Hormonal contraceptives and venous thrombosis

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Chapter 3

Effect of ethinylestradiol and progestagen in combined oral contraceptives on plasma sex hormone binding globulin levels in premenopausal women

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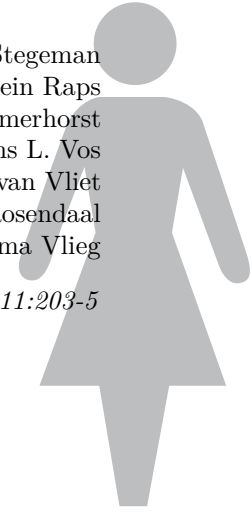
J. Thromb. Haemost. 2013;11:203-5



Abstract

Background: Sex hormone binding globulin (SHBG) levels may be a marker for the risk of venous thrombosis in oral contraceptive users. While the effects of different progestagen types on SHBG levels are well established, the association between the ethinylestradiol dose in combined oral contraceptives and SHBG levels remains to be studied.
Objectives: To determine the effect of the ethinylestradiol dose on SHBG levels.
Methods: Healthy premenopausal women using a combined oral contraceptive were included from a case-control study (MEGA study, N=181) and a cross-over study (DRSP study, N=101). Women exposed to risk factors for venous thrombosis (except for oral contraceptive use) were excluded. Mean differences with 95% confidence intervals were estimated, adjusted for confounders and depending on the analysis adjusted for the progestagen used.
Results: A total of 282 women were included from the MEGA and DRSP study. The mean SHBG level in these women was 139.5 nmol/L (95%CI: 131.2 to 147.8). After restriction to 30 µg ethinylestradiol, users of desogestrel, gestodene, or drospirenone had about 100 nmol/L higher SHBG levels than levonorgestrel users. SHBG levels were higher in users of ≥35 µg ethinylestradiol (mean difference: 136.4, 95%CI: 64.5 to 208.3) and in users of triphasic contraceptives (mean difference 50.9 nmol/L, 95%CI: 20.7 to 81.1) than in users of 20 µg ethinylestradiol. No difference was observed between users of 20 µg and 30 µg ethinylestradiol.
Conclusions: An increase in ethinylestradiol dose is associated with an increase in SHBG levels in combined oral contraceptive users.

Introduction

The use of combined oral contraceptives, containing an estrogen (i.e. ethinylestradiol) and a progestagen, is associated with an increased risk of venous thrombosis[1–5]. Because the estrogen compound in combined oral contraceptives was thought to cause the increased risk of venous thrombosis, the dose of ethinylestradiol has over time been reduced from ≥100 µg via 50 µg to 30 µg or 20 µg, indeed resulting in a lower risk of venous thrombosis[6–9]. The type of progestagen in combined oral contraceptives also affects the risk of venous thrombosis, e.g., the risk of venous thrombosis is higher in users of third generation combined oral contraceptives (containing desogestrel or gestodene) and in users of cyproterone acetate than in users of second generation combined oral contraceptives (containing levonorgestrel)[8–11]. Furthermore, in users of preparations containing ethinylestradiol and drospirenone (introduced in 2001) a sixfold increased risk of venous thrombosis compared with non-users was observed[8,9], which was later confirmed in two other studies[12,13].

Results from recent studies have suggested that the effect of a combined oral contraceptive on sex hormone binding globulin (SHBG) levels could be an indicator for the risk of venous thrombosis[14–16]. SHBG is a plasma glycoprotein that binds the sex steroid hormones testosterone and 17β-estradiol but not ethinylestradiol. SHBG is primarily produced in hepatocytes and variation in its plasma levels is due to multiple regulating factors such as age, body weight, sex steroids, and insulin. Users of combined oral contraceptives containing a third generation progestagen have higher SHBG levels than users of a second generation progestagen[14,15,17–19] reflecting the difference in venous thrombosis risk. In accordance with the hypothesis that SHBG levels are a marker of the risk of venous thrombosis, SHBG levels in combined oral contraceptives users are positively associated with thrombin generation-based activated protein C (APC) resistance[16]. APC resistance is the relative inability of protein C to

cleave activated factor V or activated factor VIII thereby leading to a more prothrombotic state. APC resistance has been shown to predict venous thrombosis risk in both men and women[20].

