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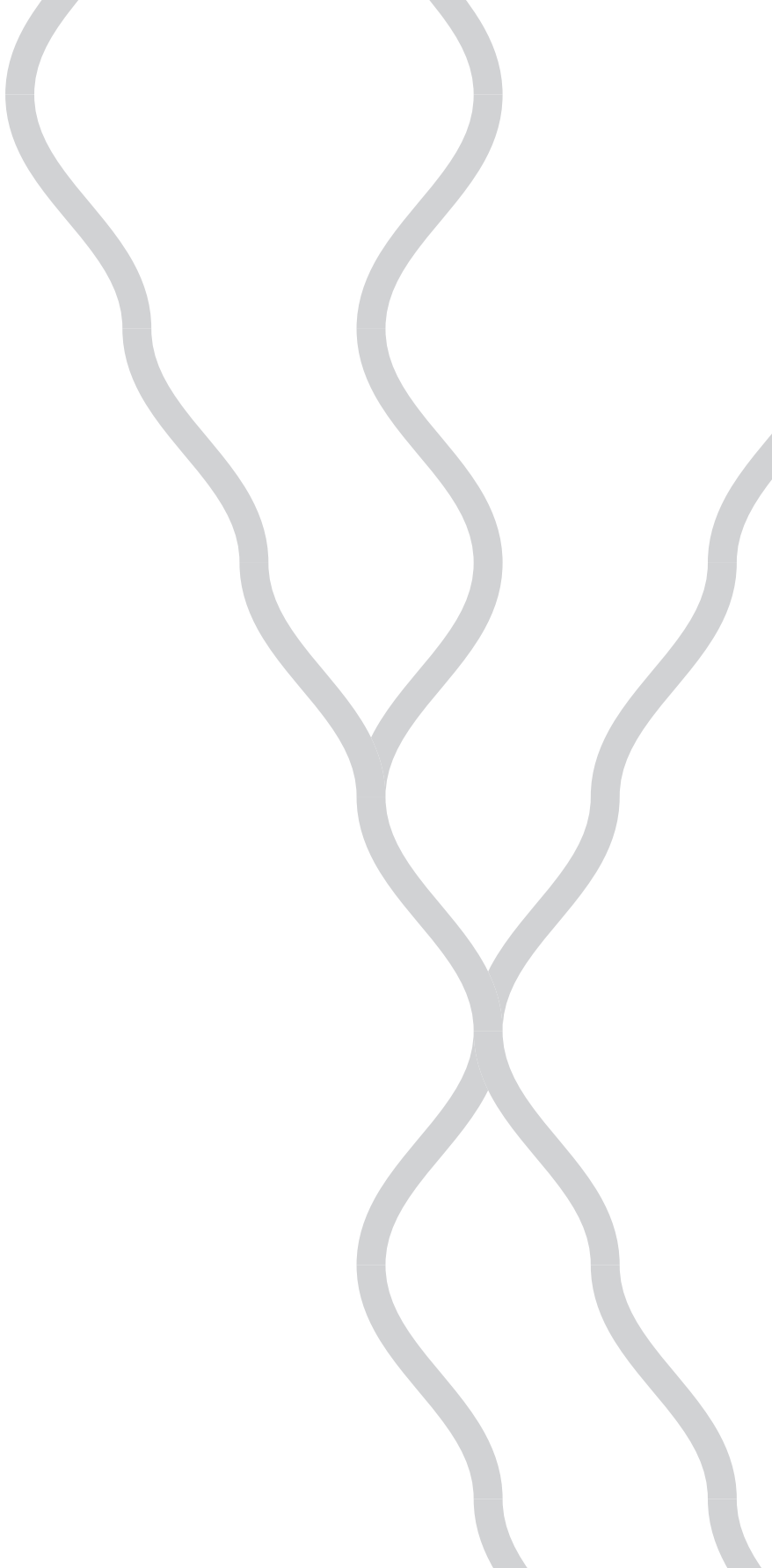


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Author: Raps, Marjolein

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Resistance to APC and SHBG levels
during use of a four-phasic oral
contraceptive containing dienogest
and estradiol valerate:
a randomized controlled trial

Marjolein Raps

Frits R. Rosendaal

Bart E.P.B. Ballieux

Jan Rosing

Stella Thomassen

Frans M. Helmerhorst

Huib A.A.M. van Vliet

Abstract

Background

The use of combined oral contraceptives is associated with a 3- to 6-fold increased risk of venous thrombosis. This increased risk depends on the estrogen dose as well as the progestogen type of combined oral contraceptives. Thrombin generation-based activated protein C resistance (APC resistance) and sex hormone-binding globulin (SHBG) levels predict the thrombotic risk of a combined hormonal contraceptive. Recently, a four-phasic oral contraceptive containing dienogest (DNG) and estradiol valerate (E2V) has been marketed. The aim of this study was to evaluate the thrombotic risk of the DNG/E2V oral contraceptive by comparing APC resistance by measuring normalized APC sensitivity ratios (nAPCsr) and SHBG levels in users of oral contraceptives containing dienogest and estradiol valerate (DNG/E2V) and oral contraceptives containing levonorgestrel and ethinyl estradiol (LNG/EE). Methods: We conducted a single-center, randomized, open label, parallel-group study in 74 women using DNG/E2V or LNG/EE, and measured nAPCsr and SHBG levels in every phase of the regimen of DNG/E2V.

