Rapid activation of haemostasis after hormonal emergency contraception

Marianne van Rooijen¹, Angela Silveira², Stella Thomassen³, Lars-Olof Hansson⁴, Jan Rosing³, Anders Hamsten², Katarina Bremme¹

¹Department of Woman and Child Health, Division of Obstetrics and Gynecology, and ²Department of Medicine, Atherosclerosis Research Unit, King Gustaf V Research Institute, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden; ³Department of Biochemistry, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, The Netherlands and ⁴Department of Clinical Chemistry and Pharmacology, University Hospital, Uppsala, Sweden

Summary
Hormonal emergency contraception (EC) is a well established contraceptive method, recommended to all women, although the effects on haemostasis are not fully evaluated. The aim of this study was to evaluate whether exposure to EC has effects on well established cardiovascular risk factors, and also to examine whether differences exist between two EC treatments. In a prospective randomized cross over design 11 women used two different EC methods, one with estrogen and levonorgestrel (EE-EC) and one with levonorgestrel only (LNG-EC). Plasma concentrations of haemostatic factors (APC resistance, anti-thrombin, fibrinogen, prothrombin fragment 1+2, free protein S, factor VII and PAI-1), sex-hormone-binding globulin (SHBG), the apolipoprotein (apo)B/apoA1 ratio and C-reactive protein (CRP) were followed frequently during the following 48 hours.

A rapid haemostatic activation was induced with both treatments, although more pronounced with EE-EC. Already two hours after EC, the plasma concentrations of haemostatic parameters and SHBG were significantly different from baseline concentrations. An ETP-based APC-resistance method showed increased APC resistance with EE-EC and decreased APC resistance with LNG-EC. The ApoB/ApoA1 ratio was affected in a favourable direction with EE-EC. CRP increased slightly regardless of treatment. Even a very short exposure to exogenous sex hormones causes prompt effects on hepatic protein synthesis and the coagulation system. This must be taken into consideration whenever exogenous steroid hormones are administered, especially to individuals with a genetic predisposition to thrombosis or transiently disturbed haemostasis.

Keywords
APC resistance, estrogen, levonorgestrel, SHBG

Introduction
Hormonal emergency contraception (EC) with a combination of estrogen and progestogen was first introduced in the 1970s. In the original method, known as Yuzpe’s method, a combination of 100 µg ethinylestradiol (EE) and 1 mg dl-norgestrel was administered twice with an interval of twelve hours (1). During the 1990s levonorgestrel (LNG) alone was shown to be more effective and have fewer side effects than the Yuzpe regimen (2). Therefore, the recommended regimen today is 750 µg LNG given twice with an interval of 12 hours (h) or 1.5 mg administered as a single dose. Irrespective of method, treatment has to be started within 72 h after unprotected intercourse.

Today EC is offered to all women asking for it, including women with risk of deep vein thrombosis (DVT), even though the effects of EC on the coagulation system remain to be fully evaluated. There is, as far as we know, only one published report on venous thromboembolism (VTE) in relation to EC; a case report on retinal vein thrombosis occurring the day after use of 1,000 µg norgestrel and 100 µg EE twice within 12 h (3). Conversely in a large American cohort study including 73,302 EC users without cardiovascular risk factors no case of VTE was reported (4).

On the other hand, treatment with conventional combined oral contraceptives (COC), containing both EE and progestagen, is a well-known risk factor for VTE. COCs elevate the plasma
concentrations of coagulation factors and initiate changes in the natural anticoagulant system. The effect on haemostatic parameters is more pronounced with compounds containing higher estrogen dosages. The haemostatic changes during COC use are also dependent on the type of progestin used, i.e. the progestin is supposed to modify the estrogenic effect of EE on haemostasis (5, 6). Plasma from women using more estrogenic COCs (so-called third generation COCs which contain the progestins desogestrel or gestodene) is less sensitive to the anticoagulant effect of activated protein C (APC), i.e. is more APC-resistant than that of women using less estrogenic COCs (so-called second generation COCs containing LNG) (6). APC resistance in the presence (7) and absence of FV Leiden (8) as well as acquired APC resistance during oral contraceptive (OC) use (9) increase the risk of DVT.

Progestin alone used in different hormonal treatments has been reported to change haemostatic parameters, particularly when administered at a very high dose, e.g. as in treatment of gynaecological malignancies (10). However, the role of progestin treatment in promoting VTE risk in these situations is difficult to assess, since gynaecological malignancies themselves enhance the thromboembolic risk. Use of progestogen-only contraceptives, i.e. pills (POP) or implants, is not associated with thrombotic complications (11) and seems to be a good contraceptive method for women with increased risk of VTE (12).

