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Clotting Factor Changes during the First Cycle of Oral Contraceptive Use

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Abstract

Objectives—The risk of venous thromboembolism (VTE) is highest during the initial months of oral contraceptive (OC) use. We sought to evaluate the extent of hemostatic variable changes during the initial OC cycle and if such changes are related to systemic ethinyl estradiol (EE2) exposure.

Study Design—Participants provided multiple blood samples during a 21-day OC cycle (30 mcg EE2; 150 mcg levonorgestrel) and after a single dose following a wash-out period. Analytes included D-dimer, factor VIII activity, protein C total antigen and the hepatic proteins corticosteroid- and sex-hormone-binding globulins (CBG and SHBG). EE2 pharmacokinetic analyses related to the 24 hours after the first OC tablet (OC1) and at steady state (OC21).

Results—Seventeen women completed the study. D-dimer more than doubled by OC6 ($p = 0.013$) and remained elevated at OC21 ($p=0.012$). D-dimer levels within women varied widely from day-to-day. Factor VIII increased 27% by OC2 ($p < 0.001$), but declined to a 9% increase by OC21. Protein C increased only 6%. EE2 steady-state area-under-the-curve ranged from 488 to 1103 pg·h/mL; higher levels were not correlated with greater increases in clotting variables. CBG and SHBG increased significantly, but were not significantly correlated with levels of EE2 or with the hemostatic variables.

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Conclusions—D-dimer increases during the first OC cycle were at least as great as increases seen with longer OC use. These results provide support for the increased VTE risk during initial OC use. The extreme variability in D-dimer levels may be an important component of this risk.

Keywords

oral contraceptives; venous thromboembolism; D-dimer; factor VIII; protein C

1. Introduction

The risk of VTE within the first 3 months of oral contraceptive (OC) use may be more than double the risk after the first year, with the risk gradually decreasing between the first 3 months and 1 year [1–3], although this has not been invariably found [4]. Despite this, hemostatic variable changes before 3 months of use have not been reported. We therefore designed the study reported here to measure hemostatic changes during the first OC treatment cycle.

Numerous studies have assessed the effects of OC use on the coagulation system [5–7]. The large ‘Seven OC Study’, measured 24 hemostatic variables after 3 and 6 OC cycles in 707 women [6]. D-dimer concentration, a global marker of fibrinolysis associated with future venous thromboembolism (VTE) risk [8, 9], increased approximately 50% after 3 and 6 cycles of all OC regimens [6]. Factor VIII activity, another independent risk factor for VTE [10–15], increased approximately 20% after 3 and 6 cycles [6]. Neither EE2 dose or progestin type had a clear effect on these increases. The significance of the observed changes in D-dimer and factor VIII to the increased VTE risk among healthy OC users has not been studied. We chose to measure D-dimer concentration and factor VIII activity levels due to their association with risk of future VTE and their change with OC use.

OCs may also dis-equilibrate the coagulation system through increased synthesis of hepatic proteins. Protein C, a hepatic clotting factor, increased ~15% after 3 and 6 OC cycles [6]. We chose to measure protein C total antigen as our representative hepatic clotting factor, even though it is an anti-coagulant, as its short half-life (6–7 hours) [16, 17] may facilitate detection of short-term changes. We also studied how changes in these measures correlated with corticosteroid-binding globulin (CBG) and sex-hormone-binding globulin (SHBG) [18, 19].

Epidemiological studies show that higher OC doses of EE2 are associated with a greater increase in VTE risk [20–22]. We, therefore, also explored whether a woman’s systemic EE2 level during the first OC cycle was related to the magnitude of her clotting system changes.

2. Materials and methods

This single-arm, open-label pilot study took place at Columbia University Medical Center (CUMC) after Institutional Review Board approval. Participants provided written informed consent prior to enrolment. Women were eligible if aged 18–35 years and self-identified as white. We excluded women with any medical contraindication to use of OCs [23].

Additional exclusion criteria included: use of medications known to affect the CYP450 system; use of injectable contraception in the past 6 months or use of other hormonal contraceptives within the past month; pregnancy within the past six weeks; smoking; and a body mass index ≥ 30.0 kg/m^2. We instructed participants to abstain from ibuprofen, aspirin and grapefruit juice throughout the study, alcohol within 24 hours, and caffeine within 1 hour of study visits as suggested by the European Concerted Action on Thrombosis Manual [24].

