INFLUENCE OF GESTODENE AND DESOGESTREL AS COMPONENTS OF LOW-DOSE ORAL CONTRACEPTIVES ON THE PHARMACOKINETICS OF ETHINYL Estradiol (EE2), ON SERUM CBG AND ON URINARY CORTISOL AND 6β-HYDROXYCORTISOL


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ABSTRACT

A randomized controlled clinical trial was undertaken over a 6-month treatment period with two low-dose combined oral contraceptives (OC) to investigate whether the metabolism and elimination of ethinyl estradiol (EE2) is differently influenced by the two progestational components gestodene (G) and desogestrel (D), an issue which has been very controversial recently. The two formulations contained 30 µg EE2 each, together with either 75 µg G or 150 µg D. Of the 40 young women recruited for each formulation, 31 of each group were available for statistical evaluation.

The pharmacokinetics of serum EE2 were studied on day 1, 10 and 21 of cycle 1, 3 and 6. There were no significant differences between the two groups in any cycle with respect to parameters measured. This was true for the distinct intracyclical rise in the mean EE2 serum levels from day 1 to day 10 and the smaller further increase between day 10 and day 21, with no change in this respect between the cycles studied. Respective changes were seen with regard to the area under the EE2 serum concentration curve up to 4 and 24 hours (AUC0-4 and AUC0-24), Cmax and tmax of serum EE2. The estrogen-dependent corticoid-binding globulin (CBG) increased similarly in the two groups intracyclically and slightly also intercyclically at all times tested. Except for the first treatment cycle, urinary...
excretion of cortisol and 6β-hydroxycortisol displayed a tendency to lower values intracyclically as well as intercyclically, again with no differences between the two groups. Also, the 6β-hydroxycortisol-to-cortisol ratio was not different between the groups, showing a slight tendency to rise from about 4 at the beginning of the medication to around 5.5 at the end of the 6th treatment cycle in both groups.

It is concluded that G and D as components of low-dose OCs exert comparable effects on the metabolism and elimination of EE2.

INTRODUCTION

With low-dose combination-type OCs being the predominant formulation in use for some years, differences in the pharmacological profile of the contraceptive progestogens are now generally felt to be less important. However, in 1989 Jung-Hoffmann and Kuhl (1) found that the plasma concentrations of EE2 were significantly higher in 11 women taking 30 µg EE2 with 75 µg G compared to a similar group of 11 women taking 30 µg EE2 with 150 µg D. The increased levels of EE2 were noted from the first day of the first cycle of use and the authors suggested that a stronger inhibition of hepatic EE2 metabolizing enzymes by G than by D was the most probable explanation of the effect; this suggestion was apparently supported by the in vitro findings on liver preparations of Guengerich (2). The results of the study of Jung-Hoffmann and Kuhl (1) might be of clinical importance because they seemed to indicate that EE2 pharmacokinetics were differentially influenced by progestogens. This issue being clearly controversial prompted several re-investigations of various design (3-7), all of which failed to confirm the original findings (1).

As a contribution to settle this clinically important issue, it was felt appropriate to mount a two-centre study, in which the general experimental design was essentially the same as in the original study except for the number of volunteers which was more than three times as high as previous and for the total time of pill usage which was reduced to 6 months. The main target variable was the area under the EE2 serum concentration-time curve to 4h (AUC0-4), with other target variables being Cmax (maximum concentration), tmax (time of Cmax) and AUC0-24 of serum EE2 as well as changes in serum CBG and in urinary excretion of cortisol and 6β-hydroxycortisol during treatment. The rules of "Good Clinical Practice for Trials on Medicinal Products in the European Community" (GCP; 8) were strictly observed.
MATERIALS AND METHODS

Volunteers: 80 women were recruited at the outset of study; 40 in each of the two centres (Marburg and Essen). After extensive verbal and written information, all subjects gave full-informed written consent as part of the protocol according to the Declaration of Helsinki. The protocol was approved by the ethical committee of the Klinikum Steglitz of the Freie Universität Berlin.

