Oral contraceptives, venous thrombosis and the role of coagulation defects

Kitty W. M. Bloemenkamp

ORAL CONTRACEPTIVES, VENOUS THROMBOSIS AND THE ROLE OF COAGULATION DEFECTS

PROEFSCHRIFT

ter verkrijging van de graad van Doctor
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STELLINGEN

behorend bij het proefschrift
"Oral contraceptives, venous thrombosis and the role of coagulation defects"

I

The risk of venous thrombosis with the use of current low-dose brands of oral contraceptives still carries a risk of venous thrombosis which is not explained by diagnostic suspicion and referral bias.

This thesis

II

If a woman develops venous thrombosis in the first year of oral contraceptive use this may indicate the presence of an inherited coagulation defect.

This thesis

III

Variation in the susceptibility of the individual woman holds the key to provide a biologically plausible explanation of why oral contraceptives cause venous thrombosis.

This thesis

IV

Third generation oral contraceptives should not be prescribed as a first choice to women; as a second choice, it should only be prescribed after giving full information about the venous thrombosis risk.

This thesis

V

The marketing of third generation oral contraceptives for "starters and switchers" is unacceptable, because there is evidence that these women are especially vulnerable to the thrombogenic nature of these oral contraceptives.

This thesis

VI

Myocardial infarction in women can be prevented more effectively by advising women to stop smoking than to stop using the pill.

VII

"Birth control is essentially an education for women".

Margaret Sanger, The Pivot of Civilization, 1922.

VIII

The postcoital test should not be included in the standard fertility investigation.

IX

Better methods to estimate the risks of a vaginal breech delivery should be developed because "very few situations are as anxiety-provoking for the obstetrician or as dangerous for the fetus as entrapment of the aftercoming head with a breech delivery".

After: L.C. Gilstrap III, 1995.

X

If there was a male contraceptive method available that carries the same small risks as oral contraceptives do, these risks would probably not be accepted by the male users.

XI

Don't mind about your make up, you'd better make up your mind now.

After: Sweet d'Buster, Smashs The Mirro, Dutch band, 1979.

XII

It is men rather than women who were liberated by the pill and emancipation.

After: W. Leith. Can't commit, won't commit. The Observer 12 September 1999.

XIII

In the Netherlands, condoms are not made more readily available by condom-dispensers which only accept five-guilder coins.

Leiden, 24 november 1999

Kitty W.M. Bloemenkamp

It is not a disaster to be unable to capture your ideal,
but it is a disaster to have no ideal to capture.
It is not a disgrace not to reach the stars,
but it is a disgrace to have no stars to reach for.
Not failure, but low aim is sin.

Origin unknown

'.....human kind cannot bear very much reality'

T.S. Elliot. Burnt Norton, 1935

For my parents

CONTENTS

Chapter 1

GENERAL INTRODUCTION

General introduction

Oral contraceptives have been available since the 1960s and are now used by more than 100 million women throughout the world[1]. In the Netherlands, oral contraceptives are the most commonly used contraception method. In 1997, 43.3% of the women who were aged between 16 and 50 years, and up to 73.7% of the women who were aged between 20 and 25 years used oral contraceptives[2]. The effects of oral contraceptives continue to be of immense interest to women, their medical advisors, the media and increasingly to the legal profession, because oral contraceptives are the most frequently used drugs by healthy women in the developed world. Besides having beneficial effects, the highly effective protection against pregnancy, like all medicines, oral contraceptives also show adverse effects. Ever since oral contraceptives have been marketed, reports have appeared on links between oral contraceptive use and cardiovascular disease, including both venous and arterial thrombosis[3-5]. Oral contraceptives influence the hemostatic- (see addendum I), carbohydrate-, lipid-, and endothelium systems, mechanisms regulating blood pressure and probably as yet unknown systems, as a result of which there is an increased risk of cardiovascular disease[5-12].

Cardiovascular side effects can be distinguished in arterial thrombosis (acute myocardial infarction, ischaemic stroke and hemorrhagic stroke) and venous thrombosis (deep-vein thrombosis, pulmonary embolism, ischaemic stroke)[5,13]. The work in the present thesis was undertaken to investigate the influence of oral contraceptives on the hemostatic system, in a renewed attempt to understand why oral contraceptives cause cardiovascular disease, in particular venous thromboembolism. In this introductory chapter some background information will be given on oral contraceptives and venous thrombosis.

History of oral contraceptives

The earliest demonstration that fertility could be influenced by hormonal manipulation is attributed to Ludwig Haberlandt, professor of physiology at the University of Innsbruck, Austria, who in 1921 performed ovarian transplants between pregnant animals and non-pregnant animals of the same species. He was able to render

five out of eight rabbits and three out of eight guinea-pigs infertile[14]. In the same year Fellner reported infertility in twenty-seven out of thirty rabbits following injection of a lipid extract of the ovary. As a result Fellner suggested that estrogen could be used for fertility control[15]. By 1931, Haberlandt had proposed the administration of ovarian and placental hormones for birth control, and an extract was produced, named Infecundin. Haberlandt was convinced that hormonal contraception was feasible: "das aber die auf rein biologischen Pinzip aufgebaute zeitweilige hormonale Sterilisierung .... für die praktische Medizin und ihre künftigen Aufgaben der Geburtenregelung als die ideale zu bezeichnen ist, bedarf wohl keinen näheren Begründung." However, his early death in 1932 brought an end to his efforts[16,17].

One of the problems was that for the extraction and isolation of a few milligrams of the sex steroids, litres of urine or thousands of kilos of organs were needed. For example, in 1935, ovaries from 2500 pregnant pigs were required to produce 1 mg progesterone[18,19]. In 1942, the chemist Russell Marker succeeded in synthesising progesterone in kilogram quantities from diosgenin (Figure 1), a steroid glycoside from the root of the wild Mexican yam, *cabeza de negro* [20]. Meanwhile in 1937, Makepeace and co-workers had reported inhibition of ovulation in rabbits following progesterone injection[21], and in 1938 Hans Inhoffen produced a potent synthetic estrogen, ethinyl estradiol, and the progestogen ethisterone by the addition of an acetylene group at the 17-carbon position of the naturally occurring hormones, estradiol (the major estrogen secreted by the ovaries) and testosterone[22], respectively.

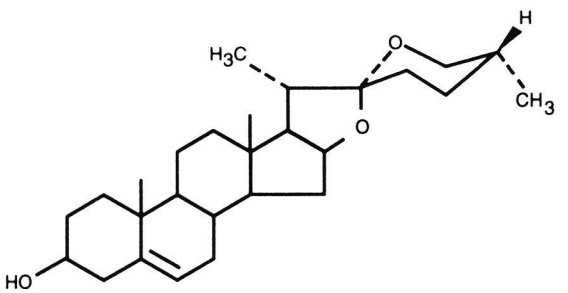


Figure 1. Diosgenin, a natural steroid precursor from Mexican yam roots

The major obstacle to the use of naturally occurring sex steroids for contraception was the inactivity of the compounds when given orally. The addition of an ethinyl group at the 17 position made estradiol orally active. Ethinyl estradiol is a very potent oral estrogen (Figure 2) and was introduced as the estrogen compound in oral contraceptives; the other estrogen used was the 3-methyl ether of ethinyl estradiol, mestranol (which is converted to ethinyl estradiol in the body)[17-19].

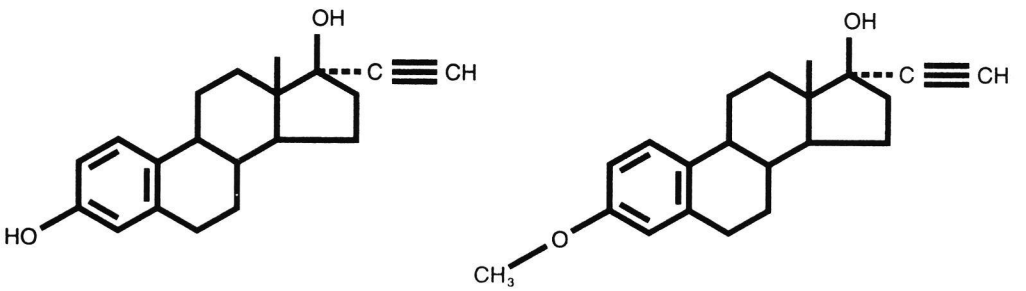


Figure 2. Ethinyl estradiol and mestranol

In 1951 Luis Miranontes (working with Djerassi) removed the 19-methylgroup from Inhoffen's ethisterone, producing 19-nor-17α-ethinyltestestorone or norethisterone[23]. Carl Djerassi worked at the Syntex laboratory in 1951 and synthesised the potent, orally active progestational compound norethindrone[24,25]. A year later, Frank Colton, working with the G.D. Searle company synthesised norethynodrel[26]. After a visit from Margaret Sanger in 1951, Gregory Pincus in Massachusetts, having spent many years studying mammalian fertilisation became interested in contraception. Sanger, president of the International Planned Parenthood Federation, pointed out that Pincus' animal experiments suggested a method of oral contraception for women, and she provided a research grant[27-32].

Norethidrone and norethynodrel were first tested on animals in 1953-1954 and the first human trial was performed in Puerto Rico in 1956[30]. The initial progestogen products were contaminated with about 1% mestranol, adding up to 50-500 micrograms mestranol,

a sufficient amount of estrogen to inhibit ovulation itself. When efforts to lower the estrogen content yielded breakthrough bleeding, it was decided to retain the estrogen for cycle control, thus establishing the principle of the combined estrogen-progestogen oral contraceptive[17-19]. In 1957, the U.S Food and Drug Administration approved a combination of 9.85 mg of norethynodrel and 0.15 mg of mestranol (Enovid; G.C. Searle and Company, Chicago, Illinois) for use in menstrual disorders and, subsequently in 1959, as a contraceptive. A norethisterone-containing preparation (licensed by Syntex to Parke Davis) was also approved for menstrual regulation in 1957, and as an oral contraceptive in 1962[33].

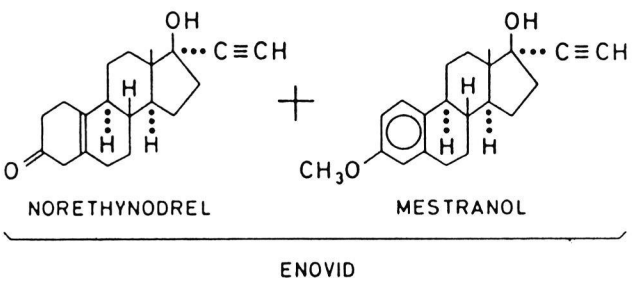
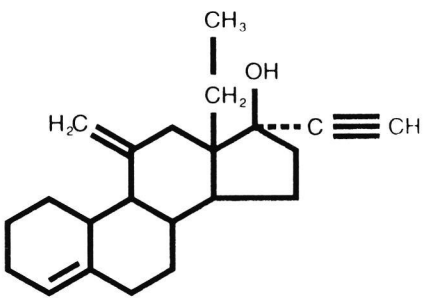


Figure 3. The first progestogen-estrogen combination contraceptive: Enovid

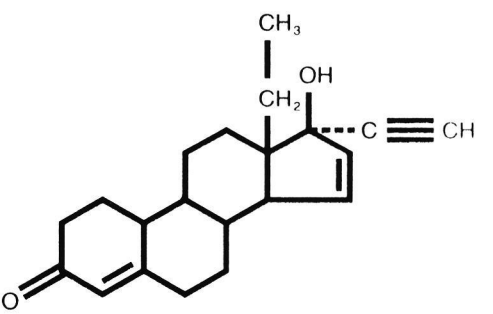
The discovery of ethinyl substitution and oral potency (at the end of the 1930s) led to the preparation of ethisterone, an orally active derivative of testosterone. The removal of the 19 carbon from ethisterone to form norethindrone did not affect the oral activity, and most importantly, it changed the predominant androgenic characteristic to that of a progestational feature. Accordingly, the progestational derivatives of testosterone were designated as 19-nortestosterones (denoting the missing 19 carbon). The androgenic properties of these compounds, however, were not totally eliminated and minimal anabolic and androgenic potential remained[17-19]. Clinically, androgenic and estrogenic activities of the progesterone component are insignificant due to the low dosage in the currently used oral contraceptives. The norethindrone family contains the following

19-nortestosterone progestogens: norethindrone, norethynodrel, norethindrone acetate, etynodiol diacetate, lynestrenol, norgestrel, levonorgestrel, norgestimate, desogestrel, and gestodene (see addendum II). Levonorgestrel is the active isomer of norgestrel[19].

Unfortunately there is no recognised classification system of oral contraceptives based on their pharmacologic properties. One system used is that the progestogens are classified as first, second, third and fourth generation. The first generation includes: ethynodiolacetate, lynestrenol, norethisterone (acetate) and norethynodrel. The second generation includes: norgestrel, levonorgestrel and norgestrione. The third generation includes: desogestrel, gestodene and norgestimate (whether norgestimate should be included in the second generation progestogens is a point of discussion, because norgestimate is partly converted to levonorgestrel). The fourth generation includes: chlormadione and cyproterone acetate (both C21 derivatives). A miscellaneous group includes mainly C21 steroids, such as dydrogesterone, medrogestone, medroxyprogesterone acetate and the natural progesterone. In currently available oral contraceptives ethinyl estradiol in the amount of 20 μg, 30 μg, 35 μg or 50 μg is combined in several ways with the different progestogens, either mono-, bi or triphasic, and in various dosages[6,7,18,19].



Desogestrel



Gestodene

Figure 4. Third generation progestogens

Mechanism of action of oral contraceptives

Oral contraceptives prevent pregnancy primarily by suppressing ovulation through the combined actions of an estrogen and a progestogen. Both sex steroids act upon many different organ systems and produce several effects, both contraceptive and other[34,35].

Progestational effects

The most important part of the contraceptive effect of oral contraceptives is contributed by the progestogen component which inhibit ovulation by suppression of luteinizing hormones. Furthermore the cervical mucus is thickened and the transport of sperm is reduced (although this is probably not an important contraceptive effect). Capacitation of sperm may be inhibited and implantation is hampered by production of a decidualized endometrial bed with exhausted and atrophied glands[34,35].

Estrogenic effects

Estrogens inhibit ovulation in part by the suppression of follicle stimulating hormone (FSH) and luteinizing hormone (LH). This suppression mimics the changes that occur during pregnancy, so the pituitary gland does not release hormones to stimulate the ovary. Secretions and the cellular structure of the endometrium within the uterus is altered, leading to areas of edema alternating with areas of dense cellularity. Luteolysis, the degeneration of the corpus luteum, may occur when high levels of estrogen (higher than levels found in current oral contraceptives) alter local prostaglandins. This effect may help explain how estrogens work as postcoital oral contraceptives[34,35].

Venous thrombosis

Venous thrombo-embolism is a common disease and the overall annual incidence of venous thrombosis is estimated 1 in 1000 persons[36]. Venous thrombosis is unwanted clot formation in a vein. The most frequent forms are deep-vein thrombosis of the legs and pulmonary embolism. Less frequent are thrombosis of the retinal veins, mesenteric veins, cerebral sinus veins, arm veins and the portal vein (Budd-Chiari-syndrome)[37]. The pathogenesis of venous thrombosis is complex and not totally understood. Virchow's

trias is still helpful for global understanding: changes of the vessel wall, slowing of the bloodstream and alterations in composition of blood cells or plasma factors[38]. As a result of many investigations during the last decades it has become clear that the causes of venous thrombosis include hereditary and acquired factors (Table 1), although there is still a group in which venous thrombosis occurs in the absence of obvious predisposing factors, the so-called idiopathic cases[39-41]. The incidence of venous thromboembolism in young women is low and depends on predisposing factor(s) such as pregnancy, oral contraceptive use, carriership of a coagulation defect etc. or combinations of risk factors. Oral contraceptive users are generally healthy women with a low background incidence of major diseases. When serious adverse events occur in oral contraceptive users, this has great implications and the modest elevations in risk have the potential to affect a large number of women[5].

Table I. Risk factors for venous thrombosis

Acquired	**Inherited**
surgery	antithrombin deficiency
malignancies	protein C deficiency
trauma	protein S deficiency
immobilisation	APC resistance/
pregnancy, puerperium	factor V Leiden (506Arg to Gln) mutation
use of oral contraceptives	dysfibrinogenemia
	hyperhomocysteinemia
	high factor VIII levels
	prothrombin (20210 A) mutation

Oral contraceptives and venous thrombosis

The aim of our study is to gain more insight into the association between oral contraceptives, venous thrombosis and inherited coagulation defects. More particularly,

we set out to find answers to the question: "Why do oral contraceptives cause venous thrombosis?" In chapter 2 an overview is given on the association between oral contraceptive use and venous thrombosis and their interaction with coagulation defects, this overview was written in 1996 and an update will be given in the discussion. Chapter 3 describes the results of a case-control study in which we investigated how a type of progestogen plays a role in the association with venous thrombosis, especially when taking factor V Leiden and family history into account. In chapter 4, we investigated whether women with inherited coagulation defects who use oral contraceptives develop their venous thrombosis at an earlier stage compared with women without known inherited coagulation defects. Elevated plasma levels of factor VIII are a strong risk factor for venous thrombosis and in chapter 5 we investigated the joint effect of high factor VIII levels and oral contraceptive use in the occurrence of venous thrombosis. The magnitude of the relative risk of venous thrombosis due to low-dose oral contraceptives is still debated because previous studies might have been influenced by diagnostic suspicion and referral bias. In chapter 6 we describe the results of a case-control study in which the effect of diagnostic suspicion and referral bias could be excluded. In chapter 7 we describe a randomised experiment in which healthy women taking two oral contraceptives containing 20 μg ethinyl estradiol and either gestodene or desogestrel are compared in their effect on the hemostatic-, carbohydrate- and lipid systems. Most studies on the effect of oral contraceptives are conducted with volunteers who are presumed healthy and with a uniform background risk. But what happens when we take their genetic differences into account? Because the genetic "make-up" of a person influences the response to coagulation, the effect on hemostatic variables during oral contraceptive use is studied in chapter 8. Almost invariably studies on the effect of oral contraceptives on the hemostatic system are done with healthy young women, but what happens when we study diseased women? The results of a comparison of the effect of continued use of oral contraceptives on hemostatic variables in venous thrombosis patients (thrombosis while using oral contraceptives) with the effect in healthy control subjects are given in chapter 9. In chapter 10 a case report is presented in which identical twins who are carriers of factor V Leiden started using oral contraceptives.

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Chapter 2

EVIDENCE THAT CURRENTLY AVAILABLE PILLS ARE ASSOCIATED WITH VASCULAR DISEASE: VENOUS DISEASE

Evidence that currently available pills are associated with vascular disease: venous disease

Kitty WM Bloemenkamp, Frits R Rosendaal, Frans M Helmerhorst, Jan P Vandenbroucke

Adapted from: Hannaford PC, Webb AMC. Evidence-guided Prescribing of the Pill 1996: 61-76. (Carnforth, UK: Parthenon Publishing)

Thirty years of epidemiological research

From the early days of the use of combined oral contraceptives, reports have emerged associating use of oral contraceptives with the development of venous thromboembolism. After the report of Jordan[1], numerous case control-[2-10] and cohort-[11-16] studies followed. In an attempt to assess the thrombogenic potential of new preparations introduced during the last decade through the assessment of surrogate end-points, young healthy volunteers have been recruited to randomised trials in which the effect of the new oral contraceptive on variables in (anti)-coagulation-, fibrinolysis- and lipid metabolism were investigated, mostly in comparison with an older preparation. Several authors, however, have expressed doubts about the clinical relevance of this type of study, or whether they can reasonably be expected to reflect the risk of thrombosis during actual, large-scale use of a new type of oral contraceptive[17,18].

Observational case-control and cohort studies have to be criticised. Not every study has been able to adjust for the influence of putative confounders or effect modifiers, such as smoking, family history of venous thrombosis, history of varicose veins, body mass index, duration of oral contraceptive use, parity, age, blood group. Furthermore, some observational studies were not specifically designed to look at the side effects of oral contraceptives. Other problems affecting comparability of data between studies include some authors reporting on fatal, and others on non-fatal venous thromboembolism; and some authors differentiating between types of thrombosis, such as superficial, deep venous thrombosis or pulmonary embolism. In some studies, objective investigations for the diagnosis (such as venography, ultrasound, impedance plethysmography, ventilation-perfusion scans, post-mortem examinations) were missing. Randomised studies with clinical end-points overcome other flaws of observational studies, such as selection, diagnostic, referral, recall and prescription bias. However, the low incidence of venous thrombosis during oral contraceptive use, ethical and financial reasons prevent us from studying this association by a randomised trial. Consequently, we have to draw our conclusions from observational and laboratory studies.

For some time there has been (near) consensus that there is an association between oral contraceptive use and venous thrombosis. Reports from various case-control and cohort studies attribute this risk to the estrogen content of the oral contraceptives, an effect which has not consistently been shown to be related to dose[19,20]

and unrelated to duration of pill use[4,5,21]. The risk disappears once the oral contraceptive is stopped; there is no elevated risk among past users. Women with bloodgroup non-O appear to have higher risks than women with bloodgroup O[22,23]. Smoking does not appear to be a risk factor for venous thrombosis[4,7,11,12,24]; obesity[4,25] and varicose veins[11] are at most weak risk factors. Prescribers, therefore, cannot readily identify women at risk.

The estimated relative risk of developing thromboembolism during oral contraceptive use has been reported between 2 and 11. Case-control studies have usually yielded a higher relative risk than cohort studies. The risk estimates have been larger when associated with idiopathic events than when other risk factors were also involved[3,4]. Furthermore, the more certain the diagnosis[4,5,13,21,25] or the more severe[11,13] the venous thromboembolic events, the larger the estimated relative risks. Even though every study is likely to be affected by at least one bias, nearly all have reported elevated risk estimates, increasing the plausibility of a relationship between current oral contraceptive use and venous thrombosis. Finally, it is a characteristic of true association that this shows up whatever the shortcomings of studies set up to investigate it (although an important flaw common to all studies can still produce erroneous conclusions).

In an attempt to lower the cardiovascular side effects of oral contraceptives, newer generations of pills, containing lower doses of ethinyl estradiol and lower doses, and new types, of progestogens, were introduced. The decrease in the amount of ethinyl estradiol from 50 μg to 35 μg, 30 μg and even 20 μg was expected by some to result in a reduced risk of venous thrombosis[11,13,25,26]. Since most studies were conducted in the 1960s and 1970s, there is little information about clinical end-points for these newer preparations. Two case-control studies[9,10] and one cohort study[16] using clinical end-points were conducted in the late 1980s or early 1990s, when the newer preparations were on the oral contraceptive market. These studies compared current users with non-users and reported relative risks of 2.1 (95% confidence interval (CI) 0.8-5.2) for fatal venous thromboembolism and pulmonary embolism[10], 3.8 (95% CI 2.4-6.0) for non-fatal deep vein thrombosis (age-adjusted risk 6.0 (95% CI 3.4-10.6))[9] and 2.7 (95% CI 1.8-4.2) for superficial venous thrombosis, deep vein thrombosis, pulmonary embolism, and venous thrombosis[16]. These data suggest that oral contraceptives with less than 50 μg ethinyl estradiol may still have a thromboembolic risk.

Inherited coagulation defects

One of the studies reported a new insight into the relation between oral contraceptive use and thromboembolism[9]. An interaction between a newly discovered inherited coagulation disorder[27] and oral contraceptive use was described. Factor V Leiden mutation, which leads to resistance to activated protein C and which is commonly found among patients with venous thrombosis (up to 20% of patients with deep vein thrombosis are carriers)[28], displays a strong interaction with use of oral contraceptives. In non-carriers who use oral contraceptives, the risk of venous thrombosis is increased four-fold, in carrier non-users the risk is increased eight-fold, but in users of oral contraceptives who also carry the factor V Leiden mutation the risk rises 30-50-fold[9]. Other inherited coagulation defects, protein C, protein S and antithrombin deficiency, which are themselves risk factors of venous thrombosis, also appeared synergistically to lead to an excess risk of venous thrombosis among oral contraceptive users[29]. The prevalence of factor V Leiden in the Caucasian population is estimated between 3-5%, with much lower levels for the other inherited clotting abnormalities[30-33].

Screening

It is questionable if the routine screening for genetic clotting disorders before starting oral contraceptives is useful or feasible[34]. Part of the problem is the lack of evidence concerning this issue. In general, however, family history is a strong risk factor for thromboembolism in healthy young people. It *may* be useful, therefore, to screen potential users when there is a family history of inherited thrombophilia in a first-degree relative. On the other hand, it is not yet clear whether it is helpful to screen women with a positive family history, or even personal, history of thrombosis. A major problem is that venous thrombosis is relatively common when viewed over the history of a life time. Thus, many people have a positive history without having a genetic defect[35].

New progestogens

In October 1995 the Committee on Safety of Medicines in the United Kingdom warned, in a letter to all doctors[36], of a differential risk between oral contraceptive types. Based on at the time unpublished data, the Committee advised that several of the so-called third generation preparations had a greater risk of venous thrombosis than older preparations. In December 1995 and January 1996, four studies were published which showed that women using oral contraceptives containing desogestrel and gestodene had, on average, twice the risk of developing venous thrombosis than users of older levonorgestrel-containing preparations[37-41]. Previous to these unexpected results, only two epidemiological studies had reported on the effects of the type and dose of progestagens used in oral contraceptives[11,19]. Other studies which examined surrogate and intermediate end-points distinguished between combined or progestogen-only oral contraceptives, different preparations, dose of ethinyl estradiol or mestranol used, and type and dose of progestogen used[18]. However, as already stated, this type of study has now been discredited since results about surrogate end-points have proved irrelevant when predicting future risk of venous thrombosis.

The four studies

The different studies by the World Health Organization (WHO), Jick and colleagues, Bloemenkamp and co-workers and Spitzer and colleagues will now be briefly discussed (Tables 1 and 2).

World Health Organization Study

The WHO conducted a case-control study in 21 hospitals in 17 countries[37]. Some 1143 women aged between 22 and 44 years with a history of idiopathic venous thromboembolism were recruited as cases. The control group consisted of 2998 age-matched women. In the European countries, use of the pill was associated with an overall relative risk of 4.15 (95% CI 3.09-5.57); in non-European countries the relative risk was 3.25 (95% CI 2.59-4.08). The risk estimates were generally higher for deep vein

thrombosis than for pulmonary embolism, but no consistent trend was found for certainty of diagnosis (definite, probable, possible). An increased risk was apparent within 4 months of starting oral contraceptives, was unaffected by duration of current episode of oral contraceptive use, and had disappeared within 3 months of stopping the pill. The relative risks were not related to age, history of hypertension (except during pregnancy) or smoking. A body mass index (BMI) of more than 25 kg/m^2 appeared a weak risk factor. In a subgroup analysis on oral contraceptive type the risk was found to be highest among users of oral contraceptives containing desogestrel and gestodene. This prompted a more detailed analysis of the WHO data which included all women for which these preparations had been prescribed during the study period[38]. A total of 769 cases were compared with 1979 age-matched hospital controls, and, in one centre, with 246 community controls matched on age and general practice. Compared with non-users, those using oral contraceptives containing levonorgestrel had a three-fold elevated risk of thrombosis (Odds Ratio (OR) 3.5 (95 % CI 2.6-4.7)) and those using desogestrel- or gestodene-containing pills in a nine-fold risk (OR 9.1 (95% CI 4.9-17.0) and OR 9.1 (95% CI 4.9-16.7), respectively). After adjustment for BMI, the ORs were 3.4, 7.3 and 10.2, respectively. Direct comparison of desogestrel- and gestodene-containing oral contraceptives with levonorgestrel-containing preparations revealed risk estimates of 2.2 and 3.3, respectively (adjusted for body mass index).