The study OC contained 30 mcg EE2 and 150 mcg levonorgestrel (LNG) packaged with 21 active and 7 placebo tablets (Portia®, Teva Pharmaceuticals, Philadelphia, PA). Treatment began within 7 days of the start of menses [25]. Participants selected a particular time to take her daily OC, and we directly observed OC intake at this particular time on study visit days. Participants underwent multiple blood draws to measure hemostatic variables over 4 weeks immediately before each OC was taken on days 1 ($OC1_0$), 2 ($OC1_{24}$), 3 ($OC2_{24}$), 4 ($OC3_{24}$), 7 ($OC6_{24}$) and 21 ($OC21_0$); and at the same time on day 22 ($OC21_{24}$) and day 28. After completing the OC pack, each participant returned for a single OC pill at her next spontaneous menses and we collected blood samples over the following 4 days (noted as days 60–63). Participants sat quietly for 30 minutes prior to each blood draw using a 21 gauge butterfly needle in the antecubital vein. Each participant was admitted for 24 hours on days 1 and 21 to collect 14 timed samples for pharmacokinetic (PK) analyses, as previously described [25]. At each visit, participants answered questions about use of concomitant medications, caffeine/alcohol intake, and adverse events since the last visit. All study visits were conducted in winter 2012–2013, to minimize seasonal variation in hemostatic variables [26].

Samples for clotting factor analyses were collected in a citrated vacutainer and centrifuged at 3000 rpm at 4°C for 10 minutes; plasma was transferred and frozen in 1 mL aliquots at −80°C until analysis in batches. The ARUP National Reference Laboratories (Salt Lake City, UT) measured D-dimer concentration, factor VIII activity and protein C total antigen. D-dimer was measured by immunoturbidimetric assay using the STA Compact analyzer (Diagnostica Stago Inc., Parsippany, NJ); factor VIII activity by a clotting assay using the STA-R analyzer (Diagnostica Stago Inc., Parsippany, NJ) and protein C total antigen by an enzyme-linked immunosorbent assay (ELISA) using EIA Reader 520 (ARUP, Salt Lake City, UT). The within-run precision for each assay was 1.9% for D-dimer at levels around 2 mcg/mL, and 5.8% and 3% for factor VIII and protein C, respectively. The between-run precision for each assay was 0.9% for D-dimer at levels around 2 mcg/mL, and 4.6% and 5.0% for factor VIII and protein C, respectively. The lower limits of detection were 0.2 mcg/mL, 1% and 10% respectively. We set D-dimer results that were below the detection limit as 0.2 mcg/mL for analysis; this produces a conservative bias in the measurement of increases in low level D-dimer concentration with OC use.

The CUMC Biomarkers Core Laboratory performed CBG radioimmunoassays (IBL-America, Minneapolis, MN) and SHBG chemiluminescence immunoassays on an automated immunochemistry analyzer (Immulite 1000, Siemens Healthcare Diagnostics Inc., Deerfield, IL) from serum collected at baseline and on days 21 and 28 and after the wash-out period.

We measured EE2 serum concentrations using liquid chromatography-tandem mass spectrometry and conducted standard PK analyses [25].

The D-dimer, factor VIII and protein C measures were log-transformed for analysis, but all results are presented in the original units for ease of interpretation. We normalized factor VIII activity and protein C total antigen measurements to mean baseline values of 100% [6]. To reduce random variation at steady state we averaged the values of the hemostatic variables immediately before and 24 hours after OC21, except in Figures 1 and 2 where we show these values separately. We summarized the levels of hemostatic variables and binding globulins using descriptive statistics. We conducted matched-pairs t-tests to evaluate changes over time in D-dimer, factor VIII and protein C. We used linear regression to assess the relationship between (untransformed) steady-state 24-hour EE2 area-under-the-curve (EE2-AUC21) and the change in hemostatic variables from baseline to OC21 (note: absolute changes in log transformed values are equivalent to ratios of untransformed values). Confidence intervals for Pearson correlation coefficients (r values) were calculated using Fisher's z transformation. Statistical analyses were conducted using Stata 12 (StataCorp, College Station, TX). All significance levels (p values) quoted are 2-sided.

3. Results

Of 24 eligible women who consented to participate, we withdrew 3 due to scheduling conflicts. An additional 3 women withdrew after consent but prior to receiving study treatment: one due to fear of needles, one from use of an exclusionary medication and one withdrew consent. Another participant withdrew due to poor venous access at her first study visit. Thus, 17 women participated in this study completing 163 of 170 scheduled visits. Three participants missed the day 28 visit, and one participant missed the last four visits after the OC free period. Table 1 shows their baseline characteristics. The 7 participants who withdrew were similar in baseline characteristics to those who continued in the study.

All 17 participants took the first study OC within 7 days of the start of menses. Compliant OC use during the study was confirmed by questioning and observing that the CBG changes from baseline to day 21 were consistent with good compliance [27].