Women younger than 18 or older than 35 yrs of age were not eligible. Smokers (<10 cigarettes) were allowed to participate in the study only when younger than 30 yrs. Body weight was not allowed to exceed the normal range by more than 20%. Women were not included in the study when regularly taking co-medications of any kind, including vitamins.

Women were also not included if they - during the last 30 days- had had any medication known to influence liver enzyme activity. In particular, hormonal medication within the last two months before the study was considered an exclusion criterion. The generally accepted contraindications against OCs were strictly observed.

During the study, there were 6 drop-outs. Two women being placed on G/EE2 stopped the trial following a campaign in the media referring to the health risks associated with the study medication. One woman dropped out for a hematoma under G/EE2 and another one for cephalgia, nausea and hypermenorrhea under D/EE2. In addition, two women were considered drop-outs due to obvious lack of compliance.

Design of study: Volunteers entered the study after having passed a thorough medical examination addressing all potential contraindications to OC use. They were then randomly allocated to the two treatment groups, i.e. for a 6-month treatment with one of two combination type OCs, each containing 30 μg EE2 plus either 75 μg G or 150 μg D/per pill. Mean age, weight and height of the two groups of participants were comparable as shown in Table I.
Table I: Demographic data of the two groups of volunteers taking either a gestodene (G/EE$_2$) or desogestrel (D/EE$_2$) containing pill

<table>
<thead>
<tr>
<th>Preparation</th>
<th>G/EE$_2$</th>
<th>D/EE$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 31 (range)</td>
<td>n = 31 (range)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>27 ± 3 (21 - 23)</td>
<td>25 ± 4 (19 - 34)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169 ± 6 (154 - 180)</td>
<td>168 ± 5 (158 - 180)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>64 ± 10 (43 - 82)</td>
<td>62 ± 8 (50 - 79)</td>
</tr>
</tbody>
</table>

After randomization, each volunteer entered a pretreatment control cycle followed by six cycles on their study medication. The subjects started pill intake on the first day of menstrual bleeding and continued for 21 days. Following a 7-day break, the next pill cycle began as usual.

Blood collection was carried out on day 1, 10 and 21 of the first, third and sixth cycle of medication. Blood was drawn at zero-time in the morning (between 7 and 9 a.m.) after an overnight fast, and at 0.5, 1.0, 1.5, 2.0, 4.0 and 24 hours after intake of the pill in the treatment cycles 1, 3 and 6. After centrifugation, the sera were kept frozen at -20° C until EE$_2$ analysis in Liverpool (D.J.B.).

At each zero-time, additional blood was collected for the determination of a variety of hormonal and safety parameters including CBG, the latter being assayed in Essen (A.E.S.). Urine collection was started at each zero-time and completed after 24 hours. After measuring the total volume, aliquots (50ml) were stored at -20° C until the determination of cortisol and 6β-hydroxycortisol in Marburg (E.D.).

Progress of the study was supervised according to GCP by Staticon, Munich. Once monthly, representatives of this Clinical Research Organization (CRO) visited the two centres to monitor the proper performance of the investigation according to the protocol, to check the completeness of the files, the correctness of labelling and the storing of the samples. The CRO was also responsible for logistics and administration.
The code was broken only after all analyses were carried out, incomplete as well as obviously incorrect data were excluded by the study group, and the statistical examination for differences between the two data sets was accomplished in Innsbruck (A.N.).

Radioimmunoassay of ethinylestradiol (EE₂): EE₂ analysis was performed blind. Samples obtained from each woman during cycles 1 and 3 were analysed in the same assay. Samples obtained from 2 women during cycle 6 were analysed in the same assay. Thus, each assay comprised 48 serum samples. Samples collected during cycles 1 and 3 were analysed in 78 assays followed by 21 repeats. Samples collected during cycle 6 were analysed in 39 assays followed by 12 repeats. Altogether 150 EE₂ assays were run.

