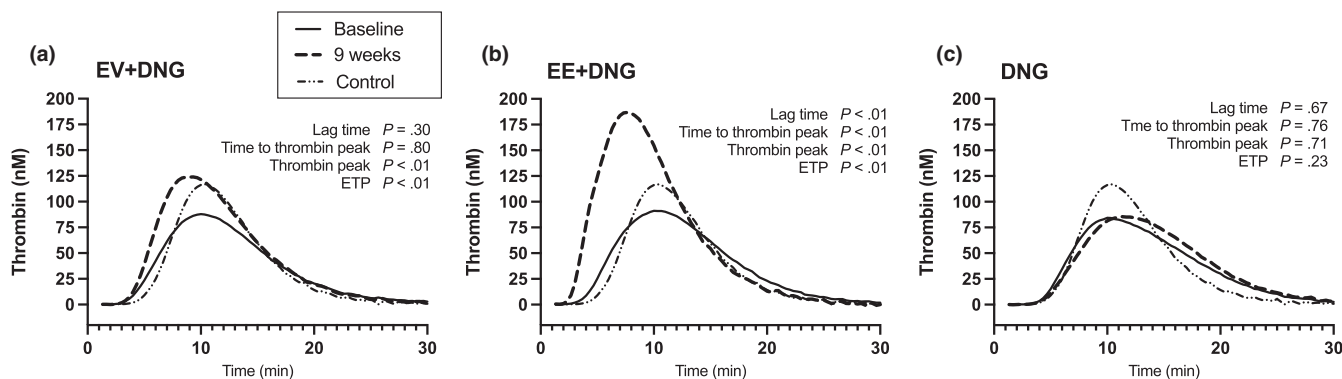
PANEL B
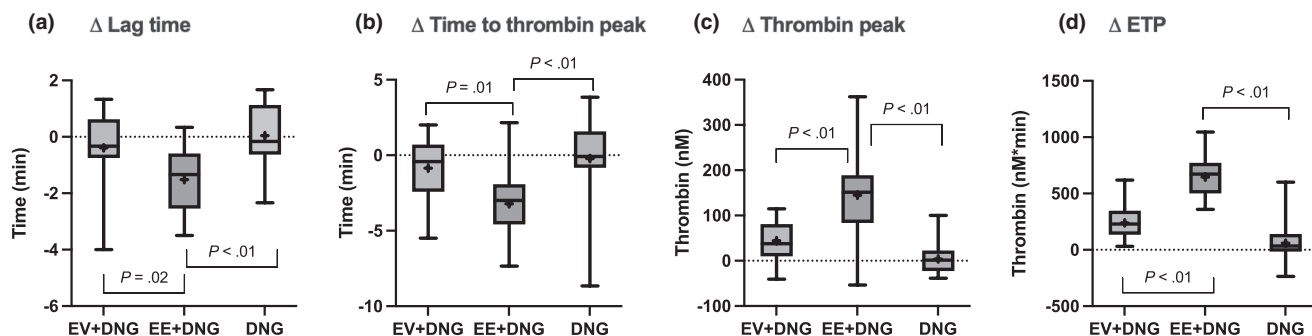
FIGURE 2 Thrombin Generation. (A) The mean results of the calibrated automated thrombogram (CAT) at baseline and after 9 weeks of the study shown as thrombin generation curves. (a) estradiol valerate+diengoest (EV + DNG); (b) ethinylestradiol+dienogest (EE + DNG); (c) dienogest alone (DNG). The thrombin generation curves depict the formation and subsequent inactivation of thrombin (nM) over time (min) after initiation of coagulation with 1 pM tissue factor in the study groups. The variables include lag time, time to thrombin peak, thrombin peak, and endogenous thrombin potential (ETP), which refers to the area under the curve. The change from baseline was analyzed by paired samples *t* tests. Eleven healthy individuals (not age- or sex-matched) were included in the plot as control. (B) The change from baseline in thrombin generation variables in the three study groups EV + DNG, EE + DNG, and DNG alone and comparisons between the groups. (a) Lag time; (b) time to thrombin peak; (c) thrombin peak; (d) ETP. The data are shown as boxplots (median and mean [+], interquartile range, min–max) and analyzed by Kruskal-Wallis test with post hoc paired comparisons (Dunn test). The EV + DNG vs DNG-alone comparison did not differ for any variable.