Table 2 shows measurements of the hemostatic variables over the study period. Mean (geometric mean of untransformed values) D-dimer concentration more than doubled from 0.31 mcg/mL at baseline ($OC1_0$) to 0.81 mcg/mL at day 6 (OC6; $p = 0.013$) and remained elevated at 0.72 mcg/mL at the last active pill (OC21; $p = 0.012$). Figures 1 and 2 show the increase and the substantial intra-individual variability in D-dimer levels. At OC21, 12 of the 17 participants had D-dimer values ≥ 0.4 mcg/mL, which exceeds the normal range of D-dimer for this laboratory and 10 had values ≥ 0.5 mcg/mL compared to only 3 of the 17 exceeding these values at baseline (10/17 vs 3/17; $p = 0.012$). D-dimer levels measured during OC use were poorly correlated with baseline D-dimer levels (Table 2). By 7 days after the last active pill was taken, mean D-dimer decreased to 0.45 mcg/mL (95% CI: 0.27, 0.75) not significantly different from the baseline value ($p = 0.28$). After the one-month wash-out, mean D-dimer concentration was 0.44 mcg/mL (95% CI: 0.27, 0.73; $p = 0.27$). D-dimer did not increase 24, 48 and 72 hours after taking the single pill after the 1-month

washout period (data not shown). EE2-AUC21 varied from 488 to 1103 pg·h/Ml (data not shown, [25]); change in D-dimer concentration from baseline to OC21 was somewhat correlated with the individual systemic EE2-AUC21 (r = 0.36; 95% CI: −0.21, 0.75; p = 0.21). Figure 3 shows that this association was, however, largely due to the 2 participants whose D-dimer levels decreased substantially after entry to the study; removing these individuals reduced this correlation substantially (r = 0.15; 95% CI: −0.46, 0.67; p = 0.65).

Factor VIII activity varied widely between individuals, but increased little from baseline to OC21 (p = 0.24) (Figure 4). Factor VIII did, however, increase briefly after the second and third OC tablets; at $OC2_{24}$ the mean increased to 126 (p < 0.001) and at $OC3_{24}$ to 127 (p = 0.011). Factor VIII levels during OC use were strongly correlated with baseline levels (Table 2). Seven days after the last active OC, mean factor VIII was 103. Following the single OC after the 1-month washout, factor VIII activity at 24 hours was 109, and then decreased to 107 and 103 at 48 and 72 hours (p = 0.065, 0.040 and 0.19, respectively). Systemic exposure to EE2 from the first OC (EE2-AUC1) was not correlated with the change in factor VIII activity from baseline to $OC3_{24}$ (r = 0.22; 95% CI: –0.29, 0.63; p = 0.40); analysis of factor VIII levels at $OC1_{24}$ and $OC2_{24}$ yielded similar results. Greater EE2-AUC21 was not associated with increases in factor VIII from baseline to OC21, rather the reverse (r = −0.57; 95% CI: −0.85, −0.06; p = 0.032).

We found little average change in protein C total antigen levels at any point in the OC cycle and little variability (Figure 5). Protein C levels during OC use were moderately to strongly correlated with baseline levels (Table 2). We found no association between EE2-AUC21 and the individual changes in protein C at OC21 (r = −0.13; 95% CI: −0.62, 0.43; p= 0.67).

The geometric mean CBG and SHBG increased from baseline to $OC21_{24}$ (52.4 to 135.1 mcg/mL, p < 0.001; and 44.7 to 73.4 nmol/L, p < 0.001, respectively), and all participants showed values consistent with good OC compliance. These increases were closely mirrored when calculations were made with untransformed values (50.7 to 140.7, and 46.5 to 77.2, respectively). Changes in untransformed CBG and SHBG from baseline to day 21 were unrelated to baseline values, and all further calculations with were made with untransformed values. The changes in CBG and SHBG levels were not statistically significantly correlated with EE2-AUC21, or with the changes in D-dimer, factor VIII or protein C.

4. Discussion

In this pilot study D-dimer levels increased gradually during a single cycle of OC use at least as much as the increases reported after 3 and 6 cycles of OC [6]. Similarly, we found a statistically significant increase in factor VIII activity after as little as 1–2 days of OC use; these transient increases were similar in magnitude to those reported after 3 and 6 cycles of OC use. We chose to focus on these particular factors because prospective studies have shown that both D-dimer and factor VIII levels are related to future VTE risk [8, 14]. These results may provide a physiological explanation to support the epidemiological studies that have reported an increased VTE risk among OC users seen even during the very first months of use [3]. As shown in Figure 2, participants demonstrated great variability in day-to-day D-dimer levels and in their apparent response to the OC with several participants

experiencing extreme increases in D-dimer; in this small study however we cannot assess whether a subset of women might be uniquely susceptible to an OC-mediated disequilibrium of hemostasis.

A number of epidemiological studies indicate that OC-related VTE risk is less in women using an OC with a lower dose of EE2 [20–22]. The level of EE2 in a tablet is, however, a poor indicator of a woman's individual exposure due to individual differences in absorption and metabolism. Among the women in this study, all taking the same OC, we found the expected greater than two-fold range in steady state 24-hour EE2 exposure (488–1103 pg·h/mL) [25]. However, contrary to expectation, higher individual EE2 exposures were not associated with greater changes in either D-dimer or factor VIII activity. These results may indicate that changes in D-dimer and factor VIII may not be the most relevant markers of OC-associated VTE risk. Future, larger studies should further evaluate EE2 exposure and consider its effect on other aspects of the clotting system.