Results

86 During the pill cycle SHBG levels did not differ between DNG/E2V users and LNG/EE users. nAPCsr levels were overall slightly lower in DNG/E2V users than in LNG/EE users, mean difference 0.44 (95% CI, 1.04 to 0.17) for day 2, 0.20 (95% CI, 0.76 to 0.37) for day 7, 0.27 (95% CI, 0.81 to 0.28) for day 24 and 0.34 (95% CI, 0.91 to 0.24) for day 26.

Conclusion

No statistical significant differences in nAPCsr and SHBG levels were found between users of the oral contraceptive containing DNG/E2V and LNG/EE, suggesting a comparable thrombotic risk.

Introduction

Use of combined oral contraceptives is associated with a 3- to 6-fold increased risk of venous thrombosis. This increased risk depends on the estrogen dose as well as the progestogen-type of combined oral contraceptives (1). So-called 'high-dose' combined oral contraceptives containing 50 µg or more ethinyl estradiol (EE) are associated with a 2-fold higher risk of thrombosis than 'low-dose' combined oral contraceptives containing 20–30 µg EE (2;3). Furthermore, combined oral contraceptives containing the progestogens gestodene (GTD), desogestrel (DSG), cyproterone acetate (CPA) or drospirenone (DRSP) increase the risk of venous thrombosis by a factor two compared with combined oral contraceptives containing levonorgestrel (LNG) (1–10).

The differences in the risk of venous thrombosis can at least partially be explained by the different effects of various combined oral contraceptives on the resistance to activated protein C (APC) as measured with the thrombin generation-based APC resistance test, and quantified via a normalized APC sensitivity ratio (nAPCsr) (11–13). High nAPCsr indicates increased APC resistance, which is a risk factor for venous thrombosis (14). The thrombin generation-based APC resistance test has been validated in a case–control study by Tans *et al.* (14) and discriminates well between oral contraceptives with a high risk of venous thrombosis (i.e. containing GTD, DSG, CPA or DRSP) and oral contraceptives with a lower risk of venous thrombosis (i.e. containing LNG) (3;6;9–13;15).

Sex hormone binding globuline (SHBG) is another marker that differentiates between combined oral contraceptives with a high and low risk of venous thrombosis (15–19). SHBG is a carrier protein, which is produced in the liver and binds estrogen and testosterone (20). Estrogens cause a dose-related increase of SHBG, whereas progestogens induce a decrease of SHBG, which depends on both the dose and type of progestogen (21;22). The effect of a hormonal contraceptive on SHBG is the net result of the estrogenic effect of estradiol and the antiestrogenic effect of the progestogen, yielding the total estrogenicity of that hormonal contraceptive. This estrogenicity serves as a marker for the thrombotic risk of a hormonal contraceptive (15–18).

Recently, a new, four-phasic, combined oral contraceptive containing dienogest and estradiol valerate (Qlaira; Bayer Schering Pharma, Berlin, Germany) was marketed. Dienogest (DNG) is a progestogen derived from the estrane structure and has antiandrogenic and no androgenic properties (23). Estradiol valerate (E2V) is an ester of the natural female hormone 17β-estradiol. The risk of venous thrombosis of this new oral contraceptive containing DNG/E2V is unknown.

In order to estimate this thrombotic risk we conducted a randomized controlled trial in which we compared APC resistance and SHBG levels during use of the four-phasic oral contraceptive containing DNG/E2V with a monophasic oral contraceptive containing LNG/EE. Our hypothesis was that the new oral contraceptive containing DNG/E2V has comparable levels of nAPCsr and SHBG as the oral contraceptive containing LNG/EE.

Material and methods

Study design and participants

We conducted a single-center, randomized, open label, parallel-group study in Leiden, the Netherlands. Participants were recruited between May 2010 and January 2011 by advertising in local newspapers and in public and university buildings. The study was approved by the Medical Ethics Committee of the Leiden University Medical Center. The trial registration number is NTR2354. The study was sponsored by the Department of Clinical Epidemiology of the Leiden University Medical Center.

Eligible participants were women aged 18–35 years who were willing to use either one of the studied combined oral contraceptives. Exclusion criteria were contraindications for oral contraceptive use as stated by the World Health Organization (24), pregnancy occurring up to 3 months before the study, use of anticoagulants or platelet aggregation inhibitors, and chronic or serious acute illness.