EE₂ concentrations in the serum were determined in duplicate following an extraction of 0.5 ml serum with diethylether (2.5 ml). The standards, which were also measured in duplicate, contained blank serum and EE₂ at a concentration range of 10-160 pg/0.5 ml. Quality control samples for the assessment of precision and accuracy of the assay were prepared by adding known quantities of EE₂ to blank serum samples. A specific antiserum raised in rabbits against EE₂-6β-CH₂-CMO-BSA (Schering AG, Berlin) was used; its characteristics were as described previously (1, 9). 6,7-³H-EE₂ (specific activity 2.2 TBq mmol⁻¹; New England Nuclear) was used as tracer. Test samples, standards and quality control were processed in the same way. Extracts from serum samples were dissolved in buffer; antiserum (1:40,000) and tracer (6000 cpm per tube) were added and the mixture incubated overnight at 0°C. Subsequently, a charcoal suspension (1.25 % dextran-coated charcoal in the presence of gelatin) was added and incubated for a further 10 min at 4°C. The tubes were then centrifuged at 2000 g for 10 min and the supernatant decanted and subjected to radiometric scintillation counting.

The sensitivity of the standard curve was about 3-4 pg EE₂ and the lower limit of quantitation was 8 pg ml⁻¹. Blank values were not subtracted. Intraassay precision (n=10) was determined with two different control samples, containing a nominal concentration of 30 and 100 pg EE₂/0.5 ml serum, respectively. Experimentally measured concentrations were 31.1 ±2.8 and 96.2 ±7.6 pg/0.5 ml. The coefficients of variation were 9.0 and 7.9%, respectively. Interassay precision was determined with control samples containing 50 and 100 pg EE₂/0.5 ml plasma. Measured concentration values were 46.6 ±6.2 and 96.1 ±12.1 pg/0.5 ml plasma in 78 assays comprising samples collected during cycle 1 and 3. Corresponding concentrations measured in 39 assays comprising samples collected during cycle 6 were 51.1 ±6.2 pg and 102.0 ±12.6 pg/0.5 ml, respectively.
Interassay precision was between 12.6 - 13.2% (cycles 1 and 3) and 12.0 - 12.3% (Cycle 6). Deviation of measured from nominal values was between 3.9 - 6.8% (Cycles 1 & 3) and 2.0 - 2.2% (Cycle 6), respectively.

EE₂ concentrations in each group were evaluated for C₉₉₉₉ (maximum concentration), t₉₉₉₉ (time of C₉₉₉₉) and AUC which was calculated to 4 and 24 hours.

Radioimmunoassay of corticosteroid-binding globulin (CBG, transcortin): A tracer amount of ¹²⁵I-transcortin was incubated with a monoclonal transcortin antiserum. The incubation also contained known amounts of unlabeled human transcortin (reference curve) or a given volume of the unknown serum samples. All reagents for the radioimmunoassay were provided from Medgenix, Fleurus, Belgium (10). After 2 hours of incubation at room temperature, anti-mouse gammaglobulin antiserum covalently linked to microcrystalline cellulose (immunabsorbent) was added to all tubes. After a further 20 min incubation at room temperature, all the complexes were bound to the immunabsorbent. The tubes were then centrifuged and the cellulose-bound radioactivity determined. The transcortin concentration of the samples was determined by dose interpolation from a standard curve between 0-8 µg/ml. Sensitivity of the method was calculated to be 0.25 ±0.02 µg/ml. Interassay and intraassay variation were 5.0% and 3.7%, respectively. Normal values for women in the reproductive age were 43.25 ±25.65 µg/ml (n=58).

Radioimmunoassay of urinary cortisol: Cortisol was determined using the coated tube radioimmunoassay, Cortisol Bridge Kit, Code no.14394, Biodata/Serono. Samples were assayed in duplicate. The standard curve covered 0-1000 ng/ml urine, sensitivity being about 8 ng/ml. Intraassay variation was 3.7% for low values and 4.0% for high values; interassay variation was 11% and 10.9%, respectively. The normal range of urinary cortisol excretion is 30-130 µg per 24 hours. Cross-reactions are known to be 0.1% for desoxycorticosterone, 8.6% for 11-desoxycortisol, 2.5% for cortisone and 0.7% for corticosterone. Creatinine excretion was measured to check that urine collection over the 24-hour period was complete.