Previous studies report moderate changes in protein C during OC use; however, we found no significant increases in protein C during the first OC cycle. Protein C (an anticoagulant factor) was of interest in this study because it is a hepatic protein with a short half-life (thus a brief study might be able to identify changes), and because of the hypothesis that the OC primarily affects coagulation due to an effect on hepatic proteins. We did not study other hepatic coagulation factors because their longer half-lives could reduce the chances of identifying a change within 21 days, particularly in such a small study. We found the expected increases in the hepatic proteins SHBG and CBG. The OC effects on hepatic proteins are demonstrably variable rather than uniform -- i.e., we found a small not-significant change in protein C, a larger, but still modest increase in SHBG and a much larger increase in CBG.

The small number of participants and small number of hemostasis variables tested are a substantial limitation of this pilot study. However, even in this small study we demonstrated that OC-mediated coagulation changes begin and are readily detectable during the first OC cycle – a finding that may be useful in the design of future studies. This study used an immunoturbidometric D-dimer assay, which is less sensitive than an ELISA assay. The D-dimer changes found here, however, were large enough to detect even with this less sensitive assay. Strengths of the study included a homogeneous sample and careful adherence to guidelines for collection of specimens for studying hemostasis, as well as deliberate minimization of menstrual cycle or seasonal variation.

SHBG increases have been suggested as a marker of VTE risk, an unsettled association [28–34]. In the present study, we found little association between SHBG or CBG increases and changes in the hemostatic variables or the systemic EE2 exposure. This limits our enthusiasm for SHBG as a surrogate variable in explaining aspects of OC-mediated VTE risk.

Short-term effects of the OC on hemostatic variables found here support reports of early increases in VTE risk from OC use. A short-term study such as this is relatively easier to

carry out than 3- or 6-month studies. This approach may thus be useful for the study of additional hemostatic variables, and for making comparisons among different OCs.

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Implications

This study showed that increases in D-dimer are clearly evident in the first cycle of OC use and may be larger than are seen after a longer duration of use, and thus provide biological support for the increased VTE risk during initial OC use found in epidemiological studies.