GPRD (General Practice Research Database) Study

Jick and collegues[39] presented data from 238130 women derived from 365 general practices in a study on the risk on non-fatal thromboembolism. In the non-users group, 3.8 cases of non-fatal thromboembolism per 100000 women-years were observed. In the oral contraceptive users group, the rate in users of levonorgestrel-containing pills was 16.1 per 100000 women-years, in the desogestrel group 29.3 per 100000 women-years, and in the gestodene group 28.1 per 100000 women-years. Thus, the relative risk associated with gestodene and desogestrel pills was approximately twice that of levonorgestrel preparations. In a nested case-control analysis, the adjusted matched relative risk estimates were 2.2 (95% CI 1.1-4.4) and 2.1 (95% CI 1.0-4.4) for desogestrel and gestodene users, respectively, compared with users of levonorgestrel. The excess risk for

non-fatal venous thromboembolism associated with the oral contraceptives containing desogestrel or gestodene compared with levonorgestrel was estimated to be 16 per 100000 woman-years.

Table 1. The characteristics of the studies examining the association between oral contraceptive use and venous thrombosis, which compared newer with older types of progestogen contained in the preparation

Database	First author	Type of study	Source data	Period	Countries	Age (years)	Event
WHO	Farley[38]	case-control	hospital	1989-1993	nine	20-44	DVT, PE F, NF
GPRD	Jick[39]	cohort + case-control	hospital	1991-1994	UK	<40	case-control: DVT, PE, NF
LETS	Bloemenkamp[40]	case-control	anticoagulation clinics	1988-1992	NL	15-49	DVT, NF
TRANS	Spitzer[41]	case-control	hospital	1993-1995	UK,GER	16-44	DVT, PE F, NF

WHO: World Health Organisation, GPRD: General Practice Research Database, LETS: Leiden Thrombophilia Study, TRANS.: Transnational Study
UK: United Kingdom, NL: The Netherlands, GER: Germany
DVT: Deep venous thrombosis, PE: Pulmonary Embolism, SVT: Superficial venous thrombosis
F: Fatal, NF: non-fatal

LETS (Leiden Thrombophilia Study)

Using data from a previously published case-control study (Leiden Thrombophilia Study)[40], we compared 126 women, aged 15-49 years, with an objective diagnosis of deep venous thrombosis with 159 control subjects. Compared with non-use, the highest age-adjusted relative risk was found among current users of desogestrel pills (OR 8.7 (95% CI 3.9-19.3)). The numbers of gestodene, norgestimate-containing oral contraceptives were too small to arrive at meaningful conclusions. The relative risks of levonorgestrel-,

lynestrenol- and norethisterone-containing oral contraceptives ranged between 2.2 and 3.8. In a direct comparison, users of desogestrel-containing oral contraceptives had a 2.5-fold higher risk (95% CI 1.2-5.2) than users of all other oral contraceptive types combined. The relative risk for the desogestrel-containing oral contraceptive was similar among women with and without a family history. The excess risk could also not be explained by previous pregnancy, and it was highest in the youngest age categories, where we could expect most new users. The age-adjusted relative risk for the desogestrel-containing oral contraceptive was 9.2 (95% CI 3.9-21.4) among non-carriers of the factor V Leiden mutation and 6.0 (95% CI 1.9-19.0) among carriers of the mutation. This latter risk is superimposed on the eight-fold increased risk of venous thrombosis for carriers of the factor V Leiden mutation. The risk of carriers using the desogestrel-containing oral contraceptive as compared with non-carrier non-users may therefore be increased almost 50-fold.

Transnational Study

After the publication of results of a pharmacokinetic study in Germany suggesting that gestodene may increase the risk of vascular events[42,43] (results which were not subsequently confirmed by other researchers[44-49], an international study was started: the Transnational Study of Oral Contraceptives and the Health of Young Women[41]. In this case-control study, 471 cases were compared with 1772 control subjects, matched for hospital and age. The adjusted odds ratio for venous thromboembolism for use of any oral contraceptive versus no use was 4.0 (95% CI 3.1-5.3). The authors made a different subdivision between first, second and third generation preparations to that used in the other studies. The adjusted odds ratio for desogestrel- and gestodene-containing oral contraceptives versus second- generation oral contraceptives was 1.5 (95% CI 1.1-2.1) for both preparations.

Table 2. Relative risks (95% CI) for venous thrombosis according to type of oral contraceptive

Database	First author	OCs vs non-use	DSG vs LNG	GSD vs LNG	Remarks
WHO	Farley[38]	4.1 (3.3-5.1)	2.6 (1.4-4.8)	2.6 (1.4-4.8)	adjusted for BMI respectively: 4.0 (all), 2.2 (DSG) and 3.0 (GSD)
GPRD	Jick[39]	-	2.2 (1.1-4.4)*	2.1 (1.0-4.4)*	non-fatal venous thromboembolism
LETS	Bloemenkamp[40]	6.0 (3.4-10.6)†	2.2 (0.9-5.4)†	-	DSG vs all others: 2.5 (1.2-5.2)†
TRANS.	Spitzer[41]	4.0 (3.1-5.3)‡	1.5 (1.1-2.2)‡	1.5 (1.0-2.2)‡	DSG and GSD were compared to second-generation products as defined in their study

WHO: World Health Organisation, GPRD: General Practice Research Database, LETS: Leiden Thrombophilia Study, TRANS.: Transnational Study

DSG: Desogestrel-containing oral contraceptives, GSD: Gestodene-containing oral contraceptives, LNG: Levonogestrel-containing oral contraceptives, BMI: body mass index

* Adjusted for smoking and BMI

† Adjusted for age

‡ Adjusted for age, smoking, alcohol use, study centre, BMI, and duration of exposure to oral contraceptives used before current oral contraceptive

Reactions to the investigations

Authorities, the public, industry and researchers in several countries have reacted differently to these findings[50-58] and the scientific discussion is still developing. Among the comments so far are remarks that none of the studies have been able to adjust for all possible confounders, that mainly Caucasian population was studied, that no distinction was made between monophasic, biphasic and triphasic preparations, that different studies had different criteria for defining first-, second- and third-generation progestogens, and that selective prescribing, healthy user effects, and differential referral bias could have contributed to the observations. Even so, as Weiss and Mc Pherson point out[56,57], these theoretical objections may not matter given that all studies point in the same direction.

Conclusions

It can be concluded that there is still an association between oral contraceptive use and venous thromboembolism, a relationship which also may be dependent on the type of progestogen. Women using desogestrel- and gestodene-containing oral contraceptives appear to have a higher risk of developing venous thrombosis compared to users of the other types of low-dose pills. These higher risks have not been completely explained (i.e., are not limited to subgroups defined) by age, duration of use, family history of thrombosis, parity, body mass index, varicose veins or factor V Leiden mutation. To our knowledge, no study has succeeded in overcoming the bias of selective diagnosis and referral; for many preparations the number of exposed cases have been to small to provide reliable estimates and an answer to extremely relevant clinical questions of what is the recurrence rate when continuing to use oral contraceptives after a first thrombosis is still lacking[59].

The higher relative risks of desogestrel- and gestodene-containing oral contraceptives have to be balanced against possible benefits of these oral contraceptives compared with the older preparations. It has to be emphasised, however, that until now these benefits have been much discussed, but not demonstrated except in intermediate end-point studies. Other effects of oral contraceptive use, like the risk of arterial thrombosis and protection against endometrial and ovarian cancer, have still to be investigated for the newer preparations. The absolute risk of fatal thromboembolism in women using oral contraceptives is small. Indeed, all serious diseases are rare in the young. Even so, when they occur they can have a serious impact. The chronic sequel of non-fatal venous thrombosis may place a heavier burden on young and active women than on the elderly, and may afflict their lives for many more years. When safety issues between different types of oral contraceptives are discussed, it is inappropriate to compare the risks to those experienced in other situations, such as pregnancy or puerperium, since these are not the alternatives under discussion (unless fears about safety lead to non-use of any contraceptive and subsequently result in pregnancy). The only useful comparison then is between types of oral contraceptives, and the only logical choice is for the safest compatible with patient acceptance and tolerance.

The future

The story of the third-generation oral contraceptives and venous thrombosis provides an example for studies on side effects of other drugs. Clinical end-points should be investigated instead of intermediate end-points.

Although many new insights into the relationship between venous thrombosis and oral contraceptives have been reported during the past years, much remains unknown and several problems are still to be solved.

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Chapter 3

ENHANCEMENT BY FACTOR V LEIDEN MUTATION OF RISK OF DEEP-VEIN THROMBOSIS ASSOCIATED WITH ORAL CONTRACEPTIVES CONTAINING A THIRD-GENERATION PROGESTAGEN

Enhancement by factor V Leiden mutation of risk of deep-vein thrombosis associated with oral contraceptives containing a third-generation progestagen

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SUMMARY

Recent concern about the safety of combined oral contraceptives with third-generation progestagens prompted an examination of data from a population-based case-control study (Leiden Thrombophilia Study). We compared the risk of deep-vein thrombosis (DVT) during use of the newest oral contraceptives, containing a third-generation progestagen, with the risk of "older" products. We also investigated the influence of family history of thrombosis, previous pregnancy, age and the thrombogenic factor V Leiden mutation.

We selected 126 women with DVT and 159 controls aged 15-49 (mean age 34.9 years) and premenopausal and found, as compared with non-users, the highest age-adjusted relative risks to be that for an oral contraceptive containing desogestrel and 30 μg ethinyl estradiol (relative risk (RR) 8.7, 95% CI 3.9-19.3). We found lower relative risks for all other types of oral contraceptives, ranging from 2.2 to 3.8. In a direct comparison, users of the desogestrel-containing oral contraceptive had a 2.5-fold higher risk (95% CI 1.2-5.2) than users of all other oral contraceptive types combined.

The relative risk for the desogestrel-containing oral contraceptive was similar among women with and without a family history -ie, preferential prescription because of family history cannot explain our findings. Nor could the excess risk be explained by previous pregnancy, and it was highest in the youngest age categories, where we would expect most new users. The age-adjusted RR for the desogestrel-containing contraceptive was 9.2 (95% CI 3.9-21.4), among non-carriers of the factor V Leiden mutation and 6.0 (95% CI 1.9-19.0) among carriers of the mutation. This latter risk is superimposed on the 8-fold increased risk of venous thrombosis for carriers of the factor V Leiden mutation. The risk of carriers using the desogestrel-containing oral contraceptive as compared with non-carrier non-users will therefore be increased almost 50-fold.

Use of low-dose oral contraceptives with a third-generation progestagen carries a higher risk of DVT than the previous generation of oral contraceptives. The absolute risk of DVT associated with these oral contraceptives seems to be especially high among carriers of the factor V Leiden mutation and among women with a family history of thrombosis. However, the higher risk associated with oral contraceptives with a third generation progestagen compared with previous generations was also present in women without factor V Leiden and with no family history.

INTRODUCTION

Since the early 1960s it has been known that oral contraceptives increase the risk of venous and arterial thrombosis. Efforts to reduce the risk by decreasing the oestrogen content have proved successful[1-4]. Third-generation progestagens were introduced in an attempt to lower further the risk of cardiovascular side effects. These new progestagens include desogestrel, gestodene and norgestimate[5,6]. Since these progestagens had less androgenic metabolic effects and did not adversely affect the lipid profile, it was thought that they might carry a lower risk of cardiovascular diseases than older progestagens such as levonorgestrel, lynoestrenol, and norethisterone[5,7].

There is no evidence yet from studies with clinical endpoints that these new progestagens do reduce the risk of cardiovascular diseases. Most studies of combined oral contraceptives containing the new progestagens have been small comparative trials on surrogate endpoints such as coagulation pattern and fibrinolytic and lipid levels in healthy young women[5,7].

Uncertainty about the safety of the newest low-dose oral contraceptives prompted us to reanalyse data from a case control study. We focussed on the possibility that oral contraceptives with a low dose of ethinyloestradiol and a third-generation progestagen would carry a greater risk of venous thrombosis than oral contraceptives containing similar doses of ethinyloestradiol but other progestagens. We also looked for alternative explanations, by investigating the effect of a positive family history of venous thrombosis, a history of pregnancy, and age. A positive family history of deep-vein thrombosis (DVT) might lead to a preferential prescription of a new low-dose oral contraceptive. Women who have ever been pregnant have been exposed to higher estrogen levels and might differ in other aspects from those who were never pregnant. Duration of use may also be a factor so we did an analysis among the youngest women, most of whom will be new users.

Factor V Leiden mutation, which leads to resistance to activated protein C and is commonly found among patients with venous thrombosis (20% carriers), displays a strong interaction with use of oral contraceptives, all types combined. In non-carriers who use oral contraceptives the risk of thrombosis is increased 4-fold, but the risk rises to 30-50 fold in users of oral contraceptives who also carry the factor V Leiden mutation[8].

PATIENTS AND METHODS

The patients and methods have been described previously[8,9]. We invited 474 consecutive patients (both sexes) with a first episode of proven DVT, diagnosed objectively between Jan 1, 1988 and Dec 31, 1992, who were aged less than 70 and who were not known to have malignant disorders. Patients had been selected from the files of three anticoagulation clinics in the Netherlands, which monitor anticoagulant treatment in all patients within a well-defined geographical area. For each thrombosis patient we invited one age and sex matched healthy control.

For the present analysis we selected only premenopausal women, aged 15-49, (mean age: 34.9 years), who were at the time of their thrombosis (or similar date in control) not pregnant, nor in the puerperium, had not had a recent miscarriage, and had not used injectable progestagens[8].

Information on the type of oral contraceptives used at the time of the thrombosis (or indexdate in the control), was obtained from the interview supplemented with data from the hospital discharge letter. This led to complete information on oral contraceptive type in 95% of the 174 users.

We limited the analysis to types of oral contraceptives for which sufficient cases and control were available (ie 5 or more case and control). We left out of the analysis a total of 20 cases and 10 controls who used the following preparations: monophasic 30 μg ethinyloestradiol and gestodene, 50 μg mestranol and norethisterone, 35 μg ethinyloestradiol and cyproterone acetate, biphasic ethinyloestradiol and desogestrel, and ethinyloestradiol and lynoestrenol. We also discarded data from 9 women (cases) in whom the type of oral contraceptives remained unknown. Thus 29 cases and 10 controls were left out of the analysis.

Because the choice of oral contraceptive might have been influenced by the perception of an increased risk known through a family history of venous thrombosis, we took family history into account. We called a family history "positive" when venous thrombosis was reported in one or more parents or siblings by the patient or control.

Presence of the mutant factor V Leiden gene was determined[10], by technicians who did not know if the sample was from a patient or a control or from an oral contraceptive user or non-user.

We analysed data from 285 women (126 cases and 159 controls) on current use of

oral contraceptives at their thrombosis- or indexdate. The oral contraceptive types were classed as: (1) monophasic, containing 30 μg ethinyloestradiol and 150 μg desogestrel; (2) monophasic, containing 30 μg ethinyloestradiol and 150 μg levonorgestrel; (3) monophasic, containing 50 μg ethinyloestradiol and 125 μg or 250 μg levonorgestrel or 1000 μg lynestroenol; (4) triphasic, containing ethinyloestradiol and levonorgestrel or norethisterone; or 5) monophasic, containing 35 μg or 37.5 μg ethinyloestradiol and 1000 μg norethisterone or 750 μg lynoestrenol.

To assess the risk of different types of oral contraceptives among factor V Leiden positive and negative cases, we used the complete control group as a reference for frequency of oral contraceptive use, because at the time these contraceptives had been prescribed for the women in our study factor V Leiden was unknown. The complete control group thus represents the best estimate of the population use of the various types of oral contraceptives.

Although the original data were age-matched we did an unmatched analysis. Because of the inclusion criteria and the age cut-off, many pairs were no longer intact in the database for this analysis. Since the analysis was restricted to the matching factor sex, we adjusted for confounding by the other matching factor (age) by controlling for age by logistic regression. Age was entered as a continuous variable (in years), use of a categorised dummy variable model led only to trivial differences for the estimators of interest.

RESULTS

Table 1 shows the number of cases and controls using the various oral contraceptive types and age adjusted relative risks (RR, all relative to non-users). The highest RR of 8.7 for the desogestrel-containing monophasic oral contraceptive. For all other types of oral contraceptives the RR was between 2.2 and 3.8. Too few women were using contraceptives containing gestodene or norgestimate to permit meaningful conclusions. Direct comparison of two oral contraceptives with identical oestrogen content (30 μg ethinyloestradiol) but with a different progestagen (ie, desogestrel vs levonorgestrel) revealed a 2.2-fold increased risk associated with desogestrel (95% CI 0.9-5.4). When we compared the oral contraceptive containing desogestrel and 30 μg

ethinyloestradiol with all other types combined the age-adjusted RR was 2.5 (95% CI 1.2-5.2).

Table 1. Numbers of cases and controls and age-adjusted RRs (95% CI) for DVT according to type of oral contraceptive used at time of thrombosis

TYPE OF ORAL CONTRACEPTIVE		CASES/CONTROLS	RR
amount of ethinyloestradiol	type of progestagen		
30 μg	desogestrel	37/15	8.7 (3.9-19.3)
30 μg	levonorgestrel	20/18	3.8 (1.7-8.4)
50 μg	levonorgestrel or lynestrenol	8/6	3.4 (1.1-10.7)
triphasic (30-40 μg)	levonorgestrel or norethisterone	7/11	2.2 (0.8-6.5)
35 μg 37,5 μg	norethisterone lynestrenol	8/5	3.8 (1.2-12.5)
no OCs		46/104	

29 Cases and 10 controls using types of rarely used oral contraceptives for which no meaningful analysis was possible, or for whom the type of oral contraceptive used was unknown, were left out of this analysis.

A family history of thrombosis was present in 41 cases and 23 controls (RR=2.9 (95% CI 1.6-5.1)), indicating a higher baseline risk in women with a positive family history. When we restrict the analysis to patients and controls with a positive family history, the age-adjusted RR (vs non-users) for the desogestrel-containing product was 7.2 (95% CI 1.2-42.1) and for the levonorgestrel-containing product it was 3.9 (95% CI 0.6-24.6). For women with a negative family history, the RRs were 8.0 (95% CI 3.2-20.1) and

3.3 (95% CI 1.3-8.5) respectively.

Of the cases and controls with a positive family history 17 % (11/64) carried the factor V Leiden mutation, while the mutation was found in only 8 % (18/221) of cases and controls without a family history. When we adjusted for all these variables jointly, by entering age, factor V Leiden mutation, and family history in a logistic model, the RRs associated with the various oral contraceptives remained essentially the same and the 2-fold higher risk for desogestrel persisted when we contrasted the desogestrel-containing oral contraceptive to the levonorgestrel-containing oral contraceptive.

Further adjustment for history of pregnancy did not change the estimates. Among women who have never been pregnant RRs of 20.8 (95% CI 4.8-90.2) for the desogestrel oral contraceptive and 7.7 (95% CI 1.8-32.9) for the levonorgestrel product (both with similar amount of ethinyloestradiol) were found. Among ever-pregnant women we found RRs of 5.1 (95% CI 1.7-15.3) and 3.0 (95% CI 1.0-9.6), respectively, compared with non-users.

When we restricted the analysis to the youngest women, where most new users will be found, we found the highest risks for the desogestrel-containing oral contraceptive, relative to levonorgestrel. Among women aged 15-19 the risk of the desogestrel-containing oral contraceptive was 7-fold higher than that of the levonorgestrel-containing product; among women aged 20-24 the risk was 4-fold higher.

Table 2 shows the age-adjusted RRs for factor V Leiden carriers and non-carriers. We must stress that the risk of the oral contraceptive is superimposed on the baseline risk[8]: The baseline risk is much higher (8-fold) in carriers of the factor V Leiden mutation than among non-carriers, so that the RR of 6.0 (95% CI 1.9-19.0) leads to a much larger overall effect in factor V positives than in the factor V negatives (RR 9.2 (95% CI 3.9-21.4)). The risk of carriers who use the desogestrel-containing oral contraceptive, compared with non-carrier, non-users, will therefore be increased almost 50-fold. Contrasting the different oral contraceptive groups, we found that, relative to 30 μg ethinyloestradiol oral contraceptives with levonorgestrel, the newer progestagen carries a 3.5 (95% CI 0.8-15.9) higher risk among factor V positives (age adjusted analysis) and only a 2.1 (95% CI 0.8-5.3) higher risk among factor V negatives. Table 2 shows that the combined RR of all other types of progestagen is lower in factor V positives.

We found no factor V Leiden positive controls who used oral contraceptives, and 4% carriers among non-users controls. Among the cases, the factor V Leiden mutation

was present in 22% of non-users, also in 22% of users of desogestrel-containing oral contraceptives and in 15% of levonorgestrel (30 μg ethinyloestradiol) containing oral contraceptives. Even though the absence of mutant gene carriers among the users of oral contraceptives in the controls render analysis with an interaction term in a regression model impossible, these data again point to a high risk associated with the combination of factor V Leiden carriership and use of a desogestrel-containing oral contraceptive.

Table 2. Numbers of factor V positive and factor V negative cases and age-adjusted relative risks (95% CI) for DVT with total control group as reference population, according to type of oral contraceptive used at time of thrombosis.

TYPE OF ORAL CONTRACEPTIVE		CASES	CASES	RR	RR
amount of ethinyloestradiol	type of progestagen	factor V positive	factor V negative	factor V positive	factor V negative
30 μg	desogestrel	8	29	6.0 (1.9-19.0)	9.2 (3.9-21.4)
30 μg	levonorgestrel	3	17	1.9 (0.4-8.5)	4.2 (1.8-9.7)
50 μg	levonorgestrel or lynestrenol	1	7	1.8 (0.2-16.3)	3.9 (1.2-12.9)
triphasic (30-40 μg)	levonorgestrel or norethisterone	1	6	1.0 (0.1-9.4)	2.6 (0.8-8.0)
35 μg 37,5 μg	norethisterone lynestrenol	2	6	4.1 (0.7-24.0)	3.8 (1.1-13.6)
no OCs		10	36	1.0	1.0

29 Cases and 10 controls using types of rarely used oral contraceptives for which no meaningful analysis was possible, or for whom the type of oral contraceptive used was unknown, were left out of this analysis.

DISCUSSION

We found that a new-low dose oral contraceptive with a third-generation progestagen has a higher risk of DVT than oral contraceptives with previous generations of progestagens. This effect is enhanced by factor V Leiden mutation and by a family history of venous thrombosis. However, neither factor V Leiden, nor the family history explains all of the excess risk of a third-generation progestagen, since the risk was also increased in women without the factor V Leiden mutation and without a family history of thrombosis.

In this study almost all of the combined oral contraceptives with third-generation progestagens contained desogestrel. Oral contraceptives containing gestodene, norgestimate or 20 μg ethinyloestradiol were not or only rarely used in the Netherlands during the time period of the investigation. Hence, our conclusions focus on desogestrel but are not necessarily limited to oral contraceptives containing this third-generation progestagen.

One objection to the findings might be that the newest oral contraceptives have been preferentially prescribed to individuals with the highest risk of thrombosis (confounding by indication). This would mean that the excess risk might be paradoxical but expected and not a consequence of the type of oral contraceptive. A factor that could lead to such preferential prescription is a positive family history of venous thrombosis. The excess risk was also present in women with a negative family history, however, and a logistic model which was adjusted for family history, factor V Leiden mutation and age together showed the same relative risks for the various types of contraceptive. Besides family history and clotting defects, there are no generally accepted strong risk factors for DVT in healthy young persons that could lead to preferential prescription patterns; smoking seems not a risk factor for venous thrombosis, and obesity and varicose veins are at most a weak risk[13,14]. It therefore seems unlikely that preferential prescribing can explain the higher risk of DVT with an oral contraceptive containing a third-generation progestagen, since prescribers cannot readily identify women at high risk.

Because pregnancy is a risk factor for thrombosis that might affect the same women who are "sensitive" to oral contraceptives, and since women who have been pregnant may use different brands of oral contraceptive, we did an analysis adjusted for previous pregnancy (ever/never). This did not change the estimates.

A similar issue is whether part of the observed risk could have been brought about by differences between women who start oral contraception for the first time and those who have used them for some time. Among the youngest users (15-19 years), where most will be recent users, we again found the excess risk for the desogestrel-containing oral contraceptive.

Our study took place within anticoagulation clinics that serve well-defined geographical areas in the Netherlands where patients with thrombosis are routinely monitored, and consecutive patients with a first objectively diagnosed DVT were enrolled. Therefore the study itself could not have introduced any change in referral patterns, diagnostic methods, or treatment.

We previously reported an interaction between oral contraceptive use and factor V Leiden carriership which appeared to be synergistic[8]. Our current data show that this synergy seems to a large part due to a positive interaction between a desogestrel-containing oral contraceptive and factor V Leiden mutation. The number of carriers of this mutation, however, was small, especially among control users of oral contraceptives, which made an analysis of the interaction in an age-adjusted model impossible and also led to estimates with a considerable statistical uncertainty. The meaning of the finding is open to interpretation: on the one hand, it might simply imply that carriers of the mutation are at a higher risk when using oral contraceptives because of a multiplication of risks. If so the factor V Leiden mutation would only indicate a subgroup with a higher baseline risk, such as family history. On the other hand, this finding might give a clue about the mechanism of the thrombogenic nature of these contraceptives and should prompt further research- Firstly, to confirm the finding of a synergistic effect and secondly to study the effect of different types of oral contraceptives on the hemostatic balance, also in women without apparent genetic abnormalities since their risk too was increased by oral contraceptives containing a third-generation progestagen.

When we come to look back on the history of oral contraception it is certain that the decrease in the dose of ethinyloestradiol will be seen to have contributed to a reduced thrombotic risk, especially for arterial disease[3,13,14]. However, the use of a third-generation progestagen does seem to have led to an unexpected and yet unexplained return of a higher risk of venous thrombosis.

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Chapter 4

WOMEN WITH INHERITED CLOTTING DEFECTS HAVE HIGHER RISK OF VENOUS THROMBOSIS DURING FIRST YEAR OF USE OF ORAL CONTRACEPTIVES

Women with inherited clotting defects have higher risk of venous thrombosis during first year of use of oral contraceptives.

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SUMMARY

Background: Recent studies showed that the risk for venous thrombosis is highest during initial oral contraceptive use. This suggests a subgroup of women who are at immediate risk of thrombosis when exposed to oral contraceptives. We postulated that women with inherited clotting defects who use oral contraceptives develop venous thrombosis at an earlier stage compared with women without inherited clotting defects.

Methods: Analysis of the data of the Leiden Thrombophilia Study, a population based case-control study, with data on duration of oral contraceptive use and recently detected genetic coagulation disorders. The patients had a first episode of objectively proven deep-vein thrombosis. Women were called thrombophilic when they had either protein C-, protein S-, antithrombin deficiency, factor V Leiden mutation or prothrombin 20210 A mutation.