While SHBG levels have been shown to reflect the difference in venous thrombosis risk between different types of progestagen, the estrogen compound is thought to be the most important factor determining the venous thrombosis risk. If SHBG levels can be considered to be a marker for venous thrombosis and ethinylestradiol is the main compound in combined oral contraceptives causing venous thrombosis, then the ethinylestradiol dose in combined oral contraceptives should be reflected in SHBG levels. The aim of this study was to determine whether an increase in ethinylestradiol dose results in higher SHBG levels in healthy premenopausal women. Additionally, we assessed the effect of different progestagens on SHBG levels.

Methods

Participants Participants were selected from a large case-control study, i.e., the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA) study and from a crossover study, i.e., the DRSP (drospirenone/ethinylestradiol) study. In the MEGA study, participants with a first deep venous thrombosis in the leg or arm or pulmonary embolism were recruited between 1 March 1999 and 31 August 2004 (N=4930). Controls were either the partners of the patients or were recruited via random digit dialling (RDD) (N=6287). All participants were asked to fill in a questionnaire and to provide a blood or a buccal swab sample. Details of the study have been described elsewhere[21]. In the DRSP study, healthy women using the same type of combined oral contraceptive for at least four cycles were recruited between July and November 2002 (N=156). In this study, women were asked to switch from their current contraceptive to an oral contraceptive containing either levonorgestrel or drospirenone. All women were asked to fill in a questionnaire and

to provide a blood sample. Blood was drawn between days 18 and 21 of the pill-cycle. Only data on contraceptive use at baseline and blood samples collected before the switch were used for the current analysis. Women were excluded if there were contraindications for combined oral contraceptive use as stated by the World Health Organization, i.e., women with a history of deep venous thrombosis or pulmonary embolism, women with current deep venous thrombosis or pulmonary embolism or women undergoing major surgery and prolonged immobilization. Details of the study have been described elsewhere[16].

From the MEGA study, we selected premenopausal women without venous thrombosis (i.e., controls); both partner controls and controls recruited through RDD were included (N=1689). Women with known environmental thrombotic risk factors were excluded, i.e., women who had any type of cancer (N=26), had been hospitalized (N=154), underwent surgery (N=109), had bone fractures (N=28), or had injuries (N=274) in the twelve months before the index date. Because some women were exposed to one or more environmental risk factors for venous thrombosis, a total of 467 women were excluded . We also excluded women who were pregnant (N=65), were within four weeks postpartum (N=1) or were using hormone replacement therapy (N=13) at the index date or experienced a miscarriage (N=8) in the twelve months before the index date. Because we were interested in the effect of ethinylestradiol on SHBG levels, women who were using a progestagen-only contraceptive were also excluded (N=23). Blood samples were needed for the SHBG measurement; therefore women who did not provide a blood sample were excluded (N=661). We excluded women who did not use a combined oral contraceptive at the time of venipuncture (N=279). For the current analysis, this resulted in the inclusion of 181 healthy premenopausal women using combined oral contraceptives of which 73 women were partners of cases and 108 were recruited through RDD.

From the DRSP study, we excluded women exposed to known

environmental thrombotic risk factors, i.e., women who had any type of cancer (N=2), had been hospitalized (N=21), underwent surgery (N=10), had bone fractures (N=2), or had injuries (N=31) in the twelve months before the index date. All women were using a combined oral contraceptive at the index date and had given a blood sample. For the current analysis, this resulted in the inclusion of 101 healthy premenopausal women.

Laboratory measurements In the MEGA study, the day of a woman's four week cycle of pill use (3 weeks of pill use followed by a pill-free week) was not taken into account when inviting her to the clinic for a blood sample. Therefore, blood was drawn randomly during the four week cycle of pill use; however, whether the women were menstruating at venipuncture was recorded. In the DRSP study, blood was drawn between days 18 and 21 of the four week cycle of pill-use.

Collection and processing of blood samples have been described previously[16,21]. In short, for both studies, blood was drawn after an overnight fasting for food, caffeine and alcohol and collected in vacuum tubes containing 0.106 mol/L trisodium citrate as anticoagulant. Blood was centrifuged to retrieve cell-free, citrated plasma.

