CONTRACEPTION

Effect of a combined oral contraceptive containing 20 \(\mu g\) ethinyl estradiol and 75 \(\mu g\) gestodene on hemostatic parameters

JOSE´ MENDES ALDRIGHI1, LUIS SALVONI CARNEIRO DE CAMPOS1, OTÁVIO CELSO ELUF GEBARA2, CARLOS ALBERTO PETTA3, & LUIS BAHAMONDES3

1Department of Obstetrics and Gynecology, School of Medicine, Santa Casa of São Paulo, São Paulo, Brazil, 2Department of Cardiology, School of Medicine, University of São Paulo, São Paulo, Brazil, and 3Department of Obstetrics and Gynecology, School of Medicine, Universidade Estadual de Campinas (UNICAMP), Campinas, Brazil

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Abstract
The effects of a combined oral contraceptive (COC) containing 20 \(\mu g\) ethinyl estradiol (EE) and 75 \(\mu g\) gestodene (GSD) on prothrombin activity (PA), activated partial thromboplastin time (APTT), thrombin time (TT), platelet number, fibrinogen, antithrombin III (ATIII), protein C, protein S and D-dimer were evaluated over 6 months in 23 young, healthy women. Laboratory assessments were performed prior to initiation of COC use (pretreatment) and after 3 and 6 months of use. Results showed no significant changes in fibrinogen, protein C, ATIII or D-dimer during COC use, compared with pretreatment values. The increase in platelet count, decreases in protein S level, PA and APTT, and the prolongation of TT were significant. In conclusion, the use of a COC containing 20 \(\mu g\) EE and 75 \(\mu g\) GSD did not cause any significant changes in the hemostatic parameters studied that could be suggestive of a higher prothrombotic risk. Further studies with a larger sample size are necessary in order to obtain conclusive data.

Keywords: Combined oral hormonal contraceptives, hemostasis, prothrombotic risk

Introduction
The use of combined oral contraceptives (COCs) has been responsible for various adverse effects, among them venous thromboembolism (VTE) and pulmonary embolism, which are principally a consequence of the estrogenic component of the COC [1]. When the estrogen dose of the first contraceptive formulations was decreased from 75–100 \(\mu g\) of mestranol to 50 \(\mu g\) of ethinyl estradiol (EE), this resulted in a 25% decrease in deep VTE events. Subsequently, the dose was further decreased to 30–35 \(\mu g\) and, currently, COCs containing 15–20 \(\mu g\) EE are available on the market [2–5].

Studies performed at the beginning of the 1990s showed that users of low-dose COCs had a lower risk of developing VTE because they presented an adequate hemostatic balance, i.e., an increase in fibrinolytic activity as well as an increase in prothrombotic activity [6–8].

At the end of the 1990s, the progestins contained in the formulations of the so-called third-generation COCs were also implicated in the genesis of VTE because users of COCs containing desogestrel (DSG) or gestodene (GSD) presented a two-fold higher risk of VTE than users of COCs containing levonorgestrel (LNG) [7]. Jung-Hoffmann and Kuhl [9] succeeded in explaining the higher thrombogenic risk in users of COCs containing DSG or GSD, compared with users of COCs containing LNG, by demonstrating that GSD could inhibit cytochrome P-450 at the hepatic microsome and reduce the metabolism of EE that provoked increased estrogen serum concentrations.

Many studies have evaluated the influence of the progestin components of COCs on several coagulation and fibrinolytic parameters [5,10,11]. Nevertheless, there is little in the literature with respect to the possible synergism between GSD and EE in post-thrombotic changes [12–17]. Therefore, the objective of the present study was to evaluate risk markers for thromboembolic disease in users of COCs containing 20 \(\mu g\) EE and 75 \(\mu g\) GSD, based on measurements of hemostatic parameters: procoagulation factors (prothrombin activity (PA), activated partial thromboplastin time (APTT), thrombin time...
(TT), platelet count and fibrinogen), coagulation inhibitors (antithrombin III (ATIII), proteins C and S) and fibrinolytic system activation (D-dimer).

