Effectiveness and side effects of hormonal contraceptives

ISBN/EAN: 978-90-9028295-4

Cover design and lay-out: Studio van Holland, Breda, The Netherlands.

Printed by: UFB/GrafiMedia, Leiden, The Netherlands

Financial support for the publication of this thesis was kindly provided by:

Raad van Bestuur Medisch Centrum Haaglanden

Leiden University Medical Centre

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Proefschrift

ter verkrijging van

de graad van Doctor aan de Universiteit Leiden,

op gezag van Rector Magnificus prof.mr. C.J.J.M. Stolker,

volgens besluit van het College voor Promoties

te verdedigen op dinsdag 17 juni 2014

klokke 13.45 uur

door

Marjolein Raps

geboren te Heemstede

in 1985

Promotiecommissie

Promotores

Prof.dr. F.M. Helmerhorst

Prof.dr. F.R. Rosendaal

Co-promotor

Dr. H.A.A.M. van Vliet, Catharina Ziekenhuis, Eindhoven

Overige leden

Prof.dr. P. Reitsma

Prof.dr. S. Middeldorp, Academisch Medisch Centrum, Amsterdam

Dr. O. Dekkers

Alles kan, als je maar wil

Contents

CHAPTER 1

General introduction

Effectiveness and side effects of hormonal contraceptives

Shortly after the introduction of the contraceptive pill in 1960, the first case of venous thrombosis during use of an oral contraceptive was reported (1;2). The incidence of venous thrombosis in the general population is low, approximately 3 per 10 000 women-years among women in reproductive age. Despite the low incidence, use of oral contraceptives frequently causes venous thrombosis since millions of women worldwide use an oral contraceptive (3;4). Overall, combined oral contraceptive use is associated with a two- to six-fold increased risk of venous thrombosis (5-7). The aim of this thesis is to study the risk of venous thrombosis during use of different hormonal contraceptives, focusing on the pathogenesis, the evaluation of a marker to estimate the risk of venous thrombosis, and the assessment of effectiveness, side effects including the risk of venous thrombosis and acceptability of a new hormonal contraceptive.

Hormonal contraceptives and venous thrombosis

After the introduction of the first oral contraceptive pill, new prescriptions were developed to lower side effects like venous thrombosis, while maintaining the benefits of contraception and cycle control. The attempts that have been made to achieve this can be classified in four categories:

1. Lowering the hormone dose,
2. New formulas and schedules,
3. New steroids and
4. New routes of administration.

Lowering the hormone dose

Because the estrogen compound in combined oral contraceptives was thought to cause the increased risk of venous thrombosis, the estrogen dose was stepwise reduced from 150 µg to 80 µg, 50 µg, 30 µg, to 20 µg currently (8-11). Lowering this dose reduced the risk of venous thrombosis (5;8;9;12). The currently most prescribed combined oral contraceptives contain 20-30 µg estrogen in the form of ethinylestradiol.

New formulas and schedules of administration

Furthermore, new formulas and schedules of administration were introduced. The first oral contraceptives contained a fixed dose of estrogen and progestogen in a 21-day schedule, the monophasic contraceptives. The multiphasic approach (biphasic and triphasic) was developed to mimic the natural cycle and improve acceptability (13). In these schedules, estrogens and progestogens are administered in varying dosages in two or three phases during the cycle. The manufacturer claimed that the multiphasic approach imitates the natural menstrual cycle resulting in a better bleeding profile (14). However, the biphasic and triphasic preparations were evaluated and

compared with monophasic contraceptives in three systematic reviews which showed that the multiphasic approach had no advantages over the monophasic approach in contraceptive effectiveness, side effects and cycle control and continuation rates (15-17). The thrombotic safety of multiphasic oral contraceptives compared with monophasic oral contraceptives was studied by Lidegaard *et al*. They compared monophasic with biphasic oral contraceptives containing levonorgestrel and ethinylestradiol and found no significant differences in thrombotic safety (adjusted rate ratio 1.07, 95% CI 0.75 to 1.52) (18).

New steroids

Another attempt to lower side effects of oral contraceptives was adjusting the chemical structure of progestogens. Progestogens were originally classified as first, second or third generation progestogens, based on the order of introduction and depending on their chemical structure. First generation progestogens (norethynodrel, norethisterone and lynestrenol) were developed in the 1960s and are derived from the estrane steroids. Second generation (norgestrel and levonorgestrel) and third generation (desogestrel and gestodene) progestogens were developed in the 1970s and 1980s and are derived from gonane steroids. Dienogest and drospirenone have been synthesized in the last 20 years and may be considered as fourth generation progestogens in a temporal fashion, although they do not share a common chemical structure (19). Drospirenone is derived from 17α-spironolactone, and was marketed internationally in 2000 (20). Dienogest is derived from the estrane steroids and was firstly marketed in 1995 in Germany, but only available in the Netherlands since 2008 (20). Cyproterone acetate is not included in the classification by generations. It was developed in the late 1980s and is derived from pregnane steroids. Since the characteristics of progestogens differ even within the generation groups, and the risk of venous thrombosis is dependent on the type of the progestogen instead of the generation, it is probably best to abstain from any classification of progestogens (21).

In 1995, three studies reported a two-fold increased risk of venous thrombosis in women using combined oral contraceptives containing the third generation progestogens gestodene and desogestrel compared with women using second generation oral contraceptives (5;7;22-26). Subsequently, cyproterone acetate containing oral contraceptives were also reported to have a two-fold increased risk compared with second generation combined oral contraceptives (23). In 2009, the combined oral contraceptive containing drospirenone was found to increase the risk of venous thrombosis 1.7-fold compared with second generation oral contraceptives (23). Both oral contraceptives showed altered concentrations of coagulation factors and increased APC resistance before the increased risk of venous thrombosis was identified in clinical trials. The ORs of different combined oral contraceptives known from recent literature are summarized in table 1.

Recently, a new combined oral contraceptive was marketed with a new estrogen: estradiol valerate (Qlaira ®, 2009, Bayer Schering Pharma, Berlin, Germany), which is an ester of the natural female hormone 17ß-estradiol. The progestogen compound is dienogest, a fourth generation progestogen. The oral contraceptive is administered in a four-phasic (quadriphasic) schedule (20;27), with

an estrogen step-down and a progestogen step-up scheme and contains 26 acting tablets and two placebos on days 27 and 28 of the cycle. The manufacturer claimed that estradiol valerate and the quadriphasic scheme is more physiological, mimics the natural cycle and should therefore lead to fewer side effects and better acceptability (28). In *chapter 2* the results of a Cochrane systematic review are presented in which the quadriphasic schedule of the combined oral contraceptive containing dienogest and estradiol valerate was compared with monophasic oral contraceptives, in terms of effectiveness, bleeding pattern, side effects and acceptability.

New routes of administration

A third attempt to lower side effects was to develop new routes of administration of hormones, such as the vaginal ring and the transdermal patch. The rationale behind non-oral administration was a continuous release of hormones which bypass the first-pass liver effect. This should allow a lower estrogen and progestogen dose, less loss of bioavailability and fewer peaks in serum levels resulting in increased acceptability (29-34).

The levonorgestrel-containing intra-uterine device (LNG-IUD, Mirena®, Bayer Schering Pharma, Berlin, Germany) is also a non-oral hormonal contraceptive, and available since 1990. It is inserted in the uterine cavity for a maximum of five years. It contains 52 mg levonorgestrel which is released continuously; the daily dose released in vivo is 14-20 µg per day and the circulating levonorgestrel plasma levels are 150-200 pg/mL. Use of levonorgestrel-only contraceptive pills results in higher levonorgestrel plasma levels of 800 pg/mL (35-37). The LNG-IUD showed no increased risk of venous thrombosis; reported odds ratios (ORs) vary from 0.3 to 0.57 (Table 1) (38;39).

The transdermal patch (Ortho-Evra ®/Evra®, Ortho-McNeil-Janssen Pharmaceuticals, Raritan, NJ, USA) was marketed in 2002. It is a thin adhesive square of 20 cm^2 which is applied on the skin (29;40) and continuously releases a daily dose of 203 µg norelgestromin (the active metabolite of norgestimate, a third generation progestogen (41)) and 33.9 µg ethinylestradiol for one week. According to the prescription, the patch is used in three consecutive weeks, followed by a patch-free interval of one week in which a withdrawal bleeding may occur (40).

The vaginal ring (NuvaRing®, Organon, Oss, The Netherlands) was marketed in 2001 and contains etonogestrel (a third generation progestogen) and ethinylestradiol. It is a flexible, soft, 4 mm thick ring of 5.4 cm in diameter which can easily be inserted into and removed from the vagina by the woman herself (31;42). The ring contains 11.7 mg etonogestrel and 2.7 mg ethinylestradiol and continuously releases a daily dose of 120 µg etonogestrel and 15 µg ethinylestradiol over a period of 3 weeks (42).

Despite attempts to reduce side effects such as venous thrombosis, the transdermal patch and vaginal ring did not lead to a lower thrombotic risk than with use of oral contraceptives containing levonorgestrel: the Food and Drug Administration (FDA) reported an OR of 7.9 (95% CI 3.5 to 17.7) during use of the transdermal patch and an OR of 6.5 (95% CI 4.7 to 8.9) during use of

the vaginal ring (Table 1) (43) compared with four low-estrogen combined hormonal contraceptives. These increased risks were later confirmed by Lidegaard *et al.*, in a large Danish cohort (39). The high thrombotic risk of the vaginal ring may be caused by its release of a third generation progestogen which is associated with an increased thrombotic risk, as described above. During use of the transdermal patch, 60% higher estrogen levels were measured than with a combined oral contraceptive containing the same steroids, which is associated with a higher risk of venous thrombosis (40;44).

Table 1: *Odds ratios and 95% confidence intervals of hormonal contraceptives. All oral contraceptives All for preparations that contain 30-37.5 µg ethinylestradiol.*

Contraceptive		OR	95% CI	Reference
Non use (reference)		1.0	-	-
Combined oral contraceptive	Lynestrenol	5.6	3.0 – 10.2	(23)
	Norethisterone	3.9	1.4 – 10.6	(23)
	Levonorgestrel	3.2	2.5 – 3.9	(21)
	Desogestrel	5.5	4.4 – 6.8	(21)
	Gestodene	4.6	3.6 – 5.9	(21)
	Norgestimate	3.9	2.9 – 5.4	(21)
	Cyproterone Acetate	5.5	3.9 – 7.7	(21)
	Drospirenone	6.0	4.1 – 8.9	(21)
Transdermal Patch	Norelgestromin	7.9	3.5 – 17.7	(43)
Vaginal ring	Etonogestrel	6.5	4.7 – 8.9	(43)
Levonorgestel - IUD	Levonorgestrel	0.6	0.4 – 0.8	(38;39)

Biological explanation

The exact biological mechanism of the increased risk of venous thrombosis during oral contraceptive use and the differences between the different oral contraceptives is unknown. Various studies showed altered levels of clotting factors during use of combined oral contraceptives. Increased levels of the procoagulant factors prothrombin, factor VII, VIII, IX, X, XI, XII, von Willebrand factor and fibrinogen, and decreased levels of the anticoagulant factors protein S and antithrombin were observed (45-48). The interpretation of the changes in clotting factors differed between researchers, since the net effect of changes of the haemostatic system was not known.

APC resistance

A major step forward in the biological understanding of the increased risk of venous thrombosis during use of oral contraceptives was the observation that oral contraceptives induce an acquired form of resistance to Activated Protein C (APC) (49;50). APC is a physiological anticoagulant which inactivates factor V and thereby inhibits coagulation. APC resistance is the relative inability of activated protein C to cleave activated factor V or factor VIII, leading to a prothrombotic state.

It was first described by Dählback *et al*. in a family with a hereditary tendency for venous thrombosis (51). A year later, Bertina *et al*. identified the factor V Leiden mutation (replacement of the amino acid Arg506 by Gln), which is the most common form of hereditary APC resistance (52). APC resistance not caused by the factor V Leiden mutation is also an important risk factor for venous thrombosis (53;54).

APC resistance was originally measured by the aPTT-based APC resistance test, which measures the effect of APC on the clotting time of plasma in which coagulation is initiated via the intrinsic pathway (55). In 1995 the thrombin generation-based APC resistance test was developed by Rosing and Hemker (56;57). The test quantifies the effect of APC on the time integral of thrombin formation in plasma in which coagulation is initiated via the extrinsic coagulation pathway. The test is a global assay, which includes the effects on individual clotting factors (procoagulant and anticoagulant factors) and combines them into a net effect (56;57). Women using combined oral contraceptives were found to be resistant to APC by this thrombin generation-based APC resistant test. More importantly, differences in APC resistance between users of second and third generation combined oral contraceptives were shown, and the test provided an explanation for the differences in venous thrombosis risk between the oral contraceptives (58;59). The thrombin generation-based APC resistance test was validated in the Leiden Thrombophilia Study and predicts the risk of venous thrombosis in users of combined oral contraceptives as well as in non-users and men (60).

TFPI and Protein S

The two main determinants of the thrombin generation-based APC resistance test are free Protein S and free Tissue Factor Pathway Inhibitor (TFPI) (61-63). Hereditary and acquired deficiencies of Protein S and low plasma levels of TFPI are associated with an increased risk of venous thrombosis (64-67). Moreover, Protein S-deficient individuals also have decreased TFPI levels, probably due to common mechanisms regulating biosynthesis of both proteins (64). Van Vliet *et al*. observed that women using combined oral contraceptives with the highest risk of venous thrombosis (i.e. containing desogestrel, cyproterone acetate or drospirenone) had lower free Protein S and free TFPI levels compared with women using the combined oral contraceptive with the lowest risk of venous thrombosis (i.e. containing levonorgestrel). The study concluded that the differences in APC resistance, induced by combined oral contraceptives, can at least partially be explained by differences in free Protein S and free TFPI (61).

Chapter 3 describes a study which evaluates whether the different risks of venous thrombosis caused by different hormonal and non-hormonal methods of contraception are reflected in the levels of free TFPI and free Protein S. The association of these levels with APC resistance measured by the thrombin generation-based APC resistance test, and with relative risks as reported in the literature is examined.

Predicting thrombotic risk by use of markers

When a new hormonal contraceptive is introduced, an estimation of the thrombotic risk is required in order not to expose women to an unnecessary high risk of venous thrombosis by prescribing this new contraceptive. As the result of the high number of participants required for a clinical study due to the low incidence of venous thrombosis, assessment of the thrombotic risk of new contraceptives in clinical preregistration studies is unfeasible. In order to demonstrate a doubling of the risk of venous thrombosis between two different combined hormonal contraceptives with a power of 80% and a significance level of 5%, a cohort of approximately 500 000 women would need to be followed for 1 year (68).

To predict the risk of venous thrombosis of a new hormonal contraceptive before market authorization, one can revert to a study with markers. A marker is a proxy, usually a laboratory test, for a clinical outcome. They make it possible to perform a study with smaller sample sizes and shorter observation periods than necessary for true clinical outcomes. Preferably, a marker is validated in a study in which both the marker and the clinical endpoint are assessed (69). An important, and validated marker for venous thrombosis during use of hormonal contraceptives is APC resistance, measured by the thrombin generation-based APC resistance test (expressed in normalized APC sensitivity ratios, nAPCsr), since it differentiates well between "low-risk" and "high-risk" oral contraceptives (60).

In search of other markers, Odlind *et al*. suggested Sex Hormone-Binding Globulin (SHBG) as marker of "estrogenicity" and thereby venous thrombosis (70). SHBG is a carrier protein produced in the liver and transports testosterone and estrogen. The effect of an oral contraceptive on SHBG levels can be interpreted as the sum of the dose-related estrogenic effect of ethinylestradiol and the dose- and type-related anti-estrogenic effect of the progestogen, resulting in the total "estrogenicity" of the oral contraceptive (70). In two observational studies, an association between APC resistance and SHBG levels was found in users of different hormonal contraceptives and non-users which support the "estrogenicity" hypothesis (71;72).

To determine whether SHBG is a useful marker for the risk of venous thrombosis of combined oral contraceptives, we conducted an observational study assessing SHBG levels in non-users, users of the hormonal and non-hormonal IUD, and users of different oral contraceptives. The results of the comparison between the SHBG levels, nAPCsr, and the risks of venous thrombosis as reported in the literature are described in *chapter 4*. Besides, we investigated whether an ethinylestradiol-dose related increase in SHBG levels is present during use of different combined oral contraceptives, which is described in *chapter 5*.

The absolute and relative risk of venous thrombosis during use of the above mentioned recently introduced combined oral contraceptive is unknown. *Chapter 6* describes a randomized controlled trial in which APC resistance and SHBG levels during use of the combined oral contraceptives containing dienogest/estradiol valerate and levonorgestrel/ethinylestradiol were compared.

Thyroid parameters and venous thrombosis

There are many risk factors for venous thrombosis, which can be broadly defined in those related to immobilization and to changes in coagulability. The latter can be divided in genetic abnormalities in the coagulation system, and acquired factors, amongst which are cancer and use of oral contraceptives. Besides the use of hormonal contraceptives, hyperthyroidism is also associated with an increased risk of venous thrombosis (73-77) and causes a hypercoagulable state with increased levels of procoagulant and anticoagulant factors. Hyperthyroidism increases antifibrinolysis and induces changes in the inflammatory pathway through complement C3 which induces a hypercoagulable state (45;77). The hypercoagulable state is probably due to high free Thyroxine (T4) levels which influence the coagulation system (73-75).

During use of hormonal contraceptives, Thyroxine-Binding Globulin (TBG) levels are increased (70;78-83). TBG is a hepatic globulin which transports thyroid hormones. Higher TBG levels lead to higher total T4 and total Tri-iodothyronine (T3) levels. Like SHBG, TBG levels are associated with the increased risk of venous thrombosis during use of hormonal contraceptives (70;71). Whether the increased levels of thyroid parameters during use of hormonal contraceptives are associated with the increased risk of venous thrombosis during use of these contraceptives has not yet been studied.

In *chapter 7* we questioned whether there is an association between the levels of TBG, FT4 and TSH during use of hormonal contraceptives and the risk of venous thrombosis. The study examines whether an association can be found between thyroid parameters and APC resistance measured with the thrombin generation-based APC resistance test and the thrombotic risks as reported in the literature.

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CHAPTER 2

Quadriphasic versus monophasic oral contraceptives for contraception

Huib A.A.M. Van Vliet
Marjolein Raps
Laureen M. Lopez
Frans M. Helmerhorst

Cochrane Database Syst Rev 2011, CD009038

Abstract

Background

Quadriphasic oral contraceptives have been developed to reduce the adverse effects of oral contraceptives and are presented as more physiological since theymimic the natural cycle. However, suggested disadvantages of quadriphasic oral contraceptives include a possible increased risk of pill-taking errors caused by the array of different color pills, complicated directions for catching up when a pill is missed, the higher price and potential inferiority in terms of side effects.

Objectives

To compare the contraceptive effectiveness, bleeding pattern, minor side effects and acceptability of quadriphasic contraceptive pills versus monophasic contraceptive pills.

Search methods

We searched CENTRAL, MEDLINE, EMBASE, POPLINE, ClinicalTrials.gov and ICTRP for trials comparing quadriphasic pills with monophasic pills. We contacted researchers and manufacturers of quadriphasic oral contraceptives to identify additional studies.

Selection criteria

Randomized controlled trials (RCTs) comparing quadriphasic with monophasic oral contraceptives. Trials had to report on contraceptive effectiveness, bleeding patterns, minor side effects, ease of use or trial discontinuation. We excluded studies where the intervention was primarily used as a treatment for disorders or was administered for fewer than three consecutive cycles.

Data collection and analysis

Two authors abstracted and entered data into RevMan. We critically appraised the methodological quality of the included trials. For continuous variables, we computed the mean difference with 95% confidence interval (CI) using the random-effects model. For dichotomous variables, we calculated the risk ratio with 95% CI using the random-effects model.

Main results

We included one double-blind, double-dummy RCT comparing a quadriphasic oral contraceptive composed of dienogest and estradiol valerate with a monophasic oral contraceptive composed of levonorgestrel and ethinylestradiol. Contraceptive effectiveness, intracyclic bleeding and discontinuation due to side effectswere similar for quadriphasic andmonophasic pills. The number of women experiencing withdrawal bleeding was higher in the monophasic group compared to the quadriphasic group.Users of quadriphasic pills reported fewer bleeding/spotting days and fewer bleeding/spotting episodes than users of monophasic pills but the report did not specify whether

the bleeding/spotting was scheduled or unscheduled. More women using quadriphasic oral contraceptives reported breast pain compared to women using monophasic oral contraceptives.