Hence, reference values would ideally be established for the specific populations investigated and for the assay conditions used. Recently, a new fully automated thrombin generation method with established reference ranges has been developed to minimize inter- and intra-assay variation.[38] However, this method was not available for the present analyses. For the CAT, as population-based reference values are lacking, we used plasma samples from 11 healthy, not age- or sex-matched individuals to control for the assay. The women in the present study had small interindividual variation in baseline thrombin generation measures and showed consistent increases in thrombin generation from baseline values after the intervention, demonstrating an effect of COC use. The laboratory used is standardized for the CAT, but as the result of technical challenges of the routine in our large tertiary hospital, we deviated from the protocol by centrifuging the samples only once. The samples were collected and frozen at –70°C between 2015 and 2018 and were analyzed in one batch in September 2020. The single-centrifugation and long storage time might have affected our results. However, the pre-analytical conditions were equal for the baseline and 9-week samples and the baseline results were comparable to the controls, indicating stability of the samples, and that the changes observed in thrombin generation were due to the interventions and not to pre-analytical factors.

The number of participants was somewhat limited, but the consistent results indicate a sufficient sample size for the comparisons between the EV + DNG and EE + DNG groups. However, the study may not be powered to detect differences between the EV + DNG and DNG-alone groups. In addition, the generalizability of our results is limited owing to the strict inclusion criteria resulting in participant characteristics not necessarily reflecting typical COC users. Further studies should assess COCs containing low-potency estrogens in women with underlying VTE risk factors, for instance higher age, obesity, and polycystic ovary syndrome.

FIGURE 3 Schematic overview of the coagulation system and changes induced by hormonal contraceptives. In the present study, coagulation activation in vivo was assessed by prothrombin fragment 1+2 (F1+2), a degradation product of prothrombin arising during its conversion to thrombin, and D-dimer, a marker of fibrin turnover. Thrombin generation was measured in vitro using calibrated automated thrombogram (CAT) in response to tissue factor (TF). Plus and minus signs denote the direction of the changes in individual pro- and anticoagulant factors or their activity observed during hormonal contraceptive use.[2,36] ATIII, antithrombin; F1+2, prothrombin fragment 1+2; PC, protein C; PS, protein S; TFPI, tissue factor pathway inhibitor; TM, thrombomodulin. Figure adapted from reference [37] and created with Biorender.com.
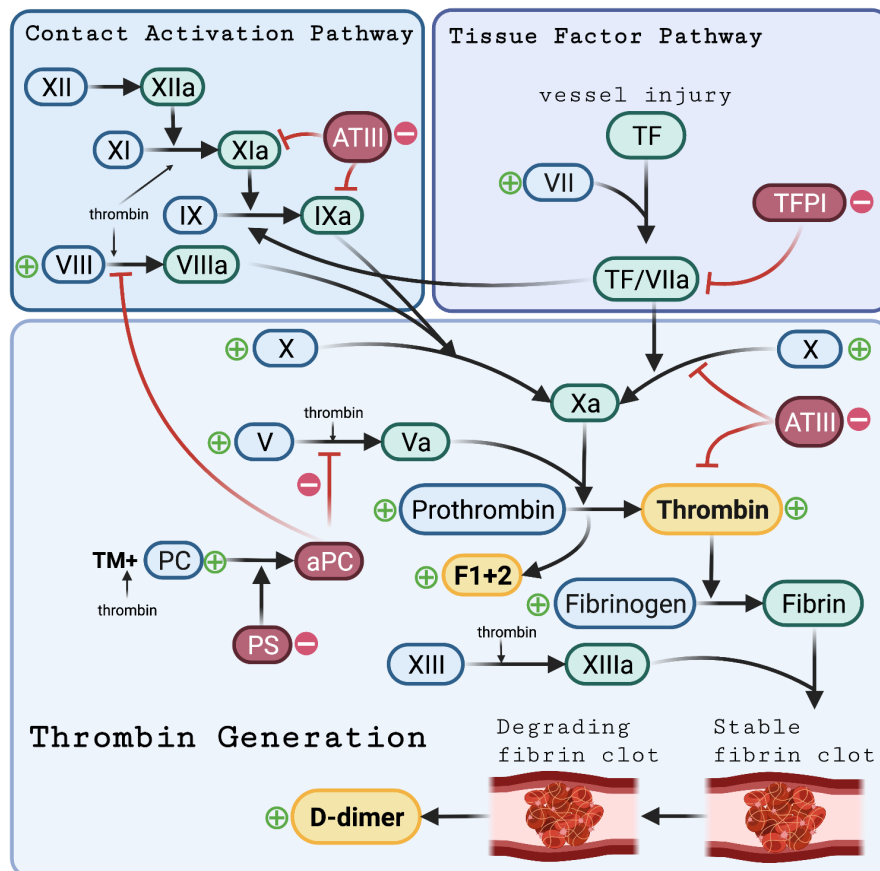
5 | CONCLUSION