Participants were randomly assigned in a 1:1 ratio to either the four-phasic combined oral contraceptive containing DNG/E2V or the mono-phasic combined oral contraceptive containing LNG/EE. The four-phasic DNG/E2V contraceptive pill contains no DNG and 3 mg E2V on days 1–2, 2 mg DNG and 2 mg E2V on days 3–7, 3 mg DNG and 2 mg E2V on days 8–24, no DNG and 1 mg E2V on days 25–26 and a placebo on days 27–28 of the cycle. The mono-phasic LNG/EE contraceptive pill contains 150 µg LNG and 30 µg EE on days 1–21 and was not taken on days 22–28. Participants used the contraceptives according the prescription for three consecutive cycles and all started on the first day of their menstrual cycle. After inclusion, women completed a standardized questionnaire covering questions on risk factors for venous thrombosis.

Randomization was done by a computer-generated random allocation sequence. The treatment allocation sequence was concealed by sequentially numbered, opaque, sealed and stapled envelopes. The envelopes were kept by an independent person at a central office, outside of the department and not involved in the study. After inclusion of a participant by the researcher, the first following numbered envelope was opened at the central office; on the card inside was described whether the patient was randomized to the LNG/EE group or the DNG/E2V group. This information was given to the researcher and participant, and was sent to the pharmacy to provide the medication. Researchers and allocated participants were aware of the allocated arm; laboratory technicians were kept blinded to the allocation.

Eighty-eight participants were included (Fig. 1). Nine participants abandoned the trial before completion, of whom four participants discontinued because of side effects, two participants did not want to use an oral contraceptive anymore, two participants were lost to followup and one participant used the oral contraceptive incorrectly. All these participants abandoned the trial before the blood draws in the third cycle of use. Three participants turned out to be carriers of the factor V Leiden (FVL) mutation, and two participants were carriers of the prothrombin mutation

and were therefore excluded. The analysis was performed according to the per protocol principal, because we only want to use data during use of the contraceptive and missing data cannot be replaced. The number of participants who were lost to follow-up is balanced in both groups. In our final analysis, we used the data of 74 participants: 35 users (47%) of DNG/E2V and 39 users (53%) of LNG/EE.

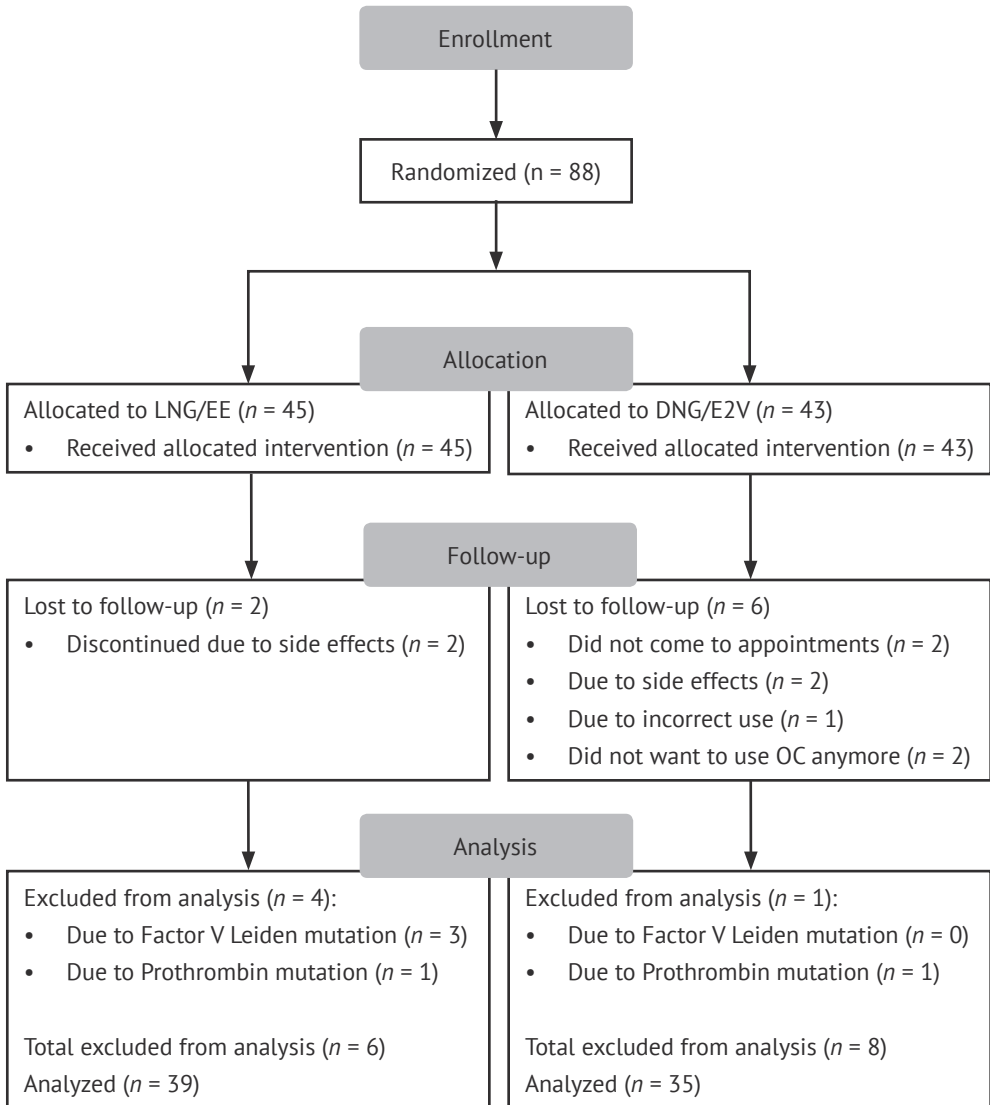


Fig. 1. Flow diagram of exclusions.