Authors' conclusions

The available evidence is insufficient to determine whether quadriphasic differ from monophasic oral contraceptives in contraceptive effectiveness, bleeding pattern, minor side effects and acceptability. Studies that compare quadriphasic and monophasic oral contraceptives with an identical progestogen and estrogen type are needed to determine whether the quadriphasic approach differs from the monophasic approach. Studies that compare quadriphasic pills with monophasic pills containing 30 µg ethinylestradiol are indicated to determine whether quadriphasic oral contraceptives have an advantage over the current, first choice oral contraceptive. Until then, we recommend monophasic pills containing 30 µg estrogen as the first choice for women starting oral contraceptive use.

Plain language summary

Birth control pills with three phases versus one phase

Standard birth control pills contain two hormones: progestogen and estrogen. One-phase birth control pills contain the same dose of progestogen and estrogen every day. Four-phase birth control pills contain different amounts of progestogen and estrogen on different days. This review looked at how well one-phase birth control pills and four-phase birth control pills work to prevent pregnancy, how often they cause bleeding problems, how often users experience side effects and how many women stop using the pills.

We did a computer search for randomized controlled trials comparing four-phase birth control pills with one-phase birth control pills. We also wrote to researchers and makers of birth control pills to find other trials. Studies had to report on pregnancy, bleeding problems, side effects or stopping the use of pills. We did not include studies where the pills were used as a treatment for disorders like acne, hirsutism, polycystic ovary syndrome, bleeding problems or endometrioses, or where the pills were administered for less than three months. We assessed whether the studies were conducted properly.

We included one study comparing a four-phase pill composed of the progestogen dienogest and the estrogen estradiol valerate with an one-phase pill composed of the progestogen levonorgestrel and the estrogen ethinylestradiol. Four-phase birth control pills and onephase birth control pills had similar pregnancy rates. The number of women with blood loss in the period between two menstruations was similar for four-phase pills and one-phase pills. More women using one-phase birth control pills had a menstruation compared to women using four-phase birth control pills. The number of women who stopped using the pills because of side effects was similar for four-phase pills and one-phase pills. Breast pain was reported more frequently by women who used four-phase birth control pills than women who used one-phase birth control pills.

The presence of only one study made it impossible to adequately compare four-phase birth control pills with one-phase birth control pills. More studies are needed to determine whether four-phase pills have advantages over one-phase pills. Until then, we recommend one-phase pills containing 30 µg estrogen for women starting to use birth control pills.

Background

Since the introduction of combined oral contraceptives in the 1960s, the development of new hormonal contraceptives has focused on reducing the adverse effects while maintaining the benefits. Four approaches have been used to reduce the adverse effects of oral contraceptives and so increase compliance: (I) lowering of the steroid dose, (II) development of new steroids, (III) development of new formulas and schedules of administration, and (IV) development of new routes of administration.

Initially oral contraceptives contained a fixed dose of estrogen and progesterone for 21 days: so-called monophasic preparations. In order to provide better cycle control, biphasic and triphasic oral contraceptives were developed in the 1970s and 1980s (1). These preparations consist of two or three phases, each with a different progesterone dosage and in some preparations estrogen dosage. In the first phase progestogen levels are low, followed by a higher dose of the steroids in the second and third phases. To date, no benefits of the bi- and triphasic approach compared to the monophasic approach have been demonstrated (2;3).

Recently, the first quadriphasic oral contraceptive has been introduced. The rationale behind the development of the quadriphasic approach was to improve the unsatisfactory bleeding patterns observed with 17ß-estradiol-containing, mono- and biphasic oral contraceptives (4-6). Further, the quadriphasic approach is presented as more physiological since it mimics the natural cycle (7;8). Limited data are available on the contraceptive effectiveness, bleeding pattern and adverse effects of quadriphasic oral contraceptives.

Description of the intervention

Combined oral contraceptives consist of a progestogen component and an estrogen component. Monophasic preparations contain the same dose of progestogen and estrogen every day. In multiphasic oral contraceptives, the progestogen dosage, and in some preparations the estrogen dosage, varies over the cycle. Currently there is one quadriphasic preparation on the market. This oral contraceptive contains estradiol valerate 3 mg on days 1 and 2; dienogest 2 mg and estradiol valerate 2 mg on days 3 to 7; dienogest 3 mg and estradiol valerate 2 mg on days 8 to 24; estradiol valerate 1 mg on days 25 and 26; and placebo on days 27 and 28.

How the intervention might work

Combined oral contraceptives prevent ovulation by inhibiting gonadotropin secretion (1). Ovulation is primarily suppressed by the progestogen component, which prevents the LH-surge by inhibiting luteinizing hormone (LH) secretion. The estrogen component suppresses follicle-stimulating hormone (FSH) secretion but its major role is stabilizing the endometrium to minimize spotting and breakthrough bleeding. Other contraceptive effects of the progestogen component include thickening of the cervical mucus and altering the endometrium in a decidualized bed with atrophied glands.

Why it is important to do this review

Limited data are available on the contraceptive effectiveness, bleeding pattern and adverse effects of quadriphasic oral contraceptives. Suggested benefits of the quadriphasic oral contraceptive containing dienogest and 17ß-estradiol include better bleeding patterns and favorable effects on metabolic and hemostatic variables (4;9-11). However, disadvantages of quadriphasic oral contraceptives include a possible increased risk of pill-taking errors caused by the array of different color pills, complicated directions for catching up when a pill is missed, the higher price and potential inferiority in terms of side effects (7).

We systematically reviewed the literature to summarize the available evidence on the benefits and disadvantages of the quadriphasic approach. The results of the systematic review can assist healthcare providers in counseling women making contraceptive choices. A summary of the available evidence may also be useful for researchers in planning future studies.

Objectives

The aim of this review was to compare the contraceptive effectiveness, bleeding pattern, minor side effects and acceptability of quadriphasic oral contraceptive pills versus monophasic oral contraceptive pills.

Methods

Criteria for considering studies for this review

Types of studies

We included randomized controlled trials in any language. We excluded non-randomized trials.

Types of participants

We included all women of reproductive age enrolled in the randomized controlled trials. Eligibility criteria were those used by the researchers. We included women starting oral contraceptives as well as women switching oral contraceptives.

Types of interventions

Interventions included any quadriphasic oral contraceptive pill compared to any monophasic oral contraceptive pill when used to prevent pregnancy. We excluded studies where the intervention was primarily used as a treatment for disorders, e.g. acne, hirsutism, polycystic ovary syndrome, dysmenorrhea, menorrhagia or endometriosis. Interventions had to be applied for a minimum of three consecutive cycles to be eligible for inclusion.

Types of outcome measures

PRIMARY OUTCOMES

The main outcome was pregnancy. We did not include studies which focus on follicular growth or ovulation.

SECONDARY OUTCOMES

Other outcomes were bleeding patterns, minor side effects, ease of use and trial discontinuation. We excluded studies which primarily focus onmetabolic and hemostatic outcome measures. The definitions of bleeding indices were those specified by the authors.

Search methods for identification of studies

Electronic searches

We searched the Cochrane Central Register of Controlled Trials (CENTRAL),MEDLINE, EMBASE and POPLINE for publications comparing quadriphasic withmonophasic oral contraceptive pills. In addition, we searched for current trials through Clinical- Trials.gov and the International Clinical Trials Registry Platform (ICTRP). The search strategies are shown below.

MEDLINE

contraceptives, oral [mesh] AND (quadrophasic OR four phasic OR four-phasic OR quadri-step OR quadro-step OR quadro step OR "four phasic" OR four-phase OR "four phase" OR "dynamic dosing")

Limited to: humans, female, clinical trial, meta-analysis, randomized controlled trial, review, comparative study, controlled clinical trial, multicenter study

POPLINE

(oral contraceptive*/oral contraceptive agent*) & (quadriphasic/quadrophasic/four phasic/four-phasic/"quadri step"/quadri-step/"quadro step"/ quadro-step)

EMBASE

(oral contraceptive or oral contraceptive agent) and (quadriphasic or quadrophasic or four phasic or four-phasic or "quadri step" or quadri-step or "quadro step" or quadro-step)

CENTRAL

oral contraceptives and phasic

ClinicalTrials.gov

oral contraceptives and quadriphasic or quadrophasic or phasic or quadro-step or quadro step or step

ICTRP

oral contraceptives and quadriphasic

Searching other resources

We reviewed the reference lists of identified studies, review articles and book chapters for additional trials. We contacted the authors of the included trials and pharmaceutical companies marketing quadriphasic oral contraceptives to inquire whether they were aware of any published or unpublished studies which we have missed with our search.

Data collection and analysis

Selection of studies

One author assessed for inclusion or exclusion all titles and abstracts identified during the literature searches under unblinded conditions.

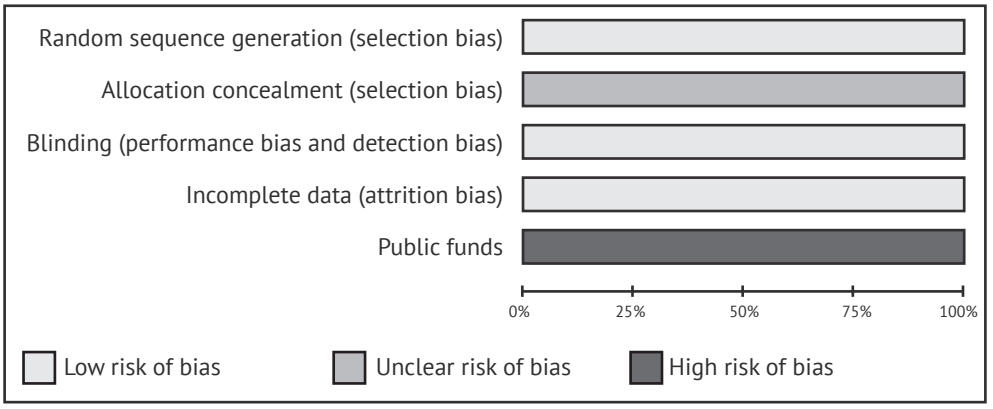
Data extraction and management

One author extracted the data from the included studies under unblinded conditions and entered the data into RevMan (12). In addition to the methodological quality of the study and outcome measures, we extracted data on participants, inclusion and exclusion criteria, study sites, duration of study, study medication, method of collecting the data and funding source. Another author performed a second, independent data abstraction and verified the correct entry of the data. No disagreements about the extracted and entered data occurred.

Assessment of risk of bias in included studies

We critically appraised the methodological quality of the trials according to the recommended principles described in the Cochrane Handbook (13). We focused on the method of generating the allocation sequence, the use and method of allocation concealment, the use and method of blinding, exclusion of participants after randomization, discontinuation and loss to follow-up. Limitations in study design are presented in Risk of bias in included studies, Characteristics of included studies, Figure 1 and Figure 2, and are discussed in the Quality of the evidence section.

Figure 1. *Methodological quality graph: review authors' judgements about each methodological quality item presented as percentages across all included studies.*



Dealing with missing data

We contacted the authors of the eligible and possibly eligible trials for additional information about the study methods and the various outcome measures.

Assessment of heterogeneity

Since only one study was eligible for inclusion we could not assess heterogeneity by the Chi^2 test or assess the impact on the meta analysis using the I^2 statistic.

Data synthesis

We compared the contraceptive effectiveness, bleeding patterns, minor side effects and trial discontinuation between quadriphasic oral contraceptives and monophasic oral contraceptives. The included study reported the number of women who became pregnant, the mean number of bleeding/spotting days with standard deviation, the mean number of bleeding/spotting episodes with standard deviation, the number of women discontinuing early due to side effects and the number of women reporting a particular adverse effect. In addition, the authors provided us the number of women with intracyclic bleeding and the number of women with withdrawal bleeding per cycle. No data on ease of use were mentioned in the paper or provided by the authors. For continuous variables, we computed the mean difference with 95% confidence interval using the random-effects model. For dichotomous variables, we calculated the risk ratio with 95% confidence interval using the random-effects model.

Subgroup analysis and investigation of heterogeneity

Since only one study was included we could not conduct a subgroup analysis by examining only studies with high methodological quality, i.e. studies with an adequate method of generating the allocation sequence, an adequate method of allocation concealment, an adequate method of

blinding and less than 20% loss to follow-up. A subgroup analysis including only starters/switchers was also not performed because the outcomes were not reported according to whether the woman was a starter or switcher.

Results

Description of studies

See: Characteristics of included studies.

Results of the search

The search strategy yielded 31 papers. Four studies were nonrandomized. Twenty-six studies did not meet our inclusion criteria or focused on outcomes not included in this review.

Included studies

One study met the inclusion criteria for this review (A). The study compared a quadriphasic oral contraceptive composed of estradiol valerate 3 mg on days 1 and 2; dienogest 2 mg and estradiol valerate 2 mg on days 3 to 7; dienogest 3 mg and estradiol valerate 2 mg on days 8 to 24; estradiol valerate 1 mg on days 25 and 26; and placebo on days 27 and 28, with a monophasic oral contraceptive composed of levonorgestrel 100 µg and ethinylestradiol 20 µg on days 1 to 21 and placebo on days 22 to 28. The trial included 804 randomized women of whom 402 were allocated to the quadriphasic group and 402 to the monophasic group. The study lasted seven cycles and included 20 822 womenyears. The main objective of the study was to compare bleeding pattern and cycle control with the two preparations. Secondary outcome measures were the number of unintended pregnancies, satisfaction with the treatment and safety. Detailed information regarding participants, inclusion and exclusion criteria, study sites, duration of study, study medication and outcome measures is presented in the Characteristics of included studies.

Excluded studies

There are no excluded studies.

Risk of bias in included studies

Allocation

Randomization was done by a computer-generated random allocation sequence generated at the sponsor's central randomization service. The study did not report the use and method of concealing the treatment allocation sequence. Communication with the authors revealed no extra information.

Blinding

The study is described as a double-blind, double-dummy trial. The paper does not specify who was kept unaware of the oral contraceptives assigned and does not provide information regarding successful implementation of blinding. Communication with the authors revealed no extra information.

Incomplete outcome data

The study reported detailed information on number and reasons for discontinuation. Of the 402 women in the quadriphasic group, 37 women (9%) discontinued early, as did 40 women (10%) of the 402 women in the monophasic group. Three women in both groups did not receive the oral contraceptives after randomization. Fourteen women in the quadriphasic and 15 women in the monophasic group were withdrawn because of protocol violations. No women were lost to follow-up. Communication with the authors indicated analysis according to the intention-to-treat principle without further specification.

Other potential sources of bias

Funding

The trial was supported by the manufacturer of the studied quadriphasic and monophasic pills.

Effects of interventions

Contraceptive effectiveness

No significant difference in contraceptive effectiveness was observed between the quadriphasic and monophasic pills (Analysis 1.1). The study describes one unintended pregnancy caused by a method failure in the monophasic group.

Cycle control and bleeding pattern

Intracyclic bleeding

Additional data on intracyclic bleeding was provided by the authors. Overall, the number of women experiencing intracyclic bleeding did not differ between the quadriphasic and monophasic preparation (Analysis 1.2 to Analysis 1.15). During the fourth cycle the proportion of women having intracyclic bleeding was higher in the quadriphasic group compared to the monophasic group (risk ratio (RR) 1.45; 95% confidence interval (CI) 1.01 to 2.10).

Withdrawal bleeding

Data on withdrawal bleeding not described in the paper were provided by the authors. During each treatment cycle the proportion of women with withdrawal bleeding was higher in the monophasic group compared to the quadriphasic group (Analysis 1.16 to Analysis 1.22 and Figure 2;

at cycle 3 RR 0.88; 95% CI 0.83 to 0.93; at cycle 6 RR 0.89; 95%CI 0.84 to 0.94). Further, the paper describes that the duration and intensity of withdrawal bleeding was shorter and lighter in women using quadriphasic oral contraceptives compared to women using monophasic oral contraceptives. The median length of withdrawal bleeding was 4.0 days for users of quadriphasic pills versus 5.0 days for users of monophasic pills (reported P < 0.05). Themedian intensity score of withdrawal bleeding was 3 (light) for women using quadriphasic oral contraceptives compared to 4 (normal) for women using monophasic oral contraceptives.

Spotting/bleeding

Women using quadriphasic oral contraceptives reported fewer bleeding/spotting days (Analysis 1.23; Analysis 1.24; Figure 3; reference period one mean difference (MD) -4.20; 95%CI -5.52 to -2.88; reference period twoMD-2.50; 95%CI -3.65 to -1.35) and fewer bleeding/spotting episodes (Analysis 1.25; Analysis 1.26; Figure 4; reference period one MD -0.40; 95% CI -0.56 to - 0.24 reference period two MD -0.10; 95% CI -0.26 to 0.06) than women using monophasic oral contraceptives. The report did not specify whether the bleeding/spotting was scheduled or unscheduled.

Discontinuation

The number of women who discontinued due to adverse effects did not differ between quadriphasic and monophasic oral contraceptives (Analysis 1.27). No discontinuations because of bleeding disorders occurred.

Side effects

During the study period significantly more women using quadriphasic oral contraceptives reported breast pain compared to women using monophasic oral contraceptives (Analysis 1.30; Figure 5; RR 3.25; 95% CI 1.07 to 9.88). The number of women reporting headache, acne, alopecia, migraine and increase in body weight did not differ between the two preparations (Analysis 1.28; Analysis 1.29; Analysis 1.31 to Analysis 1.35).

Discussion

Summary of main results

We identified only one trial including 804 women which compared a quadriphasic oral contraceptive composed of estradiol valerate 3 mg on days 1 and 2; dienogest 2 mg and estradiol valerate 2 mg on days 3 to 7; dienogest 3 mg and estradiol valerate 2 mg on days 8 to 24; estradiol valerate 1mg on days 25 and 26; and placebo on days 27 and 28, with a monophasic oral contraceptive composed of levonorgestrel 100 µg and ethinylestradiol 20 µg on days 1 to 21 and placebo on days 22 to 28 (A).

The outcomes were contraceptive effectiveness, bleeding pattern, side effects and discontinuation due to side effects. Users of quadriphasic oral contraceptives were less likely to experience a withdrawal bleeding compared to users of monophasic oral contraceptives. Additionally, the duration and intensity of the withdrawal bleeding was lower in the group of women using quadriphasic oral contraceptives. In the group of quadriphasic pill users more women reported breast pain compared to the group of monophasic pill users. The contraceptive effectiveness, intracyclic bleeding and discontinuation due to side effects did not differ between the two groups.

Overall completeness and applicability of evidence

The presence of only one study including 804 women for seven treatment cycles made it impossible to adequately compare the quadriphasic approach with the monophasic approach in terms of contraceptive effectiveness, bleeding pattern, side effects and discontinuation due to side effects. The two studied preparations differed in progestogen and estrogen content. Since the progestogen as well as the estrogen type is thought to affect cycle control, the observed differences in bleeding pattern might be (partially) explained by the differences in progestogen and estrogen type rather than the phasic approach. In addition, the quadriphasic oral contraceptive was compared with a monophasic oral contraceptive containing 20 µg ethinylestradiol. Contraceptive pills containing 20 µg ethinylestradiol have been shown to result inmore bleeding disturbances and discontinuation due to side effects than contraceptive pills containing more than 20 µg ethinylestradiol (14).

Quality of the evidence

The study featured an adequate method of generating a random allocation sequence, was reported to be double-blinded and had low discontinuation rates. Limitations of the methodological quality of the trial included no description of the use or method of allocation concealment, no specification of who was kept unaware of the assigned treatment, exclusion of participants after randomization and funding of the trial by the manufacturer of the quadriphasic pill. Inadequate methods of allocation concealment and exclusion of participants after randomization may lead to bias (15;16). Studies sponsored by pharmaceutical companies are more likely to have outcomes favoring the sponsor than studies funded by other sources (17;18).

Potential biases in the review process

No potential biases in the review process were evident.

Agreements and disagreements with other studies or reviews

Beside the included randomized controlled trial, two multicentre, non-comparative trials assessing the contraceptive effectiveness, bleeding pattern, minor side effects, early discontinuation and satisfaction during use of the quadriphasic dienogest/estradiol valerate oral contraceptive have been conducted (19;20). Both studies were funded by the manufacturer of the quadriphasic pill. One study has been published in a peer-reviewed journal (20). In this study 13 pregnancies occurred during 23,368 cycles of quadriphasic dienogest/estradiol valerate use (Pearl Index 0.73). No data on bleeding pattern were reported. The paper describes that 272 of the 1377 users of the dienogest/estradiol valerate oral contraceptive (19.8%) reported adverse effects related to the treatment; breast pain was most commonly mentioned (50 users; 3.6%). During the study 140 participants (10.2%) discontinued early due to adverse effects. All reviews, which are narrative, rely on the included randomized trial and the two non-comparative trials (4-6).

