Thyroid function, activated protein C resistance and the risk of venous thrombosis in users of hormonal contraceptives

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Thromb Res 2014, 133: 640-644

Abstract

Introduction

Use of combined hormonal contraceptives is associated with a three- to eight-fold increased risk of venous thrombosis compared with non-use. The thrombotic risk depends on the estrogen dose as well as the progestogen type. Use of hormonal contraceptives leads to resistance to activated protein C (APC), which may serve as marker for the risk of venous thrombosis. Hyperthyroidism is also associated with an increased risk of venous thrombosis, due to increased free Thyroxine (FT4) levels which cause a hypercoagulable state.

Materials and methods

The objective of this study was to evaluate the effects of hormonal contraceptives on levels of FT4, thyroid stimulating hormone (TSH) and thyroxine binding globulin (TBG), and to investigate the effects on APC resistance per contraceptive group. We measured FT4, TBG and TSH levels and APC resistance in 231 users of oral contraceptives.

Results

Users of the most thrombogenic hormonal contraceptives, i.e. containing desogestrel, cyproterone acetate or drospirenone, had higher TBG levels than users of less thrombogenic hormonal contraceptives , i.e. the levonorgestrel-containing intrauterine device. TSH levels were not significantly elevated and FT4 levels did not change. TBG levels were also associated with APC resistance.

Conclusion

Use of hormonal contraceptives lead to elevated TBG levels, slightly elevated TSH levels and unchanged FT4 levels without causing a hyperthyroid state. Thus, the increased thrombotic risk during the use of hormonal contraceptives cannot be explained by a hyperthyroid state caused by use of these hormonal contraceptives.

Introduction

Use of combined hormonal contraceptives is associated with a three- to eight-fold increased risk of venous thrombosis (1-3). The risk depends on the estrogen dose as well as the progestogen type (1). So-called 'high-dose' combined oral contraceptives containing 50 µg or more ethinylestradiol (EE) are associated with a two-fold higher risk of venous thrombosis than 'low-dose' combined oral contraceptives containing 20 to 30 µg EE (2;4). Combined oral contraceptives containing the progestogens gestodene (GTD), desogestrel (DSG), cyproterone acetate (CPA) or drospirenone (DRSP) increase the risk of venous thrombosis two-fold more than combined oral contraceptives containing levonorgestrel (LNG) (1;2;4-13). The contraceptive vaginal ring containing etonogestrel (ENG) and EE, and use of the contraceptive transdermal patch containing norelgestromin (NGMN) and EE, are both associated with a seven- to eight-fold increased risk of venous thrombosis compared with non-users (14). Use of an intrauterine device (IUD), either levonorgestrel-releasing or without hormones, is not associated with an increased risk of venous thrombosis (15).

The differences in the risk of venous thrombosis can at least be partially 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) (16-18). High nAPCsr indicates increased APC resistance, which is dose-dependently associated with venous thrombosis (19). The thrombin generation-based APC resistance test was validated in a case-control study by Tans *et al*. (19) and discriminated well between highly thrombogenic oral contraceptives (i.e., containing GTD, DSG, CPA or DRSP) and oral contraceptives with a lower risk of venous thrombosis (i.e., containing LNG) (2;7;10;18).

Oral contraceptives influence the blood plasma concentrations of several binding globulins produced by the liver. The concentrations of these binding globulins are dependent on the effect of EE which enhances hepatic globulin synthesis (20-25), and the effect of the progestogen compound which degrades hepatic globulin syntheses. This results in an overall effect known as "estrogenicity". For example, use of combined hormonal contraceptives increases levels of sex hormone binding globulin (SHBG), which is the resultant of a dose-related increase induced by estrogens and a type- and dose-related decrease induced by progestogens (20;26). SHBG levels are associated with nAPCsr and the risk of venous thrombosis during use of hormonal contraceptives (27;28) and therefore serve as surrogate marker for the thrombogenicity of a hormonal contraceptive. SHBG measurement is recommended for estimation of the thrombotic risk before a new hormonal contraceptive is marketed (29).

