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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

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BACKGROUND: Epidemiological studies have shown that both the estrogen dose and progestogen type of oral contraceptives contribute to the increased risk of thrombosis in oral contraceptive users. Thrombin generation-based activated protein C (APC) sensitivity is a global test for the net prothrombotic effect of oral contraceptives and predicts the thrombotic risk. Our objective was to test the usefulness of sex hormone-binding globulin (SHBG) as a marker for the thrombotic risk of an oral contraceptive. METHODS: We measured SHBG and APC resistance in 156 healthy users of various types of oral contraceptives. RESULTS: Users of oral contraceptives with a moderately increased risk of thrombosis (gestodene and desogestrel pills) had higher SHBG levels than users of low-risk oral contraceptives containing levonorgestrel. Similarly, for higher doses of estrogen in oral contraceptives we found higher SHBG levels. Women using oral contraceptives with the highest thrombotic risk (cyproterone acetate pills) rendered the highest SHBG levels. Users of oral contraceptives containing gestodene, desogestrel or cyproterone acetate were more resistant to APC than users of levonorgestrel pills. SHBG levels were positively associated with the increased APC resistance. CONCLUSIONS: Our findings support the hypothesis that the effect of an oral contraceptive on SHBG levels might be a marker for the thrombotic risk.

Key words: APC resistance/oral contraceptives/SHBG/venous thrombosis

Introduction

Epidemiological studies have shown that both the estrogen dose as well as the progestogen type of oral contraceptives contribute to the increased risk of venous thrombosis in oral contraceptive users (Vandenbroucke *et al.*, 2001). So called 'high-dose' oral contraceptives containing $\geq 50\,\mu g$ ethinylestradiol are associated with a higher risk of thrombosis than 'low-dose' oral contraceptives containing $20-30\,\mu g$ ethinylestradiol (Rosendaal *et al.*, 2003). Further, combined oral contraceptives containing the third-generation progestogens gestodene and desogestrel or the progestogen cyproterone acetate are more thrombogenic than oral contraceptives containing the second-generation progestogen levonorgestrel (Kemmeren *et al.*, 2001; Vasilakis-Scaramozza and Jick, 2001).

Use of oral contraceptives causes changes in procoagulant, anticoagulant and fibrinolytic parameters, resulting in a net prothrombotic effect (Vandenbroucke *et al.*, 2001). This prothrombotic effect can be measured globally by a thrombin generation-based APC resistance test (Rosing *et al.*, 1999). The test outcome predicts the risk of venous thrombosis, in users of oral contraceptives as well as in non-users and men (Tans *et al.*, 2003). Supporting the epidemiological observations, users of oral contraceptives containing desogestrel, gestodene or cyproterone acetate were found more resistant to the anticoagulant action of activated protein C (APC) by this test than users of oral contraceptives containing levonorgestrel (Rosing *et al.*, 1999; van Vliet *et al.*, 2004). We found the same for users of a new oral contraceptive containing drospirenone, for which no post-marketing data are currently available, but which safety with regard to thrombosis has been questioned (Sheldon, 2002; van Vliet *et al.*, 2004).

Recently a literature study and a randomized controlled trial postulated that the effect of an oral contraceptive on sex hormone-binding globulin (SHBG) levels could be an indicator for the thrombotic risk of that oral contraceptive (Odlind *et al.*, 2002; van Rooijen *et al.*, 2004). SHBG produced in the liver is a carrier protein for estrogen and testosterone.

Estrogens cause a dose-related increase in SHBG, whereas progestogens induce a decrease of SHBG, the extent of which depends on both dose and type of progestogen (Anderson, 1974; El Makhzangy *et al.*, 1979; van der Vange *et al.*, 1990; Knopp *et al.*, 2001). The type-related differences in progestogen-induced decrease of SHBG might be interpreted as differences in anti-estrogenic properties of progestogens. Thus, the effect of an oral contraceptive on SHBG levels can be seen as the sum of the estrogenic effect of ethinylestradiol and the anti-estrogenic effect of the progestogen resulting in the total estrogenicity of the pill (van Kammen *et al.*, 1975; Odlind *et al.*, 2002).