SHBG levels (nmol/L) were measured with an immunometric assay (Immulite; DPC, USA). The sensitivity is 0.2 nmol/L and has a long-term variation of 6% both at levels of 5 nmol/L and 80 nmol/L. The within-assay variation is 3 to 4% and the between-assay variation 3.5 to 6%. The samples were analysed in one series in random order. SHBG levels were measured without knowledge of the type of oral contraceptive used or any other of the participant's characteristics.

Statistical analysis The ethinylestradiol dose was categorised into four categories, i.e., 20 µg, 30 µg, ≥35 µg per pill and triphasic preparations. Triphasic contraceptives have varying ethinyl-

estradiol and progestagen doses per pill over 21 days. In the MEGA study, women were using triphasic contraceptives with the following regimen: 30 µg of ethinylestradiol in the first six days, 40 µg for five days and 30 µg for the last ten days. In the MEGA study, one woman used a biphasic contraceptive; during 21 days the dose of ethinylestradiol is 50 µg, the progestagen is only included in the preparation in the last fourteen days. Because the dose of ethinylestradiol does not change over 21 days, this woman was categorised in the ≥35 µg group. In the DRSP study, all women were using a monophasic contraceptive. For nine women, information on the ethinylestradiol dose in the combined oral contraceptive was not available, however information on progestagen used was available. For descriptive purposes, the progestagens used in the contraceptives were divided into second generation (i.e., levonorgestrel), third generation (i.e., gestodene, desogestrel, and norgestimate) and other progestagens (i.e., cyproterone acetate, drospirenone and first generation progestagens lynestrenol and norethisteron). For the calculation of mean differences in SHBG levels, the progestagens were not grouped by generation but separately evaluated.

The effect of the progestagen and dose of ethinylestradiol on SHBG levels was assessed using linear regression analysis. The analysis was adjusted for study and to ensure that the effect of the ethinylestradiol dose on SHBG levels is independent of the progestagen used, we adjusted this analysis for the progestagen used in the combined oral contraceptive. The analysis of the effect of the progestagen in combined oral contraceptives in association with SHBG levels was restricted to subjects taking 30 µg ethinylestradiol per contraceptive pill.

To reduce random variation in SHBG levels, the analyses were adjusted for multiple variables which can influence SHBG levels. The data were adjusted for whether women were menstruating at the time of venipuncture, and for age and BMI, which are known determinants of SHBG levels in non-users [22–24]. Results were expressed as the mean difference with 95% confi-

dence interval. Statistical analyses were performed with STATA, version 11.2 (Statacorp LP, College Station, TX, USA).

Results

Overall, 282 women using a combined oral contraceptive from the MEGA study (N=181) and the DRSP study (N=101) were included. The general characteristics of the combined population and separate per study are displayed in table 3.1. On average, women from the MEGA study were 8 years older than women from the DRSP study (mean difference: 8, 95%CI: 6 to 10). Women from both studies had a BMI of about 23 kg/m^2. In the MEGA study, 60% (N=109) of the women were using a second generation progestagen, while only 35% (N=35) of the women from the DRSP study were using this progestagen. The most frequently used dose of ethinylestradiol was 30 µg per pill in both studies (100 (58%) and 65 (64%) women in the MEGA and DRSP study, respectively). 32 women (19%) from the MEGA study were using a triphasic contraceptive. The mean SHBG plasma level was about the same in both studies (MEGA study: 143.5 nmol/L, 95%CI: 132.9 to 154.0, IQR 94.8, range 31.2 to 390.9 & DRSP study: 132.3 nmol/L, 95%CI: 118.9 to 145.7, IQR 106.0, range 28.0 to 284.0). The mean SHBG plasma level in women of both studies was 139.5 nmol/L (95%CI: 131.2 to 147.8, IQR 99.8, range 28.0 to 390.9). Both studies are combined in further analyses which were adjusted for study and potential confounders (i.e., age and BMI).

The results in table 3.2 show that not all combinations of ethinylestradiol dose and progestagen were present. A combined oral contraceptive containing 30 µg ethinylestradiol was most often combined with the second generation progestagen levonorgestrel, whereas a contraceptive with 20 µg ethinylestradiol with a third generation progestagen (i.e., desogestrel, gestodene, or norgestimate).