Enzyme immunoassay of urinary 6β-hydroxycortisol: 6β-hydroxycortisol was measured by a competitive enzyme immunoassay in two steps, using the test kits from Stabiligen S.A., Nancy, France. Samples were assayed in duplicate. The urine had to be diluted 1:1071. The standard curve ranged from 0.05 to 1.0 ng/ml. The sensitivity was about 0.05 ng/ml. Intraassay variation was 5.2% for low values and 3.1% for high values. Interassay variations were 15.5% and 12.0%, respectively. Cross-reactions are known to
be 5.8% for cortisol, 10.0% for 6β-hydrocortisone, 0.5% for corticosterone and 0.1% for 11-desoxycorticisol. The normal range of urinary 6β-hydrocortisol excretion is 200-400 µg/24h (11).

**Statistical Analysis:** Single missing values within an EE2 curve were substituted by means of linear interpolation between contiguous measurements. If at least one of the nine curves of EE2 was missing, the patient was excluded from the statistical analysis of EE2.

The same rule was applied to missing values within the nine measurements of the other parameters. Thus, groups for comparison of CBG, cortisol and 6β-hydrocortisol contain less subjects than the AUC0-4 and AUC0-24 groups. AUC values were computed using the trapezoidal-rule (linear interpolation between measurements).

To account for the approximate log-normal distribution seen in all variables, the data were log transformed before confirmatory statistical analysis was done.

Between group treatment effects were analysed using the parametric (generalized least squares) test statistic for comparing samples with multiple endpoints suggested by O'Brien (12). The null hypothesis was formulated as follows: mean values at all nine time points are equal for both treatment groups. The advantage of O'Brien's test lies in its sensitivity to departures from the null hypothesis which are consistent among the nine endpoints.

A result was called statistically significant if the corresponding p-value was less than or equal to 0.05.

**RESULTS**

**Clinical findings:** The two formulations were well tolerated. No major adverse reactions were observed. Minor adverse reactions, namely headache, breast tension, acne and nausea, were noticed at usual frequency and with no obvious difference between the two groups of patients.

**Overall results:** The statistical evaluation of the overall results are given in Table II. Differences between the groups could be detected in none of the parameters studied.
Table II: Overall comparison of the two groups of volunteers using either 75 µg gestodene (G) or 150 µg desogestrel (D) in combination with 30 µg ethinylestradiol (EE₂): Results from the statistical test proposed by O'Brien (GLS version; 1984)

<table>
<thead>
<tr>
<th>Assay</th>
<th>Size of group</th>
<th>O'Brien</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G/EE₂</td>
<td>D/EE₂</td>
</tr>
<tr>
<td>EE₂ - AUC₀₋₄₀</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td>EE₂ - AUC₀₋₂₄</td>
<td>29</td>
<td>30</td>
</tr>
<tr>
<td>EE₂ - Cₘₐₓ</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td>EE₂ - tₘₐₓ</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td>CBG</td>
<td>30</td>
<td>31</td>
</tr>
<tr>
<td>Cortisol</td>
<td>29</td>
<td>27</td>
</tr>
<tr>
<td>6β-OH-Cortisol</td>
<td>29</td>
<td>27</td>
</tr>
</tbody>
</table>

*Degrees of freedom of the O'Brien F statistic

Pharmacokinetics of EE₂: Mean serum EE₂ concentrations (± S.D.) on days 1, 10 and 21 of the first, third and sixth treatment cycles are shown in Figure 1.

No differences in serum levels between the groups could be detected. In accordance, the pharmacokinetic parameters Cₘₐₓ, tₘₐₓ, AUC₀₋₄₉, AUC₀₋₂₄ were not different significantly between the 2 groups (Table III).