The literature study demonstrated a relationship between the known thrombotic risk of second-generation, third-generation and cyproterone acetate-containing oral contraceptives and the effect of the various types of oral contraceptives on SHBG (Odlind *et al.*, 2002). In agreement with the increased risk of thrombosis, gestodene- and desogestrel-containing contraceptive pills were more estrogenic, i.e. increased SHBG more, than levonorgestrel-containing pills (Odlind *et al.*, 2002). Oral contraceptives containing cyproterone acetate were associated with the highest SHBG levels (Odlind *et al.*, 2002). A randomized controlled trial comparing SHBG levels in women using desogestrel-containing oral contraceptives and women using levonorgestrel-containing oral contraceptives confirmed the higher levels of SHBG in desogestrel-containing pill users (van Rooijen *et al.*, 2004). In addition, an association between SHBG levels and the resistance to APC measured with the classical activated partial thromboplastin time-based APC resistance test was reported (van Rooijen *et al.*, 2004).

To test the usefulness of SHBG as a marker for the thrombotic risk of an oral contraceptive, we compared the plasma levels of SHBG and the resistance to APC determined with a thrombin generation-based APC resistance test in users of oral contraceptives containing either second- or third-generation progestogens or drospirenone or cyproterone acetate. Our *a priori* hypotheses were: (i) the plasma levels of SHBG in women using oral contraceptives known to confer an increased risk of venous thrombosis are higher than in women using contraceptive pills with levonorgestrel; and (ii) the resistance to APC in women using oral contraceptives follows the same pattern and is associated with SHBG levels.

Materials and methods

Study design and participants

Healthy women using the same type of oral contraceptive for at least four cycles were recruited by advertising in local newspapers, public and university buildings, student houses, pharmacies and general practitioners' waiting rooms. Exclusion criteria were age (<18 years) and contraindications for oral contraceptive use as stated by World Health Organization (1996). After inclusion, women completed a standardized questionnaire covering questions on risk factors for venous thrombosis. Blood samples were drawn between days 18 and 21 of the menstrual cycle. After the blood donation, drospirenone- or cyproterone acetate-containing oral contraceptive users were requested to switch to a second-generation oral contraceptive composed of 150 μg levonorgestrel and 30 μg ethinylestradiol (Microgynon-30®; Schering) and second- or third-generation oral contraceptive users were asked to switch to the drospirenone-containing oral contraceptive. A second blood sample was taken between days 18 and 21 of the fourth cycle after the change of type of oral contraceptive.

The Medical Ethics Committee of the Leiden University Medical Center, Leiden, The Netherlands approved the study. All volunteers gave written informed consent.

Risk ranking per oral contraceptive

Based on the risk of thrombosis reported in the literature, we can rank oral contraceptives by estrogen dose and progestogen type. High-dose oral contraceptives containing ≥ 50 μg ethinylestradiol have been associated with a higher risk of thrombosis than low-dose oral contraceptives containing 20–30 μg ethinylestradiol (Gerstman *et al.*, 1991). Data from the ongoing Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA) study indicate a 1.9-fold higher risk of thrombosis with a 10 μg increase in estrogen dose (van Hylckama Vlieg, 2003). Concerning the progestogen component, it was shown that combined oral contraceptives containing the third-generation progestogens gestodene and desogestrel or the progestogen cyproterone acetate are more thrombogenic than oral contraceptives containing the second-generation progestogen levonorgestrel. A recent meta-analysis concluded that gestodene- and desogestrel-containing oral contraceptives increases the risk of thrombosis by a factor 1.5–1.7 compared to levonorgestrel-containing oral contraceptives (Kemmeren *et al.*, 2001). Oral contraceptives containing cyproterone acetate confer the highest thrombotic risk, 3.9-fold higher than oral contraceptives containing levonorgestrel (Vasilakis-Scaramozza and Jick, 2001).

Laboratory methods

Blood samples were taken from the antecubital vein in the morning after an overnight abstinence from intake of food, caffeine and alcohol. Nine parts of blood were collected in one part 0.106 mol/l sodium citrate (pH 5.8). Cell-free, citrated plasma was prepared by centrifuging plasma at 2100*g* for 10 min at 18°C, coded and centrally stored at −80°C.

Normalized APC sensitivity ratios (nAPCsr) were determined by quantifying the effect of APC on thrombin generation (thrombin generation-based or ETP-based APC resistance test) as described before (Rosing *et al.*, 1997). SHBG was measured with an immunometric assay (Immulite; DPC, USA). The sensitivity is 0.2 nmol/l; the variation coefficient according to the manufacturer ranges from 5.8 to 13% for the inter-assay variation from high to very low levels.