**Material and methods**

The study was conducted at the School of Medicine of the Santa Casa of São Paulo, São Paulo, Brazil and the protocol was approved by the Ethics Committee of that institution. All participants signed an informed consent form. Twenty-three healthy women requesting contraception were included and followed up for 6 months. The majority (92%) were white and between 18 and 33 years of age. Approximately half the participants had been delivered of one child, while the other half had two previous deliveries. Smokers and women with contraindication to COCs were excluded from the study [18].

All participants were instructed to use COC with 20 µg EE and 75 µg GSD (Femiane\textsuperscript{\textregistered}; Schering, São Paulo, Brazil), initiating pill intake on the first day of the cycle. Subsequent cycles were initiated after a 7-day pill-free interval. Clinical and laboratory assessments were carried out prior to initiation of medication (pretreatment) and after 3 and 6 months of COC use.

All participants were submitted to a blood collection from a forearm peripheral vein to perform the following laboratory examinations: PA, APTT, TT, platelet count, fibrinogen, ATIII, protein C, protein S and D-dimer. Other examinations were carried out prior to initiation of COC use and in the final cycle of the study, and included hemogram, serum glucose, urea, creatinine, alkaline phosphatase, calcium, total bilirubin and its fractions, total protein and its fractions, total cholesterol and its fractions, and triglycerides.

**Laboratory methods**

Blood samples were centrifuged at 2500 rev/min for 15 min to separate the platelet-rich and platelet-poor plasma. The platelet-poor plasma was used immediately for the measurement of PA, APTT, TT and fibrinogen; and its remaining aliquot was then frozen at −20°C and stored for a period of no longer than 30 days until the other tests were carried out (ATIII, protein C, protein S and D-dimer).

Platelet count was measured using an automated method (Celldyn; Abbott Laboratories, Abbott Park, IL, USA). PA was measured using the Simplastin Excel commercial kit (Organon Teknika Corp., Durham, NC, USA). APTT was measured using the Platelin LS kit (Organon Teknika Corp.). TT was evaluated using lyophilized bovine albumin that permitted measurement of the time required for the sample to coagulate after it was added to the plasma being tested. Fibrinogen was measured by the Fibriquik kit (Organon Teknika Corp.). ATIII was measured by a synthetic chromogenic substrate method (Stachrom ATIII; Diagnostica Stago, Gennevilliers, France) [17]. Proteins C and S were evaluated using the Staclot Protein C and S kits (Diagnostica Stago). D-dimer was evaluated using the D-Di Test kit (Diagnostica Stago).

**Statistical analysis**

The Friedman $\chi^2$ test for paired samples was used for numerical variables to compare values of the coagulation factors at three time intervals (pretreatment and after 3 and 6 months of COC use). If significant differences were found between the values at the three times, the Wilcoxon test for two correlated samples with Bonferroni correction was used. For the analysis of D-dimer, which is a qualitative variable, the Cochran test was used to evaluate differences in percentages of several categories at the three time intervals. Significance was established at $p < 0.05$. SPSS was the statistical package used for all the analysis.

**Results**

PA showed a significant decrease at 3 months of COC use, returning to pretreatment values at 6 months. APTT was significantly lower at 3 and 6 months of treatment compared with the baseline level; TT and platelet count were significantly higher at 3 and 6 months of treatment. Fibrinogen values were higher at 3 and 6 months of treatment but this difference was not statistically significant. Protein C showed no changes throughout the 6 months of treatment; however, protein S was significantly lower at 3 and 6 months of COC use. ATIII and D-dimer showed no changes throughout COC use (Table I).

**Discussion**

Several studies have reported changes in hemostatic balance in COC users [19–22]. However, a critical analysis of these trials showed methodological limitations such as heterogeneity of the populations studied, the laboratory examinations used in the evaluation, the type of COC evaluated, and frequent association with other risk factors such as smoking [21–23].

The estrogen components of COCs are the main ones responsible for the thromboembolic phenomena found in users of this kind of hormonal contraceptive, although, recently, progestins have also been implicated. Actually, both GSD and DSG can act synergically with EE to precipitate changes in hemostasis and coagulation [24], thereby justifying the interest in studying hemostatic variables in users of COCs containing associations of 20 µg EE with these progestins. Studies on COCs containing DSG have shown an adequate hemostatic balance, i.e. any increases in procoagulation parameters are counteracted by high fibrinolytic activity [7,14,25,26].
Studies on COCs containing GSD plus 20 μg EE are few and their results are not in agreement with those obtained in this study, i.e. minimal changes in coagulation function such as a reduction in prothrombin activity at 3 months of use returning to pretreatment values at 6 months [8,16,17,27–29]. Moreover, our results also showed a significant reduction in APTT during treatment, suggesting a tendency towards coagulation activation. However, oscillations in both variables remained within the normal range throughout the study.