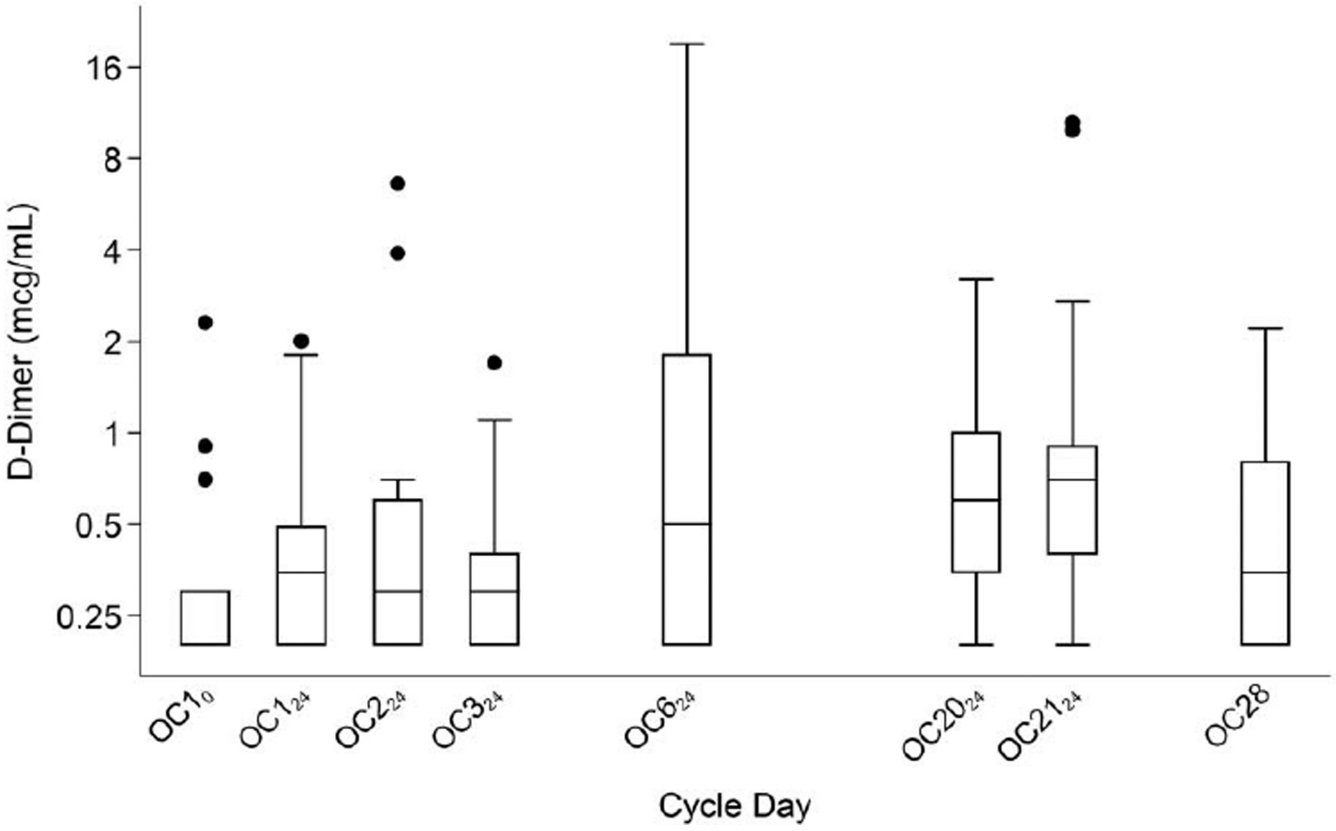

Fig. 1.

D-dimer levels during the OC cycle. Boxes show medians and interquartile ranges (IQR); lower whiskers denote the smallest values ≥ (25th percentile − 1.5 × IQR); upper whiskers denote the largest values ≤ (75th percentile + 1.5 × IQR); and individual points denote D-dimer values outside the whiskers [35]. Detection limit of the assay was 0.2 mcg/mL.

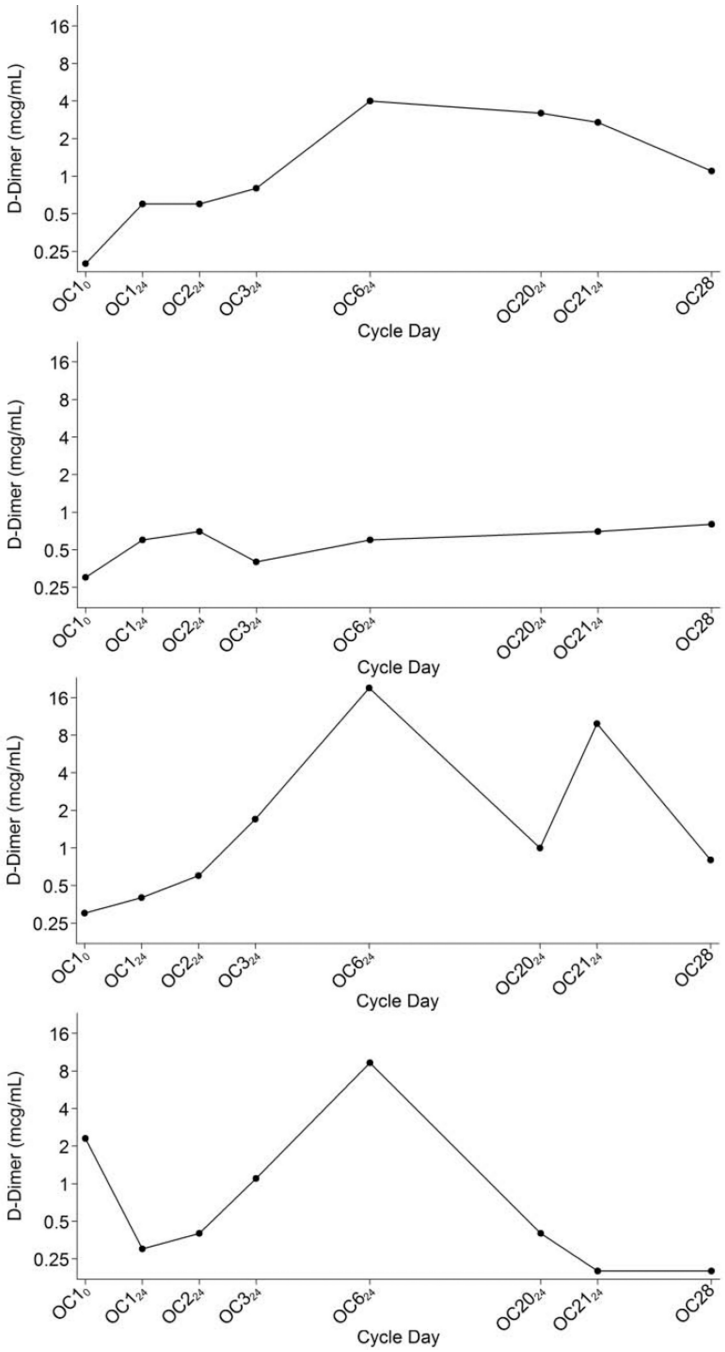

Fig. 2.
D-dimer levels of four representative individuals.