Hyperthyroidism is associated with an increased risk of venous thrombosis (30-34) and causes a hypercoagulable state. This is due to high free thyroxine (T4) levels which influence the coagulation system (30-32). During use of hormonal contraceptives thyroxine binding globulin (TBG) levels are increased (20-26). TBG is, as SHBG, a hepatic globulin, which in this case transports thyroid hormones. Higher TBG levels lead to higher total T4 and total tri-iodothyronine (T3) levels.

We conducted an observational study to assess the effects of various hormonal contraceptives with various routes of administration and different thrombotic risks on TBG, TSH and FT4 levels as parameters of thyroid function. In addition, we evaluated whether TBG, TSH and FT4 levels are associated with the nAPCsr as marker of venous thrombosis, and with the thrombogenicity of a contraceptive as reported in the literature.

Materials and Methods

Study design and participants

We conducted an observational study. Plasma samples of participants from three different, previously performed studies collected between July 2002 and December 2005 in Leiden, the Netherlands, were used for analysis (17;35-37). Participants of two observational studies on the use of the levonorgestrel containing IUD (LNG-IUD) and copper containing IUD (Cu-IUD) and on the use of different combined oral contraceptives on markers of venous thrombosis were included. The third study was a randomized controlled trial with cross-over design on the effect of the vaginal ring and transdermal patch compared with the LNG and EE containing combined oral contraceptive on markers for venous thrombosis. All participants used their contraceptive for at least three consecutive months; blood draws were performed at baseline and in the third month of use. A more detailed description can be found in the original articles (17;35-37).

Inclusion criteria of all participants were: healthy women using a hormonal contraceptive for at least three cycles. Exclusion criteria were age <18 years, and contraindications for combined hormonal contraceptive use as stated by the World Health Organization (3). No participants had a history of thyroid disease, or used medication for a thyroid disorder.

Participants who were carriers of the factor V Leiden mutation were excluded from the analysis, since this mutation causes resistance to APC without affecting levels of TBG, FT4 and TSH (n = 36). Users of contraceptives with less than 10 users were not included in the final analysis: users of the combined oral contraceptives containing GTD (n = 3), norgestimate (NGM, n = 1) and norethisterone (NET, n = 2), users of the transdermal patch containing NGMN and EE (n = 7) and users of the vaginal ring containing ENG and EE (n = 5). To exclude effects of the estrogen dose, we only used data from users of combined oral contraceptives containing 30-35 µg of EE; users of preparations with other amounts of EE were excluded (n = 23).

The final analysis included 231 participants: 154 users of a combined oral contraceptive (containing 30-35µg EE and LNG, DSG, CPA or DRSP), 60 users of the LNG-IUD and 17 users of the Cu-IUD.

Written informed consent was given by all participants and the studies were all approved by the Medical Ethics Committee of the Leiden University Medical Center, The Netherlands.

Laboratory methods

The plasma samples from the studies were taken, processed and stored identically. Blood samples were taken from the antecubital vein in the morning in a fasting state and collected in 0.106 M sodium citrate (pH 5.8), and in tubes with a clotting activator to obtain serum. Cell-free, citrated plasma and serum were prepared by centrifuging blood at 2100 g for 10 minutes at 18ºC, and were coded and centrally stored at -80 ºC. All samples were measured in a single run, using one lot of reagents by technicians who were unaware of which participant or contraceptive a sample belonged to.

Normalized APC sensitivity ratios (nAPCsr) were determined in duplicate by quantifying the effect of APC on thrombin generation in the thrombin generation-based APC resistance test, as described before (16).

TBG (nmol/L) was measured with a competitive chemiluminescent immunoassay (Immulite 2000; Siemens, USA). Sensitivity of the assay is 29 nmol/L with an imprecision of 7%. Values between 330 and 760 nmol/L are considered within reference range.

TSH (mIU/L) was measured with a chemiluminescent sandwich ELISA and FT4 (pmol/L) was measured with a competitive chemiluminescent assay (Roche Cobas 6000, E-platform, Switzerland). Imprecision of the TSH assay is 1.3%, with a sensitivity of 0.01 mIU/L. Reference values applied at the institution are 0.27 to 4.2 mIU/L for adults. The FT4 assay has an imprecision of 3% and a sensitivity of 0.3 pmol/L. The institution uses a 10 to 24 pmol/L reference range for adults.