Results: The risk of developing deep-vein thrombosis was greatest in the first six months and the first year of use. In comparison with prolonged use, the risk to develop deep-vein thrombosis was 3-fold higher in the first six months of use (95% CI 0.6-14.8) and 2-fold in the first year of use (95% CI 0.6-6.1). Patients who developed venous thrombosis in the early periods of use were more often thrombophilic. Among women with thrombophilia, the risk to develop deep-vein thrombosis during the first six months of oral contraceptive use (as compared to prolonged use) was increased 19-fold (95% CI 1.9-175.7), and in the first year of use it was increased 11-fold (95% CI 2.1-57.0).

Conclusions: Women with inherited clotting defects who use oral contraceptives develop venous thrombosis not only more often, but also sooner. Venous thrombosis in the first year of oral contraceptive use may indicate the presence of an inherited clotting defect.

INTRODUCTION

Recent studies have shown that the risk for venous thrombosis is highest during the initial period of oral contraceptive use[1-4]. This confirms a clinical impression that until now has not been studied extensively[5-7]. We previously reported that factor V Leiden mutation, which leads to resistance to activated protein C and was found in about one in five patients with venous thrombosis, displays a strong interaction with use of oral[3,8]. Other inherited clotting defects which are themselves risk factors for venous thrombosis, such as protein C-, protein S-, antithrombin deficiency and prothrombin 20210 A mutation, may also lead to a high risk of venous thrombosis in combination with oral contraceptive use[9].

A higher risk of venous thrombosis during the first months of oral contraceptive use might be specific to women with genetic risk factors. To test this hypothesis we analysed the data of the Leiden Thrombophilia Study. In the original study, oral contraceptive use led to a 4-fold risk of venous thrombosis (6-fold upon age adjustment). In the present analysis, with new data on duration of use and recently detected genetic risk factors, we first looked whether women with venous thrombosis were more often in their first six months or first year of oral contraceptive use in comparison with control subjects who also used oral contraceptives. Secondly, we investigated whether women who developed venous thrombosis early on did more often have hereditary clotting defects in comparison with women who developed venous thrombosis during prolonged use.

MATERIAL AND METHODS

Study Setting

The patients and methods of our study have been described previously[10]. We invited 474 consecutive patients (both sexes, aged less than 70 years and without a known malignant disorder) with a first episode of proven deep-vein thrombosis (diagnosed by established objective methods) between Jan 1, 1988 and Dec 31, 1992. Patients had been selected from the files of three Anticoagulation Clinics in the Netherlands, which monitor

anticoagulant treatment in all patients within well defined geographical areas. Each thrombosis patient invited one age- and sex- matched healthy control subject (friend or acquaintance; if not possible, volunteering partners of patients were age- and sex-matched to serve as controls), age matching was within 5-year bands. Patients were seen after anticoagulant treatment was discontinued for at least three months for a structured interview about risk factors for venous thrombosis and blood collection. Controls were seen around the time of enrolment of the cases, with the same interview and blood collection.

In the present analysis we selected premenopausal women, aged 15-49 years, who were at the time of their thrombosis (or the corresponding date in the control women, their index-date, see below) not pregnant, nor in the puerperium, did not have a recent miscarriage, and were not using injectable progestogens. Data about current use of oral contraceptives at the thrombosis or index date (see below) were available from 155 cases and 169 controls.

Time Window Assessment

Information on the duration of oral contraceptive use was newly abstracted from the interview data, supplemented with data from the hospital discharge letters and original investigation records (for cases and controls). We analysed all periods of oral contraceptive use (different types), and compared first-ever use with prolonged use of oral contraceptives. For the present analysis we checked whether the date of venous thrombosis was in the first six months or first year of oral contraceptive use for cases. For the controls, we used their index date, i.e., the date of the venous thrombosis of their corresponding case in the original study. To the control person this amounts to an arbitrary date, but it assures that controls also reflect oral contraceptive use of the same calendar period as the cases. Of this index date we ascertained whether it fell within the first six months or first year of oral contraceptive use of the control person. In this way, we could verify whether oral contraceptive using cases were more often in their early periods of pill use in comparison with oral contraceptive using controls. When a woman who had used oral contraceptives at time of thrombosis or index date had temporarily stopped using them in the year before this date, this renewed use was not counted as first use, since there had been exposure to oral contraceptives before; such use was categorized

as "prolonged" since the woman had already had her first exposure to oral contraceptives more than one year before the thrombosis or index date (this happened in 12 women). This categorization will, if anything, lower the effect of early use in our data.

Genetic risk factors

Blood was collected from all participants and plasma was stored at -70°C. High-molecular-weight DNA was isolated from leucocytes and stored at 4°C. Presence of the mutant factor V Leiden gene, protein C-, protein S-, antithrombin deficiency and prothrombin 20210 A mutation[11-14] was determined by technicians who did not know if the sample was from a patient or a control subject or from an oral contraceptive user or non-user. The criteria for diagnosis of clotting deficiencies were used as described before [11-13].

Statistical Analysis

Because of the age cut-off and other restrictions in this analysis (non-pregnant, premenopausal etc, see above), we had to break the original one-to-one matching. However, we stratified for age in the analysis because of confounding by age: new oral contraceptive users are very often young women and long-term users are mostly older. Without adjustment for age this will lead to underestimation of the effect of new use, since older persons have a higher risk of venous thrombosis. Since the age-matching was in five year age bands, an analysis that stratifies for age takes potential confounding as well as the effect of matching into account.

Firstly, we restricted the analysis to cases and controls who had been using oral contraceptives (at date of thrombosis or index date), to investigate the influence of duration of oral contraceptive use. We analysed whether oral contraceptive using cases had their venous thrombosis more often during the first six months or first year of oral contraceptive use, in comparison to the index date of the controls, by estimating the odds ratio (95% CI) of being in an early time window of use.

Secondly, we restricted the analysis to cases who used oral contraceptives, to investigate whether women who had developed venous thrombosis in the early periods of use, more often had thrombophilia in comparison with women who developed venous

thrombosis during prolonged use, also by calculating the odds ratio. Women were called thrombophilic when they had either protein C-, protein S-, antithrombin deficiency, factor V Leiden mutation or prothrombin 20210 A mutation.

Multivariate analysis by unconditional logistic regression was used to adjust for possible confounders, e.g. age, family history of venous thrombosis, history of pregnancy. Age was entered into the models as a continuous variable (in years), after assessing that using a categorized dummy variable model led only to trivial differences for the estimators of interest. Family history and history of pregnancy were entered as dichotomous variables.

RESULTS

Of the 155 premenopausal women with deep venous thrombosis, aged 15-49, 109 used oral contraceptives at the time of thrombosis. Of the 169 control women, 65 used contraceptives at their index date. On average cases who were using oral contraceptives were slightly older than oral contraceptive using control subjects (32.2 (SD 9.6) vs 29.8 (SD 8.9) years of age, mean age), long-term users were older than short-term users (32.1 (SD 9.1) vs 24.4 (SD 9.6) years of age (at the cut-off point of one year of use). The stratification of oral contraceptive use by duration of use is shown in Table I. The date of venous thrombosis fell more often in the first six months or first year of use, than the corresponding index date of the controls. The age adjusted odds ratio (95% CI) of oral contraceptive use, compared with longer use, for women using oral contraceptives up to six months was 3.0 (95% CI 0.6-14.8). When one year was taken as a cut-off point, the age adjusted odds ratio for use shorter than one year became 1.9 (95% CI 0.6-6.1). Further adjustment for history of pregnancy, or positive family history did not change the estimations. Prolonged users (i.e. more than one year of use) had an age-adjusted 5-fold increase in risk, relative to non-users of oral contraceptives (data not shown). In the original study, the age adjusted odds ratio of oral contraceptive use (all time periods together) was 6-fold[8].

Table 1. Numbers of cases and controls using oral contraceptives at time of thrombosis- or index-date according to different time windows

		case	control
users	1-6 months	8	2
	6-12 months	4	3
	≥ 12 months	97	60
total		109	65

Of the 109 oral contraceptive using cases, 37 women were thrombophilic; 5 women had a protein C-, 3 had a protein S-, 2 had an antithrombin deficiency, 25 women proved to have factor V Leiden and 4 women had the prothrombin 20210 A mutation (2 women had the combination of factor V Leiden and prothrombin 20210 A mutation). Of the 65 oral contraceptive using control subjects 10 were thrombophilic; 5 women had a protein S deficiency, 2 women proved to have factor V Leiden and 3 women had the prothrombin 20210 A mutation. Table 2 shows that among women who developed venous thrombosis during early use, thrombophilia was more often present than amomg women who developed venous thrombosis during prolonged use. The age adjusted odds ratio for coagulation defects was 18.5 (95% CI 1.9-175.7) for use up to six months. For the cut-off point of one year the odds ratio was 11.0 (95% CI 2.1-57.3).

Table 2. Numbers of cases with or without inherited clotting defects according to duration of oral contraceptive use

Duration of episode of use	**Inherited clotting defect**		**total**
	yes	**no**	
1-6 months	7	1	8
6-12 months	3	1	4
≥ 12 months	27	70	97
total	37	72	109

Four cases who developed deep-vein thrombosis in the first year of use, used preparations containing monophasic 30 μg ethinyl estradiol and desogestrel, and two used 30 μg ethinyl estradiol and levonorgestrel containing oral contraceptives; among the control subjects these numbers were one and two respectively. While these numbers are too small to arrive at stable conclusions, they are in line with the literature about difference in venous thrombosis risk for different types of contraceptives[15-17]. They also indicate that the "starter effect" does not explain the difference between different contraceptives[17].

DISCUSSION

In this case-control study we firstly confirm the high risk of venous thrombosis during the early stages of oral contraceptive use. Secondly, we find that the high risk in the first six months and first year of use can be explained in part by the presence of inherited coagulation defects.

Several potential biases that are often believed to exist in case-control studies do not apply to studies of genetic risk factors. For genetic risk factors it is not important that they are only assessed after diseases develops, since they do not change. Moreover, the most important genetic risk factors for venous thrombosis, factor V Leiden and factor II mutation, were not yet discovered at the time of data collection of the study. Even their clinical manifestation (venous thrombosis) cannot have influenced any prescription of oral contraceptives, since we only studied first venous thrombosis. The assessment of the time windows was performed retroactively on the existing data. However, it was done without knowledge of the genetic status of the patients. Finally, cases came from a routine care situation wherein all patients from a certain geographic area are given care; cases were consecutively included upon meeting the study and analysis requirements. The confidence intervals in our study remain large, despite the fact that we started with ample number of cases and controls. This is a consequence of looking at narrow time windows with specific genetic risk factors.

Earlier studies on duration of oral contraceptive use described that the association between oral contraceptive use and venous thrombosis was unrelated to duration of use[5-7,18,19]. The earlier negative findings might be explained by the use of different cut-off

points, with larger time windows. From our study we conclude that the risk of oral contraceptive use is higher during the first year of use, especially during the first six months of use. However, women who use oral contraceptives longer than one year are still at risk to develop venous thrombosis; their age-adjusted risk was still 5-fold higher when compared with non-users (data not shown). These results are similar to the results of the WHO study[1] and the Transnational Study in which women who had used oral contraceptives for the first time were compared with women who had never used them and a 10-fold increased risk during the first year of use was found, which went down to a 2-fold increase in subsequent years[20,21]. Once an oral contraceptive is stopped, the risk of venous thrombosis disappears within about 3 months; there is no elevated risk among past users[1,7,19,22,23].

Among patients who developed deep-vein thrombosis within one year after starting the use of oral contraceptives, most inherited clotting defects were found. The risk to develop deep-vein thrombosis during the first year of oral contraceptive use was 11-fold for thrombophilic women. An explanation for the higher risk is that these women have already one inherited risk factor, of which the effect is augmented by oral contraceptives. The exact nature of the biochemical interaction is at present unknown, although there are interesting leads about the role of acquired activated protein C resistance[24-28].

From this study and from others[1-4] we conclude that duration of oral contraceptive use influences the association between oral contraceptives and venous thrombosis: the relative risk is highest in first-ever users. Furthermore, we find that this starter effect is explained in part by the presence of inherited clotting defects: women with inherited clotting defects are most likely to develop venous thrombosis during oral contraceptive use in the first year of use. Together with the overall interaction between oral contraceptive use and inherited clotting defects[8,9], this implies that women with inherited clotting defects who use oral contraceptives develop venous thrombosis not only more often, but also sooner. The inherited clotting defects only explain part of the "starter effect", however. When women continue using oral contraceptives, their risk to develop venous thrombosis does not disappear, and it also is present in women without clotting defects. Also, the "starter" effect cannot explain differences between different contraceptives in observational studies[17].

It is uncertain whether routine screening for genetic clotting disorders before

starting oral contraceptives is useful or feasible, even when there is a family history of inherited thrombophilia in a first degree relative[29,30]. Still, a careful family history and information to patients about signs and symptoms of venous thromboembolism may well be in order. When a woman develops venous thrombosis during the first year of oral contraceptive use, this could be an indication that she has an inherited clotting defect.

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Chapter 5

VENOUS THROMBOSIS, ORAL CONTRACEPTIVES AND HIGH FACTOR VIII LEVELS

Venous thrombosis, Oral Contraceptives and High Factor VIII Levels

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SUMMARY

Recently, it has been described that elevated plasma levels of factor VIII are a strong risk factor for venous thrombosis. We analysed the data of the Leiden Thrombophilia Study, a population based case-control study on the causes of venous thrombosis, to verify whether the risk due to oral contraceptive use was higher in women with higher factor VIII levels. Furthermore we investigated the joint risk of high factor VIII levels and oral contraceptive use.

We selected 155 premenopausal women with deep-vein thrombosis and 169 control subjects, aged 15-49, who were at the time of their thrombosis (or similar date in control) not pregnant, nor in the puerperium, did not have a recent miscarriage, and were not using injectable progestogens. Of the patients, 109 (70%) women had used oral contraceptives during the month preceding their deep-vein thrombosis, in contrast to 65 (38%) of the control subjects (index date), yielding an odds ratio for oral contraceptive use of 3.8 (95% CI 2.4-6.0). Of the women who suffered a deep-vein thrombosis 56 (36%) had high factor VIII levels (≥ 150 IU/dl) as compared with 29 (17%) of the control subjects, yielding an odds ratio for high factor VIII of 4.0 (95% CI 2.0-8.0), relative to factor VIII levels < 100 IU/dl. The joint effect of oral contraceptive use and high factor VIII resulted in an odds ratio of 10.3 (95% CI 3.7-28.9), comparing women who had both with women who had neither. We conclude that there is an increase in risk due to oral contraceptive use in women with higher factor VIII levels and that both factors have additive effects.

INTRODUCTION

Oral contraceptive use increases the risk of venous thrombosis, with estimates of the relative risk varying from 2-11[1]. Recently, we described an interaction between oral contraceptive use and carrier-ship of factor V Leiden mutation; this combination leads to a 30-fold increase in risk of deep-vein thrombosis[2]. This interaction is relevant, since both factor V Leiden and oral contraceptive use are common.

Another common risk factor for venous thrombosis is elevated levels of pro-coagulant factor VIII[3-5]. For men and women in the Leiden Thrombophilia Study, high factor VIII levels (≥150 IU/dl vs <100 IU/dl) had an odds ratio of 6.2 (95% CI 3.4-11)[3]. This effect is not explained by elevated factor VIII levels as a post thrombotic acute phase reaction[4]. It is plausible that high levels of factor VIII have a mixed genetic and environmental origin. We investigated whether the risk due to oral contraceptive use was affected by factor VIII levels.

PATIENTS AND METHODS

The patients and methods of our study have been described previously[2,5]. We invited 474 consecutive patients (both sexes) with a first episode of proven deep-vein thrombosis (diagnosed by established objective methods) occurring between Jan 1, 1988 and Dec 31, 1992, aged less than 70 years and without a known malignant disorder. Patients had been selected from the files of three Anticoagulation Clinics in the Netherlands, which monitor anticoagulant treatment in all patients within a well defined geographical area. For each thrombosis patient we invited one age- and sex-matched healthy control subject. For patients we used the date of their deep-vein thrombosis; for the control subjects we used the date of their corresponding case in the original study (index-date). Patients were seen only after anticoagulant treatment was discontinued for at least three months.

For the present analysis we selected only premenopausal women, aged 15-49, who were at the time of their thrombosis (or similar date in control) not pregnant, nor in the puerperium, did not have a recent miscarriage, and were not using injectable progestogens.

Blood collection

Blood was collected into Sarstedt Monovette® tubes, containing 0.106 mmol/L trisodium citrate, and into a Becton-Dickinson Vacutainer® tube for blood group determinations. Plasma was prepared by centrifugation for 10 minutes at 2000 g at room temperature and stored at -70°C.

Laboratory Measurement

Factor VIII coagulant activity (FVIII:C) was measured by one stage clotting assays using factor VIII deficient plasma and automated APTT (Organon Teknica, Durham USA) on a Electra 1000c (MLA, Pleasantville, USA). Pooled normal plasma, standard calibrated against the WHO standard for factor VIII was used as a reference. The technicians did not know if the sample was from a patient or a control or from an oral contraceptive user or non-user.

Analysis and Statistics

We analysed data from 155 cases and 169 controls about current use of oral contraceptives at their thrombosis- or index-date. We started with univariate analysis by unconditional regression to estimate the odds ratio for respectively oral contraceptive use and high factor VIII (in different strata) or combinations. Multivariate analysis by unconditional logistic regression was used to adjust for possible confounders, e.g. age, family history of venous thrombosis, history of pregnancy. Oral contraceptive use was entered dichotomously (0 for non oral contraceptive user (currently) and 1 for current oral contraceptive use). Clotting factor VIII was entered dichotomously; 1 for factor VIII $\geq$ 150 IU/dl and 0 for factor VIII < 150 IU/dl and also as categorized variables (strata: factor VIII < 100 IU/dl, 100-125 IU/dl, 125-150 IU/dl and $\geq$ 150 IU/dl).

Although the original data were age-matched, we performed unmatched analysis. Due to the inclusion criteria and age cut-off, many pairs were no longer intact in the database for this analysis. Since the analysis was restricted to the matching factor sex, we adjusted for confounding by the other matching factor age by controlling for age by logistic regression. Age was entered into the models as a continuous variable (in years),

after assessing that using a categorized dummy variable model led only to trivial differences for the estimators of interest.

RESULTS

We selected from the original study 155 premenopausal women who had had a deep-vein thrombosis and 169 control subjects.

We used two types of analysis when analysing the factor VIII data, firstly we analysed simple dichotomy (factor VIII levels ≥ 150 IU/dl vs factor VIII levels <150 IU/dl), secondly we stratified in four different categories.

High factor VIII

Dichotomous analysis showed that of the 155 women who suffered a deep-vein thrombosis, 56 (36%) had high factor VIII levels (above 150 IU/dl) as compared with 29 (17%) of the 169 control subjects, yielding an odds ratio of 2.7 (95% CI 1.6-4.6). Table 1 gives the results for the categorized levels of factor VIII. It shows an increasing risk of venous thrombosis for increasing levels of factor VIII. The results for VIII are most prominent if factor VIII levels are ≥ 150 IU/dl. For levels exceeding 150 IU/dl, the risk was 4-fold increased (95% CI 2.0-8.0) as compared with women with factor VIII levels < 100 IU/dl.

Table I. Venous thrombosis risk for categories of factor VIII

FVIII:C strata	IU/dl	Patients	Controls	**Odds ratio**	95% CI
	< 100	20	41	1	
	100-125	40	55	1.5	(0.8-2.9)
	125-150	39	44	1.8	(0.9-3.6)
	≥150	56	29	4	(2.0-8.0)
		155	169		

Oral contraceptive use

Of the patients, 109 women (70%) had used oral contraceptives during the month preceding their deep-vein thrombosis, in contrast to 65 (38%) of the control subjects, yielding an odds ratio for oral contraceptive use of 3.8 (95% CI 2.4-6.0).

Oral contraceptive use and factor VIII

Table 2 shows separate effects and combined effects of factor VIII (factor VIII levels ≥ 150 IU/dl vs < 150 IU/dl) and oral contraceptive use. As the table shows, the separate effects of oral contraceptive use and high factor VIII levels are about the same: 4.9-, respectively 4.5-fold increased risk compared with those with normal factor VIII levels who did not use oral contraceptives. The risk of the combination of oral contraceptive use and high factor VIII, compared with women with low factor VIII who did not use oral contraceptives, was 8.8-fold increased.

Table 2. Distribution of women with deep-vein thrombosis and control subjects by oral contraceptive use (OC) and presence of high factor VIII (factor VIII ≥ 150 IU/dl vs factor VIII < 150 IU/dl)

		Patients	Controls	**Odds ratio**	95% CI
OC (-)	FVIII (-)	26	89	1	
OC (+)	FVIII (-)	73	51	4.9	(2.8-8.6)
OC (-)	FVIII (+)	20	15	4.5	(2.1-10.2)
OC (+)	FVIII (+)	36	14	8.8	(4.1-18.8)
		155	169		

This subdivision for oral contraceptive use was also performed for the different strata of factor VIII (100-125 IU/dl, 125-150 IU/dl and ≥ 150 IU/dl) in comparison with low levels of factor VIII (< 100 IU/dl). The results of the combined effects of oral contraceptive use and factor VIII (two extreme strata of factor VIII; ≥ 150 IU/dl compared with < 100 IU/dl) are shown in Table 3, showing a slightly more pronounced effect of high factor VIII levels.

Table 3. Distribution of women with deep-vein thrombosis and control subjects by oral contraceptive use (OC) and presence of high factor VIII (factor VIII ≥ 150 IU/dl vs factor VIII < 100 IU/dl)

		Patients	Controls	**Odds ratio**	95% CI
OC (-)	FVIII (-)	7	28	1	
OC (+)	FVIII (-)	13	13	4.0	(1.3-12.4)
OC (-)	FVIII (+)	20	15	5.3	(1.8-15.5)
OC (+)	FVIII (+)	36	14	10.3	(3.7-28.9)
		155	169		

The logistic model

The age-adjusted odds ratio for oral contraceptive use was 5.5 (95% CI 3.2-9.6). The age adjusted odds ratio for factor VIII differed only slightly from the crude odds ratio, with an odds ratio for those with high factor VIII levels (≥ 150 IU/dl) and oral contraceptive use of 13.8 compared with those with low levels of VIII (< 100 IU/dl) and not using oral contraceptives (Table 3). Adjustment for family history of venous thrombosis or history of pregnancy did not change the estimators of interest.

Incidence of population

The combined effects of factor VIII levels and oral contraceptive use can be seen best by back-calculation to the population incidence rates, as shown in Table 4. To show the absolute effect of the cumulation of risk factors, we estimated the population incidence of thrombosis in young women with the four possible combinations of high factor VIII levels and use of oral contraceptives. We estimated the total number of person-years (py) that had yielded the cases and partitioned these person-years according to the distribution of oral contraceptive use and high factor VIII levels in the control group. Since we know that in the original study 117 female patients aged 15-49 came from the Leiden anticoagulation clinic, which has a geographical source population of 109824 women in that age group (data provided by the municipal administration), firstly the

thrombosis incidence among young women without underlying disease in the Netherlands can be estimated over the 5 years of our study as 2.1 in 10000 women-years (117/(5×109824)). Division of the number of women with venous thrombosis by the proportional number of person-years in the categories in oral contraceptive use and high factor VIII levels (proportions taken from the control group) gives estimates of the population incidences. As we know that the population incidence in this age bracket is about 2.1/10.000 py (2), the 155 cases were generated by 740.000 women-years of follow-up. These can be partitioned according to the distribution of the control group which represents this source population (89/51/15/14). Yielding 389704 women-years for the combination of high factor VIII (≥150 IU/dl) and oral contraceptive use. The incidence of thrombosis increases from 0.7 per 10000 women per year for non-users of oral contraceptives without high factor VIII to 5.9 per 10000 for those with high factor VIII who also use oral contraceptives. The absolute increase in thrombosis risk due to oral contraceptive use (i.e., risk difference) is larger in women with high factor VIII than in women with low factor VIII (< 150 IU/dl). The joint effect of the two risk factors is additive, in women with low factor VIII there are 2.6 additional cases per 10000 women per year when women use oral contraceptives and in women with high factor VIII (non-users) there are an additional 2.3 cases per 10000 women per year. The combination of the two risk factors give an additional of 5.2 cases per 10000 person-years.

Table 4. Current use of oral contraceptives (OC) among patients and control subjects according to presence of high factor VIII (≥ 150 IU/dl)

	Patients	**Person-years***	**Incidence per 10000 person-years**
Low factor VIII			
No OC use	26	389704	0.7
Current OC use	73	223313	3.3
High factor VIII			
No OC use	20	65680	3
Current OC use	36	61301	5.9

*A total of 740000 person-years (yielding 155 patients) was partitioned according to the distribution of the control group: 89/51/15/14

DISCUSSION

We previously reported that the effect of blood group and von Willebrand factor, were both mediated through factor VIII in their effect on venous thrombosis[3,6]. In the present study we have investigated the joint effect of factor VIII and oral contraceptive use and found that their effects are additive.

In univariate analysis, high factor VIII and oral contraceptive use were associated with deep-vein thrombosis (Table 1, 2 and 3). The odds ratio for oral contraceptive use among low factor VIII was 4.0. The odds ratio for factor VIII among non-users was 5.3. From these odds ratios we can calculate what to expect under different models of interaction. Under an additive interaction model the total excess risk of oral contraceptive use and factor VIII would be 8.3 (4.0 plus 5.3 minus 1). Under a multiplicative model, total risk of joint presence of factor VIII and oral contraceptive use would be $4.0 \times 5.3 = 21.2$. The observed data are very close to the additive expectation, as we found an odds ratio of 10.3. Apparently, both oral contraceptive use and high factor VIII increase the risk of venous thrombosis, while the joint presence of both risk factors does not lead to an excess of cases. This can also be seen in the population incidences for the various risk factors combinations (Table 4).

This is different from the previously reported interaction between factor V Leiden mutation and oral contraceptive use, which interact in a way that exceeded the additive expectation[2].

When adjusting for factor V Leiden in the multivariate model with oral contraceptive use, high factor VIII, age, family history of venous thrombosis and parity (data not shown) the estimators of interest did not change. This means that the risk of high factor VIII in combination with oral contraceptive use was not affected by factor V genotype. It can be expected that the more risk factors (genetic or environmental) are present, the higher the risk of developing venous thrombosis will be[7,8].

Blood group and von Willebrand factor, are both mediated through factor VIII in their effect on venous thrombosis[3]. As we have shown previously, in univariate analysis bloodgroup (non-O), von Willebrand factor levels, and factor VIII levels were all associated with risk of venous thrombosis. In multivariate analysis, only an effect of factor VIII levels remained[3]. Our findings are in accordance with data reported in the 1960s, describing that the risk was higher in persons with blood group A as compared

with persons with blood group O, or more generally 'non-O' versus O[9-12], especially during the use of oral contraceptives or during pregnancy or puerperium[9,13-16]. In trying to understand the biochemical mechanism behind these clinical findings, lower levels of pro-coagulant factor VIII were found in normal individuals with blood group O as compared with persons with blood group non-O[17,18]. Subsequent research has shown that individuals with non-O blood group have higher levels of von Willebrand factor. Von Willebrand factor serves as the carrier protein of factor VIII, and so there is a strong correlation between von Willebrand factor levels and factor VIII levels. This led to our conclusion that factor VIII levels were the final effector of risk. It is unclear, however, by which mechanism this occurs, although in analogy to other clotting abnormalities with a gain of function, e.g. factor V Leiden, increased thrombin activation seems likely. The origin of high factor VIII levels is not entirely elucidated. There exists additional familial clustery beyond the effects of blood group and von Willebrand factor, suggesting additional genetic determinants[6]. In addition, acquired determinants are likely to play a role, too[3,4], whether oral contraceptives increase factor VIII levels is controversial[19-24].