Our results complement previous biomarker studies and show that 9 weeks of continuous exposure to EV+DNG resulted in lower thrombin generation measures than EE+DNG. Furthermore, EV+DNG did not enhance in vivo thrombin generation in contrast to EE+DNG. We suggest that the inclusion of E_2/EV in COCs could be beneficial because of the lowered coagulation potential compared with traditional COCs containing EE.

AUTHOR CONTRIBUTIONS

JTS, TP, and OH designed the primary study. AH, MKH, TP, KL, and OH collected the data. LH, SS, RL, and TS planned the assays and contributed to analyzing the data. AH conducted the statistical analyses and wrote the first version of the manuscript including the figures and tables. All authors contributed to the writing of the manuscript and accepted the final version.

CONFLICT OF INTEREST

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 no relevant conflicts of interest to disclose.

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REFERENCES

1. de Bastos M, Stegeman BH, Rosendaal FR, et al. Combined oral contraceptives: venous thrombosis. *Cochrane Database Syst Rev.* 2014;(3):CD010813.
2. Tchaikovski S, Rosing J. Mechanisms of estrogen-induced venous thromboembolism. *Thromb Res.* 2010;126:5-11.
3. Weill A, Dalichampt M, Raguideau F, et al. Low dose oestrogen combined oral contraception and risk of pulmonary embolism, stroke, and myocardial infarction in five million French women: cohort study. *BMJ.* 2016;353:i2002.
4. Klipping C, Duijkers I, Parke S, Mellinger U, Serrani M, Junge W. Hemostatic effects of a novel estradiol-based oral contraceptive: an open-label, randomized, crossover study of estradiol valerate/dienogest vs ethinylestradiol/levonorgestrel. *Drugs R D.* 2011;11:159-170.
5. Gaussem P, Alhenc-Gelas M, Thomas JLL, et al. Haemostatic effects of a new combined oral contraceptive, nomegestrol acetate/17β-estradiol, compared with those of levonorgestrel/ethinyl estradiol. *Thromb Haemost.* 2011;105:560-567.
6. Raps M, Rosendaal F, Ballieux B, et al. Resistance to APC and SHBG levels during use of a four-phasic oral contraceptive containing dienogest and estradiol valerate: a randomized controlled trial. *J Thromb Haemost.* 2013;11:855-861.
7. Ågren UM, Anttila M, Mäenpää-Liukko K, et al. Effects of a monophasic combined oral contraceptive containing nomegestrol acetate and 17β-oestradiol compared with one containing levonorgestrel and ethinylestradiol on haemostasis, lipids and carbohydrate metabolism. *Eur J Contracept Reprod Heal Care.* 2011;16:444-457.
8. Junge W, Mellinger U, Parke S, Serrani M, Serranf M. Metabolic and haemostatic effects of estradiol valerate/dienogest, a novel oral contraceptive. *Clin Drug Investig.* 2011;31:573-584.
9. Dinger J, Do Minh T, Heinemann K. Impact of estrogen type on cardiovascular safety of combined oral contraceptives. *Contraception.* 2016;94:328-339.