Author's conclusions

Implications for practice

We recommend monophasic oral contraceptives as the first choice for women starting oral contraceptive use, given the absence of proven advantages of the quadriphasic approach, the greater complexity of quadriphasic pill regimens, the higher costs of quadriphasic oral contraceptives and the sparse experience with quadriphasic pills. At first prescription, monophasic pills containing 30 µg estrogen are preferred over monophasic pills containing 20 µg since the latter cause more bleeding disturbances and discontinuation (14). Women experiencing heavy menstrual bleeding may benefit from quadriphasic oral contraceptives but continuous use of a monophasic oral contraceptive to avoid menstrual bleeding may also be an alternative.

Implications for research

All new contraceptive preparations require comparison of contraceptive effectiveness, bleeding pattern, side effects, discontinuation rates and beneficial effects with a gold standard in large, adequately reported, high-quality, randomized controlled trials. Studies that compare quadriphasic and monophasic oral contraceptives with an identical progestogen and estrogen type are needed to determine whether the quadriphasic approach differs from the monophasic approach. Trials that compare quadriphasic pills with monophasic pills containing 30 µg ethinylestradiol are indicated to determine whether quadriphasic oral contraceptives have an advantage over currently and widely used oral contraceptives with which providers and consumers have extensive experience.

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** Indicates the major publication for the study*

Characteristics of studies

Characteristics of included studies [ordered by study ID]

Ahrendt 2009

Methods	Double-blind randomized controlled trial
Participants	846 women at 34 sites in Europe Inclusion criteria were healthy women aged 18 to 50 years Exclusion criteria were pregnancy; lactation; fewer than 3 menstrual cycles following childbirth, abortion or lactation; current use of an IUD; BMI more than 30 kg/m2; use of long-acting progestins within 6 months prior to the study entry; hypersensitivity to study drug ingredients; known or suspected malignant or pre-malignant disease; more than 10 cigarettes per day when aged 18 to 30 years or smoking when aged older than 30 years; use of other sex steroids Starters and switchers were included in the study
Interventions	Quadriphasic dienogest/estradiol valerate (E2V 3 mg on days 1 and 2, DNG 2 mg and E2V 2 mg on days 3 to 7, DNG 3 mg and E2V 2 mg on days 8 to 24, E2V 1 mg on days 25 and 26 and placebo on days 27 and 28) versus monophasic levonorgestrel/ethinylestradiol (LNG 100 µg and 20 µg EE on days 1 to 21 and placebo on days 22 to 28). Women were instructed to take the tablets at the same time each day and to take any missed tablets as soon as remembered. If the interval between taking two consecutive tablets was more than 36 hours a non-hormonal contraceptive had to be used
Outcomes	The primary outcome measures are cycle control and bleeding patterns Secondary outcomemeasures include the number of unintended pregnancies, satisfaction with treatment and safety Scheduled bleeding was defined as a bleeding or spotting episode that began during the hormone-free period or started not more than 4 days before the progestin withdrawal in any cycle that continued through into the progestin-free interval. Absence of scheduled bleeding was defined as no bleeding until day 20 of consecutive cycles with the quadriphasic preparation and day 17 with the monophasic preparation Unscheduled bleeding was defined as all other bleeding episodes A bleeding/spotting episode was defined bleeding/spotting days bounded on either end by equal or more than 2 days of no bleeding/spotting Use of daily diary cards to collect data on pill intake and cycle control. Data on side effects were recorded if reported spontaneously
Notes	The report does not provide an a priori hypothesis. The report states a sample size which was chosen to obtain an acceptable estimate of the number of women required to permit acceptably precise comparisons between groups for the number of bleeding/spotting days per reference period. Study duration: 7 cycles

Risk of bias		
Bias	*Authors' judgement*	*Support for judgement*
Random sequence generation (selection bias)	Low risk	Randomization by a computer-generated random allocation sequence
Allocation concealment (selection bias)	Unclear risk	The use of allocation concealment is not described. Communication with the authors revealed no extra information
Blinding (performance bias and detection bias) All outcomes	Low risk	The study is reported as a double-blind, double-dummy trial. Who was kept unaware of the oral contraceptives assigned is not described. Communication with the authors revealed no extra information
Incomplete outcome data (attrition bias) All outcomes	Low risk	37 women in the quadriphasic group and 40 women in the monophasic group discontinued early. The reasons for discontinuation are described. 3 women in each group did not receive the oral contraceptives. No women were lost to follow-up. 14 women in the quadriphasic and 15 women in the monophasic group were withdrawn because of protocol violations. Unclear whether the analysis was according to the intention-to-treat principle. Communication with the authors indicated an analysis according to intention-to-treat without further specification
Public funds	High risk	The trial was supported by the manufacturer of the studied quadriphasic and monophasic pills

BMI: body mass index; DNG: dienogest; E2V: estradiol valerate; EE: ethinylestradiol; IUD: intrauterine device; LNG: levonorgestrel

Data and analyses

Comparison 1. *3 mg E2V on days 1-2; 2 mg DNG/2 mg E2V on days 3-7; 3 mg DNG/2 mg E2V on days 8-24; 1 mg E2V on days 25-26; and placebo on days 27-28 versus 100 µg LNG/20 µg EE on days 1-21 and placebo on days 22-28*

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Pregnancy	1	798	Risk Ratio (M-H, Random, 95% CI)	0.33 [0.01, 8.16]
2 Proportion of women with intracyclic bleeding at cycle 1	1	784	Risk Ratio (M-H, Random, 95% CI)	1.09 [0.81, 1.47]
3 Proportion of women with intracyclic bleeding at cycle 2	1	780	Risk Ratio (M-H, Random, 95% CI)	1.38 [0.97, 1.97]
4 Proportion of women with intracyclic bleeding at cycle 3	1	773	Risk Ratio (M-H, Random, 95% CI)	0.92 [0.64, 1.31]
5 Proportion of women with intracyclic bleeding at cycle 4	1	762	Risk Ratio (M-H, Random, 95% CI)	1.45 [1.01, 2.10]
6 Proportion of women with intracyclic bleeding at cycle 5	1	748	Risk Ratio (M-H, Random, 95% CI)	1.06 [0.70, 1.61]
7 Proportion of women with intracyclic bleeding at cycle 6	1	746	Risk Ratio (M-H, Random, 95% CI)	1.06 [0.69, 1.62]
8 Proportion of women with intracyclic bleeding at cycle 7	1	743	Risk Ratio (M-H, Random, 95% CI)	1.26 [0.84, 1.88]
9 Number of intracyclic bleeding episodes at cycle 1	1	784	Mean Difference (IV, Random, 95% CI)	0.0 [-0.07, 0.07]
10 Number of intracyclic bleeding episodes at cycle 2	1	780	Mean Difference (IV, Random, 95% CI)	0.1 [0.04, 0.16]
11 Number of intracyclic bleeding episodes at cycle 3	1	773	Mean Difference (IV, Random, 95% CI)	0.0 [-0.06, 0.06]
12 Number of intracyclic bleeding episodes at cycle 4	1	762	Mean Difference (IV, Random, 95% CI)	0.1 [0.04, 0.16]
13 Number of intracyclic bleeding episodes at cycle 5	1	748	Mean Difference (IV, Random, 95% CI)	0.0 [-0.06, 0.06]
14 Number of intracyclic bleeding episodes at cycle 6	1	746	Mean Difference (IV, Random, 95% CI)	0.0 [-0.05, 0.05]
15 Number of intracyclic bleeding episodes at cycle 7	1	743	Mean Difference (IV, Random, 95% CI)	0.0 [-0.05, 0.05]
16 Proportion of women with withdrawal bleeding at cycle 1	1	784	Risk Ratio (M-H, Random, 95% CI)	0.88 [0.83, 0.94]
17 Proportion of women with withdrawal bleeding at cycle 2	1	780	Risk Ratio (M-H, Random, 95% CI)	0.84 [0.79, 0.89]
18 Proportion of women with withdrawal bleeding at cycle 3	1	773	Risk Ratio (M-H, Random, 95% CI)	0.88 [0.83, 0.93]
19 Proportion of women with withdrawal bleeding at cycle 4	1	762	Risk Ratio (M-H, Random, 95% CI)	0.90 [0.85, 0.95]
20 Proportion of women with withdrawal bleeding at cycle 5	1	748	Risk Ratio (M-H, Random, 95% CI)	0.86 [0.81, 0.92]

21 Proportion of women with withdrawal bleeding at cycle 6	1	746	Risk Ratio (M-H, Random, 95% CI)	0.89 [0.84, 0.94]
22 Proportion of women with withdrawal bleeding at cycle 7	1	743	Risk Ratio (M-H, Random, 95% CI)	0.87 [0.82, 0.92]
23 Number of bleeding/spotting days in reference period 1	1	798	Mean Difference (IV, Random, 95% CI)	-4.20 [-5.52, -2.88]
24 Number of bleeding/spotting days in reference period 2	1	798	Mean Difference (IV, Random, 95% CI)	-2.5 [-3.65, -1.35]
25 Number of bleeding/spotting episodes in reference period 1	1	798	Mean Difference (IV, Random, 95% CI)	-0.40 [-0.56, -0.24]
26 Number of bleeding/spotting episodes in reference period 2	1	798	Mean Difference (IV, Random, 95% CI)	-0.10 [-0.26, 0.06]
27 Number of women discontinuing due to adverse effects	1	798	Risk Ratio (M-H, Random, 95% CI)	1.0 [0.47, 2.13]
28 Number of women reporting an adverse event	1	798	Risk Ratio (M-H, Random, 95% CI)	1.06 [0.84, 1.34]
29 Number of reported adverse events per total number of women	1	798	Risk Ratio (M-H, Random, 95% CI)	1.09 [0.92, 1.28]
30 Number of women reporting breast pain	1	798	Risk Ratio (M-H, Random, 95% CI)	3.25 [1.07, 9.88]
31 Number of women reporting acne	1	798	Risk Ratio (M-H, Random, 95% CI)	0.56 [0.19, 1.64]
32 Number of women reporting migraine	1	798	Risk Ratio (M-H, Random, 95% CI)	0.4 [0.08, 2.05]
33 Number of women reporting headache	1	798	Risk Ratio (M-H, Random, 95% CI)	1.0 [0.35, 2.82]
34 Number of women reporting alopecia	1	798	Risk Ratio (M-H, Random, 95% CI)	0.75 [0.17, 3.33]
35 Number of women reporting increase in body weight	1	798	Risk Ratio (M-H, Random, 95% CI)	0.5 [0.09, 2.71]

CHAPTER 3

The effect of different hormonal contraceptives on plasma levels of free Protein S and free TFPI

Marjolein Raps
Frans M. Helmerhorst
Kathrin Fleischer
Anders E. A. Dahm
Frits R. Rosendaal
Jan Rosing
Pieter Reitsma
Per Morten Sandset
Huib A. A. M. van Vliet

J Thromb Haemost 2013 109, 606-613

Abstract

Background:

Use of combined oral contraceptives is associated with a three- to six-fold increased risk of venous thrombosis. Hormonal contraceptives induce acquired resistance to activated protein C (APC), which predicts the risk of venous thrombosis. The biological basis of the acquired APC resistance is unknown. Free protein S (PS) and free Tissue Factor Pathway Inhibitor (TFPI) are the two main determinants APC. Our objective was to assess the effect of both hormonal and non-hormonal contraceptives with different routes of administration on free TFPI and free PS levels.

Methods:

We conducted an observational study in 243 users of different contraceptives and measured APC sensitivity ratios (nAPCsr), free TFPI and free PS levels.

Results:

Users of contraceptives with the highest risk of venous thrombosis as reported in recent literature, had the lowest free TFPI and free PS levels, and vice versa, women who used contraceptives with the lowest risk of venous thrombosis had the highest free TFPI and free PS levels. An association was observed between levels of free TFPI and nAPCsr, and between free PS and nAPCsr.

Conclusion:

The effect of oral contraceptives on TFPI and PS is a possible explanation for the increased risk of venous thrombosis associated with oral contraceptives.

Introduction

Use of combined oral contraceptives is associated with a three- to six-fold increased risk of venous thrombosis. This increased risk depends on the estrogen dose as well as the progestogen type of oral contraceptives (1). So called 'high-dose' combined oral contraceptives containing 50 µg or more ethinylestradiol are associated with a two-fold higher risk of thrombosis than 'low-dose' combined oral contraceptives containing 20 to 30 µg ethinylestradiol (2;3). Further, third-generation combined oral contraceptives, containing the progestogens gestodene or desogestrel, and combined oral contraceptives containing cyproterone acetate or drospirenone increase the risk of venous thrombosis by a factor of two compared with combined oral contraceptives containing levonorgestrel (1;3;4).

Use of combined oral contraceptives causes changes in procoagulant, anticoagulant and fibrinolytic parameters (5). The thrombin generation-based APC resistance test is a global assay that is sensitive to the levels of individual clotting factors and combines these into a net effect (6-8). In line with epidemiological observations, users of 'high risk' combined oral contraceptives containing desogestrel, cyproterone actetate and drospirenone have been found more resistant to the anticoagulant action of APC in this test than users of 'low risk' combined oral contraceptives containing levonorgestrel. (3;4;6;8-11).

The two main determinants of the thrombin generation-based APC resistance test are free protein S (PS) and free tissue factor (TF) pathway inhibitor (TFPI) (12-14). The differences in APC-resistance induced by oral contraceptives can at least in part be explained by different effects on free PS and free TFPI levels (12).

TFPI is a Kunitz-type protease inhibitor that down-regulates the extrinsic (TF-induced) coagulation pathway. It is mainly synthesized by endothelium cells (15). Approximately 80% of intravascular TFPI is bound to the vessel wall, whereas 20% circulates in plasma. Only 10% (~0.25 nM) of TFPI in plasma circulates as free full length TFPI, which is the most active form. TFPI down-regulates thrombin generation by inhibiting activated factor X (FXa) via the formation of a TFPI/FXa complex which subsequently inhibits the TF/activated factor VII (TF/FVIIa) complex by forming an inactive TFPI/FXa/TF/FVIIa quaternary complex (16). TFPI is a slow inhibitor of thrombin generation, which is most effective at low TF concentrations or when thrombin generation is slowed down through the intrinsic pathway (17).

PS is a vitamin K dependent protein which acts as a non-enzymatic cofactor of APC in the proteolytic inactivation of the activated factors Va (FVa) and VIIIa (FVIIIa) (18). It is synthesized in the liver and in endothelial cells. Approximately 60% of the PS in plasma is bound to C4b Binding Protein (C4BP) in a high affinity complex, while the remaining 40% circulates in plasma in a free form. It was recently discovered that, besides its co-factor function with APC, free PS forms a complex with TFPI and stimulates formation of the TFPI/FXa complex, thus enhancing the down-regulation of thrombin generation by TFPI (17;19-23).

Both hereditary and acquired PS deficiency is associated with an increased risk of venous thrombosis (20;21;21). It was observed by Castoldi *et al.* that PS deficient individuals also have decreased TFPI levels, probably due to common mechanisms regulating biosynthesis of both proteins (20). Van Vliet *et al.* (12) observed that women using combined oral contraceptives with the highest risk of venous thrombosis (e.g. containing desogestrel, cyproterone actetate or drospirenone), have lower levels of protein S and TFPI than women using the combined oral contraceptive with the lowest risk of venous thrombosis (i.e. contraceptives containing levonorgestrel).

The aim of this study was to assess if the different risks of venous thrombosis caused by different hormonal and non-hormonal methods of contraception with various routes of administration are reflected in the levels of APC resistance, and free TFPI and free PS, the main determinants of the thrombin generation-based APC resistance test.

Material and Methods

Study design and participants

We conducted an observational study. In a series of four different studies we collected samples of users of different hormonal and non-hormonal contraceptives, including oral, transdermal and vaginal combined hormonal contraceptives, the levonorgestrel-releasing intrauterine device (IUD), the copper-releasing IUD and female non-users with regular, ovulatory menstrual cycles (24-27).

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 (28). A more detailed description of the studies can be found in the original articles (24;26;27;29).

In total, we excluded 73 participants out of 316 eligible participants. Participants who were carriers of the factor V Leiden or the prothrombin 20210A mutation were excluded from the analysis, because these mutations affect resistance to APC (n = 31). Users of oral contraceptives containing 20 µg ethinylestradiol and groups with a small sample size were excluded: users of the vaginal ring containing ethinylestradiol and etonogestrel (n = 4), users of the transdermal patch containing ethinylestradiol and norelgestromine (n = 3), users of a combined oral contraceptives containing 100 µg levonorgestrel and 20 µg ethinylestradiol (n = 4), 150 µg desogestrel and 20 µg ethinylestradiol (n = 12), 75 µg gestodene and 20 µg ethinylestradiol (n = 7), 75 µg gestodene and 30 µg ethinylestradiol (n = 3), 250 µg norgestimate and 35 µg ethinylestradiol (n = 1) or containing 1 mg norethisterone and 35 µg ethinylestradiol (n = 2). Six participants were excluded because their data were not complete.

In our final analysis, we used the samples of 243 participants: 153 users of a combined oral contraceptive containing ethinylestradiol and levonorgestrel, desogestrel, cyproterone actetate or drospirenone, 60 users of the levonorgestrel-IUD, 17 users of the copper-IUD and 13 non-users (mid-cycle).

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 samples from the studies were drawn, 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). Cell-free, citrated plasma was prepared by centrifuging blood at 2,100 g for 10 minutes at 18ºC, coded and centrally stored at -80 ºC.

Protein S

Free Protein S was determined by ELISA according to Giri *et al*. (30) with modifications as follows: microtiter plates were coated overnight at 4-8 ºC with purified C4BPß (3,5 µg/mL in 0.1 M NaHCO3, 0.5 M NaCl buffer, pH 9). Wells were washed four times with buffer A (0.05 M Tris-HCl, 0.1 M NaCl, 0.1% Tween, 0.05% ovalbumin, pH 7,5) and incubated with buffer A containing 2,5% ovalbumin for 1 hour at 37 ºC to reduce background absorbance. After four washes with buffer B (0.05 M Tris-HCl, 0.1 M NaCl, 0,1% Tween, 0.05% ovalbumin, 0.005 M CaCl2, 0.01 M benzamidine-HCl, pH 7,5) the dilutions of calibrator (1/10-1/640) and test samples (1/20 and 1/40) were added and incubated for 15 minutes at room temperature. After four washes with buffer B, HRP-conjugated anti-human protein S (0.4 µg/mL) antibody was added and incubated for 1 hour at 37 ºC followed by four washes with buffer B. Subsequently, TMB (0.2 mg/mL) and H2O2 (0.01%) were added and incubated for 15 minutes at room temperature, after which the reaction was stopped by adding 4N H2SO4 and the absorbance at 450 nm was measured. Supernatant of PNP supplemented with an equal volume of 10% PEG 6000 was used as calibrator. This plasma contained 0.28 U/mL of protein S total, which is all free protein S. All sample dilutions were prepared within 10 minutes of starting the assay. Pooled normal plasma contained 0.31 U/mL free protein S and 1.0 U/mL total protein S. Inter- and intra-assay variability was 5.7% and 8.0% respectively.

TFPI

Free TFPI and total TFPI were assayed with commercial enzyme-linked immunosorbent assays (Asserachrom® free TFPI and Asserachrom® Total TFPI, Diagnostica Stage, Asnière, France) as described in detail elsewhere (31). Freeze-dried human plasmas containing known amounts of TFPI provided in the kits were used for calibration. Quality controls were performed using a control specimen containing a high amount of TFPI as well as a sample with normal level of TFPI. Inter- and intra-assay variability, measured as coefficients of variation, was 6,0% and 2,6% for total TFPI, and 9,5% and 5,3% for free TFPI, respectively.

APC resistance

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 (32).

Statistical analysis

In this observational study, we used means, mean differences, 95% confidence intervals of the mean and ranges to describe variables. Scatter diagrams and regression lines were constructed and a regression coefficient with 95% CI was estimated with free PS or free TFPI as independent variable and nAPCsr as dependent variable. A bar diagram was constructed to visualize the association between the risk of venous thrombosis during use of hormonal contraceptives as described in recent literature and the measured free TFPI and free PS levels. Statistics were computed using SPSS for Windows, version 17.0 (SPSS Inc., Chicago, IL, USA).

Results

There were no differences in BMI and age between the women using different kinds of hormonal contraceptives (Table 1).

Table 1: *Characteristics of participants after exclusion*

Contraceptive	Progestogen	Estrogen	n	Age (years)		BMI (kg/m2)	
				Mean	Range	Mean	Range
None	-	-	13	29	20-48	22	19-26
Cu-IUD	-	-	17	33	20-45	24	18-32
LNG-IUD	20 µg/day LNG	-	60	33	17-52	25	18-48
Oral contraceptive	150 µg/day LNG	30 µg/day EE	70	29	18-51	23	17-38
Oral contraceptive	150 µg/day DSG	30 µg/day EE	16	31	18-49	25	20-32
Oral contraceptive	3 mg/day DRSP	30 µg/day EE	46	29	18-47	24	18-34
Oral contraceptive	2 mg/day CPA	35 µg/day EE	21	28	19-44	22	19-26
Total			243	30	17-52	24	17-48

Free TFPI levels during use of hormonal contraceptives compared with non-use

Women using the levonorgestrel-IUD had higher free TFPI levels than non-users (5.48 ng/ml versus 4.62 ng/ml; difference 0.85 ng/ml, 95% CI 0.05 to 1.66). The mean free TFPI level of women using the copper-IUD did not differ with the mean free TFPI levels of non-users (5.06 ng/ml versus 4.62 ng/ml; difference 0.44 ng/ml, 95% CI -0.52 to 1.39).