In the present study we actually did an analyses of high factor VIII levels, bloodgroup, oral contraceptive use and venous thrombosis, but especially among users the data were to scarce to draw conclusions (data not shown). Nevertheless, we found the expected relationship between high factor VIII and bloodgroup non-O in explaining venous thrombosis during oral contraceptive use, i.e. the known effect of blood group on venous thrombosis, could in our data almost entirely be explained by high factor VIII among non-users. This is in line with the old observation that there is a deficit of patients with blood group O in subgroups of young women who develop venous thromboembolism during the use of oral contraceptives[9].

We can conclude that there is an increase in risk due to oral contraceptive use in women with higher factor VIII levels and that both factors have additive effects.

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Chapter 6

THE RISK OF VENOUS THROMBOSIS WITH USE OF CURRENT LOW-DOSE ORAL CONTRACEPTIVES IS NOT EXPLAINED BY DIAGNOSTIC SUSPICION AND REFERRAL BIAS

The risk of venous thrombosis with use of current low-dose oral contraceptives is not explained by diagnostic suspicion and referral bias

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SUMMARY

Background: The magnitude of the relative risk of venous thrombosis caused by low-dose oral contraceptive use is still debated because previous studies might have been affected by diagnostic suspicion and referral bias.

Methods: We conducted a case-control study in which the effect of diagnostic suspicion and referral bias was excluded. The study was performed in 2 diagnostic centers to which patients with clinically suspected deep-vein thrombosis of the leg were referred. History of oral contraceptive use was obtained before objective testing for thrombosis. Young females with an objective diagnosis of deep-vein thrombosis were considered case patients, and those who were referred with the same clinical suspicion but who had no thrombosis served as control subjects. Participants were seen between September 1, 1982, and October 18, 1995: 185 consecutive patients and 591 controls, aged 15 to 49 years, with a first episode of venous thrombosis and without malignant neoplasms, pregnancy, or known inherited clotting defects.

Results: The overall odds ratio for oral contraceptive use was 3.2 (95% confidence interval (CI) 2.3-4.5); after adjustment for age, family history of venous thrombosis, calendar time and center, the odds ratio was 3.9 (95% CI, 2.6-5.7). In the idiopathic group (120 patients and 413 controls, excluding recent surgery, trauma or immobilization), the odds ratio for oral contraceptive use was 3.8 (95% CI 2.5-5.9); after adjustment, the odds ratio was 5.0 (95% CI, 3.1-8.2).

Conclusions: In this study, in which patients and controls were subject to the same referral and diagnostic procedures, we found similar relative risk estimates for oral contraceptive use as in previous studies. We conclude that diagnostic suspicion and referral bias did not play an important role in previous studies and that the risk of venous thrombosis with use of current brands of oral contraceptives still exists.

INTRODUCTION

Several developments have generated renewed interest in the risk of venous thromboembolism associated with the use of oral contraceptives[1]. Results of recent large case-control studies[2-5] in different parts of the world show that the relative risk of venous thromboembolism associated with low-dose oral contraceptives is still elevated 3- to 4-fold. The risk is reported[3-6] to be even be higher for use of preparations containing newer progestins. In addition, females who carry the factor V Leiden mutation and use oral contraceptives have a venous thrombosis risk that might be elevated 30-fold or more[7] compared with non-users without such a mutation.

Although most physicians accept the reality of the association between oral contraceptive use and venous thromboembolism, most also think that the reported risks may be overestimated because of the diagnostic suspicion and referral bias[8-13]. The mechanism of these biases is that physicians would more readily suspect venous thrombosis in oral contraceptive users than in other patients, or, as stated in a textbook[14(p1283)] about hemostasis and thrombosis, "knowledge that the patient with leg pain is taking the oral contraceptive pill could easily sway the examining physician to make a clinical diagnosis of deep-vein thrombosis". If physicians preferentially diagnose or refer females taking oral contraceptives, the risk of thrombosis associated with oral contraceptive use will be overestimated. This view is echoed in a recent review[15] wherein the risk of venous thrombosis with oral contraceptive use is accepted but judged to be too high.

Calculations about risk-benefit of screening for the factor V Leiden mutation or other thrombophilic tendencies, and discussions about the risk-benefit of newer "third-generation contraceptives", become fruitless when one cannot trust the risks derived from epidemiological studies.

To overcome these and other biases, the ideal control group in a case-control study is a so called phenocopy of the disease group: (a group of) persons who originally sought care with similar signs and symptoms as a potential case patients. This ensures that the control group consists of individuals who were subject to the same referral process as patients. That situation is reached when the final diagnosis is made at the end of clinical evaluation by objective means that are completely independent from exposure and original clinical presentation[16]. We found such a situation in the diagnostic procedures

of 2 referral centers for patients with clinically suspected deep-vein thrombosis. Physicians at both centers evaluated patients who were referred by general practitioners or specialists. At entry, a nurse completed a questionnaire about the patient's antecedents and risk factors, including current oral contraceptive use. At the end of the visit, an objective diagnosis was made, by ultrasound examination, plethysmography, or venography. We included females with objectively confirmed diagnoses of deep-vein thrombosis as patients (approximately one third of the referred total) and the remainders as controls. The diagnostic situation at these facilities removes all possibility of diagnostic suspicion and referral bias and even interviewer or patient recall bias: future patients and controls are referred by the same physicians with the same diagnostic suspicion; oral contraceptive use is established without knowledge of the final diagnosis; and the final diagnosis is made by means that are independent of the diagnostic suspicion, clinical presentation, or use of oral contraceptives. Therefore, a difference in use of oral contraceptives between patients and controls in this design cannot have resulted from selective diagnosis or referral. If it is true that these biases would have resulted in too high estimates in previous studies, we expect lower relative risks in the present study.

MATERIALS AND METHODS

Study Setting

The 2 centers (the Academic Medical Center of the University of Amsterdam and the Amsterdam Thrombosis Service and Laboratory for General Practitioners have offered a diagnostic service for patients with clinically suspected deep-vein thrombosis of the legs for general practitioners and other physicians since 1982 for the larger part of Amsterdam[17-19]. Females were included in the study from September 1, 1982, until October 18, 1995, at which date the Committee on Safety of Medicines in the United Kingdom issued a statement about the differential risk of oral contraceptive types[20]. This was covered extensively in the European media and could have led to a change of prescription patterns after October 1995.

Study Assessment

At presentation, a medical history, including use of oral contraceptives, was obtained by nurses using an excisting questionnaire before clinical evaluation and diagnostic tests were performed. The medical history included questions about recent surgery, immobilization, trauma, pregnancy, puerperium and malignant neoplasms. A personal and family history of venous thrombosis was also obtained, and medication intake, use of oral contraceptives, use of sex corticosteroids other than oral contraceptives, and a previous diagnosis of coagulation abnormalities were recorded routinely by the nurses. After completion of the forms, participants were seen by a physician, and the diagnostic investigations were performed by technicians. The diagnostic tests used were serial impedance plethysmography or real-time B-mode ultrasound supplemented, if necessary, by contrast venography. In most participants, serial impedance plethysmography or real-time B-mode ultrasound examination was performed on days 1, 2, 7, and 10 and 3 months after referral[17-19].

Participants

During the study period, 1374 females aged 15 to 49 years were seen at the 2 centers. We excluded those without clinical symptoms (those who were seen because of a history of familial thrombosis or fear of recurrence, n=73), those with venous thrombosis at sites other than the legs (eg chest symptoms, suggesting pulmonary embolus without leg symptoms, n=51), those with a history of previous deep-vein thrombosis or pulmonary embolism (n=253), and those already known (at their first visit) to have inherited clotting defects (eg, antithrombin-, protein C-, protein S deficiency or factor V Leiden mutation (FV R506Q)) (n=14). Females were also excluded if they did not have complete data on oral contraceptive use at the first visit or did not have an objective diagnosis after serial impedance plethysmography and real-time B-mode ultrasound examination or for miscellaneous reasons (n=63). For the present analysis, women were also excluded if they were pregnant, postpartum or postabortum (untill 30 days after delivery, (n=137)); were known to have malignant neoplasms (n=57); used other sex corticosteroids (n=34); or had other risk factors, eg, intravenous drug use of nephrotic syndrome (n=8). The described categories are not mutually exclusive.

In the final analysis we included 776 females (185 cases and 591 controls). We subdivided these females into those with idiopathic thrombosis, and those in whom other risk factors were present, ie, recent surgery, recent trauma or recent immobilization.

Because the choice of oral contraceptives might have been affected by the perception of an increased risk through a family history of venous thrombosis, which could lead to prescription bias, we also took the family history into account. We considered family history to be positive when the referred females reported venous thrombosis in one or more relatives.

Statistical Analysis

Statistical analysis consisted of calculating odds ratios and their confidence intervals. Multivariate analysis by unconditional logistic regression was used to adjust for possible confounders, e.g. age, family history of venous thrombosis, time of first visit (calendar time) and center. Age and calendar time were entered as a continuous variables (in years); for age and calendar time the use of a categorized dummy variable model led to only trivial differences for the estimators of interest. Family history and center were entered as dichotomous variables. Finally, we analysed the thrombotic risks associated with use of different brands of oral contraceptives.

RESULTS

The study group consisted of 185 patients with deep-vein thrombosis of the legs and 591 controls. The median age of the entire group was 38 years. A total of 529 participants (68.2%) were referred by their general practitioner, the others by various specialists. Distribution between the 2 diagnostic centers was 2:1 (514 participants (66.2%) visited the Academic Medical Center and 262 women (33.8%) visited the Amsterdam Thrombosis Service), furthermore, 173 participants (22.3%) had a positive family history of venous thrombosis. We subdivided the participants into those with idiopathic thrombosis and those with other possible risk factors, ie, recent surgery, recent trauma, or recent immobilization (Table 1). About two thirds of all participants had none of these risk factors.

Table 1. Clinical risk factors of participants with and without deep-vein thrombosis

	participants, no (%)		
clinical risk factors	**with deep-vein thrombosis**	**without deep-vein thrombosis**	**total**
surgery	34 (18.4)	104 (17.6)	**138 (17.8)**
trauma	22 (11.9)	56 (9.5)	**78 (10.1)**
immobilization	9 (4.9)	18 (3.0)	**27 (3.5)**
no (idiopathic)	120 (64.9)	413 (69.9)	**533 (68.7)**
Total	**185 (100)**	**591 (100)**	**776 (100)**

*Percentages do not add to 100 owing to rounding

Total Group

At referral, 55.1% (102 of 185) of the patients and 27.6% (163/591) of the controls used oral contraceptives (Table 2). The odds ratios for current oral contraceptive use was 3.2 (95% confidence interval (CI) 2.3-4.5). After adjustment for age, family history, center, and calendar time (time of first visit), the odds ratio was 3.9 (95% CI, 2.6-5.7).

Table 2. Current use of oral contraceptives among participants with and without deep-vein thrombosis and their adjusted and unadjusted odds ratios*

	participants, no		
	with deep-vein thrombosis	**without deep-vein thrombosis**	**total**
oral contraceptive use	102	163	**265**
no oral contraceptive use	83	428	**511**
Total	**185**	**591**	**776**

* Crude odds ratio 3.2 (95% confidence interval, 2.3-4.5); adjusted odds ratio, 3.9 (95% confidence interval, 2.6-5.7), in logistic model with age, family history of venous thrombosis, calendar-time and center

Idiopathic Group

When we restricted the analysis to participants without 1 of the major risk factors for venous thrombosis, the odds ratio became slightly higher: 3.8 (95% CI, 2.5-5.9) which increased to 5.0 (95% CI, 3.1-8.2) when adjusted (Table 3).

Table 3. Current use of oral contraceptives among participants* with and without idiopathic deep-vein thrombosis and their adjusted and unsdjusted odds ratios†

	participants, no		
	with deep-vein thrombosis	**without deep-vein thrombosis**	**total**
oral contraceptive use	76	128	**204**
no oral contraceptive use	44	285	**329**
Total	**120**	**413**	**533**

*after exclusion of participants with possible other clinical risk factors for venous thrombosis, eg, surgery, trauma or immobilization

†Crude odds ratio, 3.8 (95% confidence interval, 2.5-5.9); adjusted odds ratio, 5.0 (95% confidence interval, 3.1-8.2), in logistic model with age, family history of venous thrombosis, calendar time and center

Possible Confounders

Given the possible influence of the variables- age, family history of venous thrombosis, calendar time, center, and referral by general practitioners or other specialists- on the relation oral contraceptive use and venous thrombosis, we analysed these variables in more detail.

Age: the odds ratio for current oral contraceptive use was 3.2 (95 % CI, 2.3-4.5) in

the total group and 3.8 (95% CI, 2.5-5.9) in the idiopathic group. After adjustment for age, these odds ratios increased to 3.8 (95% CI, 2.6-5.6) and 5.2 (95% CI, 3.2-8.3), respectively.

Family history: Fifty-two (28.1%) of the 185 patients and 121 (20.5%) of 591 controls had a positive family history for venous thrombosis. When analysing the total group of participants with a positive family history for venous thrombosis, the age-adjusted odds ratio for oral contraceptive use was 2.5 (95% CI 1.2-5.2); for the group of participants with no family history of venous thrombosis, this odds ratio for oral contraceptive use was 4.3 (95% CI, 2.7-6.8). The odds ratios became 3.7 (95% CI, 2.5-5.5) in the total group and 5.0 (95% CI, 3.1-8.2) in the idiopathic group after adjustment for age and family history.

Calendar-time: because of possible differences in referral, prescription and management patterns over time, we adjusted for calendar time. The odds ratios became 3.8 (95% CI, 2.6-5.6) in the total group and 5.1 (95% CI, 3.2-8.3) in the idiopathic group when we adjusted for age and calendar time.

Center: the age-adjusted odds ratios of oral contraceptive use in the total group were 5.1 (95% CI, 3.1-8.2) for the Academic Medical Center and 2.4 (95% CI, 1.3-4.6) for the Amsterdam Thrombosis Service. The odds ratios became 4.0 (95% CI, 2.7-5.8) in the total group and 5.2 (95% CI, 3.2-8.3) in the idiopathic group when we adjusted for age and center.

Referral by General Practitioners: when only those who were initially referred by general practitioners and not by other specialists were analysed, the odds ratio (adjusted for age, family history, center and calendartime) was 3.9 (95% CI, 2.4-6.3). The odds ratios became 4.2 (95% CI, 2.9-6.3) in the total group and 5.4 (95% CI, 3.3-8.9) in the idiopathic group when we adjusted for age and referral by general practitioners.

Type of Oral Contraceptives

Complete information about oral contraceptive type was available for 70.9% of 265 users. Only a few (5-6% of all patiens and controls) still used preparations with ethinyl estradiol, 50 μg, which had high relative risks (Table 4). All others used low dose

"subfifty" oral contraceptives. The adjusted odds ratio for monophasic levonorgestrel-containing oral contraceptives with ethinyl estradiol, 30 μg (3.7), was similar to our overall estimate (3.9) (Table 4). The odds ratio for the triphasic levonorgestrel preparation was also the same but with a wider CI because of smaller numbers. The odds ratio for all third-generation monophasic contraceptives were higher. In a direct comparison of monophasic third-generation (desogestrel- or gestodene-containing) oral contraceptives with monophasic levonorgestrel-containing oral contraceptives, the crude odds ratio was 1.5 (95% CI, 0.7-3.2). After adjustment for age, family history, center and calendar time, the odds ratio was 1.9 (95% CI, 0.8-4.5). The highest odds ratio was for the monophasic third-generation oral contraceptive containing desogestrel, 150 μg and ethinyl estradiol, 20 μg (Table 4). Of the six patients, one had Schönlein disease and one had hypertension and diabetes. In two patients, the family history of venous thrombosis was positive. This risk profile was not different from that of the other patients wherein several long-term ailments that are not direct risk factors for venous thrombosis were present. In our analysis, we had already removed all females with known hereditary clotting defects when they presented for diagnostic tests (see "Participants and Methods" section); otherwise, the odds ratio for this contraceptive would have been even higher. This indicates that preferential prescribing cannot completely explain the high odds ratio. A high odds ratio for this (20 μg) preparation has also been found in 2 other studies[5,21]. This high odds ratio is similar to the high relative risk for all third-generation contraceptives in new users (first-time users) in the World Health Organization study[22] and probably reflects the combination of a "starter" and a "third-generation" effect[23].

Table 4. Patients and controls taking selected types of oral contraceptives and their adjusted odds ratios

TYPE OF ORAL CONTRACEPTIVE		Patients, no	Controls, no	Odds Ratio (95 % CI)*
amount of ethinyl estradiol	**type of progestogen**			
monophasic, 50 μg	lynestrenol levonorgestrel norethisterone	8	7	8.7 (2.9-25.8)
monophasic, 30 μg	levonorgestrel	18	28	3.7 (1.9-7.2)
triphasic, 30-40 μg	levonorgestrel	8	14	3.7 (1.4-9.6)
monophasic, 30 μg	desogestrel	22	29	4.9 (2.5-9.4)
monophasic, 30 μg	gestodene	5	4	5.2 (1.3-20.6)
monophasic, 20 μg	desogestrel	6	1	24.7 (2.8-213.5)
no oral contraceptives	.....†	83	428	Reference

DISCUSSION

In this case-control study within a large data base of referred females, in wich the referral and diagnostic strategies for patients and controls were the same, we found that use of currently available oral contraceptives increases the risk of venous thrombosis 3- to 5-fold. This finding is fully consistent with that of earlier observations[2-4,7-10,24-34].

Diagnostic suspicion and referral bias is hypothesized to be the result of the awareness of females and their physicians of the association between oral contraceptive use and venous thrombosis[8-14,31-34]. Females who use oral contraceptives would seek health care more readily for certain symptoms than non-users. General practitioners would more readily refer women with complaints of the leg for diagnostic workup when they are using oral contraceptives. Physicians seeing these referred patients would be more likely to thoroughly investigate them and to apply objective diagnostic methods. As a result, females who use oral contraceptives might be more frequently and intensely investigated

than are those who do not use oral contraceptives. Therefore, the association with oral contraceptive use would seem stronger than it actually is[8-14,31-34].

The situation at the diagnostic centers in which we enrolled patients and controls removes all possibility of diagnostic suspicion and referral bias and even of interviewer or patient recall bias. The main difference between our study and previous studies is the choice of control group. In previous studies, only patients with venous thrombosis were referred because of a diagnostic suspicion: controls were not referred, and this difference in referral bias might have led to a difference in oral contraceptive use. In principle, patients in our study were referred similar to those in previous studies, but, unlike in previous studies, controls were referred in the same way as patients because of the same diagnostic suspicion. Any referral selection as to use of oral contraceptives thus was the same in future patients and controls. Furthermore, both centers in the present study formed the basis of several comparative diagnostic investigations, with high sensitivity and specificity[17-19] of the tests used. The diagnostic facilities have operated for more than 10 years under the same protocol, emphasizing the necessity of objective diagnosis in deep-vein thrombosis[17-19]. Given the high sensitivity and specificity that were attained, the amount of misclassification will be minimal. In the present study, a substantial proportion of referred females (20-30%) eventually had an objective diagnosis of deep-vein thrombosis. If diagnostic suspicion and referral biases had affected previous studies, we had expected to find clearly lower odds ratios in the present study. However, we still found a 3- to 5-fold increased risk, with reasonably small confidence intervals. Therefore, we conclude that diagnostic suspicion and referral bias did not affect previous studies to the extent that has been suggested.

That diagnostic suspicion and referral bias might have led to an overestimation of the association between oral contraceptives and venous thrombosis was hypothesized in the late 1960s[8-10]. This bias was a theoretical concept that has never actually been shown to be present in case-control and follow-up studies of oral contraceptive use and venous thrombosis. It was hypothesized that this bias would mostly affect the risk estimates among patients with least-evident disease[8] because the "clue" of oral contraceptive use might have been necessary for diagnosis. Therefore, in older studies, patients were classified by degree of certainty of the presence of thromboembolism. However, the association with oral contraceptive use was, if anything, higher among the definite and severe cases, which runs counter to the idea of diagnostic suspicion and referral bias[2,4,5,8-10]

but was not totally conclusive. In the literature, we found one recent study[35] of similar design as ours but with much fewer patients (9 patients). A relative risk of 6.4 was found but with a large CI (95% CI, 1.2-34.2).

In our study, the odds ratio for oral contraceptive use in the total group of participants was between 3- and 4-fold. Statistical adjustment for age gave higher odds ratios for oral contraceptive use. In the idiopathic group (ie, in which participants with other possible clinical risk factors, eg, surgery, trauma or immobilization, were excluded), the odds ratio for oral contraceptive use was slightly higher, about 5-fold, a difference that has been documented previously[9,25]. Only 5 to 6% of our study population still used older preparations containing ethinyl estradiol, 50 μg. Our results are fully in agreement with those of recent studies[2-7] on the relation of low-dose oral contraceptive use and venous thrombosis, which in turn indicates that the findings in those studies were not affected by diagnostic suspicion and referral bias. Thus, risk estimates from several recent studies can be used for risk-benefit analyses on low-dose oral contraceptive use and should not be seen as overestimated. Comparing the results of our study with those of older studies[1] that show odds ratios between 2 and 11, it is also clear that the introduction of low-dose combined oral contraceptives may have led to some decrease in the risk - because we also found higher risks for 50 μg preparations in our study - but that this decrease has been less than expected.

To address the possibility of another bias, the prescription bias, we removed from the present analysis females who were known to have inherited clotting defects such as antithrombin-, protein C-, or protein S-deficiency or the factor V Leiden mutation at their first visit. Furthermore, we adjusted for a positive family history, considered to be present when one or more relatives had an episode of venous thromboembolism in the past. This adjustment did not alter the estimators, which is consistent with previous findings[6] and supports the conclusion that prescription bias is unlikely to affect the findings.

Recently, it has been shown that oral contraceptives containing a newer (third) generation of progestins (desogestrel and gestodene) have a higher relative risk of venous thrombosis compared with older progestin preparations (mainly vs levonorgestrel)[3-6]. Also, for this finding, the effect of diagnostic suspicion and referral bias was considered as an explanation[21,36-39]. Altough the numbers are small and the type of contraceptive was not known in all participants, the results of our study are compatible with an increased risk of third-generation (desogestrel and gestodene as progestins) and relative to second-

generation products (levonorgestrel as progestin) products[3-6]. The excess in relative risk could not be explained by a positive family history of venous thrombosis, clotting defects or age[6].

In previous investigations[1,4,21,39] diagnostic suspicion and referral bias were the only biases that seemed impossible to overcome. In our study, in which patients and controls were subject to the same referral and objective diagnostic procedures, which also ruled out recall and information bias, we found that these biases do not play a major role in assesment of the venous thrombosis risk associated with oral contraceptive use. We conclude that the risk of deep venous thrombosis with use of current low-dose brands of oral contraceptives still exists.

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Chapter 7

A COMPARATIVE CLINICAL INVESTIGATION OF TWO LOW-DOSE ORAL CONTRACEPTIVES CONTAINING EITHER 75 μg GESTODENE OR 150 μg DESOGESTREL COMBINED WITH 20 μg ETHINYL ESTRADIOL: EFFECT ON HEMOSTASIS, LIPID METABOLISM AND CARBOHYDRATE METABOLISM

A comparative clinical investigation of two low-dose oral contraceptives containing either 75 µg gestodene or 150 µg desogestrel combined with 20 µg ethinyl estradiol: effect on hemostasis, lipid metabolism and carbohydrate metabolism

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SUMMARY

The effect on hemostatic balance and lipid and carbohydrate metabolism of a new 21-day, low-dose monophasic oral contraceptive containing 75 μg gestodene combined with 20 μg ethinyl estradiol was compared with 150 μg desogestrel/20 μg ethinyl estradiol in a multicenter, comparative clinical trial.

The study involved a total of 98 women between the age of 19 and 34 years who took the trial preparations for 12 cycles. A range of hemostatic, lipid and carbohydrate variables were measured during cycle days 17-21 at baseline, and again at cycles 3, 6, and 12. Forty-eight women in the gestodene group and 46 women in the desogestrel group completed the study and were available for analysis.

In both groups, statistically significant increases occured in both coagulant and fibrinolytic variables. Overall, changes in hemostasis were similar in the two groups, but factor VII levels were increased to a smaller extent in the gestodene group, the difference being significant at cycle 3 ($p<0.05$). With respect to lipid metabolism, results were similar for the two groups, and there were no significant changes to either HDL- or LDL-cholesterol levels. In response to the oral glucose tolerance test, there were no significant changes to either AUC glucose or AUC insulin in either group.

It is concluded that both preparations had only minimal impact on lipid and carbohydrate metabolism, and that, with respect to hemostasis, they both stimulate some pro-coagulant activity, together with an increase in anti-coagulant and fibrinolytic activity.

INTRODUCTION

It has been known since the early 1970s that combined oral contraceptives have the potential to precipitate cardiovascular events[1]. In order to reduce this risk, the estrogen dose has been progressively reduced[2]. Also, new progestogens have been introduced[3]. Compared with the early high-dose preparations, today's pills are associated with a comparatively low incidence of cardiovascular complications, especially arterial ones[4]. Nevertheless, venous thromboembolism remains an important though rare event[5] and it is vital that, for all new oral contraceptives, cardiovascular risk is investigated as thoroughly as is possible.

The mechanisms involved in the initiation of a cardiovascular event are complex, and probably not similar for arterial and venous disease. The mechanisms involved are thought to involve the hemostatic system, lipid metabolism, carbohydrate metabolism as well as localized vascular factors[6-8]. It has been advocated to investigate all these systems in an integrated approach[9]. In addition, it is now generally accepted that a subpopulation of women exists who are at particular at risk for venous thromboembolism, due to genetic and other factors such as deficiencies in coagulation inhibitors[10-14]. Assessing the safety of oral contraceptives with respect to cardiovascular risk is, therefore, far from straightforward and necessitates close examination of those systems thought to be involved. Notably, the assessment is not complete as indicated by Vandenbroucke[15] and new assessment variables may be added to the repertoire[16].

Considerable data have been generated with respect to the effect that combined oral contraceptives have on lipid and carbohydrate metabolism and on hemostasis. It is recognised that certain changes in these systems can be seen in populations known to have an elevated risk of cardiovascular disease. In particular, high levels of low density lipoprotein (LDL)-cholesterol and low levels of high density lipoprotein (HDL)-cholestrol are seen to be associated with an increased risk of cardiovascular disease in men[17], as well as in (postmenopausal) women[18]. Although the role of these various individual systems on cardiovascular risk is not precisely understood, our current knowledge allows new preparations to be assessed and a theoretical judgement made as to their relative safety.