Menstruating at venipuncture may have affected the SHBG

Table 3.1: Baseline characteristics of study participants

Variables	MEGA study (N=181)	DRSP study (N=101)	Combined (N=282)
Age, mean(range)	36 (18-50)	27 (18-51)	33 (18-51)
BMI, mean(range)	23.4 (15.7-37.9)	23.3 (18.3-37.7)	23.3 (15.7-37.9)
Menstruating at venipuncture (%)[a]	11 (6)	-	11 (4)
Progestagen type (%)			
2nd,[b]	109 (60)	35 (35)	144 (51)
3rd,[c]	46 (25)	37 (37)	83 (29)
Other[d]	26 (14)	29 (29)	55 (20)
EE dose (%)[e]			
20 µg	14 (8)	17 (17)	31 (11)
30 µg	100 (58)	65 (64)	165 (60)
≥35 µg	26 (15)	19 (19)	45 (16)
Triphasic[f]	32 (19)	-	32 (12)

BMI, body mass index; EE, ethinylestradiol

[a] Data was available of 174 women from the MEGA study

[b] Second generation progestagen only includes levonorgestrel (N=144)

[c] Third generation progestagen include desogestrel (N=55), gestodene (N=24) and norgestimate (N=4)

[d] Other progestagen include lynestrenol (N=6), norethisteron (N=2), cyproterone acetate (N=30) and drospirenone (N=17)

[e] No information was available on the ethinylestradiol dose in nine women

[f] Triphasic contraceptives contain 30 µg in the first six days, followed by 40 µg for five days and ending with ten days of 30 µg ethinylestradiol

levels. In the MEGA study, SHBG levels were compared between menstruating women versus women taking a pill at venipuncture. 11 women were menstruating at time of venipuncture and the mean SHBG level was 102.1 nmol/L (95%CI: 59.1 to 145.0) whereas the mean SHBG level of the remaining women who were taking a pill (N=163) was 145.4 nmol/L (95%CI: 134.3 to 156.6). The mean difference was 43.4 nmol/L (95%CI: -1.0 to 87.7). Therefore, in addition to age, BMI and study, the linear regression analyses were adjusted for menstruating at venipuncture.

Table 3.3 shows the association of progestagen and ethinyles-

Table 3.2: Distribution of progestagen type and ethinylestradiol dose

Progestagen		**EE dose, n (%)**			
		20 µg	30 µg	≥ 35 µg	Triphasic‡
2nd,*		3 (10)	99 (60)	2 (5)	31 (97)
3rd,†		28 (90)	49 (30)	5 (14)	1 (3)
Other	Cyproterone acetate	0	0	30 (81)	0
	Drospirenone	0	17 (10)	0	0

EE, ethinylestradiol

* Second generation progestagen only includes levonorgestrel (N=135)

† Third generation progestagen include desogestrel (N=55), gestodene (N=24) and norgestimate (N=4)

‡ Triphasic contraceptives contain 30 µg in the first six days, followed by 40 µg for five days and ending with ten days of 30 µg ethinylestradiol

tradiol dose with SHBG levels. When we restricted our analysis to women receiving 30 µg of ethinylestradiol, users of desogestrel, gestodene, and drospirenone had higher SHBG levels than users of levonorgestrel (mean difference: 112.8 nmol/L, 95%CI: 97.3 to 128.2, 80.6 nmol/L, 95%CI: 57.3 to 104.0, and 111.1 nmol/L, 95%CI: 89.8 to 132.3 for desogestrel, gestodene, and drospirenone, respectively). Adjustment for factors influencing SHBG levels did not change these results.

Additional to the progestagens levonorgestrel, gestodene, desogestrel, and drospirenone, 30 women used cyproterone acetate. In contrast with these other progestogens, a contraceptive with cyproterone acetate contains 35 µg ethinylestradiol. The mean SHBG level in users of cyproterone acetate was high at 215.9 nmol/L (95%CI: 199.7 to 232.1); much higher than in users of oral contraceptives containing levonorgestrel with 30 µg ethinylestradiol (mean difference: 135.4 nmol/L, 95%CI: 116.9 to 153.9 adjusted for study and menstruating at venipuncture).