Combined oral contraceptive users had lower free TFPI levels than non-users. Women using cyproterone actetate / ethinylestradiol had the lowest mean free TFPI level: 2.51 ng/ml, difference -2.12 ng/ml compared with non-users (95% CI -2.73 to -1.50). (Table 2)

Free PS levels during use of hormonal contraceptives compared with non-use

Women using the levonorgestrel-IUD had similar free PS levels as non-users (0.28 U/dl versus

0.28 U/dl; MD -0.001 U/dl, 95% CI -0.03 to 0.03). The mean free PS levels of women using the copper-IUD did also not differ from non-users (0.26 U/dl versus 0.28 U/dl; MD -0.02 U/dl 95% CI -0.07 to 0.02).

Women using combined oral contraceptives had lower free PS levels than non-users. The lowest mean free PS levels were measured in women using cyproterone actetate / ethinylestradiol: 0.19 U/dl , MD -0.09 U/dl compared with non-users (95% CI -0.12 to -0.05). (Table 3)

Free TFPI and free PS levels compared with levonorgestrel / ethinylestradiol

Women using combined oral contraceptives with a high risk of venous thrombosis had lower free TFPI levels than women using combined oral contraceptives with a lower risk of venous thrombosis, e.g levonorgestrel / ethinylestradiol. The mean difference between levonorgestrel / ethinylestradiol compared with other oral contraceptive users was -1.01 ng/ml (95% CI -1.81 to -0.22) for desogestrel / ethinylestradiol, -0.57 ng/ml (95% CI -1.10 to -0.03) for drospirenone / ethinylestradiol and -1.42 ng/ml (95% CI -2.12 to -0.72) for cyproterone actetate / ethinylestradiol.

The same pattern was observed for free PS levels: the mean difference in free PS of levonorgestrel / ethinylestradiol compared with desogestrel / ethinylestradiol was -0.09 U/dl (-0.12 to -0.05), compared with drospirenone / ethinylestradiol was -0.08 U/dl (95% CI -0.11 to -0.06) and compared with cyproterone actetate / ethinylestradiol was -0.13 U/dl (-0.16 to -0.10).

Association between free TFPI and APC resistance and free PS and APC resistance

In previous reports (33;34) on the resistance to APC in the same study population, we observed higher nAPCsr in the groups of women using combined oral contraceptives containing desogestrel, drospirenone and cyproterone actetate than in non-users. The nAPCsr in women using the levonorgestrel-IUD was decreased compared with non-users and users of the combined oral contraceptive containing levonorgestrel / ethinylestradiol (24).

Free TFPI levels were negatively correlated with the nAPCsr: a linear association was observed with the equation free TFPI = 6.082 – 0.629 * nAPCsr. Thus, a decrease of free TFPI with 1 ng/ml was associated with an increase of nAPCsr with 0.629 (95% CI 0.47 to 0.79).

Free PS levels were also negatively associated with the nAPCsr: a linear association was found with the equation free PS = 0.355 – 0.028 * nAPCsr: when free PS levels decreased with 1 U/dl nAPCsr increased with 0.028 (95% CI 0.022 to 0.34).

Risk ranking per contraceptive

For risk ranking, we used recent publications from van Hylckama Vlieg *et al*. (3;35) (Table 4). The odds ratio for venous thrombosis during use of the levonorgestrel-IUD compared with non-users was 0.3 (95% CI 0.1 to 1.1) (35). The risk of venous thrombosis during use of a copper-IUD is unknown, but expected not to be increased compared with non-users.

Free TFPI levels measured in this study are associated with the odds ratios reported in recent literature i.e lower free TFPI levels were present in users of contraceptives with a higher risk of venous thrombosis. Free PS showed a less pronounced, but similar pattern (Figures 1 and 2).

Total TFPI

Total TFPI levels in plasma of users of different kinds of contraceptives were comparable. No association was observed between known risk ratios and total TFPI levels (data not shown).

Discussion

In this study on the effect of different contraceptives on the two main determinants of the thrombin generation-based APC resistance test, we observed that different doses of ethinylestradiol and different types of progestogens have different effects on the plasma levels of free TFPI and free PS. Women who are according to recent literature exposed to the highest risk of venous thrombosis during use of hormonal contraceptives (i.e. users of combined oral contraceptives containing drospirenone / ethinylestradiol, cyproterone actetate / ethinylestradiol or desogestrel / ethinylestradiol), had the lowest free TFPI and free PS levels and vice versa: women who used hormonal contraceptives with the lowest risk of venous thrombosis (i.e. users of the levonorgestrel-IUD) had the highest free TFPI and free PS levels. An association was observed between free TFPI and nAPCsr, and between free PS and nAPCsr.

The observed effect of different types of oral contraceptives on free TFPI are consistent with a study by Alhenc-Gelas *et al*. (37). Plasma levels of free TFPI were found to be more decreased in women using oral contraceptives containing desogestrel / ethinylestradiol or cyproterone actetate / ethinylestradiol than in women using oral contraceptives containing levonorgestrel / ethinylestradiol.

Several studies observed that third generation oral contraceptives containing gestodene or desogestrel combined with ethinylestradiol induce a larger decrease of free PS than oral contraceptives containing levonorgestrel / ethinylestradiol (38-40). In our study similar decreases in free PS levels were found for desogestrel / ethinylestradiol users compared with levonorgestrel / ethinylestradiol users. In a trial by Kluft *et al*. (41), oral contraceptives containing drospirenone / ethinylestradiol and oral contraceptives containing desogestrel / ethinylestradiol led to similar reductions in PS total antigen levels and PS activity levels, but no data are available on free PS levels. No studies have been published on the effect of cyproterone actetate / ethinylestradiol on free PS levels .

In our study, sample sizes of the groups of users of the vaginal ring containing ethinylestradiol and etonogestrel and users of the transdermal patch containing ethinylestradiol and norelgestromine were too small for analysis. In a randomized controlled trial by Johnson *et al*. (42) patch users had lower free PS levels than users of a levonorgestrel / ethinylestradiol containing oral contraceptive. Jensen *et al*. (43) found that free PS levels of ring users were higher, and free PS levels of patch users were lower compared with use of a combined oral contraceptive. Recently, Lidegaard *et al*. (36) reported a RR of 7.9 (95% CI 3.5 to 17.6) for users of the transdermal patch and a RR of

6.5 (95% CI 4.7 to 8.9) for users of the vaginal ring compared with non-users. Both contraceptives contain an estrogen and a progestogen compound and work systemically. Increased relative risks were observed, so decreased levels of TFPI and PS are to be expected.

The use of the levonorgestrel-IUD is not associated with an increased risk of venous thrombosis (35;44). In this study, women using the levonorgestrel-IUD had higher free TFPI and similar free PS and nAPCsr levels as non-users, indicating that use of the levonorgestrel-IUD is not associated with an increased risk of venous thrombosis. It can be hypothesized that the differences in free TFPI levels are attributable to the progestogen compound of the hormonal contraceptive, since the amount of ethinylestradiol of hormonal contraceptives used in this study were similar while the progestogen types differed, and different levels of free TFPI were observed. Possibly, progestogens cause an increase in free TFPI, as observed in users of the levonorgestrel-IUD, and ethinylestradiol causes a decrease in free TFPI, reflected as a net effect. No other studies have been published on free PS and free TFPI levels in users of the levonorgestrel-IUD or copper-IUD.

Kemmeren *et al*. explained the differences in free PS induced by various oral contraceptives by the interaction between PS and C4BP. C4BP binds protein S in a high affinity complex (39). They observed that total PS was decreased by oral contraceptives containing desogestrel / ethinylestradiol but was hardly affected by oral contraceptives containing levonorgestrel / ethinylestradiol and that both oral contraceptives equally lowered C4BP. As a result, free PS levels increase in users of levonorgestrel / ethinylestradiol and decrease in users of desogestrel / ethinylestradiol.

Free PS forms a complex with free TFPI and acts as cofactor of TFPI through the extrinsic pathway (17;19-22). Inherited or acquired PS deficiency causes concomitant decreased free TFPI levels (20). A possible explanation could be that binding of free TFPI to free PS protects it from proteolytic degradation or slows down the clearance of free TFPI. So a decrease of free PS will then be accompanied by a decrease in free TFPI. The effect of hormonal contraceptives on free PS is probably only part of the mechanism of the increased risk of venous thrombosis since the effect on free TFPI-levels is usually larger than the effect on free PS. Due to this fact it is likely that TFPI production or release is also influenced by hormonal contraceptives; both proteins share the endothelium as common production site. In addition, the secretion of TFPI from endothelial cells might be coupled to PS secretion, as has recently been discovered for PS and the beta chain of C4BP by Carlsson *et al*. (20;45).

In conclusion, we observed that users of the levonorgestrel-IUD have similar levels of free PS and higher levels of free TFPI as non-users. Users of oral contraceptives containing drospirenone / ethinylestradiol, cyproterone actetate / ethinylestradiol and desogestrel / ethinylestradiol had lower free TFPI and free PS levels than users of oral contraceptives containing levonorgestrel / ethinylestradiol. A negative association between the thrombotic risks as reported in recent literature and free PS and free TFPI levels was observed. Our study confirms the hypothesis that the differences in APC resistance induced by hormonal contraceptives can be partly explained by different effects on free TFPI and free PS levels. Future studies are indicated to unravel the mechanism of the reduction of free PS and free TFPI during use of hormonal contraceptives and the exact mechanism of their relation with the risk of venous thrombosis.

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CHAPTER 4

Sex hormone-binding globulin as a marker for the thrombotic risk of hormonal contraceptives

Marjolein Raps
Frans M. Helmerhorst
Kathrin Fleischer
Anders E. A. Dahm
Frits R. Rosendaal
Jan Rosing
Pieter Reitsma
Per Morten Sandset

Huib A. A. M. van Vliet *J Thromb Haemost 2012; 10: 992–997*

Abstract

Background:

It takes many years to obtain reliable values for the risk of venous thrombosis of hormonal contraceptive users from clinical data. Measurement of activated protein C (APC) resistance via thrombin generation is a validated test for determining the thrombogenicity of hormonal contraceptives. Sex hormone-binding globulin (SHBG) might serve as a marker for the risk of venous thrombosis, and can be easily and rapidly measured in routine laboratories.

Objective:

To determine whether SHBG is a useful marker for the thrombotic risk of hormonal contraceptive users by comparing plasma SHBG levels with normalized APC sensitivity ratio (nAPCsr) values and thrombosis risks reported in the recent literature.

Methods:

We conducted an observational study in 262 users of different contraceptives, and measured nAPCsr and SHBG levels.

Results:

Users of contraceptives with a higher risk of causing venous thrombosis, i.e. combined hormonal contraceptives containing desogestrel, cyproterone acetate or drospirenone, and the transdermal patch, had higher SHBG levels than users of combined hormonal contraceptives containing levonorgestrel, which carry a lower thrombosis risk. Users of the patch had the highest SHBG levels, with a mean difference of 246 nmol/L (95% confidence interval 179–349) from that in users of levonorgestrel-containing combined hormonal contraceptives. SHBG levels were positively associated with both the nAPCsr and the risks of venous thrombosis reported in the recent literature.

Conclusion:

SHBG is a useful marker with which to estimate the thrombotic safety of a preparation.

Introduction

The use of combined oral contraceptives is associated with a three-fold to six-fold increased risk of venous thrombosis (1). This increased risk depends on both the estrogen dose and the progestogen type of combined oral contraceptives (1). So-called 'high-dose' combined oral contraceptives containing 50 µg or more ethinylestradiol (EE) are associated with a two-fold higher risk of thrombosis than 'low-dose' combined oral contraceptives containing 20–30 µg of 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 of two as compared with combined oral contraceptives containing levonorgestrel (LNG) (1–13).

The differences in the risk of venous thrombosis can be at least partially explained by the association of various combined oral contraceptives with differences in 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) (14–16). High nAPCsr indicates increased APC resistance, which is a risk factor for venous thrombosis. Thrombin generation-based APC resistance has been validated in a case–control study by Tans *et al.* (17), and predicts the risk of venous thrombosis in users of combined oral contraceptives, as well as in non-users and men, with or without the factor V Leiden mutation. The highest odds ratio (OR) of venous thrombosis in the absence of the FV Leiden mutation was observed in premenopausal women using combined oral contraceptives, lending support to the hypothesis that the prothrombotic effect of combined oral contraceptives is the result of acquired APC resistance in a thrombin generation-based test (17). Users of combined oral contraceptives with a higher risk of causing venous thrombosis, e.g. those containing DSG, CPA or DRSP, have been found to be more resistant to the anticoagulant action of APC than users of combined oral contraceptives with a lower risk of causing venous thrombosis, i.e. those containing LNG (3;6;9;10;14–16).

As the absolute risk of venous thrombosis in women using combined oral contraceptives is low, i.e. three to four per 10 000 woman-years (1), the assessment of differences in risk between an existing and a new preparation requires hundreds of thousands of users. This sample size makes a clinical study of a new hormonal contraceptive before market authorization almost impossible.

In a search for other markers that can predict the risk of venous thrombosis in users of hormonal contraceptives, Odlind *et al.* (18) postulated sex hormone-binding globulin (SHBG) as a marker for estrogenicity of a contraceptive preparation and possibly for the risk of venous thrombosis. SHBG is a carrier protein that is produced in the liver and binds estrogen and testosterone (19). The hypothesis is that estrogens cause a dose-related increase in SHBG levels, whereas progestogens induce a decrease in SHBG levels, dependent on both the dose and the type of progestogen (20–22). The type-related differences in the progestogen-induced decrease in SHBG levels can be interpreted as differences in the antiestrogenic properties of progestogens. Thus, the effect of a hormonal contraceptive on SHBG is the combined result of the estrogenic effect of EE and the antiestrogenic effect of the progestogen, yielding the total estrogenicity of that hormonal

contraceptive. This estrogenicity might serve as a marker for venous thrombosis. Several studies have shown an association between the risk of causing venous thrombosis of combined oral contraceptives, APC resistance, and SHBG levels (1–3;15;23).

To investigate whether SHBG is a useful marker for the risk of venous thrombosis of combined oral contraceptives, we determined SHBG levels in non-users and in users of different contraceptives, both hormonal and non-hormonal, and compared the SHBG levels with nAPCsr as determined via thrombin generation and with the risks of venous thrombosis as reported in the literature.

Materials and methods

Study design and participants

We conducted an observational study. In a series of four different studies, we included users of various hormonal and non-hormonal contraceptives (15;24–26). Users of different combined hormonal contraceptives, including oral, transdermal and vaginal combined hormonal contraceptives, users of LNG-releasing intrauterine devices (IUDs) (LNG-IUDs), users of copper-releasing IUDs (Cu-IUDs) and healthy female non-users with regular, ovulatory menstrual cycles were studied.

The inclusion criterion for all participants was as follows: 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 (27). A more detailed description can be found in the original articles (15;24–26).

Participants who were carriers of the FV Leiden mutation were excluded from the analysis, because this mutation causes resistance to APC without affecting SHBG levels (n = 30). The following data were not used because of a small sample size: users of a combined oral contraceptive containing GTD, norgestimate and norethisterone (n = 3 for GTD, n = 1 for norgestimate, and n = 2 for norethisterone). Furthermore, 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 = 24). For 26 participants, data were not complete, so they were excluded. In total, we excluded 86 participants.

In our final analysis, we used the samples of 262 participants: 159 users of a combined oral contraceptive (containing 30–35 µg of EE and LNG, DSG, CPA, or DRSP), 60 users of the LNG-IUD, 17 users of the Cu-IUD, seven users of the transdermal patch (containing EE and norelgestromine (NGM)), six users of the vaginal ring (containing EE and etonogestrel (ENG)), and 13 non-users (mid-cycle).

Written informed consent was given by all participants, and the studies were all approved by theMedical 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 mol/L sodium citrate (pH 5.8). 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.

SHBG (nmol/L) was measured with an immunometric assay (Immulite 2000 XPi; Siemens Healthcare Diagnostics, Tarrytown, NY, USA). The sensitivity is 0.2 nmol/L, and has a long-term variation of 6%, at levels of both 5 and 80 nmol/L The within-assay variation is 3–4%, and the between-assay variation is 3.5–6%. APC resistance was measured with the thrombin generation-based APC resistance test, as described previously (14).

nAPCsr values of plasma samples from women using an LNG-IUD or a Cu-IUD were originally measured with a variant of the thrombin generation-based APC resistance assay, by the use of using calibrated automated thrombinography (24;28). As nAPCsr values determined with calibrated automated thrombinography are higher than those determined with the classical endpoint method (16;29), the plasma samples from IUD users were reanalyzed with the endpoint method.

SHBG levels and APC resistance in non-users during midcycle were used in the analysis. The different phases in the menstrual cycle were defined by repeated measurements of progesterone and estradiol levels; mid-cycle is defined as the time when estradiol levels are high and progesterone levels are low.

Statistical analysis

We used means, mean differences, 95% confidence intervals and ranges to describe variables. We constructed a scatterplot to describe the association between SHBG levels and nAPCsr; in this figure SHBG data were logarithmically transformed to create normality and a histogram analysis of the residuals was performed to check whether this assumption is valid. A regression analysis was performed to describe the association.

Table 1: *Body mass index (BMI) and age of the research population*

Contraceptive	n	BMI (kg m^{-2})		Age (years)	
		Mean	Range	Mean	Range
None	13	21.7	19–29	29.0	20–48
LNG-IUD	60	24.5	18–47	32.6	17–52
Cu-IUD	17	24.2	18–32	32.4	20–45
LNG/EE	72	22.2	17–38	25.7	18–51
DSG/EE	18	24.0	20–32	30.2	18–49
DRSP/E	22	22.1	19–26	27.5	19–44
CPA/EE	22	22.1	19–26	27.5	19–44
ENG/EE (ring)	6	24.2	21–28	26.4	20–36
NGM/EE (patch)	7	22. 4	20–26	31.1	25–43
All	262	23.5	18–47	28.8	17–52

CPA, cyproterone acetate; Cu-IUD, copper-releasing intrauterine device; EE, ethinylestradiol; ENG, etonogestrel; DRSP, drospirenone; DSG, desogestrel; LNG, levonorgestrel; LNG-IUD, levonorgestrelre-leasing intrauterine device; NGM, norelgestromine.