The samples were analysed in one series in random order. SHBG levels and APC resistance were measured without knowledge of the oral contraceptive used or any other of the participant's characteristics. The APC resistance test was performed in duplicate.

Statistical analysis

Mean SHBG plasma levels and mean nAPCsr in users of oral contraceptives containing norethindrone, levonorgestrel, norgestimate, gestodene, desogestrel, drospirenone or cyproterone acetate were calculated. A scatter diagram and a regression line were constructed with SHBG levels as the independent variable and nAPCsr as the dependent variable. The degree of association between SHBG levels and nAPCsr was measured by Pearson's correlation coefficient.

Results

Between July and November 2002, 158 healthy women aged 18–51 years were recruited. We excluded two women, one because of a history of diabetes mellitus and one because of a history of venous thrombosis. Sixty women used a second-generation oral contraceptive containing levonorgestrel, 49 women a third-generation oral contraceptive containing gestodene, desogestrel or norgestimate, 23 women a drospirenone-containing contraceptive pill, 22 women an oral contraceptive containing cyproterone acetate and two women a first-generation oral contraceptive containing norethindrone (Table I).

Forty-six women agreed to switch from oral contraceptive type, of whom 40 returned for a second blood donation. Five women in the group of switchers discontinued due to breast tenderness, increase in acne and hirsutism, pregnancy wish, a previously unreported history of high blood pressure or surgery. One woman was lost to follow-up. One participant using a cyproterone acetate-containing pill was erroneously prescribed the drospirenone pill instead of a levonorgestrel-containing oral contraceptive and as a consequence excluded from the analyses. During the study, none of the participants experienced serious adverse events.

For the analyses of the first blood donation 156 women were included, of whom 39 were included in the analysis of the second blood donation. The various groups of oral contraceptive users did not differ with respect to age and body mass index (Table I).

Users of oral contraceptives with a moderately increased risk, i.e. gestodene- and desogestrel-containing pills, had SHBG plasma levels that were higher than for users of low-risk oral contraceptives containing levonorgestrel (Table II). Likewise, for higher doses of estrogen in oral contraceptives we found higher SHBG levels, i.e. women using oral contraceptives containing 30 μg ethinylestradiol rendered higher SHBG levels than women using oral contraceptives containing 20 μg ethinylestradiol. The difference in SHBG levels between third-generation pills containing gestodene and desogestrel and second-generation pills containing levonorgestrel was observed for both 20 μg and 30 μg ethinylestradiol oral contraceptives. In the group of women taking pills containing 20 μg ethinylestradiol, mean SHBG plasma levels were 111 nmol/l (95% CI 90–131) for users of gestodene pills and 143 nmol/l (95% CI 110–175) for users of desogestrel pills, while for users of levonorgestrel pills mean SHBG levels were 63 nmol/l (95% CI 36–91). Similarly, in the group of women taking oral contraceptives containing 30 μg ethinylestradiol, mean SHBG levels were 136 nmol/l (95% CI 66–205) for users of gestodene pills and 164 nmol/l (95% CI 144–185) for users of desogestrel pills compared to

Table I. Participants' characteristics

Progestogen	Estrogen	*n*	Age (years)		Body mass index (kg/m^2)	
			Mean	95% CI	Mean	95% CI
100 μg LNG	20 μg EE	5	22.2	18.1 to 26.3	21.9	19.8 to 23.9
75 μg GTD	20 μg EE	8	26.4	20.4 to 32.3	22.2	19.8 to 24.6
150 μg DSG	20 μg EE	13	30.1	25.0 to 35.1	24.5	21.8 to 27.2
150 μg LNG	30 μg EE	55	28.8	26.4 to 31.2	23.1	22.2 to 24.0
75 μg GTD	30 μg EE	4	26.0	14.9 to 37.1	23.1	20.6 to 25.5
150 μg DSG	30 μg EE	20	30.7	26.5 to 34.9	23.9	22.4 to 25.4
2 mg CPA	35 μg EE	22	27.5	24.4 to 30.6	22.1	21.3 to 22.9
3 mg DRSP	30 μg EE	23	27.6	24.7 to 30.4	24.4	22.9 to 25.8
250 μg NGM	35 μg EE	4	26.8	16.7 to 36.8	23.3	16.7 to 29.9
1 mg NET	35 μg EE	2	30.0	−71.7 to 131.7	20.6	19.9 to 21.2

CI = confidence interval; LNG = levonorgestrel; EE = ethinylestradiol; GTD = gestodene; DSG = desogestrel; CPA = cyproterone acetate; DRSP = drospirenone; NGM = norgestimate; NET = norethindrone.