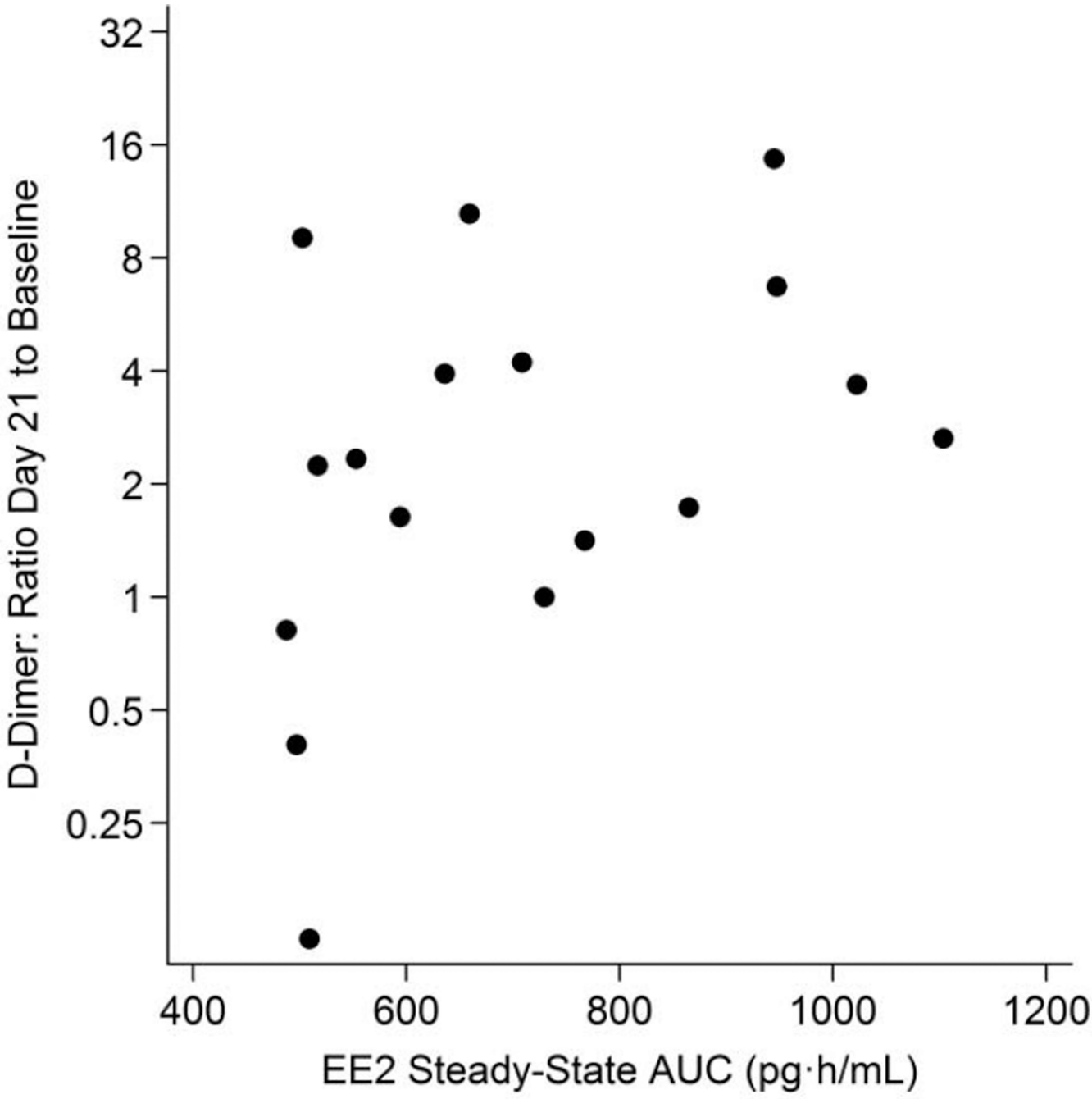

Fig. 3.
D-dimer changes from baseline to Day 21 versus ethinyl estradiol steady-state AUC.