The aim of the present study was to evaluate whether the short exposition to steroid hormones during EC has effects on the haemostatic system and to examine whether differences exist between EC preparations with and without EE. Since the rate at which the lipoprotein profile changes during hormonal therapy is unknown, we also studied the effects of the two different EC methods on serum apolipoprotein B (apoB) and A1 (apoA1) concentrations and their ratio. The latter, in particular, is now considered as an accurate lipoprotein-related predictor of cardiovascular disease (13).

Methods

Study design and subjects

Twelve healthy volunteers with regular menstrual periods were included in this prospective randomised cross-over study. Inclusion criteria were: regular menstrual periods, age less than 35 years, body mass index (BMI) 20–25 kg/m², and no contraindications to COCs. Hormonal contraceptives, pregnancy or breast feeding were not allowed within two months before the start of the study. Except for EC no other medication was allowed during the study period.

Before randomization, a pregnancy test and fasting blood samples were obtained in a normal menstrual cycle during cycle days 5–8. Participants were then randomly assigned to use one of two EC preparations at the following ovulation time. The trial medications were either a combination of 100 µg EE and 500 µg LNG (EE-EC) or 6.5 mg Torecan® (tietylperazene) together with the second dosage of each treatment. All women were served the same kind of food during the first 12 h after the second dose. The assigned EC was used for one menstrual cycle. As menstrual disturbances are common after EC use, all women had a washout period of at least one normal menstrual cycle before treatment with the alternate EC at ovulation time.

To optimize the time point for the EC treatment, sticks were used for self-measurement of the luteinizing hormone (LH)-peak (Clearblue®, Unipath Ltd, Bedford UK) from menstrual cycle day 10 to ovulation. At ovulation time the trial medication was taken twice at an interval of 12 h. Blood sampling took place before each dispensation of the medication and 2, 4, 8, 12, 24 and 48 h after the second dose. The first dose was distributed at 8 p.m. on day 1, the second at 8 a.m. the next morning (i.e. day 2). A second pregnancy test was performed three weeks after each treatment cycle to exclude pregnancy.

The local ethics committee at the Karolinska University Hospital approved the study, and all women gave their informed consent to participation.

Blood sampling

Venous blood samples were drawn from an antecubital vein after 15 minutes (min) rest in the sitting position. All samples taken in the morning, i.e. before the second dose and after 24 and 48 h, were fasting samples. The blood samples for analysis of coagulation factors were collected in vacutainer tubes containing citrate (0.13 mM) and immediately centrifuged at 2,000 g for 20 min. After removal of the cells, the plasma was re-centrifuged for another 20 min at 2,000 g. Samples for plasminogen activator inhibitor-1 (PAI-1) activity determination were drawn into acidified citrate tubes (Stabilyte, Biopol, Sweden). Blood for serum preparation was collected in plain vacutainer tubes without anticoagulants and kept at room temperature for 1 h before centrifugation at 2,000 g for 10 min. Cell-free plasma and serum samples were stored at −70°C until analysed.

Laboratory methods

C-reactive protein (CRP), apoA1 and apoB were analyzed in serum by high-sensitivity methods using particle-enhanced immunonephelometry (Behring Nephelometer Analyzer, BN Prospec; Dade Behring GmbH, Marburg, Germany) with an inter-assay variation <4% for all assays. Sex-hormone-binding globulin (SHBG) was measured using the Immulite 1000 assay (Diagnostic Product Corporation, Los Angeles, CA, USA). Prothrombin fragment 1+2 (F1+2) was determined by an enzyme immunoassay (Enzygnost F1+2 micro Dade Behring, Marburg, Germany). Fibrinogen was determined with the IL Test Fibrinogen C kit from Instrumentation Laboratory (Spa, Milan, Italy). Reference Plasma 100% from Immuno AG (Vienna, Austria) was used as control for inter-assay variation with the following coefficients of variation (CVs): 3.7% (F1+2) and 3.6% (fibrinogen). PAI-1 activity was measured by an immunoassay (Chromolize PAI-1, Biopool International, Umeå, Sweden). Inter-assay CV for the Fibrinolysis Reference Plasma (Biopool) was 5.2 %. Protein S was determined with the Coaliza Free Protein S kit, from Chromogenix Laboratory (Spa, Milan, Italy), with inter-assay CV of 8%. The heparin cofactor activity of antithrombin (AT) was quantified with the Coamatic antithrombin kit from Chromogenix with an inter-assay CV of 3%. Activated factor VII (FVIIa) was determined according to Morrissey (14). APC-sensitivity ratios (APCsr) were determined with the APTT-based
Coatest APC Resistance C from Chromogenix. CVs for the normal and abnormal control plasmas provided with the kit were 4.1% and 2.4%, respectively. Normalized APCsr (nAPCsr) were determined with the ETP-based APC-resistance test in which the effect of APC on the time integral of thrombin generation (the endogenous thrombin potential, ETP) was quantified via measurement of end-point levels of $\alpha_{2}$-macroglobulin-thrombin complex (15). The inter-assay coefficient of variation for measuring nAPCsr was 6.9%.