Recently, a new low-dose oral contraceptive preparation containing 75 µg gestodene combined with 20 µg ethinyl estradiol has been introduced. Some information

regarding the effect on metabolic variables has been published[19,20]. In order to extent our knowledge about this new gestodene containing low-dose oral contraceptive, a study was undertaken to further assess the effect on lipid and carbohydrate metabolism, and on hemostasis. In view of differences noted between progestogens[21] for 30 μg ethinyl estradiol-containing oral contraceptives, effects were compared to those with an oral contraceptive containing 150 μg desogestrel and 20 μg ethinyl estradiol.

MATERIALS AND METHODS

The effect of 75 μg gestodene/20 μg ethinyl estradiol (Harmonet, Wyeth-Ayerst) on lipid metabolism, carbohydrate metabolism and hemostasis was evaluated and compared with 150 μg desogestrel/20 μg ethinyl estradiol (Mercilon®, Organon), in a randomised, open-label, multicenter study. The study was conducted over 12 cycles, and was completed within a time period of 18 months. Subjects were instructed to begin takeing their allocated tablets on the first day of their menstrual cycle bleeding. Tablets were taken for 21 days, followed by 7 tablet-free days.

A total of 98 healthy female subjects, who were under 35 years of age and wished to use a combined oral contraceptive, were recruited to the study. To be included, subjects had to have not used an oral contraceptive for two consecutive cycles immediately prior to the pre-study screening. In addition, all subjects had to have normal pretreatments values for total cholesterol. HDL- and LDL-cholesterol, triglycerides, thyroid hormones and thyroid stimulating hormone. Exclusion criteria included the normal contraindications to oral contraceptive therapy, as well as the use of any anticoagulant within the preceding 90 days. The use of paracetamol during the study was permitted, as were other prescription treatments at the discretion of the investigator. The following were not permitted: any anticoagulant treatment; any non-steroidal anti-flammatory drug during the 14 days preceding blood sampling; sex hormones other than the trial preparations; antibiotics for more than 10 days; rifampicin; and any lipid influencing treatment.

A complete medical, gynecological and obstetric history was taken at baseline, together with a Papanicolaou smear and laboratory measurements. Weight and blood pressure were recorded at baseline and again at cycles 3,6 and 9 and at the post-visit.

Blood samples were taken between days 17 and 21 of the baseline cycle and treatment cycles 3, 6 and 12. A range of hemostatic and lipid variables, including those shown in Tables 3 and 5, were then measured. A 3-hour glucose tolerance test in response to a 75-g oral glucose load was carried out between days 17 and 21 of the baseline cycle and treatment cycles 6 and 12. From this test, the areas under the glucose and insulin curves (AUC) were calculated . All laboratory measurements were carried out using standard methodology, and each investigator used one laboratory for all determinations.

Blood collection

Blood was collected in sodium citrate (final concentration 14 mmol/L). After centrifugation (30 min, 2000 g, 4°C) the plasma was collected and stored frozen in aliquots at -70°C.

The following hemostatic variables were measured: *Coagulation:* Prothrombin (PT), fibrinogen (FBG), Thrombin-antithrombin complex (TAT), Fragment 1+2 (F12), Soluble Fibrin (SF), Intact Soluble Fibrin (ISF), factor VII FVII). *Anticoagulation:* antithrombin (AT3), protein C (PC), protein S (PS), Thrombomodulin (TM). *Fibrinolysis:* Plasminogen (PLG), Tissue-type plasminogen activator antigen (t-PA antigen), Tissue-type plasminogen activator activity (t-PA activity), Plasminogen activator inhibitor-1 antigen (PAI-1 antigen), D-dimer, Fibrin Degradatation Product (FBDP), Plasmin-anti-plasmin complex (PAP), β Thromboglobulin (BTG), Fibrinopeptide A (FPA). *Lipids:* Total Cholesterol (TC), Very low density lipoprotein cholesterol (VC), Low density lipoprotein cholesterol (LC), High density lipoprotein cholesterol (HC), High density lipoprotein-3-cholesterol (H3C), High density lipoprotein-2-cholesterol (H2C), Total Triglycerides (TTG), apolipoprotein A-1 (apoA), Apolipoprotein B (apoB), Lipoprotein (a) (LPA).

Assays for Coagulation, Fibrinolysis Factors and Lipids

Coagulation: Prothrombin was measured according to Quick[22] on a KC 10 (Amelung), the thromboplastin used was by Neoplastine Plus (Boehringer Mannheim, Almere, Netherlands), Fibrinogen was measured in the citrated plasma using a modified Clauss assay[23,24] on a MLA Electra 1000c, used thrombin (Dade, Miami, USA), Thrombin-antithrombin complexes were measured by using a commercial kit method

Enzygnost TAT micro from Behringwerke AG (Marburg, Germany), Fragment 1+2 was measured by Enzygnost F 1+2 micro from Behringwerke AG (Marburg, Germany), Soluble Fibrin was measured by Coaset Fibrin Monomer (Chromogenix, Mölndal, Sweden), Intact Soluble Fibrin was measured by Fibrinostatika Soluble Fibrin (Organon Technika, Turnhout, belgium), factor VII was determined in citrated plasma by a chromogenic assay after complete activation, representing the factor VII mass concentration and expressed relative to a pooled plasma (100%) (Chromogenix, Mölndal, Sweden)[25], *Anticoagulation:* Antithrombin was determined by using Antithrombin III (Dade, Miami, USA), protein C antigen was measured using Thrombonostika Protein C (Organon Technika, Turnhout, Belgium), protein S was measured by Thrombonostika Protein S (Organon Technika, Turnhout, Belgium), Thrombomodulin was measured by Asserachrom Thrombomodulin (Diagnostica Stago, Asnières, France), *Fibrinolysis:* Plasminogen was measured by using Coamatic Plasminogen (Chromogenix, Mölnda, Sweden), Tissue-type plasminogen activator antigen was measured using Thrombonostika t-PA (Organon Technika, Turnhout, belgium), tissue-type plasminogen activator activity was measured by using Coatest BIA t-PA (Chromogenix, Mölndal, Sweden), Plasminogen activator inhibitor-1 antigen was measured by Innotest PAI-1 (Innogenetics, Antwerp, Belgium), D-dimer was measured by using Tint Elize D-Dimer (biopool, Umea, Sweden), Fibrin Degradation Products was measured by Fibrinostatika FbDP (Organon Technika, Turnhout, belgium), Plasmin-anti-plasmin complex was measured by Enzygnost PAP micro Behringwerke AG, Marburg, Germany), β-thromboglobulin was measured by Asserachrom β-TG (Stago, Paris, France), Fibrinopeptide A was measured by Asserachrom FPA (Stago, Paris, France). *Lipids:* TC, VC, LC, HC, H3C, H2C were measured by using a kit CHOD/PAP (Boehringer), TTG were measured by GPO/PAP (Boehringer), apoA was measured by using apolipoprotein A1 reagent (Beckman), ApoB was measured by using apolipoprotein B antibody (Beckman), Lpa was measured by using lipoprotein (a) reagent (Beckman).

Statistical Analysis

For each of the efficacy variables, percentage change from baseline was calculated and an analysis of covariance performed. All tests were two-sided and used 5% level of significance. To monitor the precision of the study, 95% confidence intervals were calculated.

RESULTS

At baseline, both groups were well balanced for demographic factors, as shown in Table 1. Forty-eight women in the gestodene group and forty-six women in the desogestrel group completed the study and were available for analysis. Both study preparations were well tolerated, and no serious side effects were reported. A total of four subjects failed to complete the study, one in the gestodene group due to a protocol violation, and three in the desogestrel group, two due to minor adverse reactions (intermenstrual bleeding) and one due to pregnancy diagnosed in the first cycle.

Table 1. Demographic data at baseline

	Gestodene/ethinyl estradiol (n=49)	Desogestrel/ethinyl estradiol (n=49)
Age (years)		
Mean	24.1	23.9
Standard deviation	4.2	3.0
Minimum	19	19
Maximum	34	32
18-25 (%)	76	82
26-30 (%)	12	14
31-35 (%)	12	4
Weight (kg)		
Mean	65.3	65.9
Standard deviation	11.0	9.2
Minimum	50.0	46.0
Maximum	113.0	87.0
Previous oral contraceptive use		
Never used (%)	12	10
Former users* (%)	24	14
Recent users† (%)	63	76
Cigarette use		
Non-smoker (%)	65	67
< 10 per day (%)	31	29
10-20 per day (%)	4	4

*, No oral contraceptive in previous three cycles; †, oral contraceptive use, but not in previous two cycles

Hemostasis

Results reflecting procoagulant, anticoagulant and antifibrinolytic activity at baseline and during treatment are given in Table 2. Both preparations had a statistically significant effect on hemostatic variables. Indicators of pro-coagulant activity (fibrinogen, factor VII and prothrombin fragments I + II levels), were increased, as was the anticoagulant factor protein C antigen. In addition, there was an increase in profibrinolytic activity as measured by plasminogen activity, and fibrinolytic activity (plasmin-antiplasmin complex). Indicators of fibrinolytic turnover (fibrin degradation products and D-dimer) were also increased, confirming a shift in the dynamic state of the hemostatic system towards greater activity. Overall, there was seen to be both an increase in procoagulant activity and an increase in anticoagulant and profibrinolytic activity.

Table 2. Hemostatic variables at baseline, and after 3, 6 and 12 months: mean (standard deviation)

	Baseline		3 months		6 months		12 months	
variable	GSD/EE	DSG/EE	GSD/EE	DSG/EE	GSD/EE	DSG/EE	GSD/EE	DSG/EE
Procoagulant activity								
Fibrinogen (g/L)	2.8 (0.5)	2.7 (0.4)	3.2*** (0.5)	3.3*** (0.5)	3.2*** (0.5)	3.2*** (0.6)	3.2*** (0.7)	3.2*** (0.6)
Factor VII (%)	100.7 (19.4)	102.1 (26.1)	124.9*** (29.1)	138.2*** (35.0)	128.0*** (32.2)	139.7*** (32.3)	129.3*** (33.8)	137.1*** (34.5)
Prothrombin fragment I+II (nmol/L)	1.0 (0.3)	1.1 (0.4)	1.4** (0.8)	1.6** (1.0)	1.7*** (1.2)	1.8*** (1.0)	3.1*** (3.6)	5.2*** (7.9)
Anticoagulant activity								
Antithrombin activity (%)	104.4 (10.7)	101.4 (8.6)	107.6** (11.4)	106.3*** (9.5)	107.5* (10.2)	103.8* (9.6)	104.3 NS (10.5)	103.6 NS (9.7)
Protein C antingen (%)	90.7 (10.2)	92.7 (12.7)	95.7*** (11.7)	96.7** (10.0)	95.7*** (11.0)	94.7 NS (18.3)	99.8*** (11.3)	99.8** (13.6)
Protein S antigen (IU/L)	1.2 (0.2)	1.2 (0.3)	1.1*** (0.3)	1.0*** (0.2)	1.1*** (0.2)	1.0*** (0.2)	1.2 NS (0.3)	1.1 NS (0.2)
Profibrinolytic activity								
Plasminogen activity (%)	117.4 (16.7)	120.0 (17.8)	157.2*** (19.7)	159.2*** (22.5)	161.8*** (18.4)	158.9*** (20.2)	133.0*** (15.9)	134.0*** (18.2)
Plasmin-antiplasmin complex (µg/L)	392.9 (137.5)	366.5 (111.9)	538.3*** 144.1)	561.4*** (163.0)	549.3*** (161.2)	542.1*** (152.1)	451.6** (147.5)	430.6** (124.2)
t-PA activity (IU/ml)	0.4 (0.3)	0.3 (0.1)	0.48 NS (0.2)	0.45*** (0.2)	0.5 NS (0.2)	0.4** (0.2)	0.4 NS (0.2)	0.4* (0.2)
Antifibrinolytic activity								
PAI-1 antigen (ng/ml)	36.0 (22.6)	36.1 (24.9)	15.1*** (12.8)	14.1*** (12.2)	16.1*** (16.8)	14.8*** (13.4)	16.6*** (17.5)	17.3*** (16.8)

GSD/EE, gestodene/ethinlestradiol; DSG/EE, desogestrel/ethinyl estradiol; t-PA, tissue-type plasminogen activator; PAI-1, plasminogen activator inhibitor 1. Statistically significant difference from baseline: *, $p<0.05$;**, $p<0.01$;***,$p<0.001$;NS, not significant

Levels of the platelet factor, β thromboglobulin, increased slightly during treatment, from 50.8 ng/ml at baseline to 59.5 ng/ml at cycle 12 in the gestodene group, and from 49.3 ng/ml at baseline to 82.4 ng/ml at cycle 12 in the desogestrel group. These increases were not statistically significant and there were no significant differences between the two groups.

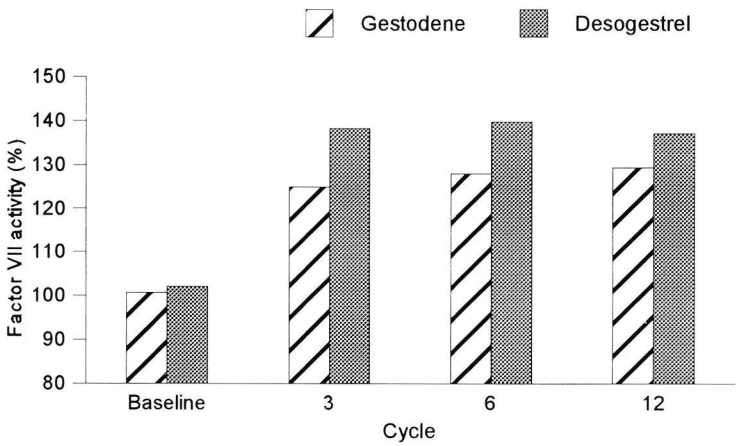
A comparison of the two groups with respect to hemostatic variables revealed that changes were similar in both groups, as shown in Table 3, which gives percentage changes from baseline to cycle 12 and between-group comparisons.

Table 3. Hemostatic variables: percentage change from baseline at study end (cycle 12)

	% change (cycles baseline-12)		
Variable	*Gestodene / ethinyl estradiol*	*Desogestrel / ethinyl estradiol*	*Group comparison*
Procoagulant			
Fibrinogen	+15.0***	+15.5***	NS
Factor VII activity	+28.6***	+34.9***	NS
Prothrombin fragment I+II	+298***	+362***	NS
Anticoagulant			
Antithrombin activity	-0.20	+0.21	NS
Protein C antingen	+4.9***	+4.0**	NS
Protein S antigen	0.00	0.00	NS
Profibrinolytic			
Plasminogen activity	+40.0***	+39.3***	NS
Tissue-type plasminogen activator activity	-7.35	+19.46*	NS
Plasmin-antiplasmin complex	+14.9**	+16.3***	NS
Antifibrinolytic			
Plasminogen activator inhibitor 1	-54.0***	-54.1***	NS
Fibrin turnover			
Fibrin degradation products	+31.3	+46.3***	NS
D-dimer	+95.5***	+73.5**	NS

Statistically significant difference from baseline: *, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.001$; NS, not statistically significant

At cycle 12, there were no statistically significant differences between the two groups with respect to any of the variables; however, factor VII was increased to a greater extent in the desogestrel group throughout the study, as shown in Figure 1. When baseline adjusted means were compared, this difference was found to be statistically significant at cycle 3 (p=0.002) and cycle 6 (p=0.018).



Statistically significant difference between groups.
*, $p<0.05$; **, $p < 0.005$

Figure 1. The effect of treatment on factor VII activity

Lipids

Lipid levels at baseline and during treatment are given in Table 4. Total cholesterol was unaffected by treatment, and there were only small changes in the levels of HDL-, HDL_2- and LDL-cholesterol. HDL-cholesterol levels were significantly increased at 6 months (gestodene, $p < 0.05$; desogestrel, $p < 0.001$), but, at 12 months, levels had decreased and were not significantly different from baseline. Triglyceride levels increased in both groups during treatment , and, at both 6 and 12 months, levels were significantly

higher than baseline. The carrierproteins, apolipoprotein A-1 and apolipoprotein B, were significantly increased in both groups, and, at the end of the study, levels of both of these proteins were significantly higher than at baseline.

Table 4. Lipid variables at baseline, and after 3, 6 and 12 months: mean (standard deviation)

	Baseline		3 months		6 months		12 months	
variable	GSD/EE	DSG/EE	GSD/EE	DSG/EE	GSD/EE	DSG/EE	GSD/EE	DSG/EE
Triglycerids (mmol/L)	1.0 (0.4)	0.9 (0.3)	1.2** (0.4)	1.2*** (0.3)	1.1** (0.3)	1.2*** (0.5)	1.1** (0.4)	1.2*** (0.3)
Total cholesterol (mmol/L)	4.4 (0.7)	4.5 (0.6)	4.2 NS (0.7)	4.4 NS (0.7)	4.4 NS (0.6)	4.5 NS (0.6)	4.3 NS (0.8)	4.5 NS (0.6)
HDL-cholesterol (mmol/L)	1.5 (0.3)	1.5 (0.4)	1.5 NS (0.3)	1.5 NS (0.3)	1.6* (0.3)	1.6*** (0.3)	1.5 NS (0.3)	1.5 NS (0.3)
HDL_2-cholesterol (mmol/L)	0.5 (0.3)	0.5 (0.2)	0.5* (0.2)	0.5 NS (0.2)	0.5 NS (0.2)	0.6* (0.2)	0.5 NS (0.2)	0.5 NS (0.2)
LDL-cholesterol (mmol/L)	2.5 (0.7)	2.6 (0.5)	2.4 NS (0.6)	2.5 NS (0.6)	2.4 NS (0.6)	2.5 NS (0.5)	2.5 NS (0.6)	2.6 NS (0.6)
Apolipoprotein A-1 (g/L)	1.4 (0.2)	1.4 (0.2)	1.5** (0.2)	1.5*** (0.2)	1.5*** (0.2)	1.5*** (0.2)	1.5*** (0.2)	1.5*** (0.2)
Apolipoprotein B (g/L)	0.9 (0.2)	0.9 (0.2)	0.9* (0.2)	1.0* (0.2)	0.9 NS (0.2)	1.0** (0.2)	0.9* (0.2)	1.0*** (0.2)

GSD/EE, gestodene/ethinlestradiol; DSG/EE, desogestrel/ethinyl estradiol. Statistically significant difference from baseline: *, $p<0.05$; **, $p<0.01$; ***,$p<0.001$; NS, not significant

Table 5 summarizes the effect of the two treatments on lipid variables, and gives percentage changes from baseline to cycle 12. It can be seen that the effects of both treatments were similar and that there were no statistically significant differences between the groups.

Table 5. Lipid variables: percentage change from baseline at study end (cycle 12)

Variable	*% change (cycles baseline-12)* *Gestodene / ethinyl estradiol*	*Desogestrel / ethinyl estradiol*	*Group comparison*
Triglycerides	+19.0**	+24.4***	
Total cholesterol	-2.3	-0.7	NS
HDL-cholesterol	-0.7	+5.4	NS
HDL_2-cholesterol	-8.4	+2.4	NS
LDL-cholesterol	-1.0	+1.0	NS
Apolipoprotein A-1	+8.3***	+12.9***	NS
Apolipoprotein B	+5.7*	+10.3***	NS

Statistically significant difference from baseline: *, $p<0.05$; **, $p<0.005$; ***, $p<0.001$; NS, not statistically significant

Carbohydrates

Changes in area under the glucose and insulin curve (AUC) in response to 75 g of glucose given orally, at cycle 6 and cycle 12, are shown in Table 6. Neither the AUC for glucose or the AUC for insulin were significantly affected by the treatment in either group, and there were no statistically significant differences between the two groups. In addition, the AUC for C-peptide was also measured, and, as for glucose and insulin, there were no significant changes from baseline and no differences between the two groups.

Table 6. Carbohydrate variables: percentage change from baseline at cycles 6 and 12

Variable	*% change from baseline* *Gestodene / ethinyl estradiol*	*Desogestrel / ethinyl estradiol*	*Group comparison*
Glucose AUC			
Cycle 6	+2.5	+1.4	NS
Cycle 12	-0.5	-4.9	NS
Insulin AUC			
cycle 6	0.0	-2.5	NS
cycle 12	+3.7	+2.1	NS

NS, not statistically significant

DISCUSSION

The findings of this study support the conclusions from earlier investigations into the metabolic influence of a combined low-dose oral contraceptive containig 75 µg gestodene and 20 µg ethinyl-estradiol[19]. The preparation was found to increase both pro-coagulant activity and anti-coagulant/profibrinolytic activity, to have only a minimal effect on lipid metabolism with no statistically significant alterations to levels of HDL- and LDL-cholesterol, and to not affect carbohydrate metabolism, as judged by alterations to glucose and insulin levels in the oral glucose tolerance test.

In the study reported here, a wide range of hemostatic variables were analysed, wherever possible focusing on molecular markers of activity, as this gives a more accurate picture of the changes to the dynamic state of the coagulation system and the continuous formation of thrombin and plasmin (activation of prothrombin and plasminogen)[20]. As many studies have previously shown, oral contraceptives have the potential to influence the coagulation system and, as a consequence, increases in the level of fibrin degradation products and D-dimer are seen normally, as well as an increase in fibrinolytic and anticoagulant activity[26,27]. The results of the study reported here, as well as previously reported findings with 75 µg gestodene/20 µg ethinyl estradiol[19,20], support this general observation. With respect to the comparison of gestodene and desogestrel, both preparations were observed to influence the hemostatic system to a similar degree. However, as has been previously reported[28,29], factor VII activity was less affected by the gestodene-containing preparation, with levels increasing to a significantly greater extent in the desogestrel group. This difference was only statistically significant at 3 and 6 months. The clinical relevance of changes in levels of factor VII is not clear for women, although it has been reported as a risk factor for coronary artery disease in men[30], but no studies have so far found any correlation between increases in factor VII and an increased risk of venous thrombosis[31].

It has previously been shown that 75 µg gestodene/20 µg ethinyl estradiol does not adversely affect lipid metabolism, and that reducing the estrogen dose from 30 µg to 20 µg has had a positive effect, reducing the impact on triglycerides[32]. Whereas, with a preparation containing 30 µg ethinyl estradiol, triglycerides were increased by 64% after 13 cycles, with the 20 µg preparation this increase was reduced to 21%. This finding is in close agreement with the results of the study reported here, which observed triglycerides

to be increased by 19% after 12 cycles. There was also no significant effect on either total cholesterol, HDL-cholesterol or LDL-cholesterol, in either group, which also supports earlier findings. With respect to apolipoprotein B, a previous 12-cycle study[33] has shown that there to be no significant difference between formulations containing either 75 µg gestodene or 150 µg desogestrel combined with 30 µg ethinyl estradiol, which increased levels by 26% and 24%, respectively. This is in agreement with the results from the study reported here, which also found no significant difference related to the progestogen.

The effect of 75 µg gestodene/20 µg ethinyl estradiol on carbohydrate metabolism has also previously been investigated, and a small but clinically insignificant effect on glucose and insulin tolerance had been reported[19]. In the study reported here, there were no significant changes from baseline for either AUC glucose or AUC insulin, and it is concluded that any effect that the gestodene preparation may have is minimal. However, for all these systems, the connection between changes in metabolic variables and the incidence of cardiovascular disease is complex and long-term epidemiological data are required to confirm the metabolic neutrality of this preparation.

It is concluded that 75 µg gestodene/20 µg ethinyl estradiol has a minimal effect on both procoagulant and anti-coagulant/fibrinolytic activity, as well as on lipid and carbohydrate metabolism. Differences between this preparation and the low-dose oral contraceptive containing 150 µg desogestrel combined with 20 µg ethinyl estradiol were minimal.

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Chapter 8

GENETIC POLYMORPHISMS MODIFY THE RESPONSE OF FACTOR VII TO ORAL CONTRACEPTIVE USE: AN EXAMPLE OF GENOTYPE-ENVIRONMENT INTERACTION

Genetic polymorphisms modify the response of factor VII to oral contraceptive use: an example of genotype-environment interaction

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Submitted

SUMMARY

Elevated plasma concentrations of factor VII and fibrinogen are well known risk factors for cardiovascular disease, especially arterial thrombosis. Oral contraceptive use increases factor VII and fibrinogen plasma levels. Recently it has been described that DNA polymorphisms are associated with the plasma levels of hemostatic variables and their regulation. The R/Q353 polymorphism in the factor VII gene and the -455G/A polymorphism in the fibrinogen ß-gene are associated with plasma levels of factor VII and fibrinogen, respectively.

We analysed data of a randomised study (n=95) in which two types of oral contraceptives were compared with regard to their effect on hemostasis (factor VII and fibrinogen), in which we also determined R/Q353 and -455G/A polymorphisms. Women were allocated randomly to either receiving a monophasic oral contraceptive containing 75 µg gestodene and 20 µg ethinyl estradiol or 150 µg desogestrel and 20 µg ethinyl estradiol. Blood was taken before treatment and after 3 months and 6 months of oral contraceptive use.

Factor VII and fibrinogen increased significantly after 3 months and 6 months oral contraceptive use; the increase in factor VII was higher in the desogestrel group than in the gestodene group. For fibrinogen there were no intergroup differences.

At baseline an association between genotype and plasma factor VII and fibrinogen levels was observed. In multivariate analysis, the R/Q353 polymorphism and the type of oral contraceptive were determinants of the effect on the change in factor VII, with the highest increase in women carrying the Q-allele and using the desogestrel containing oral contraceptive, and the lowest increase in women with the RR genotype who use the gestodene containing oral contraceptive. For fibrinogen no interaction between type of oral contraceptive, -455G/A polymorphism and change in plasma levels was observed.

We conclude that an individuals genetic variation may contribute to the respons of plasma factor VII to environmental factors, such as oral contraceptive use.

INTRODUCTION

Since the first case report of a nurse who developed pulmonary embolism after initiation of oral contraceptive as treatment for her endometriosis[1], several epidemiological studies showed that oral contraceptive use is a risk factor for cardiovascular disease, i.e. venous and arterial thrombosis[2-7]. Furthermore, studies were conducted on the effects of oral contraceptives on the hemostatic system (pro-coagulation, anti-coagulation and fibrinolysis). Oral contraceptives alter different haemostatic variables into a pro-thrombotic state[8-11]. In most studies on the effects of oral contraceptives on hemostasis, a new oral contraceptive (with a new type of progestogen or lower dose of estrogen) is compared with an older oral contraceptive[8-11]. These studies showed, that factor VII and fibrinogen levels increase during oral contraceptive use[8-11].

Elevated plasma levels of both factor VII and fibrinogen are well established risk factors for arterial thrombosis[12-14] and for fibrinogen a positive association between the plasma fibrinogen level and the venous thrombotic risk was described[15].