Laboratory methods

The primary outcomes were APC resistance measured by the thrombin-generation-based APC resistance test resulting in normalized APC sensitivity ratios (nAPCsr) and SHBG levels during the third month of use. According to two studies of Wiegratz *et al.*, SHBG levels are stable after 1 month of use (25;26). Blood samples were taken at inclusion and on days 2, 7, 24 and 26 in the third month of use. There was no wash out period. The blood samples were drawn from the antecubital vein in a fasting state in the morning and collected in 0.106 M sodium citrate (pH 5.8) and SST tubes (serum) (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Cell-free, citrated plasma was prepared by centrifuging blood at 2.100 g for 10 min at 18 °C, coded and centrally stored at 80 °C within 3 h after blood draw.

APC resistance was measured with the thrombin generation-based APC resistance test as described before (13).

SHBG (nM) was measured in serum with an immunometric assay (Immulite 2000 XPI; Siemens Healthcare Diagnostics, Tarrytown NY, USA). The sensitivity is 0.2 nM and has a long-term variation of 6% at both levels of 5 and 80 nM. The within-assay variation is 3–4% and the between-assay variation 3.5 to 6%.

90 After finishing the collection of blood samples the presence of the factor V Leiden mutation and the prothrombin G20210A mutation were measured in one run by DNA analysis. Carriers of a mutation were excluded.

Statistical analysis

Thirty-six participants had to be included in each group to detect a difference in nAPCsr of 0.52, which was previously observed between oral contraceptives containing levonorgestrel and desogestrel (27) and considered relevant. We used a significance level of 0.05, a power of 80% and an anticipated dropout rate of 10%.

We used means, mean differences, 95% confidence intervals and ranges to describe variables. We calculated P-values by performing t-tests and chi-squared tests to evaluate whether baseline characteristics were well balanced between the groups. A multivariate analysis was performed to investigate whether baseline characteristics had an influence on the outcomes. We performed t-tests to calculate mean differences of nAPCsr and SHBG levels between users of DNG/E2V and users of LNG/EE, separately calculated for the different phases of DNG/ E2V. We constructed bar diagrams to compute the figures. No interim analyses were performed. Statistics were computed using SPSS for Windows, version 20.0 (SPSS Inc., Chicago, IL, USA).

Results

There were no differences in BMI, age and smoking habits between DNG/E2V users and LNG/EE users, as shown in Table 1. There were no participants with thrombophilia, diabetes mellitus, history of venous thrombosis or other cardiovascular diseases and no participants used drugs on a regular basis (data not shown). A multivariate analysis including the variables age, BMI, smoking habit and family history shows that the outcome cannot be explained by differences in these variables (data not shown).

Over 93% of all participants used an oral contraceptive at the time of inclusion. Almost 80% of these oral contraceptives were second-generation pills containing LNG. No participants used an intrauterine device (IUD) or vaginally or transdermally administered contraceptives before inclusion. One participant used a hormonal implant before inclusion, which was removed before start of participation.

Table 1: Baseline characteristics

	LNG/EE	DNG/E2V	P-value
N (%)	39 (53)	35 (47)	-
Age, years (range)	22.7 (19–31)	21.9 (18–32)	0.43
BMI, kg/m ² (range)	22.1 (18–30)	22.3 (19–28)	0.59
Smoking (%)			0.76
Non-customer	33 (84.6)	28 (80.0)	
Current	4 (10.3)	4 (11.4)	
Past	2 (5.1)	3 (8.6)	
Contraceptive before study, n (%)			0.31
None	2 (5.1)	2 (5.7)	
Oral contraceptive	36 (92.3)	33 (94.3)	
2nd generation	32 (82)	23 (65.7)	
3rd generation	1 (2.6)	0	
DRSP	1 (2.6)	4 (11.4)	
CPA	0	3 (8.6)	
DNG	0	1 (2.9)	
Unknown	2 (5.1)	2 (5.7)	
Implant	1 (2.6)	0	
IUD	0	0	
Transdermal/vaginal	0	0	
History of venous thrombosis, n	0	0	N/A
Family history of venous thrombosis, first degree, n (%)	2 (5.1)	2 (5.1)	0.62

SHBG levels and nAPCsr in DNG/E2V users and in LNG/EE users

During the pill cycle SHBG levels did not differ between DNG/E2V users and LNG/EE users, as shown in Table 2 and Fig. 3.

nAPCsr levels were overall lower in DNG/E2V users than in LNG/EE users, mean difference 0.44 (95% CI, 1.04 to 0.17) for day 2, 0.20 (95% CI, 0.76 to 0.37) for day 7, 0.27 (95% CI, 0.81 to 0.28) for day 24 and 0.34 (95% CI, 0.91 to 0.24) for day 26 (Table 2 and Fig. 2).