Statistical methods
All statistical analyses were based on the blood sample drawn before the first treatment dose and the blood samples drawn after the treatment was completed. The data were analyzed using procedure Mixed in SAS® (16,17). A two-way repeated-measures ANOVA was performed with treatment (LNG-EC, EE-EC) and time (before treatment, 2 h, 4 h, 8 h, 12 h, 24 h, 48 h) as within-subjects variables. Patients with missing data were included in the model (30 results (or 1.8%) were missing out of 1,694 possible). The mixed procedure can accommodate missing data, assuming that data are missing at random. The treatment interaction refers to the statistical test of whether the mean change over time is the same for the two treatments. In case of a significant interaction, simple effects were examined, i.e. effects of one factor holding the other factor fixed. If the F-ratio for the time factor was significant, the six means obtained after 2, 4, 8, 12, 24 and 48 h were compared with the mean value before treatment. Since the distribution of some variables were positively skewed, log-transformation or reciprocal transformation (-1/x) were performed before analyses. $p<0.05$ was considered statistically significant.

Results
One of the women discontinued the trial due to pregnancy in the medication-free-menstrual cycle (wash-out period). Of the remaining 11 women, six started with EE-EC, and five with LNG-EC. Baseline clinical characteristics (median; range) of the study group were: age (28; 21–34 years), weight (62; 54–84 kg), BMI (21.5; 20.2–24.9 kg/m²) and blood pressure (range 95/68 – 130/80 mmHg).

| Table 1: Effects of EC on haemostatic markers, SHBG, CRP and apolipoproteins. | Values are median (interquartile range). | LNG-EC: levonorgestrel only emergency contraceptive, EE-EC: ethinyl-estradiol and levonorgestrel containing emergency contraceptive, APCr: activated protein C resistance, nAPCsr: normalized activated protein C sensitivity ratio, F1+2: prothrombin fragment I+2, FVIIa: activated factor-VII, PAI-1: plasminogen activator inhibitor 1, SHBG: sex-hormone-binding globulin; CRP: C-reactive protein, apoA1: apolipoprotein A1, apoB: apolipoprotein B. $p<0.05$, $p<0.005$, the p-values indicate values significantly different from baseline, i.e. before the first treatment dose. $\Delta p<0.05$, $\Delta p<0.005$ the p-values indicate statistically different results with the two treatment regimens. a) no difference between treatment regimens (no treatment interaction). Therefore, no separate analysis of the two treatments was performed. b) reciprocal transformation before statistical analyses. c) log transformed before statistical analyses. |
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APC resistance determined with aPTT- and ETP-based APC-resistance tests
APC resistance increased with both tests after intake of EE-EC – as reflected by the decreased APCsr values with the aPTT-based test and increased nAPCsr values with the ETP-based test. No treatment interaction was detected for the aPTT-based APCtest; i.e. there was no difference in response during the different EC regimen, although there was a change over time (Table 1). With the ETP-based test, a difference between treatment regimens was evident. The nAPCsr decreased at 12 h after the second dose of LNG-EC and remained different from baseline throughout the study period, whereas treatment with EE-EC lead to an increased nAPCsr.

Antithrombin
The median plasma concentrations of AT at baseline were 109.5% for LNG treatment and 106% for the EE-LNG treatment. Both treatments initially resulted in a reduction of AT, with the lowest plasma levels attained already two hours after the second dose; median values 98% with LNG-EC and 90.5% with EE-EC. EE-EC resulted in a sustained decrease in AT, while after LNG-alone treatment AT reverted to baseline levels at 24 h and further increased at the end of the test period. However, the difference between the treatments was statistically significant only at the last sampling point (Table 1).