10. Reed S, Koro C, DiBello J, et al. Prospective controlled cohort study on the safety of a monophasic oral contraceptive containing nomegestrol acetate (2.5mg) and 17β-oestradiol (1.5mg) (PRO-E2 study): risk of venous and arterial thromboembolism. *Eur J Contracept Reprod Heal Care.* 2021;26:439-446.
11. Heikinhemo O, Toffol E, Partonen T, But A, Latvala A, Haukka J. Systemic hormonal contraception and risk of venous thromboembolism. *Acta Obstet Gynecol Scand.* 2022;101:846-855.
12. Hemker H, Giesen P, Al Dieri R, et al. The calibrated automated Thrombogram (CAT): a universal routine test for hyper- and hypocoagulability. *Pathophysiol Haemost Thromb.* 2002;32:249-253.
13. Van Hylckama VA, Christiansen S, Luddington R, Cannegieter S, Rosendaal F, Baglin T. Elevated endogenous thrombin potential is associated with an increased risk of a first deep venous thrombosis but not with the risk of recurrence. *Br J Haematol.* 2007;138:769-774.
14. Sonnevi K, Tchaikovski SN, Holmström M, Rosing J, Bremme K, Lärfars G. Thrombin generation and activated protein C resistance in the absence of factor V Leiden correlates with the risk of recurrent venous thromboembolism in women aged 18-65 years. *Thromb Haemost.* 2011;106:901-907.
15. Westhoff CL, Pike MC, Cremers S, Eisenberger A, Thomassen S, Rosing J. Endogenous thrombin potential changes during the first cycle of oral contraceptive use. *Contraception.* 2017;95:456-463.
16. Tchaikovski S, van Vliet H, Thomassen C, et al. Effect of oral contraceptives on thrombin generation measured via calibrated automated thrombography. *Thromb Haemost.* 2007;98:1350-1356.
17. Bagot CN, Leishman E, Onyiaodike CC, Jordan F, Freeman DJ. Normal pregnancy is associated with an increase in thrombin generation from the very early stages of the first trimester. *Thromb Res.* 2017;157:49-54.
18. Bagot C, Marsh M, Whitehead M, et al. The effect of estrone on thrombin generation may explain the different thrombotic risk between oral and transdermal hormone replacement therapy. *J Thromb Haemost.* 2010;8:1736-1744.
19. Haverinen A, Luiro K, Szanto T, et al. Effect of oral contraceptives containing estradiol valerate vs. ethinyl estradiol combined with dienogest on biomarkers of coagulation a randomized clinical trial. *Endocrine Abstracts.* 2021;73:PEP4.1. doi:10.1530/endoabs.73.PEP4.1
20. Kangasniemi MH, Haverinen A, Luiro K, et al. Estradiol valerate in COC has more favorable inflammatory profile than synthetic ethinyl estradiol: a randomized trial. *J Clin Endocrinol Metab.* 2020;105:2483-2490.
21. Haverinen A, Luiro K, Kangasniemi MH, et al. Estradiol valerate vs. Ethinylestradiol in combined Oral contraceptives: effects on the pituitary-ovarian Axis. *J Clin Endocrinol Metab.* 2022;107:e3008-e3017.
22. Duarte RCF, Rios DRA, Rezende SM, et al. Standardization and evaluation of the performance of the thrombin generation test under hypo- and hypercoagulability conditions. *Hematol Transfus Cell Ther.* 2019;41:244-252.
23. Wiegratz I, Stahlberg S, Manthey T, et al. Effects of conventional or extended-cycle regimen of an oral contraceptive containing 30 mcg ethinylestradiol and 2 mg dienogest on various hemostasis parameters. *Contraception.* 2008;78:384-391.