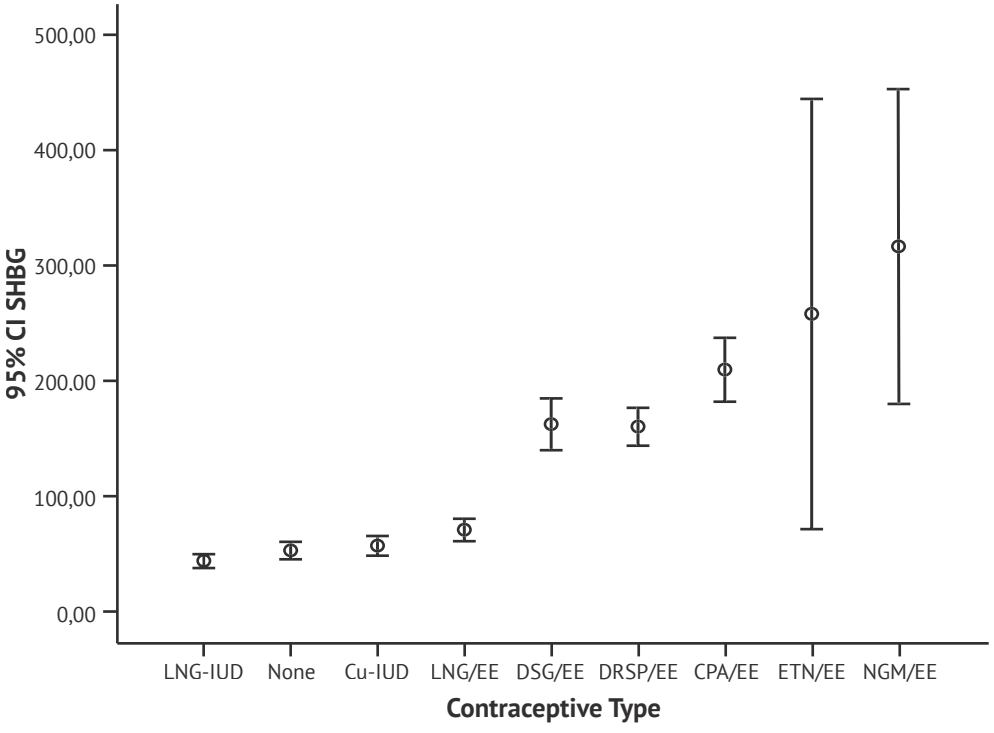

Fig. 1. *Sex hormone-binding globulin (SHBG) levels and their 95% confidence intervals (CIs) by contraceptive type. CPA, cyproterone acetate;Cu-IUD, copper-releasing intrauterine device; DRSP, drospirenone;DSG, desogestrel; EE, ethinylestradiol; ENG, etonogestrel; LNG, levonorgestrel; LNG-IUD, levonorgestrel-releasing intrauterine device; NGM, norelgestromine.*

Table 2: *Mean sex hormone-binding globulin (SHBG) and activated protein C (APC) resistance levels, mean differences (MDs) and 95% confidence intervals (CIs) for non-users as compared with levonorgestrel (LNG)/ethinylestradiol (EE) users*

Contraceptive	n	SHBG (nmol L^{-1})					APC-resistance (ratio)		
			Compared to non-use		Compared to LNG/EE		Compared to non-use		
		Mean	MD	95% CI	MD	95% CI	Mean	MD	95% CI
None	13	53.22	Ref		-17.78	-41.35 to 5.44	1.54	Ref	
LNG-IUD	60	43.77	-9.45	-22.08 to 3.17	-27.23	-39.03 to -15.44	0.85	-0.69	-1.03 to -0.36
Cu-IUD	17	57.52	4.29	-7.26 to 15.85	-13.48	-34.00 to 7.03	1.03	-0.51	-0.93 to -0.09
LNG/EE	72	71.00	17.78	-5.46 to 41.02	Ref		2.66	1.12	0.69 to 1.54
DSG/EE	18	162.78	109.55	82.98 to 136.13	91.78	69.60 to 113.96	3.94	2.40	1.93 to 2.86
DRSP/EE	47	161.04	107.82	7.10 to 139.54	90.04	72.23 to 107.85	3.53	1.98	1.49 to 2.48
CPA/EE	22	210.27	157.05	121.03 to 193.07	139.27	116.41 to 162.13	4.00	2.46	2.07 to 2.84
ENG/EE (Ring)	6	258.93	205.71	104.77 to 306.65	187.93	136.51 to 239.36	3.02	1.47	0.94 to 2.02
NGM/EE (Patch)	7	317.57	264.35	179.63 to 349.06	246.57	201.29 to 291.85	3.12	1.57	0.87 to 2.28

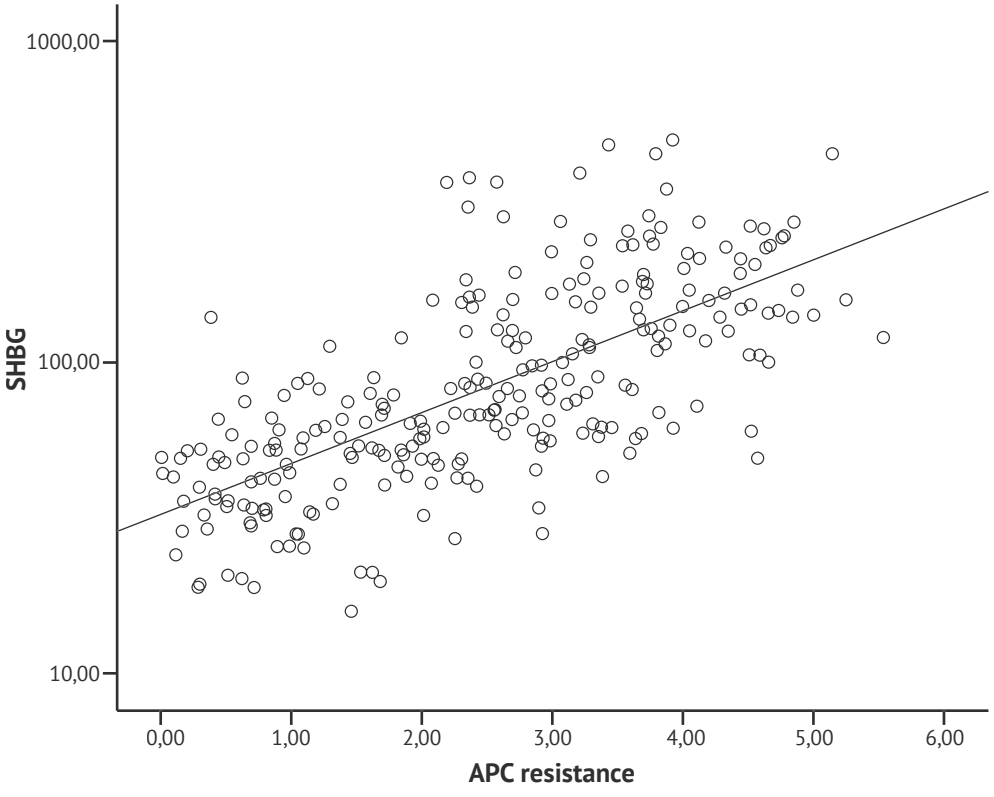

Fig. 2. *The association between sex hormone-binding globulin (SHBG) and activated protein C (APC) resistance. Equation: log10(SHBG) = 1.525 + (0.160 × nAPCsr).*

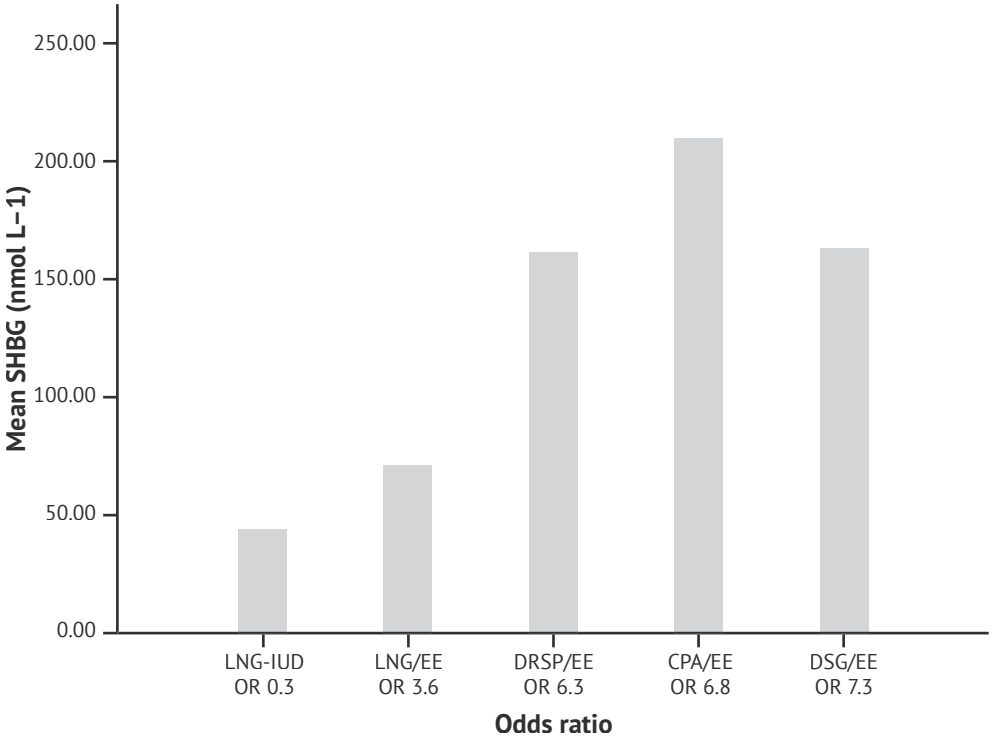

Fig. 3. *The association between odds ratios (ORs) of the risk of venous thrombosis of various contraceptives as published in the recent literature [3,31,32] and sex hormone-binding globulin (SHBG) levels of hormonal contraceptives. CPA, cyproterone acetate; DRSP, drospirenone; DSG, desogestrel; EE, ethinylestradiol; LNG, levonorgestrel; LNG-IUD, levonorgestrel-releasing intrauterine device.*

Table 3. *The odds ratios (ORs) of venous thrombosis during the use ofdifferent types of hormonal contraceptive as compared with non-users,according to the recent literature [3,31,32]*

Contraceptive	Risk		Reference
	OR	95% CI	
None	Ref		
LNG-IUD	0.3	0.1 to 1.1	(31)
Cu-IUD	-	-	
LNG/EE	3.6	2.9 to 4.6	(3)
DSG/EE	7.3	5.3 to 10.0	(3)
DRSP/EE	6.3	2.9 to 13.7	(3)
CPA/EE	6.8	4.6 to 10.0	(3)
ETN/EE	-	-	
NGM/EE	1.3 to 2.0	-	(32)

CI, confidence interval; CPA, cyproterone acetate; Cu-IUD, copperreleasing intrauterinedevice;DRSP, dros pirenone;DSG,desogestrel;EE, ethinylestradiol; ENG, etonogestrel; LNG, levonorgestrel; LNG-IUD, levonorgestrel-releasing intrauterine device; NGM, norelgestromine.

Results

There were no significant differences in BMI and age between the women using different kinds of hormonal contraceptives (Table 1).

SHBG levels during contraceptive use

SHBG levels of the studied contraceptives were compared to non-users and to users of the most used combined oral contraceptive containing LNG/EE. Users of contraceptives containing EE plus CPA, DRSP or DSG and users of the transdermal patch or vaginal ring had higher SHBG levels than users of the LNG/EE containing combined oral contraceptive. Users of the LNG-IUD or Cu-IUD had lower or comparable SHBG levels as non-users. (Fig. 1, Table 2).

Association between SHBG and APC resistance

SHBG plasma levels were positively associated with nAPCsr in users of different kinds of hormonal contraceptives (i.e. combined oral contraceptives and LNG-IUD) and non-users. An exponential association was observed according the equation: log10(SHBG) = 1,525 + 0,160 × nAPCsr. Thus, when the nAPCsr increases with 1 unit, SHBG levels increase with 45% (100.160 = 1.45) (Fig. 2).

Risk ranking per contraceptive

For risk ranking, we used recent publications by van Hylckama Vlieg *et al* (3) and Jick *et al* (30). (Table 3) The observed odds ratio for venous thrombosis during use of the LNG-IUD compared to non-users was 0.3 (95% CI 0.1 to 1.1) (3) and the observed odds ratio during use of the transdermal patch compared to use of the LNG containing combined oral contraceptives was variable and reported to be between 1.3 and 2.0 (30). The risk of venous thrombosis during use of a Cu-IUD is unknown, but expected not to be increased compared to non-users. There are no data on the contraceptive vaginal ring compared to non-users, but a study on the risk of venous thrombosis of the contraceptive ring showed an 1.56 fold increased risk compared to a group of combined oral contraceptives with low estrogen (13).

SHBG levels measured in this study are associated with the odds ratios reported in recent literature: higher SHBG levels are present in users of contraceptives with a higher risk of venous thrombosis (Table 3, Fig. 3).

Discussion

In this study we observed positive associations between the effects of hormonal contraceptives on SHBG levels, the nAPCsr and the thrombotic risk reported in recent literature. High nAPCsr in the thrombin-generation based test indicate increased resistance to APC and is reported to be a risk factor for venous thrombosis (11). Together, these observations support the hypothesis that not only the APCsr, but also SHBG levels are a marker for the risk of venous thrombosis during the use of hormonal contraceptives.

The use of the LNG-IUD did not increase SHBG levels, which is in concordance with recent clinical data. In a national cohort study by Lidegaard *et al* (12), users of the LNG-IUD had no increased risk of thrombosis compared to non-users (RR 0.83 and 95% CI 0.63 to 1.08). This was confirmed by van Hylckama Vlieg *et al* (31) who also did not find an increased risk in a recent case-control study (OR 0.3 and 95% CI 0.1 to 1.1).

Limited data are available on the thrombotic risk of the contraceptive transdermal patch and vaginal ring. Conflicting results have been reported on the thrombotic safety of the contraceptive patch with estimates of the thrombotic risk varying between 0.9 (95%CI 0.5 to 1.6) (32) to 2.4 (95% CI 1.1 to 5.5) (33) compared to oral contraceptives containing norgestimate and EE (29;30;34).

Recently, the first study on the risk of venous thrombosis of the contraceptive ring has been published by the FDA (13). Use of the vaginal ring was associated with a 1.56-fold (95% CI 1.02 to 2.37) higher risk of thrombosis compared to a group of combined oral contraceptives with low estrogen. The study also observed a 1.55-fold (95% CI 1.02 to 2.37) higher thrombotic risk during use of the transdermal patch. In our study, users of the vaginal ring and the transdermal patch had the highest SHBG levels of all contraceptive users. These results are in agreement with earlier studies, reporting an increase in SHBG of ~260% for transdermal patch users and ~150% for vaginal ring users compared to pre-treatment levels (18;26). The increased SHBG levels in women using the patch and ring compared to women using combined oral contraceptives containing LNG suggest an increased thrombotic risk.

The increased risk of the vaginal ring might be explained by the fact that etonogestrel (ENG) is the active metabolite of DSG. According to recent literature, use of combined hormonal contraceptives containing DSG is associated with a 1.82-fold (95% CI 1.49 to 2.22) higher risk of venous thrombosis compared to use of combined oral contraceptives containing LNG/EE (6). However, in women using the contraceptive ring peak serum concentrations of EE and DSG are significantly lower than in women using a combined oral contraceptive containing DSG and EE (35).

The increased risk of the transdermal patch might be explained by the 60 percent higher exposure to EE as measured by the area under the curve and steady state concentration during use of the contraceptive patch compared to use of an oral contraceptive composed of norelgestromine (NGM) and EE. NGM exposure is similar during use of the contraceptive patch and pill (36;37). Since the increased SHBG levels in users of the patch and ring in our study are based on a small number of participants, further studies are indicated to confirm these results and to draw definite conclusions.

The difference in SHBG levels between the hormone preparations was not the result of differences between women but rather between contraceptive methods as evidenced by the women who switched from one contraceptive type to another in the original studies. For example, switching from a combined hormonal contraceptive containing CPA to a combined hormonal contraceptive containing LNG, resulted in a mean decrease of SHBG by 150 nmol/L (95% CI -206 to -94) (6;19;20).

Currently, a biological explanation for the association between the changes in SHBG and APC resistance induced by hormonal contraceptives is lacking. It is known that estrogen increases the risk of venous thrombosis and that a higher dose is associated with a higher risk. We propose that SHBG reflects overall estrogenicity of a hormonal contraceptive and thereby the risk of venous thrombosis. SHBG and several coagulation factors and anticoagulant proteins are synthesized in the liver and hormonal contraceptives, which are metabolized in the liver, might interfere with the synthesis of both SHBG and coagulation factors. There are now different studies demonstrating an association between SHBG and the risk of venous thrombosis. However, the mechanism is still not known and further research is needed to unravel the association, changes in other proteins produced in the liver, changes of haemostatic parameters and the increased risk of venous thrombosis.

We acknowledge that caution is required when using surrogate markers since they can be severely misleading (38). Preferably, a surrogate marker should be validated in a prospective trial in which both the surrogate marker and the clinical endpoint are assessed. However, in case of very rare events, such as venous thrombosis during combined hormonal contraceptive use, a clinical study is almost unfeasible due to the required number of participants. In order to prospectively demonstrate a doubling of the risk of venous thrombosis between two different combined hormonal contraceptives with a power of 80% and a significance level of 5%, a cohort of approximately 500 000 women must be followed for one year (27). Case-control studies only become possible post-marketing (27;39). Such a large sample size makes it almost impossible for a pharmaceutical company to evaluate the risk of venous thrombosis of a new preparation before market authorization.

There are now reasonably reliable data of the risk of venous thrombosis from several epidemiological studies showing that the combination of EE plus LNG carries the lowest risk of venous thrombosis of all combined hormonal contraceptives (1;3;5;6). Comparing the SHBG levels in users of a new preparation with that of EE plus LNG could give an estimation of the magnitude of the risk of venous thrombosis before a new preparation is launched and should be included in the general benefit-risk analysis of the new preparation. SHBG measurement is already recommended in guidelines during clinical development of a new combined hormonal contraceptive by the European Medicines Agency (EMA).

In conclusion, our data support that SHBG could be a useful marker for estimating the risk of venous thrombosis of a new hormonal contraceptive. Preferably, the effect of a new hormonal contraceptive on SHBG should be compared with the effect of the combined hormonal contraceptive with the lowest reported risk of venous thrombosis, i.e. an oral preparation containing EE plus LNG.

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

Bernardine H. Stegeman
Marjolein Raps
Frans M. Helmerhorst
Hans L. Vos
Huib A.A.M. van Vliet
Frits R. Rosendaal
Astrid van Hylckama Vlieg

J Thromb Haemost 2013; 11: 203–205

The effect of a combined oral contraceptive (COC) on sex hormone binding globulin (SHBG) levels may be an indicator for venous thrombosis risk of the COC involved (1;2). SHBG is a plasma glycoprotein primarily produced in hepatocytes that binds the sex steroid hormones testosterone and 17β-estradiol but not ethinylestradiol. Users of COC containing a third generation progestagen have higher SHBG levels than second generation progestagen users (1;2) reflecting the difference in venous thrombosis risk. In accordance with the hypothesis that SHBG levels are a marker of venous thrombosis risk, SHBG levels in COC users are positively associated with thrombin generation-based activated protein C resistance (APC resistance) (2). APC resistance has been shown to predict venous thrombosis risk in both men and women.

If SHBG levels can be considered a marker for venous thrombosis and ethinylestradiol is the main compound in COC causing venous thrombosis, then the ethinylestradiol dose in COC should be reflected in SHBG levels. The main aim of this study was to determine whether an increase in ethinylestradiol dose result in higher SHBG levels in healthy premenopausal women.

Participants were selected from a large case-control study on venous thrombosis, i.e., the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA) study (3) and from a crossover study where women were asked to switch from their current contraceptive to either a COC containing drospirenone or levonorgestrel, i.e., the DRSP (drospirenone/ethinylestradiol) study (2). From the MEGA study, female control subjects, i.e. women without venous thrombosis were selected. From the DRSP study, data of women before switching to a specific COC were used. We excluded women with known environmental thrombotic risk factors. A total of 191 healthy premenopausal women using COC were included (181 from the MEGA study and 101 the DRSP study).

In the MEGA study, whether the women were menstruating at venipuncture was recorded; however, blood was drawn randomly during the four week cycle of pill use (3 weeks of pill use followed by a pill-free week). In the DRSP study, blood was drawn between days 18 and 21 of the four week cycle of pill-use. SHBG levels (nmol/L) were measured with an immunometric assay (Immulite; Siemens Healthcare Diagnostics, Tarrytown, NY, USA) and without knowledge of the COC used or any other of the participant's characteristics.

The ethinylestradiol dose was categorised into 20 µg, 30 µg, ⩾35 µg per pill and triphasic preparations, which have a varying ethinylestradiol and progestagen doses over 21 days. The effect of 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 ethinylestradiol dose on SHBG levels is independent of the progestagen used, we adjusted this analysis for the progestagen used in COC. Regarding the effect of progestagen in COC on SHBG levels, the analysis was restricted to subjects taking 30 µg ethinylestradiol per contraceptive pill. To reduce random variation in SHBG levels, the analyses were adjusted forwhether women were menstruating at the time of venipuncture, and for age and BMI which can influence SHBG levels. Results were expressed as mean differences with 95% confidence interval.

Overall, women were about 33 years (range: 18-49) and had a BMI of 23.3 kg/m^2 (range: 15.7-37.9). The mean SHBG plasma level was 139.5 nmol/L (95%CI: 131.2-147.8, IQR 99.8, range 28.0-390.9). 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: 59.1-145.0) whereas the level in women who were taking a pill (N=163) was 145.4 nmol/L (95%CI: 134.3-156.6); mean difference: 43.4 nmol/L (95%CI: -1.0 -87.7). When we restricted our analysis to women receiving 30 µg of ethinylestradiol, users of desogestrel, gestodene, and drospirenone had approximately 100 nmol/L higher SHBG levels than users of levonorgestrel (Table). Adjustment for factors influencing SHBG levels did not change these results. After adjustment for progestagen, users of ⩾35 µg of ethinylestradiol had higher SHBG levels than users of 20 µg (Table). Also users of triphasic contraceptives had higher SHBG levels than users of 20 µg of ethinylestradiol. The SHBG levels were only slightly higher in users of 30 µg compared with 20 µg of ethinylestradiol. Adjustment for factors influencing SHBG levels did not change these results. The same results were observed when the analysis of ethinylestradiol dose and SHBG levels was restricted to most commonly used progestagens (levonorgestrel, desogestrel and gestodene) or separately per these progestagens, although the number of women per category became very small. Furthermore, similar results were observed when the analysis was performed per study. Additional to the progestagens levonorgestrel, gestodene, desogestrel, and drospirenone, 30 women used cyproterone acetate. The mean SHBG level in users of cyproterone acetate was high at 215.9 nmol/L (95%CI: 199.7-232.1); much higher than in users of COC containing levonorgestrel with 30 µg ethinylestradiol (mean difference: 135.4 nmol/L, (95%CI: 116.9-153.9) adjusted for study and menstruating at venipuncture).