SHBG levels and nAPCsr within the cycle

The nAPCsr did not differ during the pill cycle in users of LNG/EE and DNG/E2V (Fig. 2).

In DNG/E2V users as well as in LNG/EE users SHBG levels were lower at the beginning of the pill cycle than at the end of the pill cycle (Fig. 3).

For LNG/EE users, the mean difference in SHBG was 7.10 nM (95% CI, 4.79 to 18.97) between days 2 and 7, 23.53 nM (95% CI, 10.41 to 36.65) between days 7 and 24 and 1.51 nM (95% CI, 11.78 to 14.81) between days 24 and 26.

For DNG/E2V users, the same pattern was observed: the mean difference in SHBG was 7.91 nM (95% CI, 3.48 to 19.30) between days 2 and 7, 33.44 nM (95% CI, 18.57 to 48.31) between days 7 and 24 and 2.79 nM (95% CI, 13.00 to 18.59) between days 24 and 26.

Overall, the mean difference in SHBG between days 2 and 26 was 32.14 nM (95% CI, 20.10 to 44.20) for LNG/EE users, and 44.15 nM (95% CI, 31.57 to 56.72) for DNG/E2V users.

Table 2: Means, mean differences and confidence intervals of SHBG levels and nAPCsr at different phases in DNG/E2V compared with LNG/EE

Phase	Pill	Contents	SHBG (nM)			nAPCsr		
			Mean	MD	95% CI	Mean	MD	95% CI
Phase 1, day 2	LNG/EE	EE 30 µg, LNG 150 µg,	50.87	Ref	-11.47 to 10.39	3.26	Ref	1.04 to 0.17
	DNG/E2V	E2V 3 mg	50.33	-0.54		2.83	-0.44	
Phase 2, day 7	LNG/EE	EE 30 µg, LNG 150 µg	57.96	Ref	-12.37 to 12.94	3.30	Ref	0.76 to 0.37
	DNG/E2V	E2V 2 mg, DNG 2 mg	58.24	-0.28		3.11	-0.20	
Phase 3, day 24	LNG/EE	Pill-free interval	81.49	Ref	-5.15 to 25.53	3.23	Ref	0.81 to 0.28
	DNG/E2V	E2V 2 mg, DNG 3 mg	91.68	10.19		2.97	-0.27	
Phase 4, day 26	LNG/EE	Pill-free interval	83.00	Ref	-2.31 to 25	3.21	Ref	0.91 to 0.24
	DNG/E2V	E2V 1 mg	94.47	11.47		2.88	-0.34	

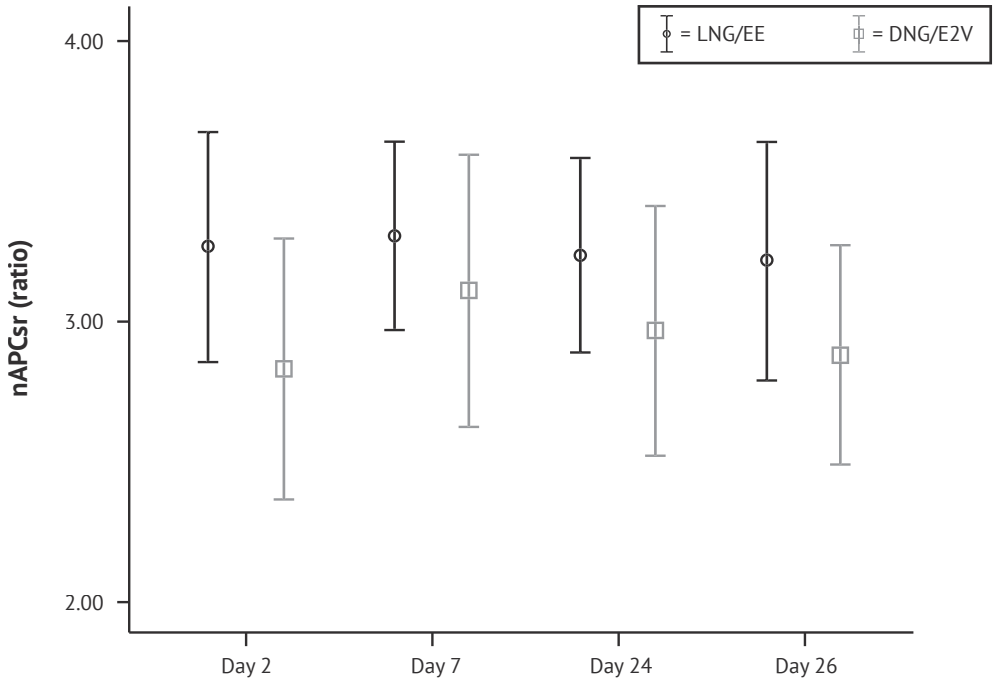


Fig. 2. Mean nAPCsr levels (ratio) and 95% confidence intervals during use of LNG/EE and DNG/E2V, subdivided by cycle day.