Statistical analysis

We used means, mean differences (MD), 95% confidence intervals (95% CI) and ranges to describe variables. We performed a normality analysis, constructed error bars and a scatter plot to describe the association between TBG levels and nAPCsr, and performed a regression analysis to study associations. Statistics were computed using SPSS for Windows, version 20.0 (SPSS Inc., Chicago, IL, USA). The odds ratios of the risk of venous thrombosis during use of different hormonal contraceptives were retrieved from recent publications of Van Hylckama Vlieg *et al*. (2;15) and were used for risk ranking. Users of the Cu-IUD were considered as non-users since the Cu-IUD does not contain hormones and does not increase the risk of venous thrombosis.

Results

Baseline characteristics

There were no statistically significant differences in age or body mass index (BMI) between the women using different kinds of hormonal contraceptives. The mean age of the total group was 29 years (range 17 to 52 years), the mean BMI was 24 kg/m^2 (range 17 to 48 kg/m^2) (Table 1). None of the participants used levothyroxine or reported a thyroid disorder. A more detailed description of the characteristics of the participants can be found in previous papers (17;35;36).

TBG, TSH and FT4 levels during use of hormonal contraceptives

The lowest TBG levels were found in women using the LNG-IUD with a mean of 331 nmol/L, compared with a mean TBG level of 366 nmol/L during use of the Cu-IUD (MD -35 nmol/L, 95% CI -71 to 0). Users of the oral contraceptives containing DSG and EE had the highest TBG levels with a mean of 629 nmol/L (MD 247 nmol/L, 95% CI 183 to 312) (Table 2).

TSH levels were also increased during use of hormonal contraceptives: the lowest levels were found in users of the Cu-IUD 2.13 mIU/L, users of the DRSP/EE containing oral contraceptive had the highest TSH level of 3.01 mIU/L. (Table 2).

FT4 levels were not significantly different during use of hormonal contraceptives compared with use of the Cu-IUD (Table 2).

Associations

TBG plasma levels were positively associated with nAPCsr in users of different kinds of hormonal contraceptives. TBG levels increased by 17% for each one-unit increase in nAPCsr (10^0.068 = 1.17), equation: log10 (TBG) = 2.494 + (nAPCsr * 0.068) (Figure 1). The Pearson's correlation coefficient was 0.714 ($p<0.001$).

Risk ranking per contraceptive

For risk ranking, we used odds ratios from recent publications of Van Hylckama Vlieg *et al.* (2;15). The TBG levels were associated with the reported thrombogenicity of oral contraceptives: users of highly thrombogenic contraceptives had higher TBG levels than users of oral contraceptives with a low thrombogenicity (Table 2).

Table 1: *Baseline characteristics*

Contraceptive	N	Age, years (range)	p value	BMI, kg/m2 (range)	p value
LNG-IUD	60	33 (17–52)	0.84	25 (18–48)	0.64
Cu-IUD	17	30 (20–45)	Ref	24 [18–32]	Ref
LNG/EE	71	29 (19–51)	0.09	23 [17–38]	0.35
DSG/EE	16	31 (18–49)	0.51	25 [20–32]	0.59
DRSP/EE	46	29 (18–47)	0.08	24 [18–34]	0.85
CPA/EE	21	28 (19–44)	0.07	22 [19–26]	0.15

BMI, body mass index; IUD, intrauterine device; LNG, levonorgestrel; Cu, copper; EE, ethinylestradiol; DSG, desogestrel; DRSP, drospirenone; CPA, cyproterone acetate.

Discussion

In this study of 231 users of various hormonal contraceptives with different routes of administration we observed different levels of TBG, TSH and FT4. TBG levels were positively associated with the thrombotic marker nAPCsr. Users of hormonal contraceptives with a known high thrombogenicity had the highest TBG levels, and users of the LNG-IUD, which is not associated with an increased risk of venous thrombosis, had the lowest TBG levels. TSH levels displayed a similar but less pronounced pattern.