One other paper evaluated the effect of different COC on SHBG levels and provided information on ethinylestradiol dose per progestagen (4). However, no direct comparisons between ethinylestradiol dose and SHBG levels were made; therefore, no conclusions could be drawn on whether the ethinylestradiol dose in different COC was reflected in SHBG levels. The positive association between ethinylestradiol dose and SHBG levels is in line with previous findings regarding venous thrombosis risk. Lidegaard *et al.* (5) reported that compared to 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: 0.9-2.8) and lower in users of 20 µg (OR 0.6, 95%CI: 0.4-0.9). In the MEGA study, we also demonstrated that within users of oral contraceptives containing levonorgestrel, the risk of venous thrombosis was higher in users of 50 µg ethinylestradiol than in users of 30 µg (OR adjusted for age 2.2, 95%CI: 1.3-3.7) (6). 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: 0.2-0.7) and desogestrel (OR 0.7, 95%CI: 0.4-1.2).

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 (7). The hours after a pill was taken do not influence the SHBG levels, because of a half-life of SHBG of about 7 days. However, data were available on factors that were previously shown to

influence SHBG levels and on whether women were menstruating at venipuncture. Furthermore, 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, the same results were observed in an analysis per study. Strengths of our study were that we included a relative large number of COC users who were using many different types of prescriptions. Furthermore, mean SHBG levels as well as the difference in SHBG levels between different progestagens in COC users were in the same range as observed in other studies.

In conclusion, SHBG levels reflect the ethinylestradiol dose used in COC independent of the progestagen used. Since ethinylestradiol is important in the pathogenesis of venous thrombosis in COC users, these findings strengthen the idea that SHBG levels in COC users may be seen as a marker for venous thrombosis risk.

Table: *Mean SHBG levels and adjusted differences per progestagen or per ethinylestradiol dose*

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*

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CHAPTER 6

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

J Thromb Haemost 2013; 11: 855–861

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

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). Socalled '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 17ß-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.

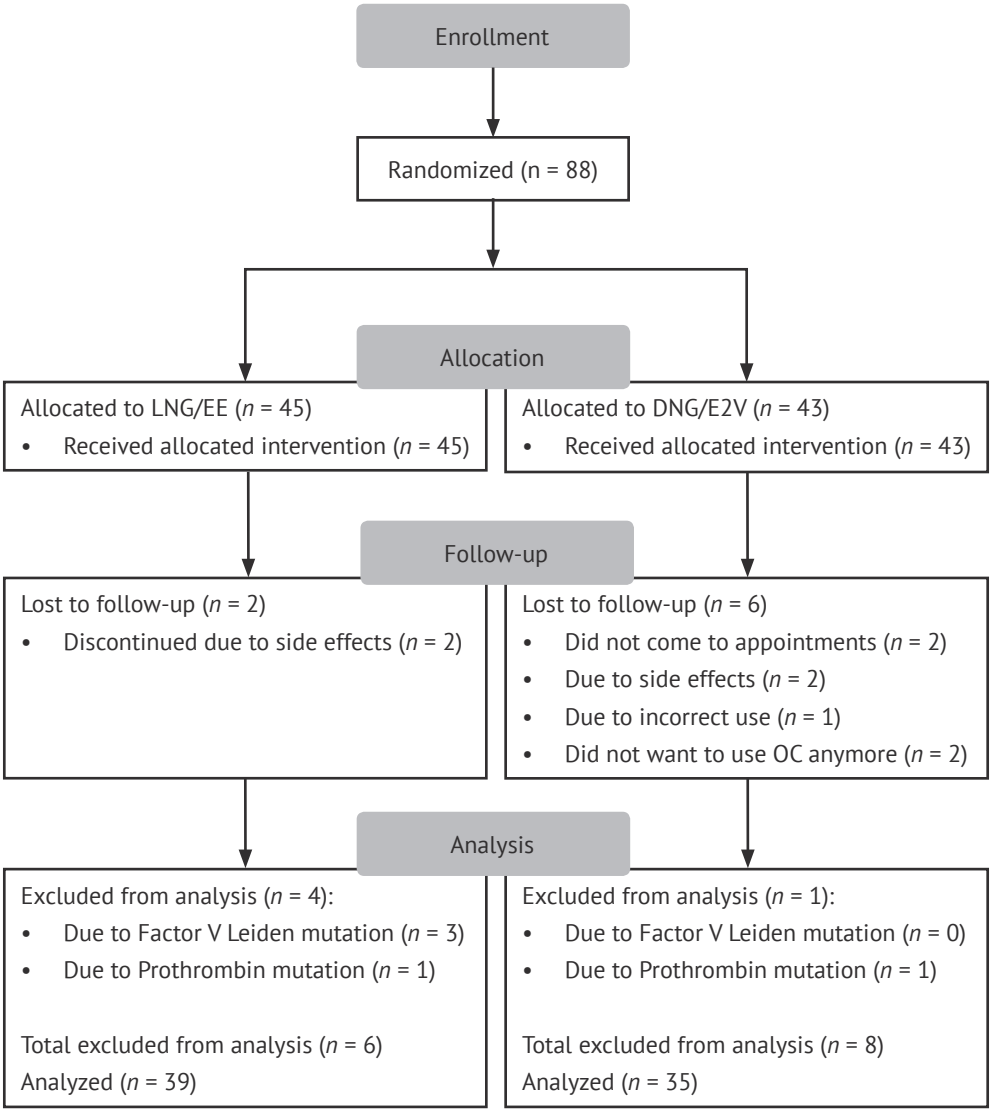

Fig. 1. *Flow diagram of exclusions.*

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%.

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/m2 (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*

			SHGB (nM)			nAPCsr		
Phase	**Pill**	**Contents**	**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	

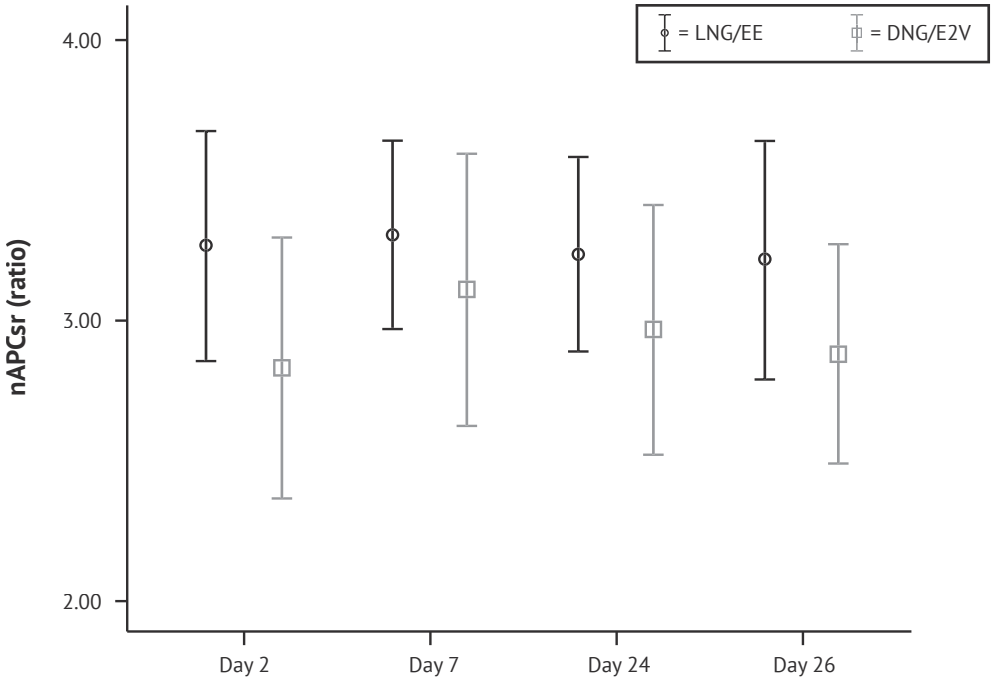

Fig. 2. *Mean nAPCsr levels (ratio) and 95% confidence intervals during use of LNG/EE and DNG/E2V, subdivided by cycle day.*

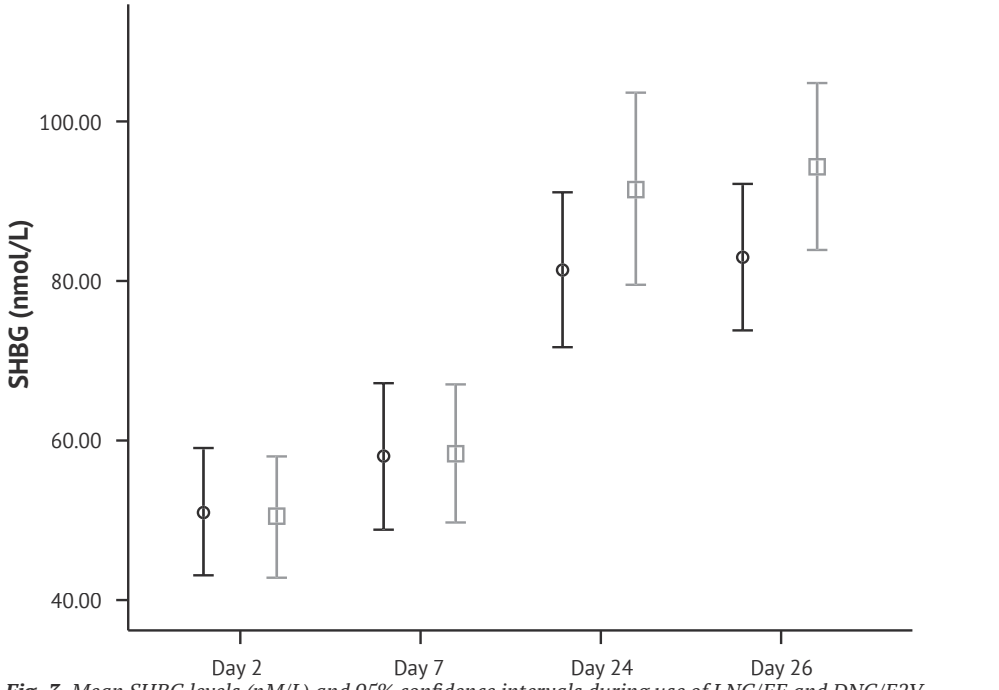

Fig. 3. *Mean SHBG levels (nM/L) and 95% confidence intervals during use of LNG/EE and DNG/E2V, subdivided by cycle day.*

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.

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.

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

Marjolein Raps
Joyce Curvers
Frans M. Helmerhorst
Bart E.P.B. Ballieux
Jan Rosing
Stella Thomassen
Frits R. Rosendaal
Huib A.A.M. van Vliet

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]

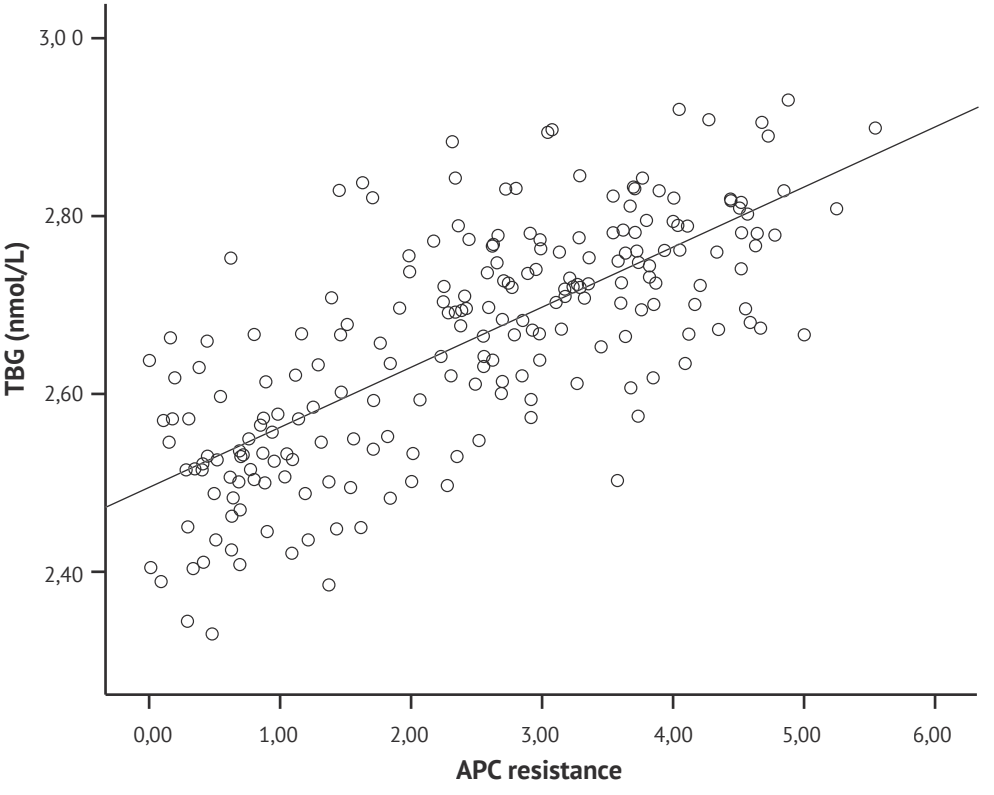

Figure 1. *The association between TBG and nAPCsr. Equation: log10(TBG) = 2.494 + (nAPCsr * 0.068). Pearson's correlation coefficient is 0.714 (p<0.001).*

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

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CHAPTER 8

General discussion and summary

Since the introduction of the first oral contraceptive pill in 1959, the development of contraceptives with new compounds and routes of administration was focused on reducing side effects, while maintaining the benefits. This thesis focuses on venous thrombosis as a rare, but serious side effect of hormonal contraceptive use. Nowadays it is known that use of different hormonal contraceptives is associated with different thrombotic risks, dependent on the estrogen dose and progestogen type of the hormonal contraceptive.

The first objective of this thesis was to investigate effectiveness, bleeding-pattern, minor side effects and acceptability during use of quadriphasic oral contraceptives compared with monophasic oral contraceptives (chapter 2). The second objective was to evaluate the levels of free TFPI and free Protein S as the main determinants of the thrombin generation-based APC resistance test, during use of different hormonal contraceptives (chapter 3). The third objective was to evaluate whether SHBG acts as a marker for venous thrombosis during use of different hormonal contraceptives, and to assess if SHBG reflects the ethinylestradiol levels of hormonal contraceptives (chapter 4, 5). The fourth objective was to investigate the thrombotic risk of a new combined oral contraceptive containing dienogest and estradiol valerate administered in a quadriphasic schedule (chapter 6). The last objective was to assess the association between the levels of TBG, FT4 and TSH during use of different hormonal contraceptives and their risk of venous thrombosis (chapter 7).

Hormonal contraceptives and venous thrombosis

New formulas and schedules of administration

In **chapter 2** we systematically reviewed the literature for randomized controlled trials comparing monophasic oral contraceptives with quadriphasic oral contraceptives, and planned to perform a meta-analysis for the outcomes on contraceptive effectiveness, bleeding pattern, minor side effects and acceptability. One double-blind, double-dummy randomized controlled trial comparing a quadriphasic oral contraceptive containing dienogest and estradiol valerate with a monophasic oral contraceptive containing 100 µg levonorgestrel and 20 µg ethinylestradiol was included. Contraceptive effectiveness, intracyclic bleeding and discontinuation due to side effects were similar for quadriphasic and monophasic pills. The number of women experiencing amenorrhea was higher in the quadriphasic group than in the monophasic group. Users of quadriphasic pills reported fewer bleeding and spotting days and fewer bleeding and spotting episodes than users of monophasic pills but it was unclear whether this was scheduled by the user or unscheduled. More women using quadriphasic oral contraceptives reported breast pain than women using monophasic oral contraceptives.

We concluded that the available evidence is insufficient to determine whether quadriphasic oral contraceptives differ from monophasic oral contraceptives in contraceptive effectiveness, bleeding pattern, minor side effects and acceptability, since only one randomized controlled trial could be included. Studies that compare quadriphasic and monophasic oral contraceptives with an

identical progestogen and estrogen type are needed to determine whether the quadriphasic approach differs from the monophasic approach. Studies that compare quadriphasic pills with monophasic pills containing 30µg ethinylestradiol are indicated to determine whether quadriphasic oral contraceptives have an advantage over the current, first choice oral contraceptive.

In the absence of proven advantages of multiphasic oral contraceptives, and because of the greater complexity of the multiphasic approach and the higher costs of multiphasic oral contraceptives, we recommend monophasic pills as first choice for women starting oral contraceptive use. Monophasic pills containing 30 µg estrogen are preferred over 20 µg estrogen since the latter causes more bleeding problems and more often lead to discontinuation of use than those containing 30 µg (5).

Biological explanation

TFPI and Protein S

In **chapter 3** we assessed the effect of hormonal and non-hormonal contraceptives with different routes of administration on free TFPI and free Protein S levels, as determinants of the thrombin generation-based APC resistance test. In addition, we measured APC sensitivity ratios (nAPCsr) by using the thrombin generation-based APC resistance test. We observed that users of contraceptives with the highest risk of venous thrombosis had the lowest free TFPI and free Protein S levels, and vice versa, women who used contraceptives with the lowest risk of venous thrombosis had the highest free TFPI and free Protein S levels. Furthermore, a negative association was observed between levels of free TFPI and APC resistance, and between free Protein S and APC resistance. Our study confirms that the different thrombotic risks associated with use of different hormonal contraceptives are reflected in the levels of free TFPI and free Protein S. Besides, our results confirm the hypothesis that the differences in APC resistance induced by hormonal contraceptives can at least be partially explained by different effects on free TFPI and free Protein S levels.

The lower free TFPI and free Protein S levels during use of third generation combined oral contraceptives or combined oral contraceptives containing cyproterone acetate or drospirenone are in concordance with several other studies on the effect of combined oral contraceptives on levels of free TFPI and free Protein S (6-11). Unfortunately, in our study the sample sizes of the groups of users of the vaginal ring and users of the transdermal patch were too small to draw conclusions. Since use of the transdermal patch and vaginal ring is associated with an increased risk of venous thrombosis with ORs of 7.9 and 6.5 respectively compared with non-use (12), decreased levels of both free TFPI and free Protein S are expected. Two studies have been performed on the levels of protein S in users of the transdermal patch or vaginal ring and the results were conflicting (13;14). A study with an adequate sample size is indicated to investigate the free Protein S and TFPI levels during use of the transdermal patch and vaginal ring.

The mechanism for the decrease in free Protein S and free TFPI has been studied, but not yet fully unraveled. Kemmeren *et al*. explained the differences in free Protein S induced by various

oral contraceptives by the interaction between Protein S and C4BP. C4BP binds Protein S in a high-affinity complex (7). They observed that total Protein S was decreased by third generation combined oral contraceptives containing desogestrel but was hardly affected by second generation combined oral contraceptives containing levonorgestrel and that both oral contraceptives equally lowered C4BP. As a result, free Protein S levels increased in users of the combined oral contraceptive with levonorgestrel and decreased in users of the combined oral contraceptive with desogestrel. Free Protein S forms a complex with free TFPI and acts as cofactor of TFPI through the extrinsic pathway (15-19). A possible explanation of the concomitant decrease in free TFPI levels could be that binding of free TFPI to free Protein S protects free TFPI from proteolytic degradation or slows down the clearance of free TFPI. A decrease of free Protein S will therefore be accompanied by a decrease in free TFPI. Further, since free Protein S and free TFPI are both produced by the endothelium it is likely that TFPI production or release is also influenced by hormonal contraceptives; both proteins share the endothelium as common production site. In addition, the secretion of TFPI from endothelial cells might be coupled to Protein S secretion, as has recently been discovered for Protein S and the beta-chain of C4BP by Carlsson *et al*. (18;20). Future studies are indicated to unravel the mechanism of the decreased free TFPI and free Protein S levels during use of hormonal contraceptives and the association of their relationship with the risk of venous thrombosis.