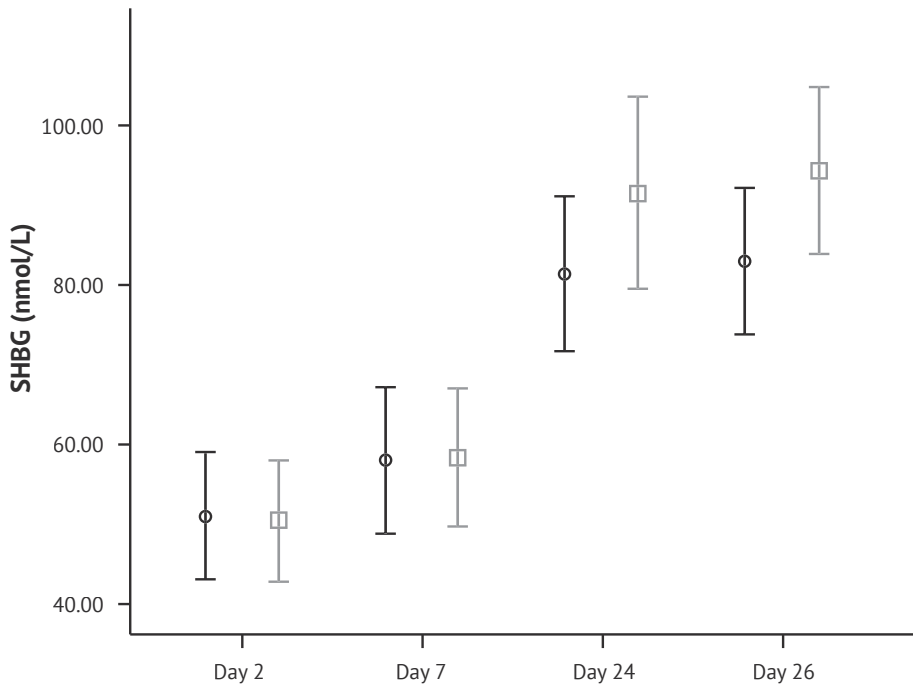


Fig. 3. Mean SHBG levels (nmol/L) and 95% confidence intervals during use of LNG/EE and DNG/E2V, subdivided by cycle day.

Discussion

In this randomized controlled trial on the effects of a four-phasic oral contraceptive containing DNG/E2V and a mono-phasic oral contraceptive containing LNG/EE on APC resistance and SHBG, we observed no significant differences between the two oral contraceptives. During the cycle, SHBG levels increased gradually in both DNG/ E2V users and in LNG/EE users. No difference in APC resistance was observed during the cycle.

The thrombin generation-based APC resistance test used in this study predicts the risk of venous thrombosis and discriminates well between oral contraceptives with a high and low thrombotic risk (14). SHBG is a marker that differentiates between combined oral contraceptives with a high and low risk of venous thrombosis (15–19). In our study we did not observe a difference in APC resistance and SHBG levels between a new oral contraceptive containing DNG/E2V and a low-risk combined oral contraceptive containing LNG/EE. These findings suggest that DNG/E2V and LNG/EE have a comparable thrombotic effect and risk of venous thrombosis. However, clinical studies assessing the absolute and relative risk of venous thrombosis in women using DNG/E2V are indicated to confirm this.

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Our study is the first clinical, independent trial in which a group with representative sample size was randomized between LNG/EE and DNG/E2V. Two studies sponsored by the manufacturer that were published during the preparation of our manuscript show lower nAPCsr and SHBG levels for DNG/E2V compared with LNG/EE. Klipping *et al.* (28) conducted a randomized, open label, cross-over study of DNG/E2V and monophasic LNG/EE and observed lower nAPCsr and lower SHBG levels in 25 users of DNG/E2V than in 25 users of LNG/EE. Junge *et al.* (29) performed a randomized, open label study and also observed less pronounced SHBG levels in 30 users of DNG/E2V than in 28 users of triphasic LNG/EE. In our study, nAPCsr levels were overall lower in users of DNG/E2V, but the differences were not statistically significant. Based on the results of these three studies, it can be stated that DNG/E2V does not lead to a more thrombogenic state compared with LNG/EE.

E2V seems to have a favorable effect on thrombotic markers compared with EE. The oral contraceptive used in our study containing DNG/E2V is the first marketed combined oral contraceptive containing E2V. Before, most combined oral contraceptives contained EE as an estrogen compound. In a study by Wiegratz *et al.* (25) users of oral contraceptives containing 2 mg DNG and 20 µg EE had higher SHBG levels than users of oral contraceptives containing 100 µg LNG and 20 µg EE. In our study no differences in SHBG were observed between users of DNG/E2V and LNG/EE. No studies that assessed the effect of DNG/EE on nAPCsr have been conducted and no studies assessing the risk of venous thrombosis due to combined oral contraceptives containing E2V as estrogen content have been performed.