Table II. Mean thrombin generation-based normalized activated protein C sensitivity ratios (nAPCsr) and mean sex hormone-binding globulin (SHBG) levels in women using different types of oral contraceptives

Progestogen	Estrogen	*n*	nAPCsr		SHBG (nmol/l)	
			Mean	95% CI	Mean	95% CI
100 μg LNG	20 μg EE	5	3.22	1.30 to 5.15	63	36 to 91
75 μg GTD	20 μg EE	8	3.40	2.52 to 4.28	111	90 to 131
150 μg DSG	20 μg EE	13	3.82	3.44 to 4.20	143	110 to 175
150 μg LNG	30 μg EE	55	2.97	2.69 to 3.25	63	59 to 67
75 μg GTD	30 μg EE	4	4.19	3.20 to 5.19	136	66 to 205
150 μg DSG	30 μg EE	20	4.24	3.68 to 4.79	164	144 to 185
2 mg CPA	35 μg EE	22	4.00	3.74 to 4.26	210	182 to 238
3 mg DRSP	30 μg EE	23	4.13	3.53 to 4.74	167	143 to 190

LNG = levonorgestrel; EE = ethinylestradiol; GTD = gestodene; DSG = desogestrel; CPA = cyproterone acetate; DRSP = drospirenone.

Table III. Mean thrombin generation-based normalized activated protein C sensitivity ratios (nAPCsr) and mean sex hormone-binding globulin (SHBG) levels in women without the factor V Leiden or prothrombin 20210A mutation using different types of oral contraceptives

Progestogen	Estrogen	*n*	nAPCsr		SHBG (nmol/l)	
			Mean	95% CI	Mean	95% CI
100 μg LNG	20 μg EE	4	2.55	1.80 to 3.31	68	33 to 103
75 μg GTD	20 μg EE	7	3.12	2.43 to 3.81	112	88 to 136
150 μg DSG	20 μg EE	12	3.91	3.55 to 4.27	144	109 to 179
150 μg LNG	30 μg EE	52	2.78	2.59 to 2.98	63	59 to 68
75 μg GTD	30 μg EE	3	4.18	2.29 to 6.08	137	4 to 270
150 μg DSG	30 μg EE	16	3.96	3.56 to 4.35	163	138 to 189
2 mg CPA	35 μg EE	21	3.96	3.70 to 4.23	209	180 to 238
3 mg DRSP	30 μg EE	19	3.69	3.22 to 4.17	168	140 to 195

LNG = levonorgestrel; EE = ethinylestradiol; GTD = gestodene; DSG = desogestrel; CPA = cyproterone acetate; DRSP = drospirenone.

Table IV. Change in thrombin generation-based normalized activated protein C sensitivity ratios (nAPCsr) and sex hormone-binding globulin (SHBG) levels in women switching from oral contraceptive type

Oral contraceptive		*n*	Mean nAPCsr			Mean SHBG (nmol/l)		
Before	After		Before	After	Difference (95% CI)	Before	After	Difference (95% CI)
100 μg LNG/20 μg EE	3 mg DRSP/30 μg EE	1	2.49	2.79	+0.30 (−)	43	120	+77 (−)
150 μg LNG/30 μg EE	3 mg DRSP/30 μg EE	13	3.18	3.66	+0.47 (+0.15 to +0.80)	63	144	+81 (+61 to +101)
3 mg DRSP/30 μg EE	150 μg LNG/30 μg EE	5	3.55	2.73	−0.83 (−1.80 to +0.15)	157	80	−77 (−129 to −25)
2 mg CPA/35 μg EE	150 μg LNG/30 μg EE	6	3.96	3.00	−0.96 (−1.66 to −0.26)	235	85	−150 (−206 to −94)
150 μg DSG/20 μg EE	3 mg DRSP/30 μg EE	4	3.50	3.90	+0.40 (−0.20 to +1.00)	189	215	+25 (+6 to +44)
150 μg DSG/30 μg EE	3 mg DRSP/30 μg EE	4	4.07	4.04	−0.03 (−0.32 to +0.27)	147	181	+34 (0 to +68)
75 μg GTD/20 μg EE	3 mg DRSP/30 μg EE	3	2.78	2.79	+0.01 (−2.45 to +2.47)	104	120	+16 (−91 to +122)
250 μg NGM/35 μg EE	3 mg DRSP/30 μg EE	2	4.61	4.93	+0.31 (−0.99 to +1.62)	123	183	+61 (−22 to +143)
1 mg NET/35 μg EE	3 mg DRSP/30 μg EE	1	3.73	2.36	−1.38 (−)	138	306	+168 (−)