24. Haverinen A, Kangasniemi M, Luiro K, Piltonen T, Heikinheimo O, Tapanainen JS. Ethinyl estradiol vs estradiol valerate in combined oral contraceptives – effect on glucose tolerance: a randomized, controlled clinical trial. *Contraception.* 2021;103:53-59.
25. Ljungkvist M, Strandberg K, Berntorp E, et al. Evaluation of a standardized protocol for thrombin generation using the calibrated automated thrombogram: a Nordic study. *Haemophilia.* 2019;25:334-342.
26. Skouby SO, Sidelmann JJ. Impact of progestogens on hemostasis. *Horm Mol Biol Clin Investig.* 2018;37(2).
27. Blanco-Molina MA, Lozano M, Cano A, Cristobal I, Pallardo LP, Lete I. Progestin-only contraception and venous thromboembolism. *Thromb Res.* 2012;129:e257-e262.
28. Heit JA, Spencer FA, White RH. The epidemiology of venous thromboembolism. *J Thromb Thrombolysis.* 2016;41:3-14.
29. Pomp ER, le Cessie S, Rosendaal FR, Doggen CJM. Risk of venous thrombosis: obesity and its joint effect with oral contraceptive use and prothrombotic mutations. *Br J Haematol.* 2007;139:289-296.
30. Vandenbroucke JP, Koster T, Briet E, Reitsma PH, Bertina RM, Rosendaal FR. Increased risk of venous thrombosis in oral-contraceptive users who are carriers of factor V Leiden mutation. *Lancet.* 1994;344:1453-1457.
31. Stanczyk FZ, Archer DF, Bhavnani BR. Ethinyl estradiol and 17β-estradiol in combined oral contraceptives: pharmacokinetics, pharmacodynamics and risk assessment. *Contraception.* 2013;87:706-727.
32. Mashchak CA, Lobo R, Dozono-Takano R, et al. Comparison of pharmacodynamic properties of various estrogen formulations. *Am J Obstet Gynecol.* 1982;144:511-518.
33. Odlind V, Milsom I, Persson I, Victor A. Can changes in sex hormone binding globulin predict the risk of venous thromboembolism with combined oral contraceptive pills? *Acta Obstet Gynecol Scand.* 2002;81:482-490.
34. The Oral Contraceptive and Hemostasis Study Group. The effects of seven monophasic oral contraceptive regimens on hemostatic variables: conclusions from a large randomized multicenter study. *Contraception.* 2003;67:173-185.
35. Rosing J, Tans G, Nicolaes GAF, et al. Oral contraceptives and venous thrombosis: different sensitivities to activated protein C in women using second- and third-generation oral contraceptives. *Br J Haematol.* 1997;97:233-238.

36. Van Vliet H, Bertina R, Dahm A, et al. Different effects of oral contraceptives containing different progestogens on protein S and tissue factor pathway inhibitor. *J Thromb Haemost*. 2008;6:346-351.

37. Hoffbrand AV, Moss PAH. Platelets, blood coagulation and haemostasis. *Essential Haematology*. 6th ed. Wiley-Blackwell; 2011:315-329.

38. Calzavarini S, Brodard J, Quarroz C, et al. Thrombin generation measurement using the ST Genesia thrombin generation system in a cohort of healthy adults: Normal values and variability. *Res Pract Thromb Haemost*. 2019;3:758-768.