Plasma levels of hemostatic variables are, in addition to oral contraceptive use, also influenced by smoking habits, age, body mass index and by genetic factors. Recently, several DNA polymorphisms[16] have been identified that are associated with the plasma levels of the clotting variables[17-23]. The R/Q353 polymorphism in the factor VII gene[20] and the -455G/A polymorphism in the fibrinogen ß-chain[18] are associated with the plasmalevels of fibrinogen and factor VII, respectively. Furthermore, associations between these polymorphisms and clinical outcome, i.e. thrombotic disease been described[15,25,26]. The polymorphisms have not only been associated with the plasma levels of factor VII and fibrinogen, but also with their regulation by environmental factors. For example, the relationship between genotype and factor VII and fibrinogen plasma levels is different in men and women[27,28] and the postprandial rise in factor VII depends on the genotype[29]. The rise in fibrinogen levels in smokers and in patients with trauma is more pronounced in subjects carrying the -455A allele[18,30], which is also associated with the highest levels in the general population. We hypothesize that the R/Q353 and -455G/A polymorphisms are determinants of the rise in plasma factor VII and fibrinogen levels in women using oral contraceptives.

The aim of our study is to investigate if there is a gene-environment interaction

between oral contraceptive use, DNA polymorphisms and plasma levels of factor VII and fibrinogen; i.e. is an individuals genotype important for the individual respons to an environmental factor, such as oral contraceptive use?

MATERIAL EN METHODS

Subjects

Ninety-five healthy women under the age of 35, were enrolled in this study, after having given informed consent. Exclusion criteria were the normal contra-indications to oral contraceptive therapies, as well as a history or presence of: known hypersensitivity to estrogens or progestogens, thrombophlebitis or thromboembolic disorders, cerebrovascular or coronary artery disease or myocardial infarction, metabolic or endocrinal disease, known or suspected clotting disorders (eg protein C, protein S or antithrombin deficiencies), pregnancy, smoking more than 10 cigarettes per day, use of anticoagulants within 90 days preceding the study or the use of other estrogens, progestogens or androgens, switching from another oral contraceptive (use of a prior oral contraceptive in the preceding cycle) or recently using an oral contraceptive (within 2 cycles before prestudy screening).

Treatment

The women were randomised into two treatment groups. One group received a 75 μg gestodene and 20 μg etinylestradiol-containing oral contraceptive (GSD/20EE), the other group received a 150 μg desogestrel and 20 μg etinylestradiol-containing oral contraceptive (DSG/20EE). After a prestudy screening, consisting of complete medical, obstetric and gynaecological history and physical examination (including breast and pelvic, routine eye and neurologic examination), sitting blood pressure, weight, height, cervical Papanicolaou smear, and serum βHCG test, the volunteers started with one tablet daily for 21 days followed by 7 days without study medication.

Blood collection

At baseline and during cycle 3 and 6, between cycle day 17 and 21 blood was collected in sodium citrate (final concentration 14 mmol/L). After centrifugation (30 min, 2000 g, 4°C) the plasma was collected and stored frozen in aliquots at -70°C. The white blood cells were stored at -20°C for DNA isolation.

Assays

Fibrinogen was measured in the citrated plasma using a modified Clauss assay[31,32]. Factor VII was determined in citrated plasma by a chromogenic assay after complete activation, representing the factor VII mass concentration and expressed relative to a pooled plasma (100%) (33). The R/Q353 and the -455G/A genotypes were determined as described previously[18,34,35]. Briefly, genomic DNA was amplified using polymerase chain reaction (PCR). Ten microliters of each PCR product were digested with the appropriate restriction enzyme (MspI for the R/Q353 and HaeIII for the -455G/A polymorphism) under the conditions described by the manufacturer. These digestion products were separated by electrophoresis through a 2% agarose gel containing ethidium bromide and visualized under UV-light. The alleles with the restriction site and the non-cleavable alleles were the R353 and Q353 for the R/Q353 polymorphism and -455G and -455A for the -455G/A polymorphism, respectively.

Statistical analysis

Changes in the factor VII and fibrinogen levels were tested by using paired-samples T-test. To compare the differences between the two treatment groups, independent samples T-test was used. To examine the genetic contribution to the total variance of the factor VII, multivariate linear regression analysis was performed with the 3-month change in factor VII as the dependent variable and contraceptive group and R/Q353 genotype as independent variables. To examine the genetic contribution to the total variance of plasma levels of fibrinogen, a multivariate linear regression analysis was performed with the 3-month change in fibrinogen as the dependent variable and contraceptive group and -455G/A genotype as independent variables. To study whether oral contraceptive type was an effect modifier, the analyses were repeated with the interaction term between genotype and oral contraceptive type. Because of the small group size for the women who were homozygous for the rare allele, the heterozygotes

and the homozygotes for the rare alleles were combined in the multivariate analysis, the type of oral contraceptive was entered as discrete variables.

RESULTS

Change during oral contraceptive use

Plasma factor VII and fibrinogen levels increased during oral contraceptive use, in both treatment groups these changes were statistically significant after 3 and after 6 months oral contraceptive use, when compared with baseline (Table 1). Comparison of the oral contraceptive groups show that plasma levels of factor VII increased less in the gestodene containing oral contraceptive group, after 3 months and after 6 months of oral contraceptive use. At the time of the first visit (baseline), there were no differences between the treatment groups. The baseline values and changes in fibrinogen after 3 and 6 months oral contraceptive use were not different for the two oral contraceptive groups.

Table 1. Effect of two types of oral contraceptives on the different hemostatic variables. Means and standard deviations are given

		GSD/20EE (n=48)	DSG/20EE (n=47)
Factor VII (%)	baseline	100.7 (19.4)	102.1 (26.1)
	3 months	124.9 (29.1)*#	138.2 (35.0)*#
	6 months	128.0 (32.2)*#	139.7 (32.3)*#
Fibrinogen (g/L)	baseline	2.8 (0.5)	2.7 (0.4)
	3 months	3.2 (0.5)*	3.3 (0.5)*
	6 months	3.2 (0.5)*	3.2 (0.7)*

GSD/20EE: oral contraceptive containing 75 μg gestodene and 20 μg ethinyl estradiol in a monophasic combination; DSG/20EE: oral contraceptive containing 150 μg desogestrel and 20 μg ethinyl estradiol in a monophasic combination

* $p < 0.05$, when compared with baseline values

\# $p < 0.05$, when comparing both oral contraceptive groups

Baseline

There were no differences in genotype distribution in the different treatment groups (Table 2). The allele frequency for the Q allele of the R/Q353 factor VII polymorphism was 0.11 (95%CI 0.07-0.17) (n=21) and the allele-frequency for the A allele of -455G/A fibrinogen polymorphism was 0.24 (95% CI 0.19-0.30) (n=45). The genotype distributions were not different from Hardy-Weinberg equilibrium.

Plasma factor VII at baseline was highest in the women with the RR353 genotype, lowest in the QQ353 and intermediate in the heterozygotes. The baseline plasma levels of fibrinogen were highest in the women with the -455AA genotype, lowest in the -455GG and intermediate in the heterozygotes (Table 3).

Table 2. Factor VII R/Q353 and fibrinogen -455G/A polymorphisms in the oral contraceptive groups

Genotype		**GSD/20EE**	**DSG/20EE**
Factor VII	RR353	37	39
	RQ353	9	8
	QQ353	2	-
Fibrinogen	-455GG	29	27
	-455GA	16	17
	-455AA	3	3

GSD/20EE: oral contraceptive containing 75 μg gestodene and 20 μg ethinyl estradiol in a monophasic combination, DSG/20EE: oral contraceptive containing 150 μg desogestrel and 20 μg ethinyl estradiol in a monophasic combination

Genotype - oral contraceptive use

During oral contraceptive use, factor VII increased more in the RR353 genotype than in the other two genotypes, irrespective of the oral contraceptive type used.

The association between oral contraceptive type, R/Q353 genotype and changes in hemostatic levels during oral contraceptive use was evaluated using a multivariate linear regression analysis. After 3 months oral contraceptive use the increase in factor VII is 12% more in women using desogestrel containing oral contraceptive than in women using the gestodene containing oral contraceptive. The increase in the factor VII was 16% more in women with the RR genotype when compared with women who carried the Q allele. In a complete multivariate model, a significant interaction between R/Q353 genotype and type of oral contraceptive on the effect on the change in factor VII during three months of oral contraceptive use was observed.

Table 3. Effect of R/Q353 genotype on plasma factor VII (%) and the effect of -455G/A genotype on plasma fibrinogen levels (g/L)

Factor VII	**QQ353** (n=2)	**RQ353** (n=17)	**RR353** (n=76)
baseline	68.5 (10.6)	80.9 (19.2)	106.9 (20.5)
3 months	81.5 (14.8)	98.4 (23.9)*	140.2 (28.7)*
6 months	66.0 (7.1)	101.5 (21.1)*	142.8 (28.2)*
Fibrinogen	**-455GG** (n=56)	**-455GA** (n=33)	**-455AA** (n=6)
baseline	2.8 (0.4)	2.7 (0.4)	2.5 (0.4)
3 months	3.3 (0.6)*	3.2 (0.5)*	3.0 (0.3)*
6 months	3.2 (0.6)*	3.1 (0.5)*	3.1 (0.3)*

Means and standard deviations are given

* $p < 0.05$, when compared with baseline values

The change in fibrinogen levels during oral contraceptive use was independent of the genotype (Table 3). For fibrinogen, the univariate linear regression model showed that the 455G/A polymorphism and the oral contraceptive type had only a small effect on the change of fibrinogen during 3 months of oral contraceptive use.

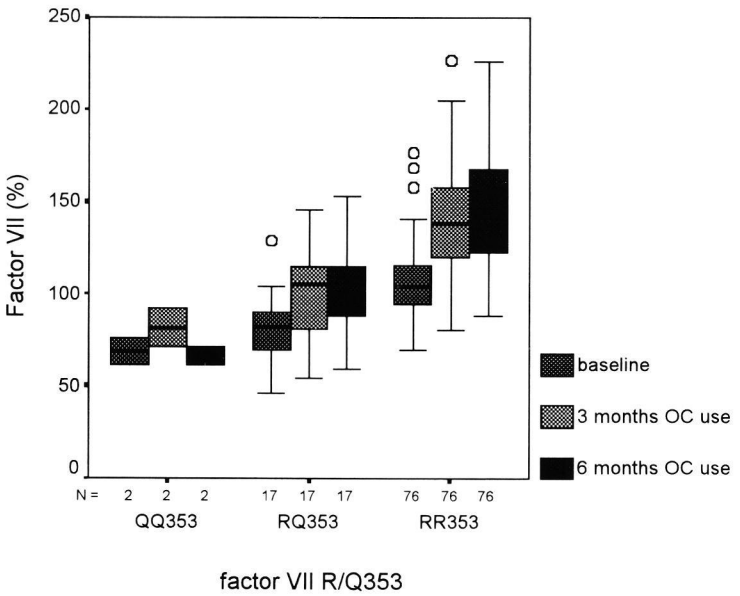

Figure 1. Boxplots of the mean plasma levels of factor VII for the different genotype groups at the different time intervals

DISCUSSION

We found in this study that the 353R/Q genotype is a determinant for the increase of factor VII during oral contraceptive use.

We previously reported that in the current study population a significant difference in the increase in factor VII between the two oral contraceptive groups was found. After 3 and after 6 months of oral contraceptive use the levels increased most in the desogestrel containing oral contraceptive group[36]. The only difference between the two types of monophasic oral contraceptives are the progestogens used. This difference in increase of factor VII levels was described before[10,37,38].

As described in literature we found in our study that baseline factor VII and fibrinogen are associated with the R/Q353 and -455G/A polymorphisms, respectively[15,17-24,34,35]. Furthermore, we can conclude that factor VII levels are also associated with the R/Q353 polymorphism during oral contraceptive use. The different effects of the oral contraceptives on factor VII are not the result of a different genotype-distribution in the oral contraceptive groups, because after adjustment for genotype in a multivariate linear regression analysis, the effect of the oral contraceptive type was still significant.

We consider it the main result of our study that the 353R/Q genotype is a determinant of the increase of factor VII during oral contraceptive use. The mechanism of this gene-environment interaction has not yet been elucidated, but the R/Q353 polymorphism is highly in linkage disequilibrium with a 10 basepair insertion/deletion polymorphism at position -323 in the promoter region of the factor VII gene. This polymorphism has been suggested to be involved in the regulation of the factor VII synthesis[39,40].

When we assume that the promoter polymorphism is the functional polymorphism and the association with the R/Q353 polymorphism is there because the two polymorphisms are strongly associated, a putative hormone responsive element in the promoter region may contribute to this gene-environment interaction.

The levels of fibrinogen increase during use of oral contraceptives, this increase was the same for both oral contraceptive groups. This is in accordance with many other studies on the effect of oral contraceptives on hemostasis[3,4]. The -455G/A polymorphism had no effect on the increase of the fibrinogen levels and no interaction was observed between oral contraceptive type, polymorphism and fibrinogen levels.

We also determined -323ins10 polymorphism and NS7(37bp)n polymorphism in the factor VII gene; 6638ins28 (Taq1) polymorphism in the fibrinogen gene; NS8ins311 polymorphism in the t-PA gene; -675(4G/5G) polymorphism in the PAI gene, respectively and their plasma levels. There was no different effect of oral contraceptive use on the plasma levels in the different genotypes (data not shown).

The present study included a relatively small number of women and conclusions should be drawn with great care. However, the concept of genotype-dependent differences in response to oral contraceptive use may have important consequences for daily clinical practice. Therefore, this gene-environment interaction needs further investigation.

It may be hypothesized that genotype-environment interaction, such as observed between factor VII polymorphism and oral contraceptive type in this study, is also present for other drugs or other environment factors. Gene-environment is important in understanding the multifactorial etiology of thrombotic disease and may explain why some individuals are at a higher risk for developing venous thrombosis when using oral contraceptives.

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Chapter 9

HEMOSTATIC EFFECTS OF ORAL CONTRACEPTIVES IN WOMEN WHO DEVELOPED DEEP-VEIN THROMBOSIS WHILE USING ORAL CONTRACEPTIVES

Hemostatic effects of oral contraceptives in women who developed deep-vein thrombosis while using oral contraceptives

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SUMMARY

Objective: Comparison of the effect of oral contraceptives on hemostatic variables in venous thrombosis patients (thrombosis while using oral contraceptives) with the effect in healthy control subjects. Our aim was to assess whether some of these effects were more pronounced in women who had suffered thrombosis, i.e., whether these were "hemostatic hyperresponders".

Study Design: A population-based case-control study, the Leiden Thrombophilia Study.

Materials and Methods: We investigated 99 pre-menopausal women, age 15-49 years, who had used oral contraceptives at the time of a first, objectively confirmed episode of deep-vein thrombosis. They were not pregnant, nor in puerperium, nor had had a recent miscarriage, and were not using injectable progestogens, nor suffering from inherited coagulation defects. The median time between occurrence of deep-vein thrombosis and venepuncture was 18 months, and 30 of the 99 women were still using oral contraceptives, while 69 had discontinued oral contraceptive use. In addition, a group of 153 control women (54 of them were oral contraceptive users and 99 were non-users) were studied. The following hemostatic variables were measured: APTT, factor VII, factor VIII, factor XII, fibrinogen, prothrombin, total antithrombin, normalised activated protein C sensitivity ratio (n-APC-sr), protein C, protein S and free protein S.

Results: We found marked and significant effects of oral contraceptive use on the levels of several clotting factors, with an increase in factor VII, factor XII, protein C and a decrease in antithrombin, n-APC-sr and protein S. Less marked effects that were non-significant or only significant in either patients or controls, were an increase in factor VIII, fibrinogen and prothrombin and a decrease in the APTT and free protein S. In the former thrombosis patients several of these effects of oral contraceptives were more pronounced than in healthy women: specifically on factor VII, antithrombin, n-APC-sr and protein C.

Conclusions: Our results of the effects of oral contraceptives generally confirm previous reports in healthy volunteers. Our data also show that in former deep-vein thrombosis patients these effects are more pronounced. Apparently some women become "high hemostatic responders" when exposed to oral contraceptives, and they may be the women most vulnerable to its thrombogenic effects.

INTRODUCTION

Since the report of Jordan in 1961[1] on the association of oral contraceptive use and venous thromboembolism, it has become generally accepted that there is an estrogen dose-dependent association between oral contraceptive use and venous thrombosis[2-5]. Recently several studies have shown that the type of progestogen also plays a role in the development of venous thrombosis during oral contraceptive use[6-10]. This resulted in renewed interest in the biological mechanisms underlying venous thrombosis during oral contraceptive use.

The natural balance between the pro-coagulant, anti-coagulant and fibrinolytic system may be disturbed by environmental factors, such as oral contraceptive use, or by hereditary defects in pro-coagulation, anti-coagulation or fibrinolysis[11-12].

Previous studies have documented that oral contraceptives may stimulate pro-coagulation, inhibit anti-coagulation and stimulate fibrinolysis[13-16]. Most studies on the hemostatic and fibrinolytic effects of oral contraceptives have been conducted in healthy volunteers. All these studies showed changes in hemostatic variables, mostly within physiological ranges and not all in the same, i.e. prothrombotic or antithrombotic, direction[13-16]. It is still unclear if and how these hemostatic changes would result in an increased risk of venous thrombosis[17-19]. From the recent clinical studies[6-10] it appears that these studies on intermediate end-points (plasma levels of hemostatic variables) have been of limited value in predicting clinical thrombosis risk.

Recently we described a gene-environment interaction between the factor V Leiden mutation[20] and oral contraceptive use. The factor V Leiden mutation, which leads to resistance to activated protein C (APC) is commonly found among patients with venous thrombosis[21]. In women who are not using oral contraceptives, this mutation increases the risk of thrombosis 7-fold[22], while the combination of use of oral contraceptives and carrier-ship of the factor V Leiden mutation increases the risk 30- to 50-fold[9,22]. For the other less frequent inherited clotting defects, i.e., protein C-, protein S- and antithrombin deficiency, oral contraceptive use may also act synergistically on the risk of development of venous thrombosis[23-27], though large data on these rare inherited clotting defects are lacking.

To explain why some women using oral contraceptives develop venous

thrombosis and others not, interactions with other coagulation risk factors may also play a role. We postulated that some women are more prone to oral contraceptive-induced hemostatic changes and thus to thrombotic disease.

The ideal design to study this question would be to randomise women with a previous thrombosis during oral contraceptive use, to either use of oral contraceptives or placebo. This offers obvious practical problems, and therefore we used a pseudo-randomised approach: we analysed hemostatic variables in the Leiden Thrombophilia Study (LETS)[28]: a population-based case-control study on risk factors for venous thrombosis. In this study patients were invited for a visit at the outpatient clinic at least six months after the thrombotic event. At that time, about one third of the women who had used oral contraceptives at the time of the deep-vein thrombosis were still using oral contraceptives; two thirds had discontinued. Since the decision to continue or discontinue oral contraceptives will depend to a great extent on the opinion of the physician, or on the feasibility of other contraception methods, and not on the hemostatic balance, this may be considered a "random" process, i.e., pseudo-randomised. In this way we assessed the effect of oral contraceptives in former thrombosis patients who developed venous thrombosis during oral contraceptive use, and contrasted this to the effect on hemostasis of oral contraceptives in healthy control women.

MATERIALS AND METHODS

Patients and control subjects

The patients and methods of our study have been described previously[22,28]. We invited 474 consecutive patients (272 women) with a first episode of objectively demonstrated deep-vein thrombosis (diagnosed by ultrasound, impedance plethysmography or phlebography) occurring between Jan 1, 1988 and Dec 31, 1992, aged less than 70 years and without a known malignant disorder. Patients had been selected from the files of three anticoagulation clinics in the Netherlands, which monitor anticoagulant treatment in all patients within a well defined geographical area. For each thrombosis patient we invited one age- and sex-matched healthy control individual.

Patients were seen after anticoagulant treatment had been discontinued for at least

3 months (median time elapsed since the thrombotic event 18 months (range 6-48)), unless treatment could not be discontinued.

For the present analysis we selected from the original study[28], premenopausal women, aged 15-49 years, who were at the time of their thrombosis (or similar date in the control women, the index date) not pregnant, nor in the puerperium, did not have a recent miscarriage, and were not using progestogen-only methods. Women receiving anticoagulant treatment on the day of blood collection were excluded (n=6). Also women with a protein C- (n=6, three of them also used anticoagulant treatment), protein S- (n=9), antithrombin (n=2) deficiency or the factor V Leiden mutation (n=41) were excluded when analysing the plasma levels of respectively: protein C activity[29], total protein S antigen and free protein S antigen[29], antithrombin activity[29] and normalised activated protein C sensitivity ratio (n-APC-sr)[28]. The criteria for diagnosis of these abnormalities have been described before[29].

149 former thrombosis patients and 169 control women were included in our analysis (mean age: 35 years). We divided this group in subgroups according to their oral contraceptive use in the month before the date of thrombosis and their oral contraceptive use on day of blood collection. 21 women of whom no data were available on the use of oral contraceptives on the day of blood collection were excluded. This resulted in the following subgroups (see figure 1): women who developed deep-vein thrombosis during oral contraceptive use and were still using oral contraceptives at blood collection (group A, n=30), women who developed deep-vein thrombosis during oral contraceptive use and did not use oral contraceptives during blood collection (group B, n=69), healthy control women using oral contraceptives during blood collection (group C, n=54) and healthy control women not using oral contraceptives during blood collection (group D, n=99). Women who did not use oral contraceptives at the time of the thrombosis are not included in this analysis (n=45).

Blood collection

Blood was collected from the antecubital vein into Sarstedt Monovette® tubes, containing 0.106 mmol/l trisodium citrate and centrifuged for 10 min at 2000 *g* at room temperature. The plasma was stored at -70 °C. High-molecular-weight DNA was isolated from leucocytes and stored at 4°C.

Laboratory Measurements

The following hemostatic variables were measured as previous described: of the pro-coagulation system; activated partial thromboplastin time (APTT)[29], factor VII activity[30], factor VIII activity[31], factor XII activity[32], fibrinogen[30], prothrombin activity[29] and of the anti-coagulation system; antithrombin activity[29], normalised activated protein C sensitivity ratio (n-APC-sr)[28], protein C activity[29], total protein S antigen and free protein S antigen[29]. Total protein S was measured by polyclonal ELISA and free protein S was measured directly in plasma by ELISA using two monoclonal antibodies specific for free protein S (Asserachrom free protein S, Stago Diagnostica, Asnières-sur-Seine, France). Results of the Activated Protein C resistance test are expressed as normalised APC-sensitivity ratios (n-APC-sr)[33]. Detection of the factor V Leiden mutation (guanine-adenine replacement at nucleotide position 1691) was performed as described previously[20]

Statistics

We performed two main comparisons by Mann-Whitney-U-test: first between women using oral contraceptives and non-users. This analysis was performed among cases and controls. Secondly, to identify women particularly sensitive to the effect of oral contraceptives, we investigated which effects were most striking in patients, as compared with controls, i.e. a comparison of the magnitude of the change in the former thrombosis patients, vs the change in the control. Since our study was in part hypothesis generating, we report significant as well as non significant results.

RESULTS

Given the complexity of the analysis a time frame is given in Figure 1. This figure describes the events which divided the women in different subgroups. Of 149 deep-vein thrombosis patients, 104 had used oral contraceptives at the time of the event, which was discontinued in 69, i.e., 30 still used it at the time of the venepuncture (data on 5 women missing). Of the 169 control women, 54 used oral contraceptives at the time of the venepuncture, and 99 did not (16 missing). This time frame enabled us to verify the effect

of oral contraceptives in former thrombosis patients whose thrombosis had happened during oral contraceptive use (group A (n=30) vs B (n=69)) and in control subjects (group C (n=54) vs D (n=99)).

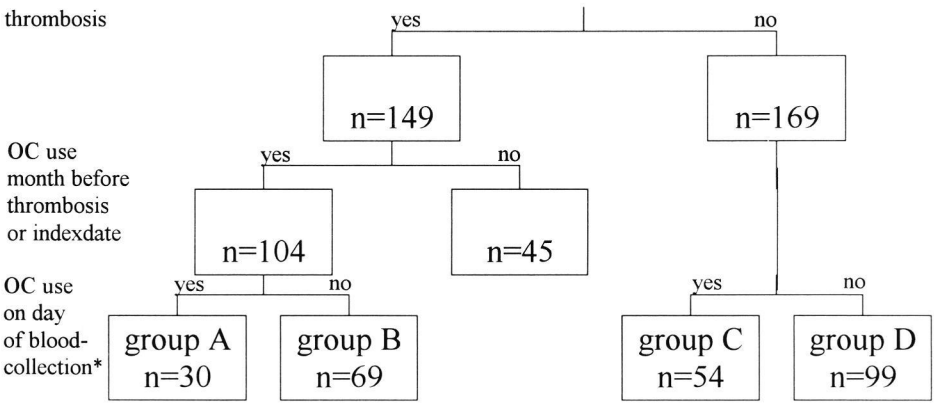

Figure 1. Subgroups of women in the Leiden Thrombophilia Study.

Exclusion criteria: women who are above the age of 50, pregnant, postpartum, menopausal, using injectable progestagens or anticoagulant treatment.
OC= oral contraceptive
* unknown for 21 women

We made several comparisons (Table 1). Firstly, we checked the effect of oral contraceptives on hemostatic variables in healthy women by comparing the values of the different hemostatic variables in the control subjects who used oral contraceptives at the time of venepuncture (n=54) with the values in the subjects of the control group who were non-users at the time of blood collection (n=99), ie group C vs group D. Of the pro-coagulant variables all, except the APTT (lower), were higher in the oral contraceptive-user group. For the anti-coagulant variables, free protein S was not really different when we compared oral contraceptive-users with non-users, protein C was higher in the oral contraceptive-user group and the other variables were lower in the oral contraceptive-user group.

Table 1. Median values (25th and 75th percentiles) of the hemostatic variables in the different subgroups of the Leiden Thrombophilia Study, i.e. among former thrombosis patients (cases) who developed their thrombosis during oral contraceptive (OC) use and healthy control subjects who continued or discontinued using oral contraceptives

	former thrombosis patients who developed their thrombosis during OC use			healthy control subjects			
oral contraceptive use during blood collection	**yes**		**no**	**yes**		**no**	
blood test	**group A**		**group B**	**group C**		**group D**	
	n=30		**n=69**	**n=54**		**n=99**	
PRO-COAGULATION							
APTT (s.)	27.2 (25.9-29.0)		27.4 (25.9-28.7)	27.7 (26.3-29.1)		28.1 (26.7-29.3)	
Factor VII (U/ml)	1.28 (1.05-1.42)	⇔*	1.03 (0.90-1.22)	1.16 (1.00-1.35)	⇔*	1.03 (0.92-1.16)	
Factor VIII (IU/ml)	1.43 (1.11-1.56)		1.37 (1.19-1.61)	1.28 (1.00-1.50)		1.12 (0.99-1.35)	B⇔D*
Factor XII (U/ml)	1.40 (1.12-1.55)	⇔*	1.05 (0.85-1.20)	1.35 (1.03-1.52)	⇔*	1.00 (0.81-1.18)	
Fibrinogen (g/L)	3.4 (2.8-4.0)		3.2 (2.8-3.5)	3.3 (3.0-3.7)	⇔*	3.1 (2.7-3.6)	
Prothrombin (U/ml)	1.06 (1.00-1.16)		1.03 (0.94-1.16)	1.06 (0.99-1.16)	⇔*	1.02 (0.93-1.11)	
ANTI-COAGULATION							
Antithrombin (U/mL)**	0.91 (0.83-1.06)	⇔*	1.00 (0.96-1.06)	0.94 (0.88-1.03)	⇔*	1.00 (0.94-1.07)	
n-APC-sr †	0.90 (0.80-0.96)	⇔*	0.95 (0.89-1.00)	0.94 (0.90-0.98)	⇔*	1.02 (0.95-1.08)	A⇔C* B⇔D*
Protein C (U/mL)‡	1.14 (0.95-1.36)	⇔*	1.00 (0.86-1.15)	1.05 (0.94-1.17)	⇔*	0.96 (0.88-1.08)	
Protein S (U/mL)§	0.90 (0.78-0.98)	⇔*	1.02 (0.90-1.13)	0.84 (0.75-0.93)	⇔*	0.96 (0.83-1.09)	B⇔D*
Free Protein S (U/mL)§§	0.85 (0.68-0.96)		0.91 (0.80-1.02)	0.88 (0.75-0.96)		0.86 (0.76-1.01)	

* 6 to 48 months after their thrombosisdate for the patients or indexdate for controls.
⇔* significantly different ($p < 0.05$) by Mann-Whitney-U-test.
** Antithrombin, n=29, 69, 54, 99 respectively
† n-APC-sr, n=22, 54, 53, 93 respectively.
‡ Protein C, n= 30, 65, 54, 98 respectively
§ Protein S, n= 29, 68, 49, 98 respectively
§§ Free Protein S, n=29, 68, 46, 93 respectively.