Fibrinogen
The median plasma fibrinogen concentration, which before treatment was 2.65 g/l for LNG-EC and 2.50 g/l for EE-EC, showed a slight decrease immediately after treatment followed by an increase, which was most pronounced with EE-EC. There were significant differences between the effects of LNG-EC and EE-EC on the plasma fibrinogen concentrations at 24 and 48 h after treatment, with the combined preparation showing a more pronounced procoagulant pattern. At the last study time-point (48 h), the plasma concentrations had increased up to 2.9 g/l for LNG-EC and 3.6 g/l for EE-EC (Table 1).

Additional haemostatic parameters
For plasma F1+2, free protein S, FVIIa and PAI-1 no treatment interactions were detected; i.e. there was no difference in response with the different EC regimen, although there were changes over time. The median plasma concentrations F1+2 before treatment were 147 nmol/ml for LNG-EC and 171 nmol/ml for EE-EC. The plasma concentrations had increased already two hours after treatment and remained increased up to 12 h. The highest median levels were recorded 8–12 h after medication; thereafter a distinct decrease occurred. The highest median plasma FVIIa concentrations were 4.7 ng/ml on EE-EC after 12 h and 5.2 ng/ml on LNG-EC after eight hours of treatment.

For PAI-1, a significant decrease in plasma concentration was detected at eight hours compared with baseline. The plasma concentrations subsequently increased from a baseline value of 1.24 IU/ml to a maximum of 2.02 IU/ml for EE-EC and from 1.57 IU/ml to 3.64 IU/ml for LNG-EC at 48 h after treatment.

SHBG
The median baseline SHBG concentration in serum before treatment was 60.8 mM for LNG-EC and 53.8 mM for EE-EC (Table 1). With EE-EC, the serum concentration did not change until 24 h after the second dose, when a significant increase occurred which persisted until the end of the study period. After LNG-EC the serum concentrations rapidly fell below baseline level and remained low throughout study period with a minimum median level of 52.5 mM at 48 h.

CRP
The median baseline CRP concentrations before treatment were 0.54 mg/l in the EE-EC group and 0.46 mg/l in the LNG-EC group (Table 1). Compared with baseline levels, the serum levels of CRP started to increase already at +8 h and the increase was significant at 24 and 48 h, in both groups. No statistically significant differences between the two treatment regimens were noted (Table 1).

Apolipoproteins B and A1
The median baseline serum concentrations for apoB were 0.66 g/l for women treated with EE-EC and 0.68 g/l for women treated with LNG-EC. The concentrations changed significantly during treatment with an initial significant decrease followed by a slight increase. At 48 h the median plasma apoB level was 0.65 g/l in the EE group and 0.74 g/l in the LNG-alone group. In contrast, the serum levels of apoA1 initially decreased from the baseline levels of 1.58 g/l for LNG-EC and 1.72 g/l for EE-EC. At four hours the serum concentrations started to increase with EE-EC, whereas they remained decreased with LNG-EC.

The different alterations in serum apoB and apoA1 concentrations lead to changes in the ratio of apoB/apoA1 with both ECs. The median baseline level of the ratio was 0.43 in both groups. EE-EC caused the ratio to decrease at eight hours and remain significantly lower throughout the study period, whereas LNG-EC caused an increase, which was only significant after 48 h at which the median ratio was 0.50 (Table 1).

Discussion
The purpose of the present study was to compare the effects of two different EC preparations, containing either estrogen and progestogen or progestogen alone, on known cardiovascular risk factors, i.e. the haemostatic balance, CRP and apolipoproteins A1 and B. To the best of our knowledge the present study is the first to thoroughly evaluate haemostatic changes and liver-related changes in serum levels of CRP and apolipoproteins during EC performed in a cross-over design.
The serum level of SHBG is a well known mirror of the estrogen level in serum. Administration of exogenous estrogen causes a rapid increase in serum level of SHBG (18), whereas LNG-EC is associated with a decrease in SHBG after 24 h (19). In the present study the plasma concentrations of SHBG increased after EE-EC, to a level similar to that described for COC containing 30 µg EE and 150 µg LNG (6), and after LNG-EC the SHBG concentration rapidly decreased and remained low. These changes support the notion that SHBG is a marker of the overall estrogenicity of different oral contraceptives (6).

An increase in serum CRP concentration was described during use of COC as well as during hormonal replacement therapy, and has been supposed to reflect the EE-induced increase of the hepatic protein synthesis rather than a general inflammatory response (20, 21). The progestogen component in COCs seems to modify the estrogen effect on CRP synthesis in the same way as it modifies the haemostatic effects of estrogen (5). No reports are available on the effects of progestogen alone on serum CPR neither during contraceptive or hormonal replacement therapy. In the present study we observed that both EE-EC and LNG-EC cause an increase in serum CRP concentrations, that started already at eight hours after treatment. The time-pattern of CRP increase is similar to the one seen after experimental induction of CRP-synthesis or after start of an inflammatory process (22). This strongly indicates that the increase in serum CRP was secondary to a de-novo synthesis of CRP and not from a release of intracellularly stored CRP.