Users of ≥35 µg of ethinylestradiol had higher SHBG levels than users of 20 µg (mean difference: 145.4 nmol/L, 95%CI:

Table 3.3: Linear regression analysis

Variable	N (%)	Mean SHBG levels* (95%CI)	Adjusted difference* (95%CI)	Adjusted difference† (95%CI)
Progestagen‡				
Levonorgestrel	99 (60)	80.3 (72.3 to 88.2)	Reference	Reference
Desogestrel	36 (22)	193.0 (179.9 to 206.2)	112.8 (97.3 to 128.2)	116.9 (101.1 to 132.7)
Gestodene	13 (8)	160.9 (138.8 to 183.0)	80.6 (57.3 to 104.0)	81.5 (56.3 to 106.6)
Drospirenone	17 (10)	191.3 (171.8 to 210.9)	111.1 (89.8 to 132.3)	114.3 (93.1 to 135.5)
EE dose				
20 µg	31 (11)	101.6 (80.4 to 122.8)	Reference	Reference
30 µg	165 (60)	115.3 (103.9 to 126.8)	13.8 (-7.1 to 34.6)	13.9 (-8.3 to 36.2)
≥35 µg	45 (16)	247.0 (200.6 to 293.4)	145.4 (87.1 to 203.7)	136.4 (64.5 to 208.3)
Triphasic	32 (12)	152.5 (132.7 to 172.4)	51.0 (22.8 to 79.1)	50.9 (20.7 to 81.1)

CI, confidence interval; EE, ethinylestradiol
* Adjusted for progestagen in the case of ethinylestradiol dose and adjusted for study
† Further adjusted for age, BMI and menstruating at venipuncture
‡ Restricted to 30 µg ethinylestradiol

87.1 to 203.7). Also users of triphasic contraceptives had higher SHBG levels than users of 20 µg of ethinylestradiol (mean difference: 51.0 nmol/L, 95%CI: 22.8 to 79.1). The SHBG levels were only slightly higher in users of 30 µg compared with 20 µg of ethinylestradiol (mean difference: 13.8 nmol/L, 95%CI: -7.1 to 34.6). Adjustment for factors influencing SHBG levels did not change these results.

The same results were observed when the analysis was restricted to most commonly used progestagens (levonorgestrel, desogestrel and gestodene) or separately per these progestagens, although the number of women per category was very small (data not shown). Furthermore, similar results were observed when the analysis was performed per study (Supplementary table).

Discussion

When restricting to combined oral contraceptive preparations with 30 µg ethinylestradiol, users of combined oral contraceptives containing desogestrel, gestodene, and drospirenone had higher SHBG levels than users of levonorgestrel. Cyproterone acetate use was also associated with higher SHBG levels than levonorgestrel use, although we cannot exclude an effect caused by the difference in ethinylestradiol dose. Women using a combined oral contraceptive with ≥35 µg ethinylestradiol or women using a triphasic contraceptive had higher SHBG levels than women using a combined oral contraceptive with 20 µg. However, SHBG levels were only slightly higher in 30 µg ethinylestradiol users than in 20 µg users.

Estrogens such as ethinylestradiol increase the synthesis of SHBG[25], whereas progestagens induce a decrease in SHBG levels depending on the type and dose used[14,26]. In women receiving 15 µg of ethinylestradiol without a progestagen, the SHBG levels increased from 213.5 nmol/L on day 1 to 661.9 nmol/L on day 21 of the pill-cycle[27]. In contrast, women using 150 µg levonorgestrel without ethinylestradiol showed a decrease in the

SHBG levels over 23 days from 40.4 nmol/L to 15.5 nmol/L[28]. The effect of a combined oral contraceptive on SHBG levels may be seen as the result of the stimulating effect of ethinylestradiol and the inhibiting effect of the progestagen in the contraceptive[14]. The final net change is sometimes referred to as the total estrogenicity of the contraceptive. It has been suggested that this may reflect the magnitude of the risk of venous thrombosis[14].