LNG = levonorgestrel; DRSP = drospirenone; EE = ethinylestradiol; DSG = desogestrel; GTD = gestodene; NGM = norgestimate; NET = norethindrone; CPA = cyproterone acetate.

63 nmol/l (95% CI 59–67) for users of levonorgestrel pills. For oral contraceptives with the highest risk of thrombosis, i.e. cyproterone acetate-containing pills, we found the highest SHBG levels (mean 210 nmol/l; 95% CI 182–238).

Women using oral contraceptives with a moderate or highly increased thrombotic risk (desogestrel, gestodene and cyproterone acetate pills) had higher nAPCsr than women using oral contraceptives containing levonorgestrel (Table II). Mean nAPCsr in the group of women using 20 μg ethinylestradiol oral contraceptives were 3.4 (95% CI 2.5–4.3) for gestodene pills, 3.8 (95% CI 3.4–4.2) for desogestrel pills compared to 3.2 (95% CI 1.3–5.2) for levonorgestrel pills. In the groups of 30 μg or 35 μg ethinylestradiol oral contraceptive users mean nAPCsr were 4.2 (95% CI 3.2–5.2) for gestodene-containing oral contraceptive users, 4.2 (95% CI 3.7–4.8) for desogestrel-containing oral contraceptive users and 4.0 (95% CI 3.7–4.3) for cyproterone acetate-containing oral contraceptive users, while for users of levonorgestrel-containing oral contraceptives the mean nAPCsr was 3.0 (95% CI 2.7–3.3). Users of the recently introduced pill containing drospirenone had a mean nAPCsr similar to that of users of third-generation oral contraceptives (mean 4.1; 95% CI 3.5–4.7). After exclusion of 14 participants with the factor V Leiden mutation and five participants with the prothrombin 20210A mutation, mean nAPCsr for gestodene, desogestrel, cyproterone acetate and drospirenone oral contraceptive users all remained markedly higher than for levonorgestrel oral contraceptive users (Table III).

In the 39 women who switched from oral contraceptive type, SHBG levels and nAPCsr altered correspondingly, e.g. SHBG levels and nAPCsr decreased when switching from a cyproterone acetate-containing pill to a levonorgestrel-containing pill, and similarly, SHBG and nAPCsr increased when switching from an oral contraceptive containing levonorgestrel to an oral contraceptive containing drospirenone (Table IV).

SHBG plasma levels were positively associated with nAPCsr in the 156 oral contraceptive users, i.e. an increase of SHBG levels of 100 nmol/l was associated with an increase of nAPCsr of 0.7 (95% CI 0.4–0.9) over the range of measurements made. Pearson's correlation coefficient was 0.4 ($P < 0.001$). After exclusion of 14 women with the factor V Leiden and five women with the prothrombin 20210A mutation, the beta of the regression equation slightly decreased, i.e. an elevation of SHBG levels of 100 nmol/l was associated with an elevation of the nAPCsr of 0.6 (95% CI 0.4–0.8) over the range of measurements made (Figure 1). Pearson's correlation coefficient increased to 0.5 ($P < 0.001$).