The lipid parameters evaluated in this study, apoA1 and apoB, were chosen because they were recently recognized as risk markers for cardiovascular disease. The apoB/apoA1 ratio decreased with EE-EC but increased with LNG-EC, in agreement with the notion that the lipid pattern improves with estrogen therapy (23). The rapid effect on the apolipoproteins is an expected consequence of the prompt effect of steroid hormones on the liver protein synthesis. The clinical relevance of the changes in CRP and apolipoproteins after EC use in the perspective of atherosclerosis is probably negligible, but they highlight that steroid-induced changes in liver protein synthesis occur rapidly even after a very brief exposure.

There are only few studies on haemostatic changes during use of EC. No differences in FVII and AT were detected 1, 3 or 7 days after treatment with 100 µg EE and 500 µg LNG twice in 12 h (24). A small study with 5 mg EE daily showed a pronounced decrease in AT after four to five days, which returned to normal values after 12 days (in one woman the plasma level AT dropped to 0.60 U/ml, which is within the range that is associated with an increased risk of thromboembolism) (25).

In the present study we observed that procoagulant alterations, as far as it concerns AT and fibrinogen, were more pronounced with EE-EC than with LNG-EC. The lowest level of AT was attained at two hours after treatment, although still within normal range. At that time point the plasma AT concentrations were lower than levels observed in women using COC but reversed after two more hours to a level corresponding to changes previously described for COC (6). The decrease in AT was partially counteracted by a decrease in PAI-1, suggesting an increased endogenous fibrinolytic activity. The increase in plasma fibrinogen concentration was slower, but 48 h after treatment levels were reached that were similar to those reported during COC use (6).

We have also observed that, due to treatment with EC, the plasma concentrations of free protein S, F1+2 and FVIIa as well as the aPTT-based APCsr changed in a direction that is indicative of a more procoagulant state. There were, however, no statistically significant differences between the treatment regimens. An increase in free protein S has been previously described in a study comparing effects of LNG COCs and LNG POP on haemostatic markers (5). Studies including women using POP mainly showed no effects on haemostatic parameters, although some investigators reported small changes in certain parameters (5, 26). The response of FVIIa to the treatments is difficult to evaluate because FVIIa increases in the postprandial period (27) and the women had regular meals during the study days.

With the ETP-based APC-resistance test, an assay which is particularly sensitive to changes in the hormonal status (28), a distinct difference between the estrogen-containing preparation and the preparation without estrogen was detected, with increased APC resistance after EE-EC and decreased APC resistance after LNG-EC. The changes in nAPCsr after EE-EC were similar to those described in users of LNG containing COCs (29, 30), and the level after LNG-EC decreased as described in users of LNG POPs (30). The degree of APC resistance in some COC users is as pronounced as in carriers of the FVLeiden mutation (29). The APCsr obtained with this test correlate remarkably well with the risk of VTE reported in epidemiological studies (31). On the basis of these observations it has been postulated that acquired APC resistance may explain the thrombotic effect of EC.

During use of COC VTE mainly occur during the first year (32), and no data are available on the number of thromboembolic events that occur already within a few days after initiation of treatment. Women with thrombophilies, who often present an already activated coagulation system, have COC-associated VTE not only more often, but also sooner than other women (32). The effects of COCs and FVLeiden on the APC-sensitivity ratio are additive (33). A large cohort study of EC users provided some reassurance that the risk of VTE attributable to EC was not substantially higher than the risk for users of traditional OC, despite the higher content of both estrogen and progesteron (4). In the approach of giving EC to all women, without considering the presence of thrombophilias, the LNG-EC should be considered as safer.

In summary, the present study indicates that even a very short and rather high exposure to exogenous sex hormones causes a prompt effect on hepatic protein synthesis. Already two hours after administration of EC AT, fibrinogen and F1+2 as well as SHBG circulate at concentrations which deviate significantly from baseline. Activation of protein synthesis also involves increase in some important procoagulant factors, which triggers the coagulation cascade and initiates an activation of coagulation and fibrinolysis.

The rapid effect on haemostasis might be of importance in various clinical situations – whenever exogenous steroid hormones are administered, even in short treatment situations like this, the risk for VTE should be considered. For individuals or patients with a genetic predisposition or a transiently disturbed haemostatic balance even small changes like the ones seen in the present study might be harmful.
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References