Dear Sir,

Since 1995, several independent studies have shown that combined oral contraceptives (COCs) containing third-generation progestogens (desogestrel and gestodene) have been associated with a risk of venous thromboembolism that is two times greater than that of COCs containing second-generation progestogens (levonorgestrel, norethisterone) (1, 2). For this reason, the progestogen type to be combined with oestrogen, and not only the dose of the latter, has been the subject of debate regarding the role of progestogens in haemostasis.

Platelets play an important role in thrombus formation, participating directly in primary haemostasis, and indirectly by secreting substances that activate coagulation. In addition, there have been no studies evaluating the effects of isolated etonogestrel, a third-generation progestogen, on platelet function. Therefore, our objective was to evaluate the effects of the use of subdermal implants of etonogestrel (the active metabolite of desogestrel) on platelet aggregation in healthy women.

We carried out an open, self-controlled, longitudinal and prospective study, in which platelet aggregation was evaluated prior to implant placement, and at one, three and six months after implant placement. The implant was inserted during the early follicular phase of the menstrual cycle in order to avoid any discrepancy in the initial platelet aggregation among women. In case of postpartum amenorrhoea, the implant was inserted at least three months after delivery.

Twenty-four healthy female volunteers were selected to participate in the study between March 2002 and September 2003. Inclusion criteria were being 20 to 35 years old, having suspended hormonal contraception at least six weeks prior to participation in the study, and having a body mass index (BMI) < 30 kg/m². Exclusion criteria were being a smoker, using alcohol or illicit drugs, presenting a systemic disease, using any medication that might interfere with blood coagulation, and having a history (personal or family) of thromboembolic events. Of the 24 women recruited, 23 completed the study, and one was excluded for having used a COC and a non-steroidal anti-inflammatory drug in order to control prolonged bleeding after implant placement. All volunteers gave written informed consent, and the study was approved by the local Ethics in Research Committee.

The statistical calculations were obtained using the GraphPad StatMate® software program (Graphpad Software, San Diego, CA, USA). Using a two-tailed test with a 0.05 level of significance and a statistical power of 80%, we would have been able to detect an alteration of 25% or greater in the median of platelet aggregation in healthy women with a sample of 20 patients.

Whole blood samples were drawn into plastic tubes containing 3.2% sodium citrate at a predetermined time of day, after an 8-hour fast. Patients did not use any medication that might alter the results of the platelet aggregation test, especially non-steroidal anti-inflammatory drugs and acetylsalicylic acid. Platelet aggregation was induced by the following agonist solution: adrenalin (50 µM; Hospital das Clinicas Pharmacy Unit, Ribeirão Preto, Brazil), adenosine diphosphate (ADP) (35 and 17.5 µM; Sigma Diagnostics®, St. Louis, MO, USA), and collagen (5 and 10 µg/ml; Helena Laboratories®, Beaumont, TX, USA). For each test, we used 450 µl of platelet-rich plasma (PRP) and 50 µl of the aggregating agent so that the final agonist concentration was 10 times lower than the initial solution. Platelet aggregation tests were carried out using at a concentration of 200,000 platelets/µl. This concentration was obtained by diluting the PRP with the respective plasma devoid of platelets. Platelet aggregation assays were conducted by spectrophotometry using a two-channel aggregometre (Model 560: Chrono-log, Havertown, PA, USA). The interval between blood collection and analysis of platelet aggregation was less than two hours.

Results

Mean age of the volunteers was 26.3 years (standard deviation = 4.0), mean BMI was 23.32 kg/m² (standard deviation = 2.3), and 35% of the volunteers were nulliparous.

In the platelet aggregation test results, we observed statically significant transitory reductions of 27%, 14% and 11% in the median percentage of maximum platelet aggregation with the

<table>
<thead>
<tr>
<th>Platelet agonist**</th>
<th>Baseline</th>
<th>1 month</th>
<th>3 months</th>
<th>6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen, 0.5 µg/ml</td>
<td>75 (67 – 79)</td>
<td>69 (52 – 74)</td>
<td>70 (55 – 76)</td>
<td></td>
</tr>
<tr>
<td>Collagen, 1 µg/ml</td>
<td>78 (74 – 83)</td>
<td>73 (68 – 78)</td>
<td>75 (73 – 83)</td>
<td></td>
</tr>
<tr>
<td>Adrenaline, 5 µM</td>
<td>73 (46 – 84)</td>
<td>63 (28 – 73)</td>
<td>67 (34 – 77)</td>
<td>72 (49 – 76)</td>
</tr>
<tr>
<td>ADP, 3.5 µM</td>
<td>77 (60 – 86)</td>
<td>72 (66 – 82)</td>
<td>75 (73 – 86)</td>
<td>78 (72 – 81)</td>
</tr>
<tr>
<td>ADP, 1.75 µM</td>
<td>66 (30 – 77)</td>
<td>68 (40 – 75)</td>
<td>63 (44 – 71)</td>
<td>63 (48 – 75)</td>
</tr>
</tbody>
</table>

**Final agonist concentration. Data are reported as median ([1st and 3rd quartiles]) percentage of maximum platelet aggregation. The implant releases, on average, 40 µg etonogestrel/day.

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use of 0.5 µg/ml of collagen ($p = 0.04$), 5 µM of adrenalin ($p = 0.02$), and 1 µg/ml of collagen ($p = 0.01$), respectively. This reduction was observed at one month after implant placement and is in comparison with the pre-placement value (Table 1). With the use of 5 µM of adrenalin, this effect was still seen at 3 months after insertion of the medication. Platelet aggregation with the use of these agonists returned to pre-placement values by month 6 of hormonal contraceptive use. The same phenomenon was not observed with the use of ADP (3.5 and 1.75 µM).

Discussion
Although there have been a few studies analyzing the effect of isolated progestogens on platelets(3–5), none have evaluated acute alterations or have employed the type of progestogen used in this study (etonogestrel). There have been no reports of alterations in platelet counts due to the use etonogestrel (6). In the present study, we established a standard concentration of 200,000 platelets/µl for each blood sample. Therefore, the transitory reduction of platelet aggregation was not caused by any decrease in the number of platelets caused by the medication used.

It has previously been demonstrated that platelet cytosol contains androgen (AR) receptors, as well as receptors type α and β for oestrogen (7, 8), although progesterone receptors have yet to be identified. Therefore, how can we explain the effect of progestogens on platelets? An explanation might be found in the ARs since progestogens have varied affinities for this receptor and may stimulate or block androgen activity (9). Although controversial, it has been shown that androgens administered in vitro and in vivo can increase platelet aggregation (10). These androgen effects may vary according to the androgenicity (10) and the dose (7) of the steroid used. Etonogestrel has an affinity for AR, producing a weak androgen effect by binding itself with this receptor (9) and may therefore decrease platelet aggregation through genomic mechanisms in megakaryocytes or through non-genomic mechanisms in platelets.

In conclusion, the fact that subdermal implant of etonogestrel did not promote platelet hyperactivation, neither did it cause changes in plasma coagulation (6, 11), is surprising, considering its thromboembolic potential when oral desogestrel is combined with oestrogen (12, 13). Further studies are necessary in order to seek an explanation to this intriguing issue.

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References