Cₘₐₓ of serum EE₂ increased from day 1 to day 10 in each cycle (range 20-55%) followed by only a further small increase between days 10 and 21 (0-11%), if any. Concomitantly, AUC₀₋₄₉ (Fig.2) as well as AUC₀₋₂₄ for EE₂ increased from day 1 to day 10 in each cycle at a 37-64% rate for G/EE₂ and at a 25-46% rate for D/EE₂. Respective changes between day 10 and 21 did not exceed 11% and were mostly negligible. In contrast to these intracyclical increases of serum EE₂, no changes were seen intercyclically in either group.
Table III: Pharmacokinetic parameters (Mean ± SD) calculated from serum EE₂ concentrations in 2 groups of OC users on days 1, 10 and 21 of cycles 1, 3 and 6; OCs used contained either 75 µg gestodene (G) or 150 µg desogestrel (D) in combination with 30 µg EE₂.

<table>
<thead>
<tr>
<th>Treatment cycle</th>
<th>Day</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (pg/ml)</th>
<th>t&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>AUC&lt;sub&gt;0-4&lt;/sub&gt; (pg·h·ml⁻¹)</th>
<th>AUC&lt;sub&gt;0-24&lt;/sub&gt; (pg·h·ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>G</td>
<td>D</td>
<td>G</td>
<td>D</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>99 ± 46</td>
<td>117 ± 56</td>
<td>2.0 ± 1.0</td>
<td>1.7 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>148 ± 59</td>
<td>144 ± 63</td>
<td>1.6 ± 0.7</td>
<td>1.5 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>152 ± 60</td>
<td>150 ± 58</td>
<td>1.6 ± 0.7</td>
<td>1.8 ± 0.9</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>107 ± 47</td>
<td>113 ± 51</td>
<td>2.0 ± 1.1</td>
<td>1.9 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>166 ± 62</td>
<td>153 ± 68</td>
<td>1.5 ± 0.3</td>
<td>1.3 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>164 ± 64</td>
<td>154 ± 68</td>
<td>1.6 ± 0.9</td>
<td>1.6 ± 0.8</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>105 ± 39</td>
<td>130 ± 57</td>
<td>1.6 ± 0.6</td>
<td>1.6 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>132 ± 46</td>
<td>156 ± 61</td>
<td>1.6 ± 0.8</td>
<td>1.6 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>147 ± 52</td>
<td>160 ± 52</td>
<td>1.6 ± 0.7</td>
<td>1.5 ± 0.7</td>
</tr>
</tbody>
</table>
Figure 1: EE<sub>2</sub> serum concentrations in two groups of women using either 75 μg gestodene (G) or 150 μg desogestrel (D) in combination with 30 μg EE<sub>2</sub>. Serum concentrations (Mean ± S.D.) were analysed after pill ingestion on the 1st, 10th and 21st day of the first, third and sixth treatment cycles.

● = gestodene-containing OC
○ = desogestrel-containing OC
mean and extreme values;

median, 25 and 75% quartile, minimum and maximum within 3 box lengths

Figure 2: Box plots of $AUC_{0-4}$ of EE$_2$ on day 1, 10 and 21 of cycle 1, 3 and 6 in two groups of women using either 75 µg gestodene (G) or 150 µg desogestrel (D) in combination with 30 µg EE$_2$.

Serum levels of CBG (Table IV): No significant differences in this parameter were seen between the groups. All CBG mean values of cycle 1 were distinctly lower than those of the corresponding days of cycle 3 and 6, but there were only minor changes in this respect between cycle 3 and 6.

An increase in CBG mean serum levels was seen within each of the three cycles tested. This increase was about two-fold between day 1 and 10 of cycle 1 and somewhat less between the corresponding days of cycle 3 and 6. Between day 10 and 21, there was a further distinct increase in cycle 1, but only a small increase in cycle 3 and 6.