Several studies have reported increases in TBG levels during use of combined oral contraceptives. Wiegratz *et al.* (20) published a randomized controlled trial with four different oral contraceptives. All four preparations led to increased serum levels of TBG, and a less pronounced rise of total T4 and T3 which was most likely the result of the increased TBG levels. They explain the elevation of TBG by an EE-induced enhancement of the hepatic TBG synthesis and a counteraction by progestogens as illustrated by the difference in users of oral contraceptives containing LNG compared with DNG (20). Ågren *et al.* (23) and Sänger *et al.* (21) also observed increased TBG levels in women using different combined oral contraceptives. In these two studies TSH and FT4 levels were only minimally affected. We also did not observe a difference in FT4 between users of hormonal contraceptives and users of the non-hormonal Cu-IUD. For TSH, a trend similar to TBG was observed: highly thrombogenic hormonal contraceptives were associated with higher TSH levels than less thrombogenic hormonal contraceptive, indicating that the pituitary-hypothalamic axis was affected to some extent. Our findings confirm the data of Ågren, Sänger and Wiegratz (21;23;38).

Limited data are available about the vaginal ring containing ENG and the transdermal patch containing NGMN. Duijkers *et al.* investigated the effect of the vaginal ring on TSH and FT4 (25). They observed comparable FT4 levels, and higher TSH levels for users of the vaginal ring than for to users of an oral contraceptive containing LNG and EE. No TBG levels were reported. White *et al.* (24) investigated the effect of the transdermal patch on TBG levels, compared with an oral contraceptive containing NGM and EE. They found higher TBG levels in users of the transdermal patch than for the oral contraceptive. We excluded users of the vaginal ring and transdermal patch due to a small sample size (n = 5 for the vaginal ring and n = 7 for the transdermal patch).

Use of hormonal contraceptives and hyperthyroidism are both associated with an increased risk of venous thrombosis (1-4;6;7;13;14;30-34). Use of hormonal contraceptives causes resistance to APC, and changes of the plasma levels of several procoagulant, anticoagulant and fibrinolytic proteins (39-43). Hyperthyroidism increases antifibrinolysis and induce changes in the inflammatory pathway through complement C3 which induces a hypercoagulable state (34;39). We observed that use of hormonal contraceptives influence thyroid parameters but does not lead to a hyperthyroid state since FT4 levels were not much affected. So, the increased risk of venous thrombosis during use of hormonal contraceptives cannot be explained by a hyperthyroid state induced by hormonal contraceptives which causes hypercoagulability. Like other hepatic binding globulins such as SHBG, TBG levels are associated with the increased risk of venous thrombosis during use of hormonal contraceptives (26;28;44).

Table 2. *Means and 95% CI of TBG, TSH and FT4, and their risk ranking according to the literature.*

Contraceptive	N	TBG (nmol/L)			TSH (mIU/L)		
		Mean	MD	95% CI	Mean	MD	95% CI
Cu-IUD	17	366	Ref		2.13	Ref	
LNG-IUD	60	331	−35	−71 to 0	2.24	0.12	−0.66 to 0.89
150mcg LNG/30mcg EE	71	487	121	79 to 162	2.72	0.59	−0.19 to 1.38
150mcg DSG/30mcg EE	16	629	263	199 to 327	2.91	0.78	−0.46 to 2.02
3 mgDRSP/30mcg EE	46	582	216	160 to 272	3.01	0.89	−0.25 to 2.02
2 mg CPA/35mcg EE	21	605	238	180 to 297	2.71	0.58	−0.64 to 1.81

TBG, thyroxine binding globulin; TSH, thyroid stimulating hormone; FT4, free thyroxine; MD, mean difference; CI, confidence interval; OR, odds ratio; IUD, intrauterine device; LNG, levonorgestrel; Cu, copper; EE, ethinylestradiol; DSG, desogestrel; DRSP, drospirenone; CPA, cyproterone acetate.

Currently, a biological explanation of the increased risk of venous thrombosis during use of combined hormonal contraceptives is lacking. It seems likely that the liver plays a modulating role in the increased risk of venous thrombosis during use of combined hormonal contraceptives: the serum levels of hepatic binding globulins are increased during use of combined hormonal contraceptives, coagulation factors are produced in the liver and hormonal contraceptives are metabolized in the liver. Possibly, combined hormonal contraceptives interfere with the synthesis of both binding globulins and coagulation factors.

Limitations of our study were the sample size of some contraceptive groups and the reference group. The sample sizes of users of various contraceptives were too small for comparison and had to be excluded from the analysis, including the non-oral contraceptives vaginal ring and transdermal patch. Besides, non-users are preferably used as reference group. However, data of non-users were not available and therefore users of the Cu-IUD served as reference group since use of the Cu-IUD is not thought to increase the risk of venous thrombosis and considered inert.