During the cycle, increasing SHBG levels were observed, even in the pill-free interval of users of LNG/ EE. This is in agreement with other studies. Devineni *et al.* (30) observed increasing SHBG levels during the cycle in users of the contraceptive patch containing norelgestromin and EE, and users of the combined oral contraceptive containing norgestimate and EE. Wiegratz *et al.* (25)

observed increasing SHBG levels throughout the cycle during use of four different combined oral contraceptives. As the biological half-life of SHBG is supposed to be around 2–4 days, this can be explained by decreasing levels of SHBG, which do not reach baseline levels during the pill-free interval. This indicates that the cyclic variability of SHBG during use of oral contraceptives should be taken into account in future studies; SHBG should be measured at the same moment in the cycle to prevent bias caused by increasing SHBG levels throughout the cycle.

Some potential limitations need to be addressed. Caution is required when surrogate markers are used, as they can be severely misleading (31). Preferably, a surrogate marker is validated in a prospective trial. nAPCsr is a validated surrogate marker; SHBG is not validated yet, but is a recommended measurement before marketing of a new hormonal contraceptive by the European Medicines Agency (EMA). In the case of very rare events, such as venous thrombosis during combined hormonal contraceptive use, a clinical study is almost unfeasible before marketing, due to the required number of participants (24;32).

Remarkably, no increasing nAPCsr levels were observed throughout the cycle and there was no correlation found between SHBG levels and nAPCsr levels (data not shown). In previous studies, a correlation between SHBG and nAPCsr was observed in users of hormonal contraceptives (15;19). This might be explained by the sample size of our study, which is probably too small to demonstrate a correlation.

In conclusion, we found similar SHBG levels and APC resistance in users of DNG/E2V and LNG/EE-containing oral contraceptives, which suggests a similar thrombotic risk for both oral contraceptives. Since oral contraceptives containing DNG/EE causes a stronger rise in SHBG levels, the similar effects found in this study might be explained by a favorable effect of E2V compared with EE. Future studies are indicated to assess whether E2V and EE have different effects on hemostatic and other parameters and on the risk of venous thrombosis. Epidemiological studies are needed to confirm the hypothesis that DNG/E2V and LNG/EE are equally safe regarding the risk of venous thrombosis.

References

- 1 Vandenbroucke JP, Rosing J, Bloemenkamp KWM, Middeldorp S, Helmerhorst FM, Bouma BN, Rosendaal FR. Oral contraceptives and the risk of venous thrombosis. *N Engl J Med* 2001; 344: 1527–34.
- 2 Rosendaal FR, van Hylckama Vlieg A, Tanis BC, Helmerhorst FM. Estrogens, progestogens and thrombosis. *J Thromb Haemost* 2003; 1: 1371–80.
- 3 van Hylckama Vlieg A, Helmerhorst FM, Vandenbroucke JP, Doggen CJ, Rosendaal FR. The venous thrombotic risk of oral contraceptives, effects of oestrogen dose and progestogen type: results of the MEGA case–control study. *BMJ* 2009; 339: b2921.
- 4 Bloemenkamp KW, Rosendaal FR, Helmerhorst FM, Buller HR, Vandenbroucke JP. Enhancement by factor V Leiden mutation of risk of deep vein thrombosis associated with oral contraceptives containing a third-generation progestagen. *Lancet* 1995; 346: 1593–6.
- 5 Effect of different progestagens in low oestrogen oral contraceptives on venous thromboembolic disease. World Health Organization Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception. *Lancet* 1995; 346: 1582–8.
- 6 Lidegaard O, Lokkegaard E, Svendsen AL, Agger C. Hormonal contraception and risk of venous thromboembolism: national follow-up study. *BMJ* 2009; 339: b2890.
- 7 Jick H, Jick SS, Gurewich V, Myers MW, Vasilakis C. Risk of idiopathic cardiovascular death and nonfatal venous thromboembolism in women using oral contraceptives with differing progestagen components. *Lancet* 1995; 346: 1589–93.
- 8 van Hylckama Vlieg A, Middeldorp S. Hormone therapies and venous thromboembolism: where are we now? *J Thromb Haemost* 2011; 9: 257–66.
- 9 Jick SS, Hernandez RK. Risk of non-fatal venous thromboembolism in women using oral contraceptives containing drospirenone compared with women using oral contraceptives containing levonorgestrel: case–control study using United States claims data. *BMJ* 2011; 342: d2151.
- 10 Parkin L, Sharples K, Hernandez RK, Jick SS. Risk of venous thromboembolism in users of oral contraceptives containing drospirenone or levonorgestrel: nested case–control study based on UK General Practice Research Database. *BMJ* 2011; 342: d2139.
- 11 Rosing J, Middeldorp S, Curvers J, Christella M, Thomassen LG, Nicolaes GA, Meijers JC, Bouma BN, Buller HR, Prins MH, Tans G. Low-dose oral contraceptives and acquired resistance to activated protein C: a randomised cross-over study. *Lancet* 1999; 354: 2036–40.
- 12 van Vliet HA, Winkel TA, Noort I, Rosing J, Rosendaal FR. Prothrombotic changes in users of combined oral contraceptives containing drospirenone and cyproterone acetate. *J Thromb Haemost* 2004; 2: 2060–2.
- 13 Tchaikovski SN, van Vliet HA, Thomassen MC, Bertina RM, Rosendaal FR, Sandset PM, Helmerhorst FM, Tans G, Rosing J. Effect of oral contraceptives on thrombin generation measured via calibrated automated thrombography. *Thromb Haemost* 2007; 98: 1350–6.
- 14 Tans G, van Hylckama Vlieg A, Thomassen MC, Curvers J, Bertina RM, Rosing J, Rosendaal FR. Activated protein C resistance determined with a thrombin generation-based test predicts for venous thrombosis in men and women. *Br J Haematol* 2003; 122: 465–70.
- 15 Raps M, Helmerhorst F, Fleischer K, Thomassen S, Rosendaal F, Rosing J, Ballieux B, van Vliet H. Sex hormone-binding globulin as a marker for the thrombotic risk of hormonal contraceptives. *J Thromb Haemost* 2012; 10: 992–7.
- 16 Stegeman BH, Raps M, Helmerhorst FM, Vos HL, van Vliet HA, Rosendaal FR, van Hylckama Vlieg A. Effect of ethinylestradiol dose and progestagen in combined oral contraceptives on plasma sex hormone binding globulin levels in premenopausal women. *J Thromb Haemost* 2013; 11: 203–5.