Urinary corticoids (Table IV): Urinary cortisol excretion values of day 1 from the three cycles tested were somewhat higher in the G/EE$_2$ group than in the D/EE$_2$; but otherwise, no differences between the groups were discernible, either in relative or in absolute terms. There was a slight decrease in cortisol excretion in the course of each cycle in both groups. Intercyclically, a distinct decrease of the
Table IV: Serum concentrations of CBG and urinary 24-hour excretion of cortisol and 6β-hydroxycortisol during treatment with either a gestodene (G) or desogestrel (D) plus 30 µg EE2 containing pill

<table>
<thead>
<tr>
<th>Treatment cycle</th>
<th>Day</th>
<th>CBG n mol/l</th>
<th>Urinary cortisol µg/24 h</th>
<th>Urinary 6β-OH-cortisol µg/24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>G</td>
<td>D.</td>
<td>G</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>772 ± 189</td>
<td>700 ± 122</td>
<td>82.5 ± 42.5</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1560 ± 342</td>
<td>1409 ± 178</td>
<td>79.6 ± 38.6</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>1858 ± 485</td>
<td>1719 ± 251</td>
<td>76.5 ± 27.1</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1360 ± 264</td>
<td>1324 ± 271</td>
<td>78.6 ± 35.7</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1847 ± 422</td>
<td>1896 ± 377</td>
<td>57.9 ± 37.9</td>
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<tr>
<td></td>
<td>21</td>
<td>2035 ± 488</td>
<td>1952 ± 375</td>
<td>63.7 ± 30.3</td>
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<tr>
<td>6</td>
<td>1</td>
<td>1401 ± 374</td>
<td>1350 ± 259</td>
<td>70.5 ± 44.8</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1958 ± 479</td>
<td>1882 ± 383</td>
<td>50.0 ± 25.9</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>2136 ± 514</td>
<td>2064 ± 415</td>
<td>57.1 ± 39.2</td>
</tr>
</tbody>
</table>
Cortisol excretion from cycle 1 through cycle 3 to cycle 6 was seen. Urinary excretion of 6β-hydroxycortisol was similar under the influence of the two OC formulations. Whereas no changes were seen in the course of the first treatment cycle, the excretion values on day 10 and 21 of the 3rd and 6th treatment cycle were somewhat lower than those of the corresponding days of the first cycle in the two groups. The 6β-hydroxycortisol-to-cortisol ratio showed no major differences between the groups. (Fig. 3) There was a slight trend in both groups to increased ratios from about 4 in cycle 1 through cycle 3 to about 5.5 in cycle 6.

Figure 3: Urinary excretion ratios (mean + S.D.) of 6β-hydroxycortisol/cortisol in women during treatment with either a gestodene- or a desogestrel-containing combined oral contraceptive.
open bars = gestodene-containing formulation
black bars = desogestrel-containing formulation
DISCUSSION

The main objective of this investigation was to examine the controversial findings of Jung-Hoffmann and Kuhl (1, 13) that the pharmacokinetics of EE₂ after ingestion of two low-dose combination oral contraceptives were differentially influenced by the progestogen components, gestodene (G) and desogestrel (D). We gave special attention to study design, study monitoring and analytical performance.

The overall study design closely resembled that of Jung-Hoffmann and Kuhl (1) with the important benefit of a more than 3-fold increase in subjects in each group. The initial recruitment of 40 volunteers to each group, ensured that the sample size was sufficiently large to rule out subject bias in disposition/metabolic characteristics. Also subject exclusions for medical or personal reasons (n=4), improper usage of the OC (n=2) or incomplete data sets (n =12) did not seriously diminish the statistical power of the study under the methodological conditions of O'Brien (12). To our knowledge, this is the largest pharmacokinetic study of OCs over a period of half a year reported to date.

Another major consideration was the monitoring of the study. This was performed according to the rules of GCP of the EC (8), by an independent CRO (Staticon, Munich). Monitors guaranteed a rigorous adherence to protocol at the two clinical centres involved and were responsible for making sure that EE₂ determinations were performed blind. The analytical work and the statistical analysis were done at different centres and the code was not broken until analysis was completed.