In conclusion, this study shows that the use of hormonal contraceptives leads to increased TBG levels and TSH levels, but has no effect on FT4 levels during use of hormonal contraceptives. Hormonal contraceptive use does not lead to a hyperthyroid state, and hence the increased risk of venous thrombosis during use of combined hormonal contraceptives cannot be explained by a hyperthyroid state induced by hormonal contraceptives which causes hypercoagulability. TBG reflects the resultant of EE and progestogens, and shows a positive association with nAPCsr and relative risks of venous thrombosis reported in the literature.

FT4 (pmol/L)			Risk Ranking		
Mean	**MD**	**95% CI**	**OR**	**95%CI**	**Reference**
13.52	Ref		1.0		
14.77	1.25	0.22 to 2.29	0.3	0.1 to 1.1	[15]
14.97	1.45	0.53 to 2.38	3.6	2.9 to 4.6	[2]
14.42	0.90	−0.15 to 1.96	7.3	5.3 to 10.0	[2]
14.23	0.72	−0.27 to 1.70	6.3	2.9 to 13.7	[2]
13.75	0.24	−0.74 to 1.21	6.8	4.6 to 10.0	[2]

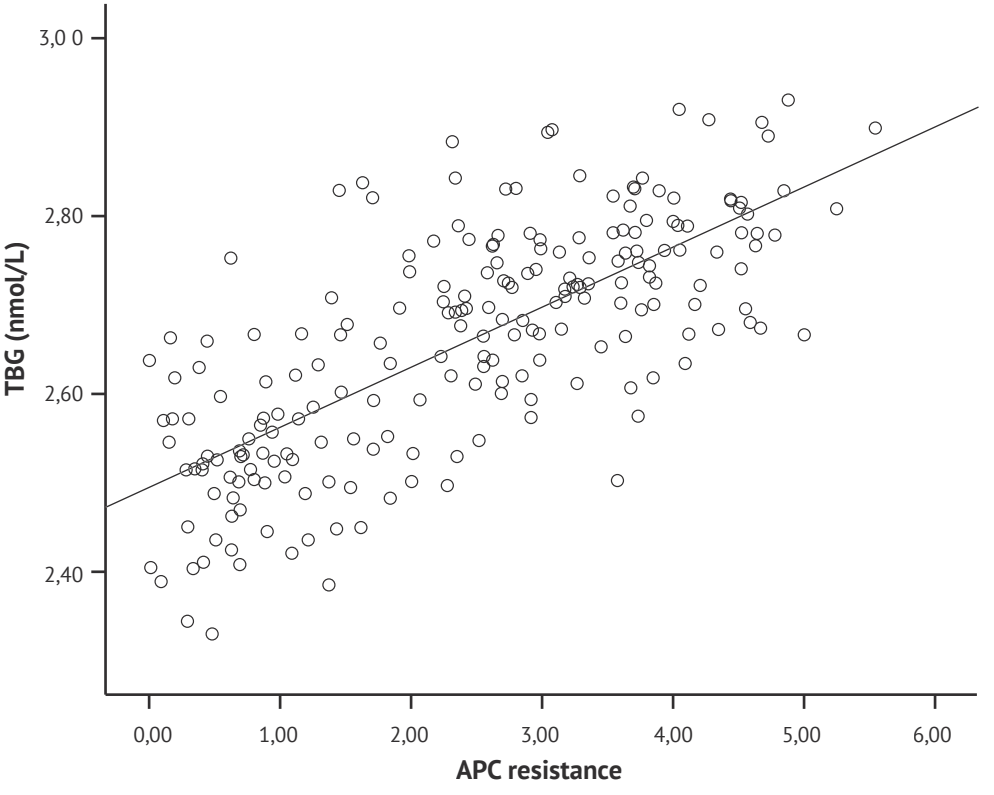

Figure 1. *The association between TBG and nAPCsr. Equation: log10(TBG) = 2.494 + (nAPCsr * 0.068). Pearson's correlation coefficient is 0.714 (p<0.001).*

TBG, Thyroxine binding globulin; nAPCsr, normalized Activated Protein C sensitivity ratio.

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