In the literature, one paper reported on the effect of different oral contraceptives as well as the effect of the ethinylestradiol dose in combined oral contraceptives on SHBG levels; however, the difference in SHBG levels before and after a contraceptive was reported. No difference in SHBG levels between different contraceptives was stated[29]. No conclusions were drawn on whether the ethinylestradiol dose in different combined oral contraceptives was reflected in SHBG levels.

The positive association between ethinylestradiol dose and SHBG levels is in line with previous findings regarding the risk of venous thrombosis. Lidegaard et al reported that compared with users of oral contraceptive preparations containing 30-40 µg ethinylestradiol, the risk of venous thrombosis was higher in users of 50 µg ethinylestradiol (OR 1.6, 95%CI: 0.9 to 2.8) and lower in users of 20 µg (OR 0.6, 95%CI: 0.4 to 0.9)[30]. In the MEGA study, we also demonstrated that within users of oral contraceptives containing levonorgestrel, the risk of venous thrombosis adjusted for age was higher in users of 50 µg ethinylestradiol (OR 2.2, 95%CI: 1.3 to 3.7) than in users of 30 µg[9]. The risk of venous thrombosis was lower in users of 20 µg than in users of 30 µg; both in users of progestagens gestodene (OR 0.3, 95%CI: 0.2 to 0.7) and desogestrel (OR 0.7, 95%CI: 0.4 to 1.2).

Unfortunately, ethinylestradiol levels could not be measured directly because the blood was drawn at random during the four week cycle of pill use in the MEGA study and without considering the hours after a pill was taken, which both have a significant influence on ethinylestradiol levels[31]. Because of a half-life of SHBG of about 7 days[32], the hours after a pill was taken do

not influence the SHBG levels. Data were available on factors that were previously shown to influence SHBG levels and on whether women were menstruating at venipuncture. Regarding the analysis between ethinylestradiol dose and SHBG levels, we would have preferred to restrict our analysis to one progestagen; however, the number of women per category became very small leading to unreliable estimates. We combined two studies that differed in their design, which may have affected our results. However, sensitivity analyses showed that this did not influence our results. Finally, although we excluded women exposed to environmental risk factors, women with a positive family history were included. Nevertheless, we do not expect that having a positive family history influenced SHBG levels. Strengths of our study were that we included a relative large number of combined oral contraceptive users who were using many different types of prescriptions. Furthermore, SHBG levels as well as the difference in SHBG levels between different progestagens in combined oral contraceptive users were in the same range as observed in other studies[33–35].

In conclusion, users of the progestagens desogestrel, gestodene, and drospirenone had increased SHBG levels compared with levonorgestrel users. An increase in the ethinylestradiol dose in the combined oral contraceptive leads to an increase in the SHBG levels in premenopausal women using these combined oral contraceptives. This study demonstrates that SHBG levels reflect the ethinylestradiol dose used in combined oral contraceptives independent of the progestagen used. Because ethinylestradiol is important in the pathogenesis of venous thrombosis among combined oral contraceptive users, these findings strengthen the idea that SHBG levels in combined oral contraceptive users may be seen as a marker for the risk of venous thrombosis.

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Supplementary data

Supplementary table Results of sensitivity analysis per study

Variable	MEGA study Adjusted difference SHBG levels* (95%CI)	DRSP study Adjusted difference SHBG levels* (95%CI)
Progestagen†		
Levonorgestrel	Reference	Reference
Desogestrel	125.9 (103.4 to 148.4)	106.6 (84.7 to 128.4)
Gestodene	91.0 (61.4 to 120.7)	44.9 (-8.3 to 98.1)
Drospirenone	94.1 (55.9 to 132.4)	124.9 (99.8 to 150.0)
EE dose		
20 µg	Reference	Reference
30 µg	4.8 (-30.1 to 39.7)	12.5 (-15.8 to 40.8)
≥35 µg	130.8 (49.8 to 211.7)	141.2 (73.3 to 209.0)
Triphasic	45.2 (4.4 to 86.0)	-

CI, confidence interval; EE, ethinylestradiol

* Adjusted for progestagen in the case of ethinylestradiol dose, study, menstruating at venipuncture, age and BMI

† Restricted to 30 µg ethinylestradiol