Secondly, we investigated the effect of oral contraceptive use in former thrombosis patients, who developed venous thrombosis during oral contraceptive use, by comparing the values of the different hemostatic variables in the former thrombosis patients who still used oral contraceptives at the time of venepuncture (n=30) with the values in the group of former thrombosis patients who were non-users at the time of blood collection (n=69), ie group A vs group B. Again, of the pro-coagulant variables all, except the APTT (lower), were higher in the oral contraceptive-user group. Of the anti-coagulant variables all median values, except for protein C (higher), were lower in the oral contraceptive using group.

Thirdly, we compared effects of oral contraceptives between women who had experienced a venous thrombosis during oral contraceptive use and healthy women. For this comparison we compared the median values of the hemostatic variables among former thrombosis patients (cases) who developed their thrombosis during oral contraceptive use and still used oral contraceptives (n=30) vs healthy control subjects who also used oral contraceptives at the time of blood collection (n=54), ie group A vs group C. The former thrombosis patients group showed higher median levels of all pro-coagulant, except the APTT (lower) and prothrombin (no difference), variables. The anti-coagulant variables antithrombin, n-APC-sr and free protein S had lower levels in the former thrombosis patients and protein C and S were higher.

Another way to compare these effects is shown in Figure 2. This figure shows the percentage of high responders in former thrombosis patients who developed their thrombosis during oral contraceptive use (n=30, group A) and in healthy control subjects (n=54, group C) who continued using oral contraceptives, i.e. women with values above the 75th percentile or below the 25th percentile (depending how the variable evolves when oral contraceptives are used). The percentiles were calculated on the group women who consisted of 149 former thrombosis patients and 169 control subjects. The purpose of this table is to identify possible high responders in this "pseudo randomised re-challenge study". For almost all hemostatic variables there were more high responders in the group of former thrombosis patients.

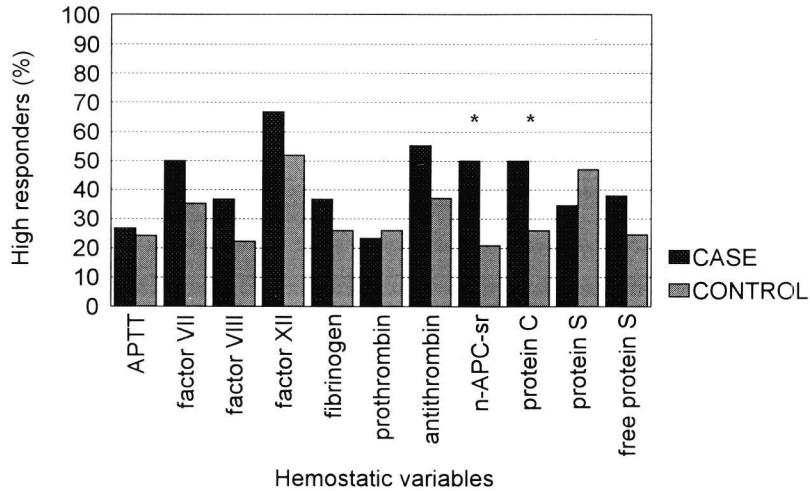

Figure 2. Percentage of high responders in former thrombosis patients who developed their thrombosis during oral contraceptive (OC) use and continued using OCs (group A) and percentage of high responders in healthy control subjects who were also using OCs at time of blood collection (group C).

* Significantly different ($p<0.05$) by Chi-Square

Fourthly, we compared the changes in hemostatic variables induced by oral contraceptives among former thrombosis patients (group A-B) with the differences induced by oral contraceptives among healthy women (group C-D), to see if women who developed venous thrombosis during oral contraceptive use react differently when using oral contraceptives in comparison with healthy women who use oral contraceptives. The changes were greater in the former thrombosis patients group for the procoagulant variables factor VII and for the anti-coagulant variables antithrombin and protein C. For the pro-coagulant variables APTT, factor VIII and for the anti-coagulant variable n-APC-sr the changes were greater in the control women.

Fifthly, to make sure that the effect in the former thrombosis patients group and still using oral contraceptives is really the effect of oral contraceptive use and not a post thrombotic state, we looked at baseline values of control women who did not use oral contraceptives (n=99) and compared these values with the values of former thrombosis

patients who discontinued using oral contraceptives (n=69), ie group D vs group B. Most pro-coagulant variables were higher in the former thrombosis patient group, except for the APTT and factor VII. For the anti-coagulant variables, protein C, protein S and free protein S the median values were higher in the former thrombosis patients, the levels of antithrombin were almost the same in both groups and n-APC-sr levels were lower in the former thrombosis group.

Sixthly, we counted the total number of variables for which a women was a high responder, for example when she was a high responder for n-APC-sr and for factor VIII her total number would be 2. The median (SD) were: for the group former thrombosis patients who developed their thrombosis during oral contraceptive use and who continued using oral contraceptives (n=30, group A); 5 (2.0), for the group former thrombosis patients who developed their thrombosis during oral contraceptive use and who discontinued using oral contraceptives (n=69, group C); 3 (1.6), for the group healthy women who used oral contraceptives at the time of venepuncture (n=54); 3 (1.7) and for the group healthy control women who were not using oral contraceptives at the time of venepuncture (n=99); 2 (1.3). When comparing the groups, the differences in group A vs the group B, group C vs group D, the group A vs group C and group B vs group D were significant.

DISCUSSION

In this pseudo-randomised study on the effect of challenging the hemostatic system by oral contraceptives in former thrombosis patients (during oral contraceptive use), we found that the hemostatic system of former thrombosis patients has a more pronounced reaction to oral contraceptives (especially for factor VII, antithrombin, n-APC-sr and protein C) in comparison to healthy control subjects.

The effects of oral contraceptive use among healthy control subjects (Table 1) are generally in agreement with the findings of many randomised studies of different types of oral contraceptives (containing different types and doses of estrogen and progestogen) and their effects on hemostasis in healthy volunteers[13-16]. Moreover this general trend of oral contraceptive effect was the same among former thrombosis patients. Only for free

protein S levels we did not observe reduced levels associated with use of oral contraceptives as has been described by others[34-36].

The comparisons in our study also show that a women who has experienced a deep-vein thrombosis, even if she is not currently using oral contraceptives, still shows characteristics of changes in the hemostatic system that are similar as if sex steroids are involved (such as in pregnant women and in women using oral contraceptives)[15,37], i.e. her hemostatic system is in a more hyper-coagulant or prothrombotic state.

A problem in our study is that we had no base-line values, so it is possible that some differences were due to the postthrombotic state. However, the time elapsed from the event to the time of blood collection was more than six months in all thrombosis patients. During these six months the values of the hemostatic variables affected by the thrombosis event are likely to have returned to the values as present before the event.

When we compare the median values of the hemostatic variables among former thrombosis patients (cases) and healthy control subjects irrespective of their oral contraceptive status, we see some of the established risk factors for venous thrombosis, such as high levels of factor VIII[31], high levels of fibrinogen[30], low levels of antithrombin [29] and low levels of n-APC-sr[28]. Some effects are not related to thrombosis risk, i.e., high levels of protein C[38], factor VII[30] and factor XII[32].

While for total protein S we found a significant decrease, we did not find a decrease of free protein S with oral contraceptive use that one usually expects. Several studies have shown decreases during oral contraceptive use, for both total and free protein S levels[34-36,39-42]. There is an important difference in design between the studies, however: most previously reported studies followed new-users for a relatively short period of time, i.e. 6 to 12 months, while our study was a cross-section of women, i.e., mainly long term users. A transient effect would therefore had been apparent in the earlier studies, but less so in our study. Several reports showed a marked effect during the first months of oral contraceptive use and a decline of that effect with prolonged use[34,35,39,42].

In a next comparison it is remarkable that the difference in the effect on hemostasis of oral contraceptives is more marked in women who developed deep-vein thrombosis during oral contraceptive use than in control subjects. There was a greater difference in former thrombosis patients for the following hemostatic variables: pro-coagulation: factor VII and anti-coagulation: antithrombin, protein C. Apparently the

hemostatic system of a former thrombosis patient, still using oral contraceptives, reacts different from a healthy control subject when using oral contraceptives. Our data suggest that some women who will suffer a thrombotic event show more pronounced effects of oral contraceptives. This may point to some women being more vulnerable to the thrombogenic effects of oral contraceptives.

Considering the various analyses, most striking are the differences in the median values of factor VII, antithrombin, n-APC-sr. Especially for these hemostatic variables it seems likely that women who developed deep-vein thrombosis during oral contraceptive use in the past, do respond differently to oral contraceptive use than their control subjects.

As was recently described by others[43-47], we found an effect on sensitivity to APC when women use oral contraceptives, also among women who did not carry the factor V Leiden mutation. This acquired APC-resistance is likely to be relevant in the pathogenesis of venous thrombosis.

At this moment the types of progestogen used in oral contraceptives are of special interest, since reports were published on the difference in risk of venous thrombosis with the different types of progestogens used in oral contraceptives[6-10]. Although both second and third generation oral contraceptives were used by a substantial number of the women in our study, the numbers in subgroups became too small to make meaningful comparisons between types of oral contraceptives possible (data not shown).

The differences in hemostatic variables in this study are between individuals, not within individuals, information of the base line values is lacking. On the other hand this study mimics the reality of the randomised trial sufficiently to infer that in some women the hemostatic system reacts more pronounced to oral contraceptives than in other women. The less likely alternative is that individuals who have had a previous thromboembolic event only become "high hemostatic responders" afterwards, when exposed to another risk factor for venous thromboembolism, i.e. oral contraceptives.

In this pseudo-randomised study some women were still using oral contraceptives after a thrombotic event (former thrombosis patients), while others were barred from using oral contraceptives. Mostly it was the physician who treated the patient, who had influenced this decision. The only influence on this decision could be the extensiveness of the deep venous thrombosis and possibly the combination with pulmonary embolus.

The data derived from the different analysis in our study may help to explain why women who use oral contraceptives, and have no other known risk factors (such as inherited clotting defects, malignancy), develop thrombosis. Apparently some women become "high hemostatic responders" when exposed to oral contraceptives, and these may be the women most vulnerable to its thrombogenic effects.

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Chapter 10

VENOUS THROMBOSIS IN A MONOZYGOTIC TWIN WHO USED ORAL CONTRACEPTIVES

Venous thrombosis in a monozygotic twin who used oral contraceptives

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Submitted

Case

Two 17-year-old girls, monozygotic twins, living in the Netherlands, went together by airplane to the United Kingdom for a short holiday. In London they climbed the stairs of St Paul's Cathedral, as part of a sight-seeing tour. During the next day one of the girls noticed increasing pain in the right calf and swelling of the leg, for which she consulted a doctor at Hammersmith Hospital. She had no shortness of breath and no chest pain. She had no relevant past medical history, no history of trauma, surgery or immobilisation (except from the short flight between Amsterdam and London). She had started using Dianette (a cyproterone acetate- and ethinyl estradiol-containing oral contraceptive) three months previously for the treatment of acne, dysmenorrhoea and pre-menstrual headache. Her father had had a venous thrombosis at the age of 20 years and her maternal grandfather had a deep-vein thrombosis after an operation.

On admission her temperature was 37.3^{0}C, pulse was 94/min., regular. Cardiorespiratory and abdominal examinations were normal. Her right leg was swollen with mild local erythema and local tenderness. An acute right deep-vein thrombosis was confirmed by an abnormal compression ultrasound test of the right leg. She was started on intravenous heparin 20,000 units for 24 hours, and analgesia. Her chest X-ray was normal and her laboratory results were all within normal limits. After 48 hours she started using warfarin and on the 6th day after admission she was discharged on warfarin 5 mg daily.

Back in the Netherlands anticoagulation treatment was discontinued after three months, but two months later she developed a deep-vein thrombosis of her left leg. Anti-coagulation treatment was restarted. Laboratory assays of the patient (no medication) were: APC resistance: 0.54, factor V Leiden mutation present (heterozygous), protein C act.; 102% (normal), protein C ag.; 96% (normal), protein S ag.; 98% (normal), anti-thrombin; 114% (normal), no anti-fospholipids detectable. She now uses Exluton (twice daily 0.5 mg lynestrenol). The other twin had started the same contraceptive on the same day as her sister, because of dysmenorrhoea. After her sister developed venous thrombosis in the United Kingdom, she also stopped using combined oral contraceptives. Like her sister, she is carrier of factor V Leiden mutation (heterozygous, APC-r; 0.58) and has no other clotting defects. Both sisters were advised not to start using combined oral contraceptives again and were counselled for other methods of

contraception e.g., progestin only pills.

Venous thrombosis is a multifactorial disease, the presence of an inherited clotting defect by itself predisposing towards thrombosis is often not enough to develop venous thrombosis. Interaction with other component causes is required (inherited or acquired) before onset of the clinical disorder, especially at young age. A gene-environment interaction between oral contraceptive use and carriership of factor V Leiden mutation has been described; the risk rises 30-50 fold among women with the combination[1,2]. In addition we have found that women with inherited clotting defects who use oral contraceptives develop venous thrombosis not only more often, but also sooner[3].

Most monozygotic twins are phenotypically similar, but there are some monozygotic twin pairs who are neither phenotypically nor genotypically identical. Mechanisms for differences in monozygotic twins are complex and not fully understood. A wide range of antenatal genetic and environmental influences can cause phenotypic and genotypic divergence[4,5]. An explanation why one of the twin developed a deep-vein thrombosis and the other not, could be that the environmental circumstances were not totally the same: for instance, one having a window seat and the other an aisle seat in the plane. Finally, they might have exerted themselves differently during their holiday.

The case, in which one half of a monozygotic twin pair developed deep-vein thrombosis after a monozygotic twin had started using oral contraceptives on the same day (both sisters were heterozygotic carriers of factor V Leiden mutation), shows us that gene-environment interaction is complex and not fully understood.

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Chapter 11

DISCUSSION AND SUMMARY

DISCUSSION AND SUMMARY

Since oral contraceptives have been introduced, it has become clear that, like all medicines, they have adverse effects. Most important is the effect on the cardiovascular system[1]. After the first case report by Jordan of a nurse who developed pulmonary embolism after oral contraceptives as a treatment for endometriosis[2], many studies were conducted on the association between oral contraceptive use and venous thrombosis, and on the effects of oral contraceptives on the hemostatic system (procoagulation, anticoagulation and fibrinolysis). Epidemiological studies (cohort- and case-control studies) showed that oral contraceptive use was a clear risk factor for venous thrombosis and studies with healthy volunteers (randomised and cross-sectional studies) showed that oral contraceptives alter various hemostatic variables, inducing a coagulation system slightly tilted towards a prothrombotic state (chapter 2). In order to reduce the cardiovascular risk during oral contraceptive use, the estrogen type was changed from mestranol into ethinyl estradiol and the estrogen content was lowered from 100 μg to 50 μg ethinyl estradiol and less. The decrease from 100 μg to 50 μg seemed effective in decreasing the risk of arterial and venous disease. Nevertheless, the lowering of the estrogen content to less than 50 μg ethinyl estradiol and the efforts to produce newer types of progestogens with the idea that they might have less impact on the hemostatic- and lipid-system did not lead to the expected further decrease in incidence of venous thromboembolism[1] (chapter 3 and 6).

In 1995 several studies showed that the newer "third generation" oral contraceptives which contained desogestrel or gestodene as progestogens have a higher risk of venous thromboembolism than the older "second generation" oral contraceptives which contain mainly levonorgestrel[3-6]. Subsequent studies had variable results[7-16]. The original studies were heavily criticised, it was hypothesised that the observed higher risk for oral contraceptives containing desogestrel or gestodene was completely explained by bias and confounding. Old and new counterarguments were put forward, such as selective prescribing (confounding by indication), referral and diagnostic suspicion bias, healthy user effect, attrition of susceptibles, an artefact of differences in duration of use, the absence of a biological explanation, inadequate statistical analysis etc. During a conference organised by the World Health Organization, a committee of independent and uninvolved researchers discussed all published and many unpublished studies in

November 1997. This resulted in the conclusion: "Combined oral contraceptives containing desogestrel or gestodene probably carry a small risk of venous thromboembolism beyond that attributable to combined oral contraceptives containing levonorgestrel" [1]. Scientific background papers, among them an excellent review by Walker in *Contraception*, came to the same conclusion[17-21] (chapter 2).

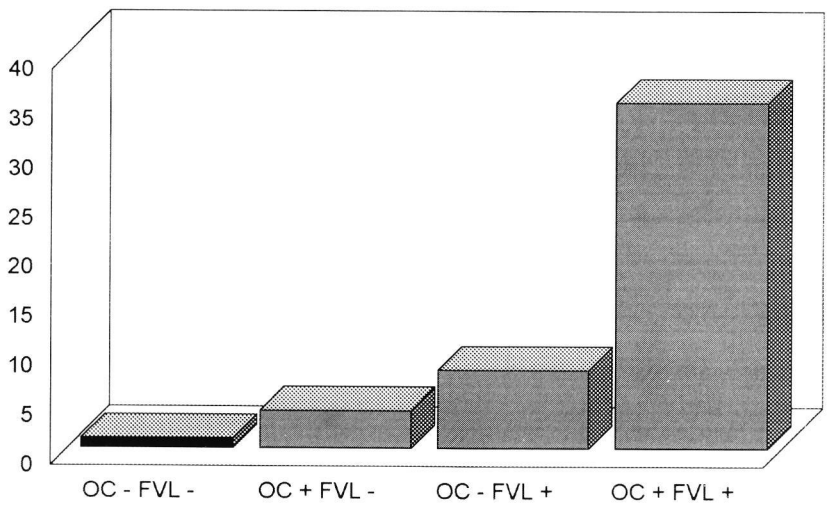
In the present thesis we describe a study in which we found that low dose oral contraceptives with a third generation progestogen have a higher risk of venous thrombosis than the previous generation of oral contraceptives. The higher risk associated with oral contraception with a third generation progestogen compared with previous generations was also present in women without factor V Leiden and without a positive family history, i.e. preferential prescription because of family history could not explain our findings (chapter 3). In a second case-control study, in the present thesis, in which we were able to exclude the diagnostic and referral bias, we also found that third generation oral contraceptives lead to a higher risk than second generation oral contraceptives. Moreover we confirmed that the risk of venous thromboembolism with currently available oral contraceptives still exists (chapter 6), and is higher than generally believed, also for preparations containing a low dose of estrogen.

As regards the arterial thrombosis it has been claimed that oral contraceptives which contain desogestrel and gestodene lead to a lower risk of myocardial infarction compared with levonorgestrel containing oral contraceptives. These conclusions were based on results from studies without clinical endpoints. The few studies with clinical endpoints, which were all quite small, led to contradictory results[22-25]. We have to wait for the result of larger ongoing studies before we can make an evidence-based decision on the indication for prescribing third generation oral contraceptives. For example, for the over 35-year-old woman who smokes[26]. Recently, the results of a large case-control study were published, in which the association between myocardial infarction and the use of different types of oral contraception in young women was determined: no benefit of third generation as compared with second generation products was found[27].

The largest benefit of the whole debate on third generation oral contraceptives is that many researchers are (again) interested in investigating the association between oral contraceptive use and venous thrombosis, a field in which during the previous decades few new insights have been reported. However, the pathogenesis of the development of venous and arterial thrombosis during oral contraceptives remains unclear. At the

moment epidemiology and biochemistry seem to meet each other: the model of gene-environment interaction is a tremendous step forward in understanding when and why oral contraceptives develop venous thromboembolism[28]. The interaction between factor V Leiden and oral contraceptive use is probably the best example of the new insights[29] (chapter 3) (Figure 1), but the other inherited clotting defects (which are themselves risk factors of venous thrombosis, protein C-, protein S-, antithrombin deficiency and newly detected prothrombin mutation[30,31]) also appear to lead synergistically with oral contraceptive use to an excess risk of development of venous thrombosis[32,33].

Figure I. Deep-vein thrombosis per year per 10000 women



OC: oral contraceptives
FVL: factor V Leiden

Interaction of oral contraceptive use and factor V Leiden

Along the line of this model we investigated the combined effects of high factor VIII levels and oral contraceptive use in the occurrence of venous thrombosis (chapter 5). Furthermore, we found that women with inherited coagulation defects, when they are

exposed to oral contraceptives, develop venous thrombosis in the early stage of use (chapter 4), which confirms an old clinical impression[34].

When investigating the effect of oral contraceptive use on hemostatic variables, we find that procoagulation is stimulated, anticoagulation is inhibited and fibrinolysis is stimulated. How to interpret these findings remains difficult. The concept that the increase in procoagulation is counterbalanced by an increase in fibrinolysis[35] seems illogical, since we do not know what the impact is of the changes in the different hemostatic systems, i.e. what the net result will be. Moreover, to date no effect of variation in the fibrinolytic system on the risk of thrombosis has been demonstrated, i.e., fibrinolysis appears to play at most a minor role here. To estimate the net effect, we have to go back to epidemiological studies which investigate clinical endpoints, instead of intermediate endpoints (plasma levels of hemostatic variables).

Nevertheless, hemostatic studies may improve our understanding. In this thesis a randomised experiment shows that current low-dose oral contraceptives stimulate procoagulation, inhibit anticoagulation and stimulate fibrinolysis (chapter 7). There is now evidence that even small functional differences in hemostatic variables can be considered as risk factors for venous thrombosis, such as elevated levels of factor II, factor VIII and fibrinogen[36-38] and decreased levels of normalised activated protein C-sensitivity ratio's (n-APC-sr)[39]. Oral contraceptive use seems to influence all parts of the hemostatic system towards a prothrombotic state, even the antifibrinolysis might be increased[40]. Maybe it is the combination of the alterations of these several hemostatic variables that leads to an increased risk of venous thrombosis. It was interesting to find that genetic polymorphisms modify the response of factor VII levels to oral contraceptive use (chapter 8). This is another example of gene-environment interaction and may be relevant to the interpretation of the effects of oral contraceptives on the hemostatic system.

Recently, several studies reported that oral contraceptive use leads to an increase in APC-resistance[41-47]. One problem when comparing these studies is that different assays to measure APC-resistance were used (intrinsic vs extrinsic pathway, "in house" vs "commercial"). Although all tests show that oral contraceptive use increases resistance to the anticoagulant action of activated protein C, the tests seem to differ in their sensitivity to sex-steroids. One assay (prothrombin-time based), developed by Rosing, is particularly promising. The assay shows high sensitivity for use of oral contraceptives, of all types,

and even distinguishes between different "generations" oral contraceptives in accordance with the epidemiological findings. Women who used third generation containing oral contraceptives were less sensitive to activated protein C than women using second generation containing oral contraceptives and had n-APC-sr that did not differ from heterozygous female carriers of factor V Leiden who did not use oral contraceptives[45-47].

Probably, investigating the effect of oral contraceptives on the hemostatic system in *healthy* women will not be sufficient for understanding the pathogenesis of venous thrombosis during oral contraceptive use. We therefore studied women who had suffered a deep-vein thrombosis during oral contraceptive use and had continued using oral contraceptives after their anticoagulation treatment had been stopped. We found that in former thrombosis patients several of the effects of oral contraceptives were more pronounced than in the healthy women: specifically on factor VII, antithrombin, n-APC-sr and protein C (chapter 9). In chapter 10 a case is presented, in which one half of a monozygotic twin pair developed deep-vein thrombosis after they had started using oral contraceptives on the same day (both sisters were heterozygotic carriers of factor V Leiden mutation). This shows us that gene-environment interaction is complex and not fully understood.

Recently major progress has been made in our understanding of the pathogenesis of venous thromboembolism during oral contraceptive use. The greatest promise may lie in the interaction between inherited tendencies for thrombosis and oral contraceptive use. Virchow's third factor, "composition of the blood", has thus to be split in an inherited and an acquired part[48]; their effects might even reinforce each other. Variation in the susceptibility of the individual woman holds the key to provide a biologically plausible explanation of why oral contraceptives cause venous thrombosis. We are confident that this knowledge will be helpful when developing new and even safer oral contraceptives and other medically used sex-steroids.

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Chapter 12

DUTCH SUMMARY / SAMENVATTING

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Orale anticonceptiva -"de pil"- zijn beschikbaar sinds de 60'er jaren en worden momenteel door meer dan 100 miljoen vrouwen in de gehele wereld gebruikt[1]. In Nederland is de pil het meest gebruikte voorbehoedmiddel, in 1997 gebruikte van de vrouwen tussen de 16 en 50 jaar, 43.3% de pil en in de leeftijdscategorie 20-25 jaar zelfs 73.3%[2]. De effecten van pilgebruik blijven in de belangstelling staan van vrouwen, de artsen, de media, in toenemende mate juristen, omdat de pil de meest gebruikte medicatie is van gezonde vrouwen in de ontwikkelde wereld. Sinds de introductie van orale anticonceptie, blijkt dat de pil behalve gunstige (bij)werkingen, zoals bescherming tegen het optreden van een zwangerschap, evenals andere medicatie, minder gunstige bijwerkingen heeft. Het belangrijkst daarbij is het effect op het cardiovasculaire systeem (het krijgen van hart- en vaatziekten)[3-5]. Orale anticonceptiva beïnvloeden de stollings- (zie addendum I), koolhydraten-, lipiden- en endotheliale systemen, mechanismen die de bloeddruk reguleren en waarschijnlijk tot nu toe onbekende systemen, waardoor er een verhoogd risico ontstaat op cardiovasculaire ziekten[5-12]. Cardiovasculaire bijwerkingen kunnen onderverdeeld worden in arteriële thrombose (hartinfarct, herseninfarct en hersenbloeding) en in veneuze thrombose (diepe veneuze thrombose, longembolie en herseninfarct)[5,13].

De in dit proefschrift beschreven onderzoekingen hadden tot doel om meer inzicht te krijgen in het effect van pilgebruik op de stolling. Het is een poging om te begrijpen waarom de pil cardiovasculaire ziekten veroorzaakt, in het bijzonder veneuze thrombose.