Discussion

In this study of SHBG plasma levels and prothrombotic effects among 156 healthy users of various types of oral contraceptives, we observed a positive association between the effect of an oral contraceptive on SHBG levels or

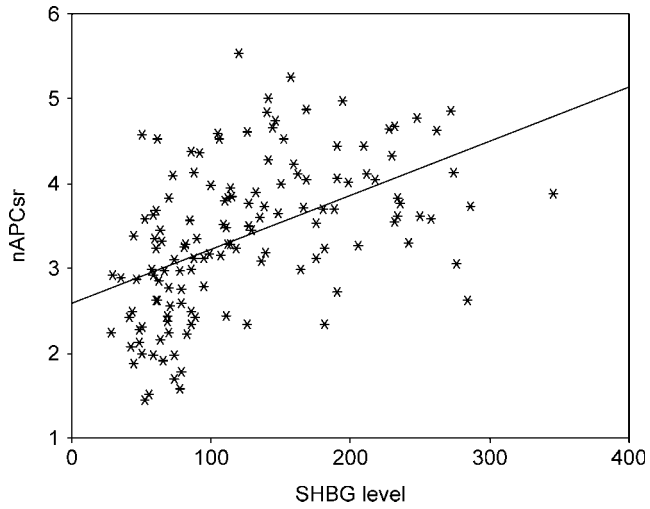

Figure 1. Scatter diagram and linear regression line of sex hormone-binding globulin (SHBG) plasma levels and nAPCsr in 137 women without the factor V Leiden and prothrombin 20210A mutation using different types of oral contraceptives. An increase of SHBG plasma levels of 100 nmol/l was associated with an increase of nAPCsr of 0.6 (95% CI 0.4–0.8). Pearson's correlation coefficient $= 0.5$ ($P < 0.001$).

estrogenicity of the formulation and the thrombotic risk of a formulation as reported in the literature. Users of oral contraceptives with a moderately increased risk, i.e. third-generation oral contraceptives containing gestodene or desogestrel, had SHBG levels that were higher than in users of low-risk, second-generation oral contraceptives containing levonorgestrel (Vandenbroucke *et al.*, 2001). Similarly, for higher doses of estrogen in oral contraceptives, we observed higher SHBG plasma levels (Rosendaal *et al.*, 2003). Users of oral contraceptives with the highest risk of thrombosis, i.e. cyproterone acetate-containing pills, also rendered the highest SHBG levels (Vasilakis-Scaramozza and Jick, 2001).

In addition, SHBG plasma levels were positively associated with the resistance to the anticoagulant action of APC determined with a thrombin generation-based APC resistance test, i.e. high SHBG levels were related to a high resistance to APC. The thrombin generation-based APC resistance test that we used in this study predicts the risk of thrombosis in users of oral contraceptives as well as in non-users and men, so the relationship between SHBG plasma levels and APC resistance supports the hypothesis that SHBG is a marker for the thrombotic risk of an oral contraceptive (Tans *et al.*, 2003).

The differences in SHBG levels and resistance to APC were not the result of differences between women rather than between type of oral contraceptive, as indicated by the results in women who switched from oral contraceptive type. Switching from a highly thrombogenic pill containing cyproterone acetate to a less thrombogenic pill containing levonorgestrel resulted in a decrease of SHBG levels as well as APC resistance.

Recently, Kemmeren *et al.*, (2004) postulated that the different effects of third- and second-generation oral contraceptives on the anticoagulant pathway might be explained by the observation that the effect of ethinylestradiol on anticoagulant parameters is less well counteracted by desogestrel than by levonorgestrel. In their study, progestogen-only pills did not induce changes of anticoagulant parameters or induce changes opposite to those of combined oral contraceptives containing the same dose of progestogen (Kemmeren *et al.*, 2004). The divergent effects of estrogen and progestogens have also been observed with regard to SHBG, i.e. progestogen-only pills decrease SHBG in contrast with combined oral contraceptives or estrogen alone which increase SHBG levels (van Kammen *et al.*, 1975; El Makhzangy *et al.*, 1979; Crona *et al.*, 1984). In agreement with the effect on anticoagulant parameters, the lowering effect on SHBG is more pronounced in pills containing only levonorgestrel compared to pills containing only desogestrel (Crona *et al.*, 1984; Barkfeldt *et al.*, 2001; Kemmeren *et al.*, 2004).

In conclusion, our findings support the hypothesis that the increase of SHBG induced by a combined oral contraceptive could be interpreted as a measure of estrogenicity of a combined oral contraceptive and that estrogenicity is a factor influencing the thrombotic risk of an oral contraceptive.

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