- 17 Odland 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: 482–90.
- 18 van Vliet HA, Rosendaal FR, Rosing J, Helmerhorst FM. Sex hormone-binding globulin: an adequate surrogate marker for venous thromboembolism in women using new hormonal contraceptives. *Contraception* 2009; 79: 328–30.
- 19 van Vliet HA, Frolich M, Christella M, Thomassen LG, Doggen CJ, Rosendaal FR, Rosing J, Helmerhorst FM. Association between sex hormone-binding globulin levels and activated protein C resistance in explaining the risk of thrombosis in users of oral contraceptives containing different progestogens. *Hum Reprod* 2005; 20: 563–8.
- 20 Gardner DG. *Greenspan's Basic & Clinical Endocrinology*, 8th edn. New York: McGraw-Hill Companies Inc., 2007.
- 21 van der Vange N, Blankenstein MA, Kloosterboer HJ, Haspels AA, Thijssen JH. Effects of seven low-dose combined oral contraceptives on sex hormone binding globulin, corticosteroid binding globulin, total and free testosterone. *Contraception* 1990; 41: 345–52.
- 22 Knopp RH, Broyles FE, Cheung M, Moore K, Marcovina S, Chandler WL. Comparison of the lipoprotein, carbohydrate, and hemostatic effects of phasic oral contraceptives containing desogestrel or levonorgestrel. *Contraception* 2001; 63: 1–11.
- 23 Sitruk-Ware R, Mishell DR. *Progestins and Antiprogestins in Clinical Practice*. New York: Marcel Dekker Inc., 2000.
- 24 WHO. Medical eligibility criteria for contraceptive use. 4th edn, Geneva: WHO, 2009. Available at http://www.who.int/reproductivehealth/publications/family_planning/9789241563888/en/index.html
- 25 Wiegatz I, Kutschera E, Lee JH, Moore C, Mellinger U, Winkler UH, Kuhl H. Effect of four different oral contraceptives on various sex hormones and serum-binding globulins. *Contraception* 2003; 67: 25–32.
- 26 Wiegatz I, Jung-Hoffmann C, Kuhl H. Effect of two oral contraceptives containing ethinylestradiol and gestodene or norgestimate upon androgen parameters and serum binding proteins. *Contraception* 1995; 51: 341–6.
- 27 Tans G, Curvers J, Middeldorp S, Thomassen MC, Meijers JC, Prins MH, Bouma BN, B€uller HR, Rosing J. A randomized cross-over study on the effects of levonorgestrel- and desogestrel-containing oral contraceptives on the anticoagulant pathways. *Thromb Haemost* 2000; 84: 15–21.
- 28 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 versus ethinylestradiol/levonorgestrel. *Drugs R D*. 2011; 11: 159–70.
- 29 Junge W, Mellinger U, Parke S, Serrani M. Metabolic and haemostatic effects of estradiol valerate/dienogest, a novel oral contraceptive: a randomized, open-label, single-centre study. *Clin Drug Investig* 2011; 31: 573–84.
- 30 Devineni D, Skee D, Vaccaro N, Massarella J, Janssens L, La-Guardia KD, Leung AT. Pharmacokinetics and pharmacodynamics of a transdermal contraceptive patch and an oral contraceptive. *J Clin Pharmacol* 2007; 47: 497–509.
- 31 Stanczyk FZ, Grimes DA. Sex hormone-binding globulin: not a surrogate marker for venous thromboembolism in women using oral contraceptives. *Contraception* 2008; 78: 201–3. 32 Cardiovascular disease and steroid hormone contraception. Report of a WHO Scientific Group. *World Health Organ Tech Rep Ser* 1998; 877: 1–89.