Predicting thrombotic risk by use of surrogate markers

In **chapter 4** we focused on SHBG as potential marker for venous thrombosis during use of hormonal contraceptives. We investigated whether a positive association could be found between levels of SHBG and APC resistance and between levels of SHBG and relative risks of venous thrombosis as reported in the literature during use of different combined oral contraceptives, LNG-IUD and copper-IUD, transdermal patch and vaginal ring. APC resistance was determined using the thrombin generation-based APC resistance test. The study demonstrates that users of contraceptives with a higher risk of venous thrombosis, i.e. third generation combined oral contraceptives, oral contraceptives containing cyproterone acetate or drospirenone, the transdermal patch and vaginal ring had higher SHBG levels than users of a second generation levonorgestrel combined oral contraceptive, which carry a lower thrombotic risk. The lowest SHBG levels were found during use of the LNG-IUD, which is not associated with an increased risk of venous thrombosis. A positive association was found between SHBG levels and APC resistance and between SHBG levels and the relative risks as reported in the literature. These results suggest that SHBG is a useful marker to estimate the risk of venous thrombosis during use of hormonal contraceptives. We recommend that the effect of a new hormonal contraceptive on SHBG should be measured before licensing, and compared with the effect on the combined oral contraceptive with the lowest risk of venous thrombosis, i.e. containing 30 µg ethinylestradiol and 150 µg levonorgestrel.

In **chapter 5** we determined the effect of the ethinylestradiol dose of combined oral contraceptives on SHBG levels, since the estrogen dose is thought to be the most important factor in

hormonal contraceptives in causing the increased risk of venous thrombosis. In this study we observed that women using a combined oral contraceptive with ≥ 35 µg ethinylestradiol had higher SHBG levels than women using a combined oral contraceptive with 20 µg ethinylestradiol. However, SHBG levels were only slightly higher in users of a combined oral contraceptive with 30 µg ethinylestradiol than 20 µg ethinylestradiol.

Our results of SHBG during use of combined oral contraceptives, the transdermal patch and the vaginal ring are in concordance with other studies on the effect of hormonal contraceptives on levels of SHBG (3;21-26). We are not aware of studies published about SHBG during use of the LNG-IUD, or studies about the effect of different ethinylestradiol levels on SHBG.

Stegeman *et al*. (27) investigated whether increased SHBG levels are causally related to venous thrombosis in women not using hormonal contraceptives. They used a Mendelian randomization approach and showed that SHBG is only a marker for venous thrombosis during hormonal contraceptive use, and not a cause for an increased risk of venous thrombosis per se.

Some researchers have questioned whether SHBG can act as a marker for venous thrombosis. They stated that SHBG has no relation with coagulation, is not validated as a marker and affected by many factors not involved by coagulation (28). Currently, a biological explanation for the association between the changes in SHBG levels and APC resistance induced by hormonal contraceptives indeed is lacking. Our study supports the hypothesis of Odlind *et al*. (21) that SHBG reflects the overall estrogenicity of a hormonal contraceptive, and thereby the risk of venous thrombosis. SHBG and several coagulation factors and anticoagulant proteins are synthesized in the liver, and hormonal contraceptives, which are metabolized in the liver, might interfere with the synthesis of both SHBG and coagulation factors. Further research is needed to understand the association between increased SHBG levels and the increased risk of venous thrombosis during use of hormonal contraceptives, which, however, is not a prerequisite for its use as a marker of thrombogenicity.

We acknowledge that clinical data on the risk of venous thrombosis of new hormonal contraceptives are in theory to be preferred over markers such as SHBG. However, a non-clinical marker has the advantage that fewer women need to suffer venous thrombosis before a decision can be made. In addition, a marker should be validated in a prospective trial in which both the marker and the clinical endpoint are assessed (28). Given the low incidence of venous thrombosis during oral contraceptive use, a high number of participants is necessary in clinical studies to provide absolute and relative risks, which would be almost impossible before market authorization (29). Hence, during the developmental phase of a new contraceptive, the thrombotic risk can be estimated by using SHBG as marker and by comparing the SHBG levels of the new contraceptive with the combined oral contraceptive with the lowest risk of venous thrombosis, i.e. containing levonorgestrel. Thrombin generation-based APC resistance is a validated marker for venous thrombosis (8), but a complex measurement which cannot be performed in every laboratory. SHBG, however, can easily be measured in every routine laboratory. The EMA therefore now recommends SHBG measurement in guidelines applying to the clinical development of a new combined hormonal contraceptive (30).

In **chapter 6** we investigated the thrombogenicity of a new oral contraceptive containing dienogest and estradiol valerate administered in a quadriphasic schedule. APC resistance and SHBG levels were measured in participants using this new oral contraceptive and compared with APC resistance and SHBG levels during use of a monophasic oral contraceptive containing levonorgestrel and ethinylestradiol. We observed no clear differences in APC resistance and SHBG as markers for venous thrombosis between the oral contraceptives. During the pill cycle, SHBG levels increased gradually in both dienogest with estradiol valerate users as well as in and in levonorgestrel with ethinylestradiol users. No differences in APC resistance were observed during the pill cycle.

These results are in concordance with two studies sponsored by the manufacturer. Klipping *et al*. (1) conducted a randomized, open label, cross-over study of dienogest with estradiol valerate and monophasic levonorgestrel with ethinylestradiol and observed lower nAPCsr and lower SHBG levels in users of dienogest with estradiol valerate than in users of levonorgestrel with ethinylestradiol. Junge *et al*. (2) performed a randomized, open label study and also observed less pronounced SHBG levels in users of dienogest with estradiol valerate than in users of triphasic levonorgestrel withethinylestradiol. In our study, nAPCsr levels were overall lower in users of dienogest with estradiol valerate, but the differences were not pronounced and not statistically significant. This can be due to a smaller sample sizes in the studies of the manufacturer. Based on the results of these three studies, it can be stated, however, that dienogest with estradiol valerate does not lead to a more thrombogenic state than levonorgestrel with ethinylestradiol. However, clinical studies assessing the absolute and relative risk of thrombosis in women using dienogest with estradiol valerate are indicated to confirm this.

Dienogest has, in addition to estradiol valerate, also been combined with ethinylestradiol in a combined oral contraceptive pill (Valette®, Bayer Schering Pharma, Berlin, Germany; not available in the Netherlands). In a study by Wiegratz *et al*. (3) users of oral contraceptives containing 2mg dienogest and 20 µg ethinylestradiol had higher SHBG levels than users of oral contraceptives containing 100 µg levonorgestrel and 20 µg ethinylestradiol. This indicates that dienogest is a less anti-estrogenic progestogen than levonorgestrel. In our study no differences in SHBG were observed between users of dienogest with estradiol valerate and levonorgestrel with ethinylestradiol. No studies have been published yet on the effect of dienogest with ethinylestradiol on nAPCsr, or the risk of venous thrombosis of combined oral contraceptives containing estradiol valerate as estrogen content. Estradiol valerate seems to have a favorable effect on the risk of venous thrombosis, but has not been combined with other progestogens before. Therefore, the impact of estradiol valerate alone on venous thrombosis remains unknown. Future research should focus on the effect of estradiol valerate as the estrogen compound of a combined oral contraceptive on the risk of venous thrombosis compared with ethinylestradiol.

In 2011, a new monophasic combined oral contraceptive composed of the progestogen nomegestrol acetate and the estrogen 17ß-estradiol (Zoely®, Merck & Co., Inc., Whitehouse Station, New Jersey USA) was marketed. Gaussem *et al*. (4) compared APC resistance and SHBG levels during use of this new combined oral contraceptive with the monophasic combined oral contraceptive

containing 100 µg levonorgestrel and 20 µg ethinylestradiol. SHBG levels were similar for the two oral contraceptives, and APC resistance levels were more favorable during use of nomegestrol acetate with estradiol than during use of levonorgestrel with ethinylestradiol. Gaussem *et al*. observed no increase in markers for the risk of venous thrombosis during use of the new combined oral contraceptive containing nomegestrol actetate with estradiol compared with levonorgestrel with ethinylestradiol. Clinical data of independent studies are indicated to assess the absolute and relative risk of venous thrombosis during use of this new combined oral contraceptive.

Thyroid parameters and venous thrombosis

In **chapter 7** we investigated whether there is an association between the levels of thyroid parameters during use of hormonal contraceptives and the risk of venous thrombosis. We measured the thyroid parameters TBG, FT4 and TSH, as well as APC resistance determined by the thrombin generation-based APC resistance test. We observed that users of different hormonal contraceptives had different levels of TBG, TSH and FT4. A positive association was found between TBG levels and nAPCsr. Users of hormonal contraceptives associated with an increased risk of venous thrombosis had higher TBG levels than users of the LNG-IUD, which is not associated with an increased risk of venous thrombosis. TSH levels showed the same, but less pronounced, trend during hormonal contraceptive use: users of hormonal contraceptives with a high risk of venous thrombosis had higher TSH levels than users of low-risk hormonal contraceptives. FT4 levels stayed within the normal range, indicating that use of hormonal contraceptives does not lead to a hyperthyroid state. Therefore, the increased risk of venous thrombosis during use of hormonal contraceptives cannot be explained by hypercoagulability caused by a hyperthyroid state as a result of hormonal contraceptive use.

The increased levels of TBG in users of combined oral contraceptives are in concordance with other studies (3;22;31-34). We found a less pronounced increase in TSH levels and hardly any changes in FT4 levels, as was also observed by Ågren *et al*. and Sänger *et al*. (32;33).

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 such as TBG and SHBG 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. Estrogens regulate levels of polymorphic glycoproteins, e.g. hepatic binding globulins, by affecting their sialylation (35). Sialic acid occupies the terminal ends of oligosaccharides of glycoproteins and prevents degradation of these molecules. A high sialic acid content leads to slower breakdown and higher circulating serum levels of glycoproteins (36). Besides binding globulins such as TBG and SHBG, coagulation factors (e.g. FV) and anticoagulation factors (e.g. APC) are all glycoproteins and therefore contain sialic acid. Hau *et al*. observed that desialylated APC was more active than normal APC, suggesting

that sialic acid inhibits the activity of APC (37). Fernández *et al*. studied the protective role of the sialic acid content of FV in its inactivation by APC and found that desialylation of FV increases its susceptibility for proteolytic inactivation by APC (38). Therefore, in the case of increased sialic acid, it is possible that FV becomes more resistant for the action of APC which increase the thrombotic risk. We hypothesize that the increased sialic acid compound of glycoproteins, as a result of estrogen administration during use of hormonal contraceptives, contributes to the increased risk of venous thrombosis associated with hormonal contraceptive use. Further research is indicated to assess the effect of different hormonal contraceptives on the sialic acid content of glycoproteins affected by hormonal contraceptive use, such as TBG, SHBG and FV.

Recommendations

Clinical

- The combined oral contraceptive with 30 µg ethinylestradiol and 150 µg levonorgestrel is recommended over other combined hormonal contraceptives.
- The new oral contraceptive containing dienogest with estradiol valerate has a similar thrombotic profile as levonorgestrel with ethinylestradiol.
- A quadriphasic regimen is not favorable compared with monophasic preparations regarding effectiveness, side effects and acceptability.
- Use of hormonal contraceptives does not lead to a subclinical hyperthyroid state.

Research

- Clinical epidemiological studies are necessary to assess the absolute and relative risk of the quadriphasic oral contraceptive containing dienogest with estradiol valerate, and to confirm the hypothesis that this quadriphasic oral contraceptive has a similar risk of venous thrombosis as the monophasic oral contraceptive containing levonorgestrel with ethinylestradiol.

- Independent laboratory studies are indicated to estimate the thrombotic risk of the new monophasic oral contraceptive containing nomegestrol acetate with estradiol by measuring nAPCsr and SHBG levels. Clinical epidemiologic studies are indicated to assess the absolute and relative risk of venous thrombosis during use of this new oral contraceptive.
- Future studies examining the mechanism of the reduction of free PS and free TFPI during use of hormonal contraceptives are indicated to gain more insight in the basis of the increased risk of venous thrombosis.
- Before a new hormonal contraceptive is licensed or used in practice, the effect of the preparation on nAPCsr and SHBG levels should be evaluated to estimate the risk of venous thrombosis. These measurements should be included in the general benefit-risk analysis of the new preparation. The new prescription should be compared with an oral contraceptive containing 30 µg ethinylestradiol and 150 µg levonorgestrel.
- Laboratory studies are indicated to assess the biological explanation for the association between the changes in SHBG levels and nAPCsr induced by hormonal contraceptives.
- Sialic acid should be measured in users of different hormonal contraceptives to investigate the role of sialic acid and its influence on glycoproteins in the etiology of venous thrombosis during use of hormonal contraceptives.

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Abbreviations and Acronyms

APC	Activated Protein C
BMI	Body Mass Index
C4BP	C4 binding protein
CI	Confidence Interval
COC	Combined Oral Contraceptive
CPA	Cyproterone Acetate
Cu-IUD	Copper containing Intrauterine Device
DNG	Dienogest
DRSP	Drospirenone
DSG	Desogestrel
E2V	Estradiolvalerate
EE	Ethinylestradiol
EMA	European Medicines Agency
ENG	Etonogestrel
FSH	Follicle Stimulating Hormone
FT4	Free Thyroxine
FV Leiden	Factor V Leiden
FVa	Activated Factor V
FVIIa	Activated Factor VII
FVIIIa	Activated Factor VIII
FVL	Factor V Leiden
FXa	Activated Factor X
GTD	Gestodene
IUD	Intrauterine Device
LH	Luteinizing Hormone
LNG	Levonorgestrel
LNG-IUD	Levonorgestrel containing Intrauterine Device
MD	Mean Difference
MEGA Study	Multiple Environmental and Genetic Assessment Study
M-H	Mantel-Haenszel
n	Number
nAPCsr	normalized Activated Protein C sensitivity ratio
NET	Norethisteron
NGM	Norgestimate
NGMN	Norelgestromine
NOMAC	Nomegestrol Acetate
OR	Odds Ratio
PS	Protein S
RCT	Randomized Controlled Trial
RR	Relative Risk, Rate Ratio
T3	Tri-Iodothyronine
T4	Thyroxine
TBG	Thyroxine Binding Globulin
TF	Tissue Factor
TFPI	Tissue Factor Pathway Inhibitor
TSH	Thyroid Stimulating Hormone

Nederlandse samenvatting

Kort na de introductie van de combinatie anticonceptiepil in 1959 werd de eerste casus van veneuze trombose tijdens gebruik van een anticonceptiepil beschreven. Veneuze trombose is een zeldzame aandoening bij vrouwen in de fertiele levensfase en heeft een incidentie van ongeveer 3 per 10.000 vrouwen per jaar. Het gebruik van orale combinatie anticonceptie is geassocieerd met een verhoogd risico op veneuze trombose: twee tot zes keer zo hoog ten opzichte van gezonde vrouwen die geen orale anticonceptie gebruiken. Ondanks de lage incidentie is het gebruik van hormonale anticonceptie toch een veelvoorkomende oorzaak van veneuze trombose, omdat wereldwijd miljoenen vrouwen een anticonceptiepil gebruiken.

De doelstelling van dit proefschrift was om het risico op veneuze trombose tijdens gebruik van verschillende hormonale anticonceptiva te bestuderen en te vergelijken, en meer inzicht te krijgen in het mechanisme dat ten grondslag ligt aan het verhoogde risico op veneuze trombose tijdens gebruik van hormonale anticonceptie.

Hormonale anticonceptie en veneuze trombose

Sinds de introductie van de combinatie anticonceptiepil werden nieuwe hormonale anticonceptiva ontwikkeld met als doel om bijwerkingen (zoals veneuze trombose) te verminderen, en tegelijkertijd de voordelen (zoals het voorkómen van zwangerschap en cyclusregulatie) te behouden. De verschillende methoden van aanpak om de risico's te verlagen zijn onder te verdelen in vier categorieën:

Het verlagen van de dosis steroïden

Aanvankelijk werd geprobeerd het risico op veneuze trombose te verlagen door de dosis steroïden te verlagen, wat vooral bij het aanpassen van de dosis oestrogeen effectief bleek te zijn. Dit verband was recht evenredig: hoe lager de dosis ethinylestradiol (het meest gebruikte synthetische oestrogeen in hormonale anticonceptiva), des te lager was het risico op veneuze trombose. Tegenwoordig bevatten de meest voorgeschreven combinatie anticonceptiepillen 20 of 30ug ethinylestradiol.

Nieuwe toedieningschema's

Vervolgens werden er nieuwe toedieningschema's ontwikkeld, zoals de tweefasen of driefasen anticonceptiepillen. Studies hebben aangetoond dat deze toedieningschema's echter geen verlaging gaven van het risico op veneuze trombose. Daarnaast werd in meta-analyses onvoldoende bewijs gevonden voor een groter gebruiksgemak of een vermindering van andere bijwerkingen (bijvoorbeeld tussentijds bloedverlies) tijdens gebruik van een twee- of driefasen preparaat.

Recent werd een nieuwe pil op de markt gebracht met een vierfasen toedieningschema. Het bevat een nieuw oestrogeen, estradiolvaleraat, en een progestageen dat nog niet eerder in Nederland werd gebruikt, dienogest. In **Hoofdstuk 2** worden de resultaten beschreven van een systematische review van gerandomiseerde studies waarin vierfasen orale anticonceptiva worden vergeleken

met eenfase orale anticonceptiva. Het doel was om het vierfasen toedieningschema van orale anticonceptiva te vergelijken met het eenfase schema op de uitkomsten van effectiviteit, bloedingpatroon en de mate waarin vrouwen het anticonceptivum verdragen.

Er was één studie die aan de inclusiecriteria voldeed, waarin een vierfasen anticonceptiepil met dienogest en estradiolvaleraat werd vergeleken met een eenfase anticonceptiepil met 100 µg levonorgestrel en 20 µg ethinylestradiol. In deze studie werden geen verschillen gevonden tussen het vierfasen preparaat en het eenfase preparaat met betrekking tot de effectiviteit en de mate waarin vrouwen het anticonceptivum verdragen. Er werd tijdens gebruik van de vierfasen pil vergeleken met de eenfase pil vaker een kortere duur van de onttrekkingbloeding of een amenorroe geconstateerd.

De conclusie was dat de literatuur onvoldoende wetenschappelijk bewijs levert om vierfasen en eenfase toedieningschema's gedegen met elkaar te vergelijken en om vast te stellen of er verschillen zijn tussen deze toedieningschema's, aangezien er slechts één studie geïncludeerd kon worden. De klassieke eenfase anticonceptiepil wordt nog steeds geadviseerd als eerste keuze voor vrouwen die starten met het gebruik van de combinatie anticonceptiepil.

Nieuwe steroïden

In de afgelopen jaren zijn diverse pillen met verschillende nieuwe typen steroïden op de markt gebracht. Deze voldeden echter niet altijd aan het gewenste doel van vermindering van bijwerkingen. Epidemiologische studies uit de jaren negentig toonden aan dat orale anticonceptiva met de derde generatie progestagenen gestodeen of desogestrel, welke werden ontwikkeld om de arteriële bijwerkingen van de pil te doen verminderen, een verdubbeling gaven van het risico op veneuze trombose ten opzichte van tweede generatie orale anticonceptiva met levonorgestrel. Later bleek dat ook orale anticonceptiva met cyproteronacetaat of drospirenon als progestagene component een verdubbeling gaven van het risico op veneuze trombose ten opzichte van de anticonceptiepillen met levonorgestrel.

Nieuwe toedieningroutes

Naast veranderingen in dosis, type steroïden en meerfasen toedieningschema's, werden ook nieuwe toedieningsroutes ontwikkeld in de vorm van het hormoonhoudende (levonorgestrel bevattende) spiraaltje, de vaginale ring en de anticonceptie pleister. Recent werd bekend dat het hormoonhoudend spiraaltje geen verhoogd risico geeft op veneuze trombose terwijl de vaginale ring en de anticonceptie pleister net als derde generatiepillen bijna een verdubbeling geven van het risico op veneuze trombose ten opzichte van de tweede generatiepil met levonorgestrel.

Biologische verklaring

Het exacte biologische mechanisme van het verhoogde risico op veneuze trombose tijdens gebruik van hormonale anticonceptie is onbekend. Diverse laboratoriumstudies toonden aan dat

de concentraties van stollingsfactoren beïnvloed worden door het gebruik van hormonale anticonceptie, maar de resultaten van de verschillende onderzoeken zijn niet eenduidig.

Een doorbraak was de ontdekking dat het verhoogde tromboserisico deels verklaard kan worden door de effecten van hormonale anticonceptie op geactiveerd proteïne C (APC), een eiwit dat de stollingscascade remt. Gebruik van hormonale anticonceptie leidt in wisselende mate tot resistentie voor de werking van APC, waardoor de noodzakelijke remming van de stollingscascade verminderd wordt. Dit leidt tot een verhoogde kans op het ontwikkelen van veneuze trombose. De mate van APC resistentie kan gemeten worden met de thrombin generation-based APC resistance test, welke een betrouwbare marker bleek te zijn voor het risico op veneuze trombose.