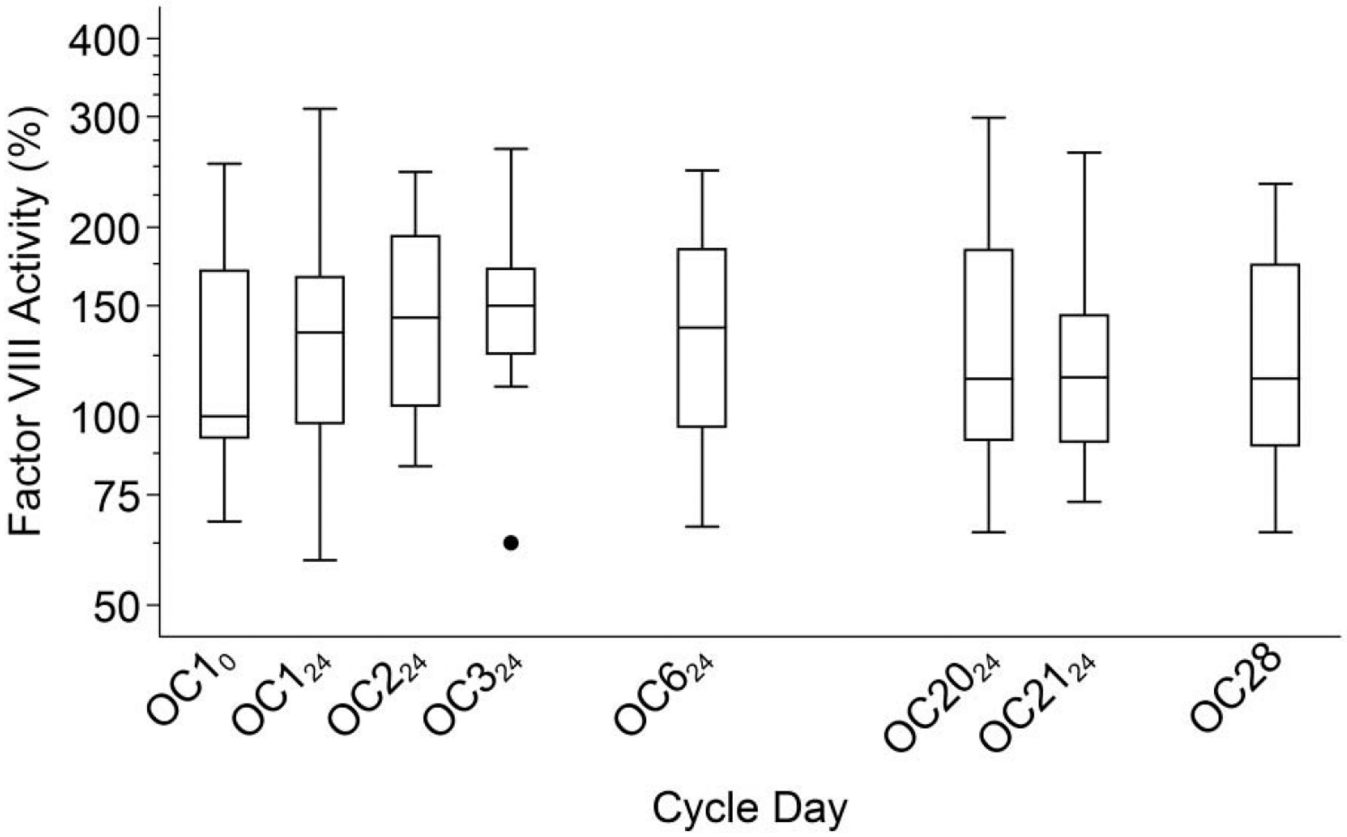

Fig. 4.

Factor VIII activity levels during the OC cycle. Boxes show medians and interquartile ranges (IQR); lower whiskers denote the smallest values ≥ (25^{th} percentile − 1.5 × IQR); upper whiskers denote the largest values ≤ (75^{th} percentile + 1.5 × IQR); and individual points denote D-dimer values outside the whiskers [35].