There was also a change in TT, as shown by a significant prolongation at 3 and 6 months of COC use compared with pretreatment; however, the change was within the normal range. As TT evaluates the function of functional fibrinogen, it can be speculated that its stability throughout the study may preclude a possible tendency to procoagulation. In fact, fibrinogen values remained unchanged during the study period.

In agreement with previous studies, platelet count showed a significant increase at 3 and 6 months of COC use, albeit always within the normal range. Moreover, this increase cannot reflect a hypercoagulability risk [8,13,29–31] as platelet count per se does not imply a higher risk. Much more important from the clinical point of view would be to evaluate the status of platelet activation by, for example, studying P-selectin, a cell adhesion molecule, and some antigens that mediate the adhesion of neutrophils and monocytes to activated platelets and endothelial cells. In addition, P-selectin reactivity to a thrombotic stimulus could be evaluated. The failure to perform these tests could be interpreted as a limitation of our study.

In conclusion, the use of a COC containing 20 μg EE and 75 μg GSD for a period of 6 months in healthy women cannot explain the increased risk of thromboembolic disease; hence caution should be exercised in considering results from this kind of clinical trial.

Table I. Different variables studied at different times of exposure.

<table>
<thead>
<tr>
<th>Variable</th>
<th>T0</th>
<th>T3</th>
<th>T6</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA (%)</td>
<td>99.1 ± 2.6</td>
<td>94.6 ± 10.5</td>
<td>99.4 ± 2.7</td>
<td>0.041</td>
</tr>
<tr>
<td>APTT (s)</td>
<td>1.04 ± 0.09</td>
<td>0.96 ± 0.12</td>
<td>0.96 ± 0.09</td>
<td>0.058</td>
</tr>
<tr>
<td>TT (s)</td>
<td>16.7 ± 2.4</td>
<td>17.2 ± 1.3</td>
<td>17.1 ± 1.1</td>
<td>0.007</td>
</tr>
<tr>
<td>Platelets/ml</td>
<td>236.0 ± 62</td>
<td>258.2 ± 57</td>
<td>272.8 ± 59</td>
<td>0.012</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>271.7 ± 83.4</td>
<td>299.3 ± 77.3</td>
<td>315.5 ± 104.7</td>
<td>0.118</td>
</tr>
<tr>
<td>Protein C (%)</td>
<td>93.1 ± 3.1</td>
<td>91.2 ± 8.3</td>
<td>92.0 ± 2.9</td>
<td>0.926</td>
</tr>
<tr>
<td>Protein S (%)</td>
<td>92.4 ± 21.5</td>
<td>79.2 ± 28.1</td>
<td>87.9 ± 19.9</td>
<td>0.009</td>
</tr>
<tr>
<td>ATIII (%)</td>
<td>98.5 ± 17.6</td>
<td>99.3 ± 27.2</td>
<td>88.7 ± 17.2</td>
<td>0.344</td>
</tr>
<tr>
<td>D-dimer &lt;0.5</td>
<td>20 (87.0)</td>
<td>17 (73.9)</td>
<td>17 (73.9)</td>
<td>0.500</td>
</tr>
<tr>
<td>D-dimer ≥0.5</td>
<td>3 (13.0)</td>
<td>6 (26.1)</td>
<td>6 (26.1)</td>
<td></td>
</tr>
</tbody>
</table>

T0, pretreatment cycle; T3, evaluation at 3 months of treatment; T6, evaluation at 6 months of treatment; PA, prothrombin activity; APTT, activated partial thromboplastin time; TT, thrombin time; ATIII, antithrombin III; values are expressed as mean ± standard deviation except for D-dimer, which is expressed as n (%).
women with no associated risk factors caused no significant changes in hemostatic parameters suggestive of a higher prothrombotic risk. The clinical significance of these findings should be evaluated in a larger cohort of women with associated risk factors such as smoking and over a longer period of COC use.

References