Tissue Factor Pathway Inhibitor (TFPI) en Proteïne S zijn net als APC fysiologische remmers van de stollingscascade. Naast hun individuele remming van de stolling zijn zij ook van invloed op de werking van APC, en zijn het belangrijke determinanten van de thrombin generation-based APC resistance test. Recent bleken de levels van TFPI en Proteïne S ook beïnvloed te worden door het gebruik van hormonale anticonceptiva, wat mogelijk bijdraagt aan de hogere stollingsneiging tijdens gebruik van deze anticonceptiva.

In **Hoofdstuk 3** worden de resultaten van een dwarsdoorsnede onderzoek beschreven waarin de effecten van gebruik van verschillende hormonale anticonceptiva op de concentraties van vrij TFPI en vrij Proteïne S (de werkzame fracties) worden onderzocht. Het doel van deze studie was om na te gaan of de verschillen in tromboserisico bij gebruik van verschillende hormonale anticonceptiva weerspiegeld werden in de concentraties van vrij TFPI en vrij Proteïne S in het bloed. Daarnaast werd de mate van APC resistentie gemeten met behulp van de thrombin generation-based APC resistance test.

Gebruiksters van hormonale anticonceptiva met het hoogste tromboserisico (orale anticonceptiva met desogestrel, cyproteronacetaat of drospirenon) hadden de laagste vrij TFPI en vrij Proteïne S concentraties. Verder hadden gebruiksters van het hormoonhoudend spiraaltje, waarvan bekend is dat het geen verhoging geeft van het risico op veneuze trombose, de hoogste vrij TFPI en vrij Proteïne S concentraties in het bloed. Lage vrij TFPI en vrij Proteïne S waarden waren geassocieerd met een verhoogde APC resistentie wat een risicofactor is voor veneuze trombose.

Helaas waren de groepen met gebruiksters van de vaginale ring en de anticonceptiepleister te klein voor een betrouwbare analyse.

De uitkomsten van deze studie laten zien dat de verschillen in risico op veneuze trombose die gepaard gaan met het gebruik van verschillende hormonale anticonceptiva weerspiegeld worden in de concentraties van vrij TFPI en vrij Proteïne S. Daarmee ondersteunen ze de hypothese dat de verschillen in APC resistentie veroorzaakt door gebruik van verschillende hormonale anticonceptiva ten minste gedeeltelijk verklaard kunnen worden door de effecten op vrij TFPI en vrij Proteïne S.

Het voorspellen van tromboserisico met markers

Zodra een nieuw hormonaal anticonceptivum op de markt komt, is het belangrijk een indicatie te hebben van het tromboserisico zodat gebruiksters niet onnodig aan een hoog risico op veneuze trombose worden blootgesteld. Omdat veneuze trombose bij vrouwen in de fertiele levensfase een lage incidentie heeft is een zeer groot aantal gebruiksters nodig om het risico op veneuze trombose tijdens gebruik van een nieuw hormonaal anticonceptivum vast te stellen (circa 500 000). Dit is nagenoeg onmogelijk om uit te voeren voordat een nieuw preparaat op de markt komt. Om toch een schatting te kunnen maken van het tromboserisico kan gebruik worden gemaakt van markers. Zoals eerder vermeld, bleek APC resistentie gemeten met de thrombin generation-based APC resistance test een gevalideerde marker voor veneuze trombose te zijn.

In 2002 werd een studie gepubliceerd waarbij het transporteiwit Sex Hormone-Binding Globulin (SHBG transporteert oestrogeen en testosteron in het bloed) wordt genoemd als marker voor "oestrogeniciteit" en daarmee als marker voor veneuze trombose. Oestrogeniciteit werd gedefinieerd als de som van de effecten van het oestrogeen en het progestageen (dosis- en typeafhankelijk), resulterend in een netto effect. In twee observationele studies werd een verband gevonden tussen APC resistentie en SHBG levels bij gebruiksters van verschillende hormonale anticonceptiva en non-users. Hiermee werd de hypothese dat SHBG als marker fungeert voor veneuze trombose tijdens gebruik van hormonale anticonceptie versterkt.

Hoofdstuk 4 beschrijft een dwarsdoorsnede onderzoek waarin bij gebruiksters van verschillende hormonale anticonceptiva SHBG levels en APC resistentie werden bepaald. Het doel van de studie was om te onderzoeken of SHBG een goede marker is voor veneuze trombose tijdens gebruik van hormonale anticonceptiva.

Gebruiksters van hormonale anticonceptiva met een hoog tromboserisico (anticonceptiepillen met desogestrel, gestodeen, cyproteronacetaat of drospirenon, de anticonceptiepleister en de vaginale ring) hadden hogere SHBG spiegels ten opzichte van gebruiksters van laag risico orale anticonceptiva zoals anticonceptiepillen met levonorgestrel. Gebruiksters van het hormoonhoudend spiraaltje, dat geassocieerd is met het laagste tromboserisico, hadden ook de laagste SHBG spiegels. Hetzelfde verband werd gevonden tussen de hoogte van de SHBG waarden en de mate van APC resistentie: gebruiksters met hogere SHBG waarden hadden ook een hogere APC resistentie.

Hoofdstuk 5 beschrijft de resultaten van een dwarsdoorsnede onderzoek naar het effect van de ethinylestradiol dosis in gecombineerde orale anticonceptiva op de SHBG spiegels. Het doel was om na te gaan of ook enkel de ethinylestradiol dosis van invloed is op de SHBG spiegels, onafhankelijk van het progestageen.

Gebruiksters van een gecombineerde anticonceptiepil met ⩾ 35 µg ethinylestradiol hadden hogere SHBG spiegels dan gebruiksters van een gecombineerde anticonceptiepil met 20 µg ethinylestradiol. Het verschil in SHBG spiegels tussen gebruiksters van 30 µg en 20 µg was echter maar klein. De resultaten toonden aan dat de dosis ethinylestradiol gereflecteerd wordt in de

SHBG spiegels van gebruiksters van gecombineerde orale anticonceptiva, onafhankelijk van de progestagene component van de anticonceptiepil.

De uitkomsten van beide studies ondersteunen de hypothese dat SHBG een goede en bruikbare marker is voor het risico op veneuze trombose tijdens gebruik van hormonale anticonceptiva. Wij adviseren dan ook om de SHBG spiegels en de mate van APC resistentie van nieuwe hormonale anticonceptiva te vergelijken met de meest gebruikte anticonceptiepil met ethinylestradiol en levonorgestrel, voordat het nieuwe preparaat op de markt wordt gebracht.

In **Hoofdstuk 6** staan de resultaten beschreven van een gerandomiseerde studie naar het tromboserisico van de nieuwe vierfase anticonceptiepil met estradiolvaleraat en dienogest. De SHBG spiegels en APC resistentie tijdens gebruik van deze nieuwe pil werden vergeleken met de eenfase pil met ethinylestradiol en levonorgestrel. Het doel van de studie was om het tromboserisico van de nieuwe pil te schatten.

Gebruiksters van de nieuwe vierfase pil met estradiolvaleraat en dienogest hadden geen hogere of lagere SHBG spiegels of APC resistentie dan gebruiksters van de eenfase pil met ethinylestradiol en levonorgestrel. Tijdens de pilcyclus werd een geleidelijke stijging van de SHBG spiegels bij alle gebuiksters gemeten. Een mogelijke verklaring hiervoor was dat SHBG een halfwaardetijd heeft van enkele dagen.

Hieruit werd geconcludeerd dat de vierfase pil met estradiolvaleraat en dienogest geen hoger tromboserisico heeft dan de onderzochte eenfase pil. In de toekomst zijn klinische studies nodig om deze hypothese te bevestigen.

Schildklierparameters en veneuze trombose

Naast gebruik van hormonale anticonceptie is hyperthyreoidie ook geassocieerd met een verhoogd risico op veneuze trombose. Patienten met hyperthyreoidie hebben onder andere hogere levels vrij Thyroxine (T4), wat het stollingssysteem beïnvloedt. Gedacht wordt dat dit de oorzaak is van het verhoogde tromboserisico.

Gebruik van hormonale anticonceptie leidt tot licht verhoogde waardes van de schildklierparameters. Of de verhoogde levels van deze schildklierparameters tijdens gebruik van hormonale anticonceptie geassocieerd zijn met het verhoogde tromboserisico tijdens gebruik van deze hormonale anticonceptie was nog niet onderzocht.

In **Hoofdstuk 7** worden de resultaten besproken van een dwarsdoorsnede onderzoek waarbij in gebruiksters van verschillende hormonale anticonceptiva de spiegels van Thyroxine Binding Globulin (TBG), T4 en Thyroid Stimulating Hormone (TSH) als schildklierparameters, en APC resistentie als marker voor veneuze trombose werden bepaald. Het doel van de studie was om te onderzoeken of de verhoogde spiegels van deze schildklierparameters tijdens gebruik van hormonale anticonceptiva geassocieerd zijn met het verhoogde risico op veneuze trombose tijdens gebruik van deze hormonale anticonceptiva.

Gebruiksters van de onderzochte hormonale anticonceptiva hadden verschillende spiegels van TBG, TSH en vrij T4. Er werd een verband gevonden tussen de TBG spiegels en de APC resistentie en tussen TBG spiegels en het risico op veneuze trombose. Gebruik van het hormonaal anticonceptivum met het hoogste risico op veneuze trombose (in deze studie de orale anticonceptiepil met cyproteronacetaat of drospirenon) gaf de hoogste TBG spiegels en de hoogste APC resistentie. Gebruiksters van het hormoonhoudende spiraaltje, wat niet geassocieerd is met een verhoogd tromboserisico, hadden de laagste TBG spiegels en de laagste APC resistentie. De vrij T4 spiegels bleven binnen de normaalwaarden bij alle gebruiksters.

De uitkomsten van deze studie toonden geen verband aan tussen het verhoogde risico op veneuze trombose bij het gebruik van hormonale anticonceptiva en de verhoogde stollingsneiging als gevolg van hyperthyreoidie bij het gebruik van deze hormonale anticonceptiva.

Authors and Affiliations

Bart E.P.B. Ballieux	Department of Chemistry, Leiden University Medical Center, Leiden, the Netherlands
Joyce Curvers	Department of Chemistry, Catharina Hospital, Eindhoven, the Netherlands
Anders E. Dahm	Department of Haematology, Oslo University Hospital, and University of Oslo, Oslo, Norway
Kathrin Fleischer	Department of Obstetrics and Gynaecology, University Medical Center St. Radboud, Nijmegen, the Netherlands
Frans M. Helmerhorst	Department of Gynaecology and Reproductive Medicine, Leiden University Medical Center, Leiden, the Netherlands; Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands
Astrid van Hylckama Vlieg	Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands
Laureen M. Lopez	Clinical Sciences, FHI 360, Research Triangle Park, North Carolina, United States of America
Pieter Reitsma	Department of Haemostasis and Thrombosis, Leiden University Medical Center, Leiden, the Netherlands
Frits R. Rosendaal	Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands
Jan Rosing	Department of Biochemistry, Maastricht University, Maastricht, the Netherlands
Per Morten Sandset	Department of Haematology, Oslo University Hospital, and University of Oslo, Oslo, Norway
Bernardine H. Stegeman	Department of Thrombosis and Haemostasis, Leiden University Medical Center, Leiden, the Netherlands; Department of Epidemiology and Public Health, University College London, United Kingdom
Stella Thomassen	Department of Biochemistry, Maastricht University, Maastricht, the Netherlands
Huib A.A.M. van Vliet	Department of Obstetrics & Gynaecology Catharina Hospital, Eindhoven, the Netherlands
Hans L. Vos	Department of Thrombosis and Hemostasis, Leiden University Medical Center, Leiden, the Netherlands

Acknowledgements

I ran to get where I was going, I never thought it would take me anywhere
- Forrest Gump

Toen ik begon aan mijn wetenschapsstage in het laatste jaar van de Geneeskunde studie had ik enkel een onderzoeksidee. Nu, vier jaar later heeft dat geresulteerd in een voltooid proefschrift. Maar niet zonder hoogte- en dieptepunten, obstakels, en zeker niet zonder alle mensen die mij hebben geholpen tijdens deze periode; zowel op wetenschappelijk als persoonlijk gebied.

Professor Helmerhorst, Frans, bedankt voor de fantastische begeleiding en het in mij gestelde vertrouwen, ik had het niet beter kunnen treffen. Ik heb je adviezen altijd enorm gewaardeerd, zowel op onderzoeks-, opleidings-, als persoonlijk vlak. Je bent een fijne begeleider en ik heb bewondering voor de manier waarop je dat doet. Dr. van Vliet, Huib, bedankt voor alle kritische en gedetailleerde feedback die ik van jou kreeg; of het nu versie 2 of 12 was, jij bleef altijd zorgvuldig en optimistisch. Ik kijk terug op een paar mooie jaren met een memorabele reis naar Miami. Professor Rosendaal, Frits, wij zagen elkaar niet vaak, maar je had wel altijd het laatste woord over mijn stukken met steeds spaarzaam, maar zeer waardevolle kritiek. Een aantal van jouw terugkerende commentaren ben ik zelfs "de regels van Frits" gaan noemen, bedankt hiervoor!

De verschillende co-auteurs wil ik graag bedanken voor hun inspanningen. Kathrin Fleischer, bedankt voor het gebruiken van je samples; Bernardine Stegeman voor het samenwerken in verschillende studies; Jan Rosing en Stella Thomassen voor jullie expertise op het gebied van de thrombin generation-based APC resistance test; Pieter Reitsma, Anders Dahm en Per Morten Sandset voor jullie expertise op het gebied van TFPI en Proteïne S; en Bart Ballieux voor het gebruiken van het laboratorium voor de SHBG bepaling.

Bedankt Femke Hilgersom, Ilona Coops en Marja Konstantinovski voor het ondersteunen tijdens de Qlaira studie. Carol Manion, thank you for creating order in the chaos of literature search!

Rosemiek Cupers, Petra Noordijk en hun collega's van het Einthoven laboratorium en Epidemiologie laboratorium wil ik graag bedanken voor de snelcursus labvaardigheden! Het langdurig en herhaaldelijk pipetteren van NaCl heeft uiteindelijk geleid tot het zelfstandig uitvoeren van een ELISA, waar ik stiekem best trots op ben!

Zonder de proefpersonen was dit onderzoek nooit gelukt. Ik wil alle vrouwen bedanken die belangeloos hebben meegewerkt aan de verschillende projecten en op deze manier hebben bijgedragen aan het vergroten van de kennis op het gebied van anticonceptie en veneuze trombose.

Lieve collega's van het Medisch Centrum Haaglanden, Groene Hart Ziekenhuis en LUMC, bedankt voor jullie interesse, gezelligheid, oppeppende woorden en een fantastische tijd de afgelopen jaren! Maatschap gynaecologie en verloskundige van het Medisch Centrum Haaglanden, bedankt voor de flexibiliteit en de mogelijkheid tot parttime werken tijdens mijn promotieonderzoek, en voor alle opleidingsmomenten die ik heb gehad, en die nog zullen komen! Collega's van de afdeling Klinische Epidemiologie, bedankt voor de gezellige en leerzame tijd.

Mijn vrienden wil ik bedanken voor hun begrip voor mijn afwezigheid de laatste periode. Clovertjes, voortaan ben ik weer present tijdens de gezellige etentjes! En Haarlemse vrienden, gelukkig heb ik weer wat meer vrije tijd nu het weer zomer wordt!

Lieve paranimfen, Marise en Pieter, jullie hebben me regelmatig over wat onderzoeks-hobbels heen geholpen en het met me gevierd als er weer een artikel gesubmit was! Marise, hopelijk ben je er bij tijdens mijn verdediging, ik zou jou als vriendin, collega en onderzoeksmaatje hierbij niet willen missen. Pieter, met jou beleefde ik enkele diepe dieptepunten, maar ook vele hoogtepunten tijdens mijn promotietraject. Bedankt voor je hulp!

Andere kleine grote broer, wat hebben we altijd een lol samen. Dankjewel voor je interesse en support! Familie Knuivers, ik ben superblij met zo'n gezellige schoonfamilie! "Kuuroord Knuivers" was meer dan eens een moment van rust na een drukke werkweek. Niels, bedankt voor je engelengeduld en het prachtige ontwerp van mijn proefschrift!

Lieve Pap en Mam, aan jullie heb ik zoveel te danken, daarvoor heb ik hier geen ruimte genoeg. Bedankt voor jullie betrokkenheid, meedenken, optimisme en alle goede zorgen. Ik hou van jullie.

Tot slot, Eelko, mijn lief, het is af! Jij hebt alle fases van het onderzoek van dichtbij meegemaakt, en hielp me opstaan als ik viel. Je zorgde dat ik steeds weer de motivatie vond om door te gaan, dankjewel voor je onvoorwaardelijke steun en positivisme. En wat hebben we het leuk samen! Ik geniet van alle leuke dingen die we samen doen en kijk uit naar alles wat nog komen gaat!

About the author

Curriculum Vitae

Marjolein Raps was born on July 20th 1985 in Heemstede, The Netherlands. She grew up in Hoofddorp and graduated from secondary school at the Kaj Munk College Hoofddorp in 2003. In the same year, she started with the curriculum Medicine at Leiden University.

During her study she participated as member of the board of the Medical Faculty of Students Association and followed two internships in Poland and Indonesia. Later during her training period, her enthusiasm for Obstetrics and Gynaecology was raised. In that period of time she had the opportunity to make the first steps that laid to her thesis on the effectiveness and side effects of hormonal contraceptives at the department of Clinical Epidemiology at the Leiden University Medical Center (supervision of Professor F.M. Helmerhorst and dr. H.A.A.M. van Vliet).

In 2011 she graduated from Medical School and worked as resident at the Obstetrics and Gynaecology department of the Medical Center Haaglanden in The Hague (head: dr. M.J. Kagie). Simultaneously, she finished her research project at the department of Clinical Epidemiology at the Leiden University Medical Center (supervised by Professors F.M. Helmerhorst and F.R. Rosendaal and dr. H.A.A.M. van Vliet). In October 2013 she started her residency training in Obstetrics and Gynaecology at the Groene Hart Hospital, Gouda (head: dr. H. van Huisseling), and since january 2014 she continued her training at the Medical Center Haaglanden, The Hague (head: dr. M.J. Kagie). Marjolein Raps lives together with Eelko Knuivers in Haarlem.

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Abstracts

Van Vliet HAAM, **Raps M**, Rosendaal FR, Helmerhorst FM, Hormonale anticonceptie, hemostase en trombose. Gynaecongres; 2010 November, Arnhem, the Netherlands.

Raps M, Helmerhorst FM, Fleischer K, Thomassen S, Rosendaal FR, Rosing J, Ballieux BEPB, Van Vliet HAAM Sex Hormone-Binding Globulin as a marker for the thrombotic risk of hormonal contraceptives. Abstracts from the 61st Annual Meeting of the Society for Gynaecologic Investigation; 2011 March; Miami, Florida, USA.

Raps M, Rosendaal FR, Ballieux BEPB, Rosing J, Thomassen S, Helmerhorst FM, Van Vliet HAAM. Het risico op veneuze trombose tijdens gebruik van een vierfasenpreparaat met dienogest en oestradiolvaleraat. Presentation VFS autumn meeting Rotterdam, The Netherlands, November 2012

Raps M, Helmerhorst FM, Fleischer K, Thomassen S, Rosendaal FR, Rosing J, Ballieux BEPB, Van Vliet HAAM. APC-resistentie en SHBG als markers voor veneuze trombose tijdens gebruik van hormonale anticonceptie. Presentatie Schellekens symposium, MCH, Juni 2013

Presentations

Sep. 2010	"Hormonal contraceptives and venous thrombosis", oral presentation researchlunch Clinical Epidemiology LUMC
Mrt. 2011	"Sex Hormone-Binding Globulin as a marker for the thrombotic risk of hormonal contraceptives", Poster presentation SGI Miami, Florida, USA
Okt. 2012	"The risk of venous thrombosis during use of the four phasic oral contraceptive containing dienogest and estradiol valerate", oral presentation VFS autumn meeting
Nov. 2012	"The risk of venous thrombosis during use of hormonal contraceptives", oral presentation LOCOG
Nov. 2012	"SHBG as marker for the thrombotic risk during use of hormonal contraceptives", oral presentation Landsteiner Institute Medical Centre Haaglanden
Nov. 2012	"Contraception", oral presentation Department of Obstetrics and Gynaecology Medical Centre Haaglanden
Jul. 2013	"Contraceptives and venous thrombosis", oral presentation Department of Obstetrics and Gynaecology HAGA hospital
Jul. 2013	"Contraceptives and venous thrombosis", oral presentation NVOG workgroup contraceptives
Jun. 2014	"APC resistance and SHBG as markers for the risk of venous thrombosis during use of hormonal contraceptives", oral presentation Schellekens symposium, Medical Centre Haaglanden