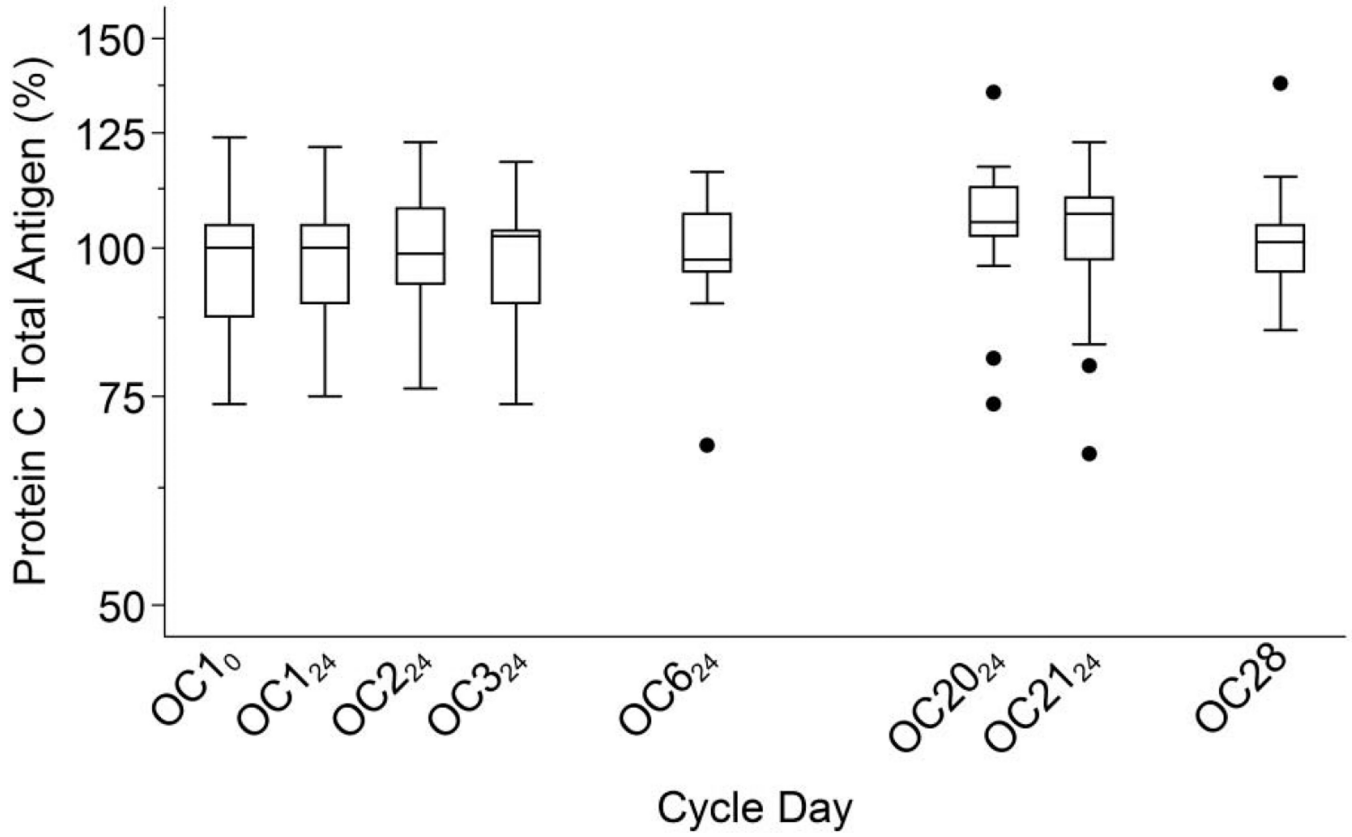

Fig. 5.

Protein C total antigen levels during the OC cycle. Boxes show medians and interquartile ranges (IQR); lower whiskers denote the smallest values ≥ (25^{th} percentile − 1.5 × IQR); upper whiskers denote the largest values ≤ (75^{th} percentile + 1.5 × IQR); and individual points denote D-dimer values outside the whiskers [35].

Table 1

Baseline characteristics of study participants (n=17).

Variable	Study Participants
Age	24.9 (±3.9)
Height (cm)	168.1 (±7.7)
Weight (kg)	63.9 (±10.5)
BMI (kg/m^2)	22.6 (±3.1)
Ever been pregnant	2 (11.8)
Ever given birth	0 (0.0)
Previously used OCs	11 (64.7)

Values are shown as mean (±SD) or n (%).

Table 2

Clotting factor values during the first OC cycle

Cycle Day	$OC1_0$	$OC1_{24}$	$OC2_{24}$	$OC3_{24}$	$OC6_{24}$	OC21[a]	OC28
D-dimer (mcg/mL)							
Geometric mean (±95% CI)[b]	0.31 (0.22, 0.44)	0.37 (0.25, 0.55)	0.42 (0.24, 0.71)	0.37 (0.26, 0.53)	0.81 (0.39, 1.71)	0.72 (0.46, 1.11)	0.45 (0.27, 0.75)
p-value[c]	-	0.47	0.31	0.36	0.013	0.012	0.28
Correlation coefficient[d] (±95% CI)[b,d]	-	0.24 (−0.29, 0.66)	0.18 (−0.33, 0.63)	0.36 (−0.15, 0.72)	0.30 (−0.21, 0.68)	−0.23 (−0.64, 0.28)	−0.16 (−0.64, 0.40)
p-value[d]		0.38	0.50	0.16	0.25	0.52	0.59
Factor VIII activity (%)							
Geometric mean (±95% CI)[b]	100 (81, 124)	109 (85, 141)	126 (105, 153)	127 (107, 149)	117 (94, 146)	109 (88, 135)	103 (82, 129)
p-value[c]	-	0.36	<0.001	0.011	0.076	0.24	0.92
Correlation coefficient[d] (±95% CI)[b,d]	-	0.66 (0.26, 0.87)	0.87 (0.67, 0.95)	0.61 (0.18, 0.84)	0.66 (0.26, 0.87)	0.74 (0.40, 0.90)	0.62 (0.13, 0.87)
p-value[d]		0.003	<0.001	0.008	0.003	<0.001	0.016
Protein C total antigen (%)							
Geometric mean (±95% CI)[b]	100 (93, 107)	101 (94, 108)	101 (94, 108)	101 (94, 108)	102 (95, 108)	106 (99, 114)	105 (98, 113)
p-value[c]	-	0.83	0.69	0.66	0.52	0.48	0.22
Correlation coefficient[d] (±95% CI)[b,d]	-	0.57 (0.12, 0.82)	0.67 (0.28, 0.87)	0.82 (0.56, 0.93)	0.70 (0.33, 0.88)	0.51 (0.04, 0.80)	0.56 (0.04, 0.84)
p-value[d]		0.015	0.002	<0.001	0.001	0.035	0.036

[a] OC21, average of values at t=0 and t=24 hrs;

[b] CI, confidence interval;

[c] Paired t-test against $OC1_0$;

[d] Correlation with $OC1_0$; CI and p-value calculated with Fisher's z-transformation of correlation coefficient.