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Endogenous thrombin potential changes during the first cycle of oral contraceptive use☆,☆☆

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Abstract

Objectives: Venous thromboembolism (VTE) risk increases within months of combination oral contraceptive (COC) initiation. Because elevated endogenous thrombin potential (ETP) has been found in several studies to be a VTE risk factor, we evaluated the extent of ETP changes during the initial cycle of an ethinyl estradiol (EE) and levonorgestrel (LNG) COC. We also assessed the relationship between ETP changes and systemic EE and LNG concentrations.

Study design: Participants provided multiple blood samples during a first 21-day cycle of a 30-mcg EE/150-mcg LNG COC and after a further 7 days without an active COC. Thrombin generation measured with and without addition of activated protein C (APC) yielded ETP_{+APC} and ETP_{-APC} and the normalized APC sensitivity ratio (nAPCsr). EE and LNG pharmacokinetic analyses were conducted over 24 h after the first COC tablet and again at steady state.

Results: Thrombin generation was determined in 16 of the 17 women who completed the study. Mean ETP_{-APC} increased steadily to 21% above baseline at 24 h after the 6th COC tablet ($COC6_{24}$; $p<.001$) and to 28% above baseline at steady state (COC21; $p<.001$). The percentage increase in mean ETP_{+APC} was considerably more — 54% at $COC6_{24}$ and 79% at steady state. Mean nAPCsr increased by 28% at $COC6_{24}$ and by 41% at steady state. Higher concentrations of EE or LNG were not correlated with greater increases in ETP.

Conclusions: ETP increases during the first COC cycle were substantial.

Implications: The early increases in ETP may provide biological support for the rapid increase in VTE risk during initial COC use. The lack of association between this clotting system perturbation and the systemic EE concentration is surprising and deserves further study.

Keywords: Oral contraceptives; Venous thromboembolism; Endogenous thrombin potential; Protein C

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☆☆ Conflicts of Interest: Dr. Westhoff receives honoraria as a data safety and monitoring board member from Merck and Bayer, both of which produce oral contraceptives, however, not the oral contraceptive studied here. None of the other authors have any conflicts to report.

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1. Introduction

The risk of venous thromboembolism (VTE) increases within the first 3 months of combination oral contraceptive (COC) use and then gradually decreases between the first 3 months and 1 year [1–3], although this has not been invariably found [4]. Rosing et al. measured activated protein C (APC) resistance in 2 women and found increases during the first week of the first COC cycle [5]. Similar increases occurred in 6 women after receiving an emergency contraceptive containing 2 high doses of ethinyl estradiol (EE) and levonorgestrel (LNG) [6]. Other studies of hemostatic changes during COC use have not evaluated early changes [7,8]. The large "Seven-OC Study" [8] measured 24 hemostatic variables, including APC resistance, at baseline and after 3 and 6 COC cycles in 707 women. In that study, D-dimer concentration, a marker of fibrinolysis associated with future VTE risk [9,10], increased approximately 50% after 6 cycles of all COC regimens tested [8]. Factor VIII activity, independently associated with risk of VTE [11–13], increased approximately 20% after 6 cycles [8]. We recently evaluated D-dimer and factor VIII changes during the first COC cycle and found changes comparable to those seen with longer use [14]. The relationship between these observed changes in D-dimer and factor VIII to the increased VTE risk experienced among COC users has not been studied directly.

The measurement of thrombin generation (TG) via the Calibrated Automated Thrombogram (CAT) is an excellent tool to determine the "thrombotic-hemostatic function of the blood" [15]. Hemker et al. developed this method to measure the time course of TG initiated with tissue factor (TF) in platelet-poor plasma and proposed that the area under the TG curve, termed the *endogenous thrombin potential* (ETP), is a global measure of the clotting potential of blood [15]. ETP has been found to be associated with VTE risk [16–21]. Although ETP is strongly affected by COC use [5,22], there has only been a single study of the association of ETP with VTE risk in COC users [23]. The ETP laboratory test we use provokes TG under several standardized conditions, including with and without the addition of APC, and these are denoted ETP_{+APC} and ETP_{-APC}, respectively. APC is a natural anticoagulant protein generated in plasma after thombin activates protein C and which, supported by its cofactor protein S, dramatically reduces TG. The increased VTE risks associated with protein C and protein S deficiencies and with so-called *APC resistance* illustrate the importance of the protein C system in down-regulating coagulation [24–30]. During COC use, the normal reduction of TG with the addition of APC is mitigated, and COC use has thus been described as causing "acquired APC resistance" [5,31]. The Seven-OC Study found a 74% increase in the normalized APC sensitivity ratio (nAPCsr) during use of a 30-mcg EE/150-mcg LNG COC at 6 months but did not report the results for ETP_{-APC} or ETP_{+APC} [8]. In the present analysis, we evaluated the changes in TG during the first cycle of use of this COC.

Because epidemiological studies show that COCs with higher doses of EE are associated with a greater increase in VTE risk [32,33], we also explored whether a woman's systemic EE concentration during the first COC cycle was related to the magnitude of her TG changes.

2. Materials and methods

2.1. Study population and blood collection

This single-arm, open-label pilot study took place at Columbia University Medical Center (CUMC) after institutional review board approval. We have previously reported details of the study [14,34]. Briefly, participants provided written informed consent prior to enrollment and were aged 18–35 years and self-identified as White. We excluded any women with medical contraindications to COC use [35]. Additional exclusion criteria included medication use known to affect the CYP450 system, injectable contraception in the past 6 months or other hormonal contraceptive use within the past month, pregnancy within the past 6 weeks, smoking and a body mass index $\geq$30.0 kg/m^2.

The study COC contained 30-μg EE and 150-μg LNG packaged with 21 active and 7 placebo tablets (Portia®, Teva Pharmaceuticals, Philadelphia, PA, USA). Treatment began within 7 days of the start of menses. Each participant selected a particular time to take her daily COC; we directly observed COC intake at this particular time on study visit days. Participants underwent multiple blood draws to measure hormone and hemostatic variables over 4 weeks: immediately before each COC was taken on days 1 ($COC1_0$), 2 ($COC1_{24}$), 3 ($COC2_{24}$), 4 ($COC3_{24}$), 7 ($COC6_{24}$) and 21 ($COC20_{24}$ = $COC21_0$) and at the same time on day 22 ($COC21_{24}$) and on day 28 (COC28). Each participant also returned to take a single COC pill within the first 5 days of her next spontaneous menses, and we collected blood samples over the following 4 days. Participants sat quietly for 30 min prior to each blood draw, which the phlebotomist performed using a 21-gauge butterfly needle in the antecubital vein. We admitted each participant for 24 h on days 1 and 21 to collect 14 timed samples for pharmacokinetic analyses of EE and LNG. All study visits occurred in winter 2012–2013.

Samples for clotting factor analyses were collected in a citrated vacutainer and centrifuged at 1200 × *g* at 4 °C for 10 min; plasma was then frozen in 1-mL aliquots at −80 °C. Normal pooled plasma used as a reference in this study was collected in Maastricht by pooling plasma of 23 healthy individuals with an average age of 34.7 years (13 men and 10 women among whom were 2 COC users) as previously described [36].

2.2. Laboratory methods

We measured ETP by CAT [36] in wells of a microtiter plate (total volume 125 μL) containing 80-μL platelet poor