Jordan beschreef de eerste casus waarbij een verpleegkundige, in aansluiting op pilgebruik als behandeling van haar endometriose een longembolie kreeg[14]. Hierna volgden er vele studies die de associatie tussen pilgebruik en het krijgen van veneuze thrombose bestudeerden en het effect van pilgebruik op het stollingssysteem (pro-coagulatie, anti-coagulatie en fibrinolyse) bekeken. Epidemiologische studies (vervolg- of follow-up en patiënt-controleonderzoeken) lieten zien dat pilgebruik een duidelijke risicofactor was voor het krijgen van veneuze thrombose. Tevens lieten onderzoekingen met gezonde vrijwilligsters (gerandomiseerde en cross-sectionele onderzoekingen) zien dat pilgebruik effect heeft op verschillende stollingsvariabelen en het stollingssyteem in de richting van een zogenaamde pro-thrombotische situatie laat gaan (hoofdstuk 2). Omdat

men bijwerkingen, zoals een verhoogde kans op hart- en vaatziekten, toeschreef aan het oestrogeen in de pil, is sinds de introductie het type oestrogeen veranderd van mestranol in ethinyl estradiol en is getracht de hoeveelheid daarvan terug te brengen van 100 µg naar 50 µg en naar 30 µg ethinyl estradiol. De vermindering van de hoeveelheid ethinyl estradiol van 100 µg naar 50 µg lijkt het risico op arteriële en veneuze thombose te verlagen. De verlaging van het oestrogeen gehalte naar minder dan 50 µg had niet het gewenste resultaat. En ook de introductie van nieuwe soorten progestagenen met de gedachte dat deze soorten minder effect zouden hebben op het stollings- en vetstofwisselingssysteem, leidde niet tot de verwachtte verdere daling in het voorkomen van veneuze thrombose tijdens pilgebruik[5] (hoofdstuk 3 en 6).

In december 1995 en januari 1996 werden vier studies gepubliceerd. Deze concludeerden allen dat vrouwen die desogestrel en gestodeen bevattende pillen (derde generatie) gebruikten, een gemiddeld twee keer zo hoog relatief risico hadden op het krijgen van veneuze thrombose als gebruiksters van oudere pillen zoals die met levonorgestrel (tweede generatie)[14-18]. Studies die volgden lieten verschillende uitkomsten zien[19-28]. De oorspronkelijke studies werden hevig bekritiseerd; er werd gesuggereerd dat het gevonden hogere risico van de desogestrel of gestodeen bevattende pillen geheel verklaard kon worden door verstorende factoren (confounders) en vertekening (bias). Oude en nieuwe argumenten werden naar voren gebracht, zoals selectief voorschrijfgedrag (confounding by indication); artsen zouden de diagnose veneuze thrombose vaker stellen bij gebruik van derde-generatiepillen (referral en diagnostic suspicion bias); "healthy user effect"; "attrition of susceptibles"; het niet corrigeren voor een verschil in duur van pilgebruik; de afwezigheid van een plausibel biologische verklaring; inadequate statistische analyse etc. Tijdens een conferentie georganiseerd door de Wereld Gezondheid Organisatie (WHO) in november 1997, discussieerden een commissie van onafhankelijke en niet betrokken onderzoekers over alle gepubliceerde en vele van op dat moment nog niet gepubliceerde onderzoekingen. Hierbij kwam men tot de conclusie dat oral anticonceptiva die desogestrel of gestodeen bevatten waarschijnlijk een klein verhoogd risico geven ten opzichte van orale anticonceptiva die levonorgestrel bevatten[5]. Wetenschappelijke achtergrondcommentaren, waaronder een uitstekend overzichtsartikel van Walker gepubliceerd in *Contraception,* kwamen tot dezelfde conclusie[4, 29-32] (hoofdstuk 2).

In dit proefschrift wordt een studie beschreven waarin we vinden dat de

moderne laaggedoseerde pillen die het derde generatie progestageen desogestrel bevatten een hoger risico op veneuze thrombose geven dan de oudere generatie pillen. Dit hoger risico geassocieerd met gebruik van een derde-generatiepil ten opzichte van oudere generatie pillen, was ook aanwezig bij vrouwen die geen draagsters van factor V Leiden mutatie zijn en vrouwen zonder een positieve familiegeschiedenis voor wat betreft veneuze thrombose. Dit betekent dat selectief voorschrijfgedrag t.a.v. familiegeschiedenis de bevindingen niet kon verklaren (hoofdstuk 3). In een tweede patiënt-controleonderzoek, beschreven in dit proefschrift, waarin we de mogelijke invloed van "diagnostic suspicion en referral" bias konden uitsluiten, vonden we opnieuw het verhoogd risico voor de derde-generatiepillen. Bovendien bevestigden we dat het risico op veneuze thrombose met de tegenwoordig beschikbare pillen nog steeds bestaat en dat dit hoger is dan algemeen werd aangenomen, ook voor de preparaten die lage doseringen oestrogenen bevatten (hoofdstuk 6).

Voor wat betreft de arteriële thrombose was er lange tijd het argument dat het slikken van pillen die desogestrel of gestodeen bevatten een voordeel hadden op het optreden van hartinfarct in vergelijking met pillen de levonorgestrel bevatten. Deze conclusies waren gebaseerd op de resultaten van onderzoekingen zonder klinische eindpunten. De weinige studies met klinische eindpunten waren allemaal tamelijk klein en gaven aanleiding tot blijvende controverse[33-36]. We moeten wachten op de resultaten van nog lopende studies om een evidence-based uitspraak te doen over wanneer er nog een indicatie bestaat om een derde-generatiepil voor te schrijven. Bijvoorbeeld, voor vrouwen ouder dan 35 jaar die roken[37]. Recent werden de resultaten van een groot patiënt-controleonderzoek gepubliceerd, waarin de associatie tussen hartinfarct en gebruik van verschillende piltypen bij jonge vrouwen werd bestudeerd. Er werd geen voordeel van derde-generatiepillen ten opzichte van tweede-generatiepillen gevonden[38].

De gehele controverse rondom de derde-generatiepillen heeft ertoe geleid dat (opnieuw) vele onderzoekers geïnteresseerd zijn geraakt in onderzoek naar pilgebruik en de associatie met veneuze thrombose, een gebied van waaruit gedurende de laatse decennia weinig nieuws werd gemeld. Desondanks is de pathogenese van het krijgen van veneuze en arteriële thrombose tijdens pilgebruik nog niet geheel opgehelderd. Op het moment lijken de epidemiologie en de biochemie elkaar te ontmoeten: het model van omgeving-geninteractie is een belangrijke stap voorwaarts in de begripsvorming van wanneer en waarom pilgebruik veneuze thrombose geeft[39]. De interactie tussen factor V

Leiden mutatie en pilgebruik is waarschijnlijk het beste voorbeeld van de nieuwe inzichten in deze materie[40] (hoofdstuk 3, zie ook blz 161, Figuur 1). Ook de andere erfelijke stollingdefecten, die op zichzelf risicofactoren zijn voor het krijgen van veneuze thrombose (proteïne C-, proteïne S-, antithrombine deficiëntie en de pas ontdekte prothrombine mutatie[41,42]) lijken -synergistisch met pilgebruik- te leiden tot een toename in het risico op het krijgen van veneuze thrombose[43,44].

In aansluiting op dit model hebben we de gecombineerde effecten van hoge plasmaspiegels van factor VIII en pilgebruik op het krijgen van veneuze thrombose bestudeerd (hoofdstuk 5). Verder ontdekten we dat vrouwen die draagster zijn van erfelijke stollingsdefecten, wanneer zij de pil gaan gebruiken, vaker veneuze thrombose krijgen tijdens kortdurend pilgebruik (hoofdstuk 4). Dit bevestigt een al langer bestaande klinische indruk[45].

Indien we de effecten van pilgebruik op stollingsvariabelen bekijken, vinden we dat de pro-coagulatie is gestimuleerd, de anti-coagulatie in verminderd en de fibrinolyse is gestimuleerd. Hoe we deze bevindingen moeten interpreteren blijft moeilijk. Het principe dat de toename in pro-coagulatie wordt tenietgedaan door een toegenomen fibrinolyse[46] lijkt onlogisch, omdat we niet weten wat de impact van de veranderingen op het gehele stollingssysteem is, met andere woorden wat het netto effect is. Bovendien is tot nu toe nog geen effect van variatie in het fibrinolyse systeem op het tromboserisico aangetoond, fibrinolyse lijkt hierin een ondergeschikte rol te spelen. Om het uiteindelijke effect in te schatten moeten we terug grijpen op epidemiologische onderzoekingen die klinische eindpunten bestuderen in plaats van intermediaire eindpunten (plasma spiegels van stollingsvariabelen).

Toch kunnen bovengenoemde onderzoekingen ons helpen. In dit proefschrift laat een gerandomiseerd experiment zien dat de moderne laaggedoseerde pillen de pro-coagulatie stimuleren, de anti-coagulatie remmen, en de fibrinolyse stimuleren (hoofdstuk 7). Er zijn thans aanwijzingen dat zelfs deze kleine functionele veranderingen in stollingsvariabelen beschouwd kunnen worden als risicofactoren voor veneuze thrombose, zoals daar zijn verhoogde prothrombine, factor VIII en fibrinogeen spiegels[47-49] en afgenomen spiegels van genormaliseerde geactiveerd proteïne C-gevoeligheids ratio's (n-APC-sr)[50]. Pilgebruik lijkt alle onderdelen van het stollingssysteem te beïnvloeden in de richting van een prothrombotische situatie, zelfs de anti-fibrinolyse kan toegenomen zijn[12]. Misschien is het de combinatie van de veranderingen van deze

verschillende stollingsfactoren die leidt tot een toegenomen risico op het krijgen van veneuze thrombose. Het was interessant te vinden dat genetische polymorfismen de respons van factor VII spiegels op pilgebruik beïnvloeden (hoofdstuk 8). Dit is een ander voorbeeld van gen-omgevinginteractie en kan belangrijk zijn bij de interpretatie van de effecten van pilgebruik op het stollingssysteem.

Recent hebben verschillende onderzoekingen beschreven dat pilgebruik meer verworven APC-resistentie geeft[10,51-56]. Bij vergelijking van deze onderzoekingen is het een probleem dat verschillende testen om APC-resistentie te meten werden gebruikt (intrinsieke versus extrinsieke stollingssysteem, -in huis testen- versus commerciële testen). Alle testen laten zien dat pilgebruik leidt tot een toename in resistentie van de anti-coagulatieactiviteit van geactiveerd proteïne C; de testen blijken verschillend in de gevoeligheid voor geslachtshormonen. Eén test, ontwikkeld door Rosing, is veelbelovend. De test laat grote gevoeligheid voor pilgebruik zien en kan zelfs het onderscheid maken tussen de verschillende generaties van pillen, in overeenstemming met de epidemiologische bevindingen. Vrouwen die derde-generatiepillen gebruikten vertoonden minder gevoeligheid voor geactiveerd proteïne C dan vrouwen die tweede-generatiepillen gebruikten en hadden n-APC-sr's die niet verschilden van heterozygote draagsters van factor V Leiden mutatie, die geen pil gebruikten[10,55,56].

Waarschijnlijk is het onderzoeken van het effect van pilgebruik op het stollingssysteem bij gezonde vrouwen onvoldoende om inzicht in de pathogenese van pilgebruik en veneuze thrombose te krijgen. Daarom hebben we vrouwen bekeken die een trombosebeen tijdens pilgebruik hadden gekregen en ons informeerden dat zij na het staken van de anti-stolling de pil waren blijven gebruiken. We ontdekten dat bij ex-thrombose patiënten de effecten van pilgebruik op het stollingssysteem meer uitgesproken waren dan bij gezonde vrouwen; speciaal gold dit voor de stollingsvariabelen factor VII, antithrombine, n-APC-sr en proteïne C (hoofdstuk 9). In hoofdstuk 10 wordt een casus gepresenteerd waarin een eeneïge tweeling, beiden draagster van factor V Leiden mutatie, tegelijkertijd dezelfde pil gaat gebruiken en één van de tweeling een trombosebeen krijgt en de ander niet. Hieruit blijkt dat gen-omgevinginteractie niet geheel begrepen en complex is.

Recent is grote vooruitgang geboekt in ons inzicht in de pathogenese van veneuze thombose tijdens pilgebruik. De grootste vooruitgang is waarschijnlijk de interactie tussen erfelijke stollingsdefecten en pilgebruik. Virchow's derde factor, "samenstelling van het

bloed", moet eigenlijk opgesplitst worden in een erfelijke en een verworven gedeelte[57]; deze effecten kunnen elkaar zelfs versterken. Variatie in de gevoeligheid van individuen is waarschijnlijk de sleutel naar een biologische plausibele verklaring waarom pilgebruik veneuze thrombose veroorzaakt. We hebben er vertrouwen in dat deze kennis in de toekomst behulpzaam zal zijn bij de ontwikkeling van nieuwere en nog veiligere anticonceptiepillen en andere hormonale preparaten.

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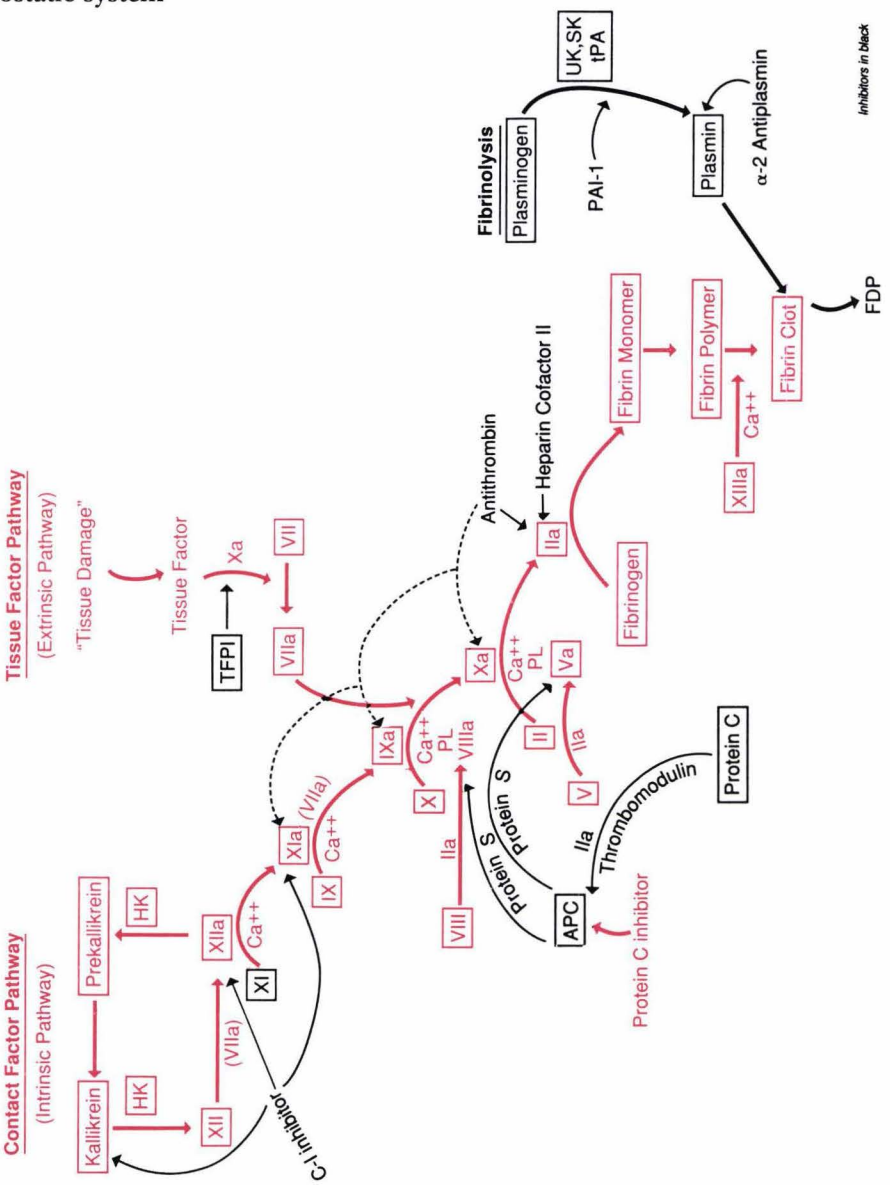
Addendum I

Hemostatic system



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Addendum II

Sex steroids used in oral contraceptives and in hormone replacement therapy

1) Estrogens

natural
synthetic
conjugated

2) progestogens

natural
synthetic (1st, 2nd, 3rd, 4th generation, misc.)

3) oral contraceptives combinations

monophasic
biphasic
triphasic

ad 1) <u>estrogens</u>

synthetic

mestranol
ethinyl estradiol (in currently available oral contraceptives)
dienestrol

natural

17 ß-oestradiol
oestriol
oestradiol valeriate

conjugated

conjugated estrogen

ad 2) <u>progestogens</u>

first generation

ethynodiolacetate
lynestrenol
norethisterone (acetate)
norethynodrel

second generation

norgestrel
levonorgestrel
norgestrinone

third generation

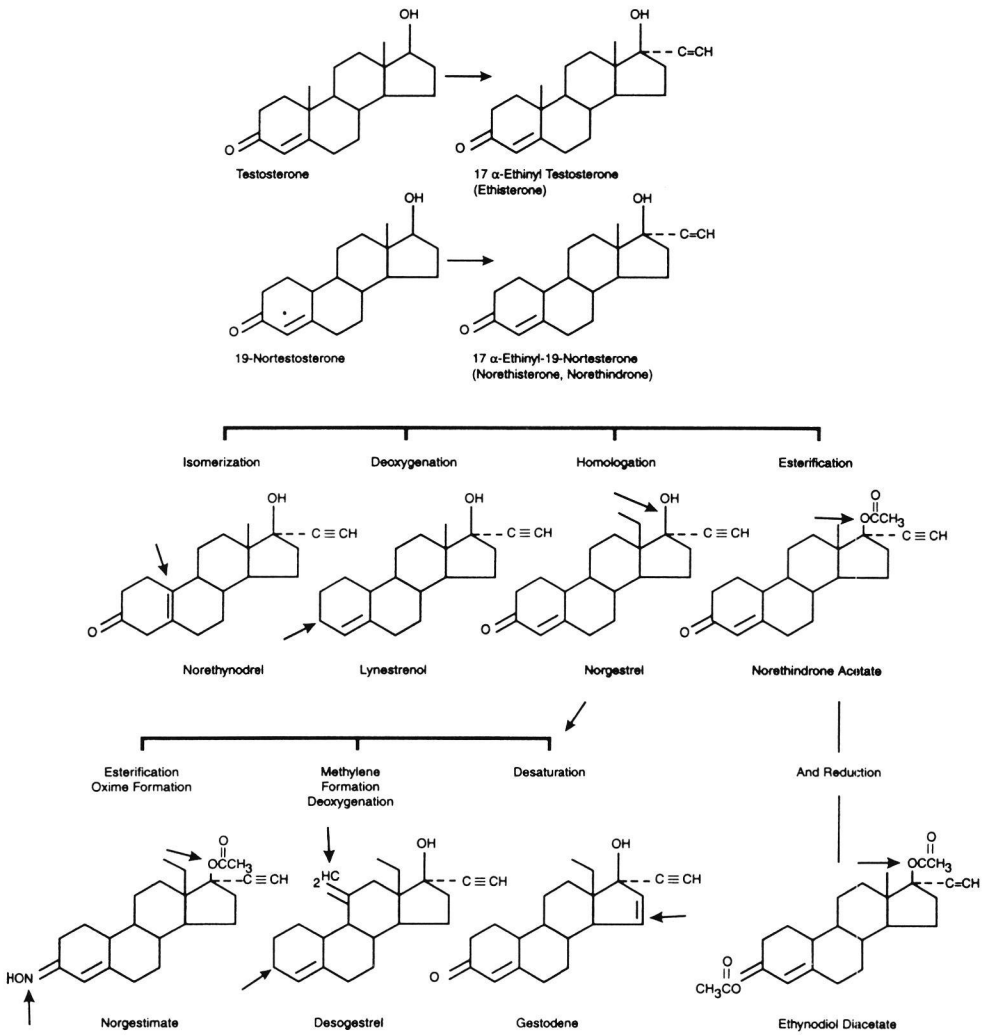
desogestrel
gestodene
norgestimate

fourth generation

chlormadinone
cyproterone

misc.

progestorone
dydrogesterone
medrogestone
medroxyprogesterone acetate
tibolone



The common progestogens. Although not synthetically accurate, if norethindrone is considered the parent progestogen, the substituent alterations in the various analogs can be appreciated.
Adapted from Peterson CM. Progestogens, Progesterone Antagonists, Progesterone, and Androgens: Synthesis, Classification, and Uses. Clin Obstet Gynecol 1995; 38(4): 815.

Addendum III

Oral contraceptives available in the Netherlands in 1999

Product name	type of estrogen and progestogen	number of tablets in one month
20 µg ethinyl estradiol		
Monophasic		
Harmonet*	ethinyl estradiol 20 µg and gestodene 75 µg	21
Meliane*	ethinyl estradiol 20 µg and gestodene 75 µg	21
Mercilon*	ethinyl estradiol 20 µg and desogestrel 150 µg	21
30 µg ethinyl estradiol		
Monophasic		
Stediril 30	ethinyl estradiol 30 µg and levonorgestrel 125 µg	21
Microgynon 30	ethinyl estradiol 30 µg and levonorgestrel 125 µg	21
Marvelon*	ethinyl estradiol 30 µg and desogestrel 150 µg	21
Femodeen*	ethinyl estradiol 30 µg and gestodene 75 µg	21
Minulet*	ethinyl estradiol 30 µg and gestodene 75 µg	21
> 30 µg ethinyl estradiol and ≤ 37.5 µg ethinyl estradiol		
Monophasic		
Ministat	ethinyl estradiol 37.5 µg and lynestrenol 0.75 mg	21
Mini Pregnon	ethinyl estradiol 37.5 µg and lynestrenol 0.75 mg	21
Neocon	ethinyl estradiol 35 µg and norethisterone 1.0 mg	21
Modicon	ethinyl estradiol 35 µg and norethisterone 0.5 mg	21
Diane 35	ethinyl estradiol 35 µg and 2 mg cyproterone acetate	21
Cilest	ethinyl estradiol 35 µg and norgestimate 250 µg	21
Triphasic		
Trinordiol/	ethinyl estradiol 30 µg and levonorgestrel 50 µg	6
Trigynon	ethinyl estradiol 40 µg and levonorgestrel 75 µg	5
	ethinyl estradiol 30 µg and levonorgestrel 125 µg	10
Trinovum	ethinyl estradiol 35 µg and norethisterone 0.5 mg	7
	ethinyl estradiol 30 µg and norethisterone 0.75 mg	7
	ethinyl estradiol 30 µg and norethisterone 1.0 mg	7
Triodeen/	ethinyl estradiol 30 µg and gestodene 50 µg	6
Tri-minulet*	ethinyl estradiol 40 µg and gestodene 70 µg	5
	ethinyl estradiol 30 µg and gestodene 100 µg	10

Product name	**type of estrogen and progestogen**	**number of tablets in one month**
50 µg ethinyl estradiol or mestranol		
Monophasic		
Ovulen 50	ethinyl estradiol 50 µg and ethynodiolacetate 1.0 mg	21
Neogynon 21	ethinyl estradiol 50 µg and levonorgestrel 250 µg	21
Stediril-d	ethinyl estradiol 50 µg and levonorgestrel 250 µg	21
Ortho-Novum 1/50	ethinyl estradiol 50 µg and norethisterone 1.0 mg	21
Ovostat	ethinyl estradiol 50 µg and lynestrenol 1.0 mg	22
Pregnon 28	ethinyl estradiol 50 µg and lynestrenol 1.0 mg	22
Microgynon 50	ethinyl estradiol 50 µg and levonorgestrel 125 µg	21
Neo-stediril	ethinyl estradiol 50 µg and levonorgestrel 125 µg	21
Biphasic		
Ovanon	ethinyl estradiol 50 µg	7
	ethinyl estradiol 50 µg and lynestenol 2.5 mg	15
Binordiol	ethinyl estradiol 50 µg and levonorgestrel 50 µg	11
	ethinyl estradiol 50 µg and levonorgestrel 125 µg	10
Fysioquens	ethinyl estradiol 50 µg	7
	ethinyl estradiol 50 µg and lynestrenol 1.0 mg	15
Ovidol*	ethinyl estradiol 50 µg	7
	ethinyl estradiol 50 µg and desogestrel 125 µg	15
Progestogen only		
Exluton	lynestrenol 0.5 mg	28
Cerazette*	desogestrel 75 µg	28

Depo-provera medroxyprogestorone acetate 150 mg 1 injection/3 months

* containing third generation progestogens, it is discussable if norgestimate should be included in this group, because it is partly converted to levonorgestrel (a second generation progestogen)

Curriculum Vitae

Kitty W.M. Bloemenkamp was born in Duiven, the Netherlands on April 15, 1965. From 1977- 1984 she was educated at College Noetsele, Nijverdal. She started medical training in 1985 at the University of Leiden, after studying biology for one year at the same university. She acquired her medical degree in 1993. During this period she participated in the following research projects: in 1990 in a fieldstudy on Onchocerciasis in Hohoe (Onchocerciasis Chemotherapy Research Centre, WHO), Ghana (dr. K. Awadzi) in collaboration with the Department of Parasitology, Leiden University Medical Centre (Prof. dr H.J. van der Kaay). In 1992 she participated in the research project: "Evaluation of the postcoitum test" at the Department of Gynaecology, Obstetrics and Reproductive Medicine, Leiden University Medical Centre, Leiden (dr S.G. Oei, Prof. dr M.J.N.C. Keirse and dr F.M. Helmerhorst). In 1993 she visited United Bulawayo Hospitals, Bulawayo, Zimbabwe for a housemanship in obstetrics and gynaecology (chairman: dr. D.A.A. Verkuyl).

From 1993 till april 1997 she worked as a IVF doctor and resident at the Department of Obstetrics, Gynecology and Reproductive Medicine, Leiden University Medical Centre, Leiden.

She started preparing the work of this thesis as a research fellow at the Departments of Clinical Epidemiology (Prof. dr J.P. Vandenbroucke, Prof. dr F.R. Rosendaal), Obstetrics, Gynecology and Reproductive Medicine (dr F.M. Helmerhorst) in collaboration with the Thrombosis and Hemostasis Research Centre (Prof. dr R.M. Bertina) at the Leiden University Medical Centre, Leiden. Part of the work was done in collaboration with the Gaubius Laboratory, TNO-PG, Leiden (Prof. dr C. Kluft) and the Amsterdam Thrombosis Service and Laboratory for General Practitioners (dr L.P. Colly) and the Centre for Hemostasis, Thrombosis, Atherosclerosis and Inflammation Research, Academic Medical Centre of the University of Amsterdam (Prof dr H. R. Büller). Further, she conducted with dr C.J.M. de Groot a case control study on the association of coagulation defects and pre-eclampsia.

In april 1997 she started her residency training programme in Obstetrics and Gynecology at Groene Hart Hospital, Gouda (head: dr. M. Helfferich) and from 1998 at the Leiden University Medical Centre, Leiden (head: Prof. dr H.H.H. Kanhai).

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