Since the original controversy centred on serum EE₂ concentrations, the analytical performance was of great importance. In previous studies (14), extraction from 1 ml of plasma was routinely used. In the present study, extraction was carried out from 0.5 ml serum. We obtained virtually identical results with both 0.5 and 1 ml (15), the correlation coefficient (r) as achieved from linear regression analysis of 58 samples being 0.93. The sensitivity, accuracy and precision of the assay as reported in the methods fulfill the criteria of acceptable routine assays. Furthermore, it should be noted that the same antiserum against EE₂ was used in all the relevant studies quoted (1, 3-7).

It is also worthwhile mentioning that after ingestion of the G-containing pill, the EE₂ serum values were in the same order of magnitude in all investigations on this subject, whereas after the ingestion of the D-containing pill, the EE₂ data of Jung-Hoffmann and Kuhl (1) were distinctly lower than those of all the other investigators (Figure 4).
Figure 4: Mean AUC\((0-24 h)\) of EE\(_2\) on day 1 of cycle 1 obtained from various studies using either a gestodene- or a desogestrel-containing low-dose oral contraceptive; investigations of Jung-Hoffmann and Kuhl (1), Kuhnz et al. (4) and Dibbelt et al. (7) being comparable in experimental design with present study.

open bars = gestodene
black bars = desogestrel

The data presented here fail to show any difference between the two groups of volunteers with regard to the serum EE\(_2\) parameters investigated. The alterations of the AUC\(_0-4\) in the course of the investigation paralleled those of the AUC\(_0-24\) within and between the groups; the same was true for the values of C\(_{\text{max}}\) of serum EE\(_2\). This is in striking contrast to Jung-Hoffmann and Kuhl (1), who found the AUC\(_0-4\) values being 37\% higher and the AUC\(_0-24\) values being 70\% higher in women using G/EE\(_2\) than in women using D/EE\(_2\).
In the course of each of the cycles studied and with both formulations, an increase in $C_{\text{max}}$, $AUC_{0-4}$ and $AUC_{0-24}$ was seen from day 1 through day 10 to day 21 which is in agreement with previous findings (1,16). In contrast to Jung-Hoffmann and Kuhl (1), however, there was no evidence of any further accumulation of serum EE$_2$ from cycle to cycle.

The findings of this study so far discussed are in accordance with two recent investigations also repeating basic parts of the original experimental design including randomization of the volunteers. One study was carried out with 83 women over a period of 3 months (7), whereas the other one was a cross-over study with 25 volunteers over a period of 6 months (5). Not taking into account additional studies with different experimental designs but basically the same results (3,4,6), a statistical body of data from 170 volunteers has now been accumulated which leave no doubt that the influence of gestodene and desogestrel on the pharmacokinetics of EE$_2$ is very much the same. Thus, the findings of Jung-Hoffmann and Kuhl (1) who used an almost identical experimental design but investigated only 11 women in each group, cannot be confirmed.

Serum CBG levels (Table IV) increased markedly during the intake of the two OCs as to be expected from the literature. In contrast to Jung-Hoffmann and Kuhl (1) who found significantly higher increases in the CBG blood level under G/EE$_2$ than under D/EE$_2$, all alterations of CBG in this study were of the same order of magnitude for the two formulations which is in accordance with the data of Dibbelt et al. (7). Since the formation of this transport protein in the liver is stimulated by estrogens in a dose-dependent manner, but not by progestogens, differences between the levels of circulating estrogens should result in respective differences in the levels of serum CBG (17). The identical increases of CBG blood levels in the two groups of volunteers of this investigation in the three cycles studied, therefore, provides further indirect evidence that the bioavailability, metabolism and elimination of EE$_2$ must be essentially the same, irrespective of the estrogen originating from a G-containing or a D-containing OC.

Finally, the urinary excretion of cortisol and 6β-hydroxycortisol (Table IV) was found to be identical in the two groups of volunteers. 6β-Oxidation of cortisol and 2-oxidation of EE$_2$ are thought to be brought about mainly by the same hepatic cytochrome P$_{450}$-dependent isoenzyme (2). Thus, if different influences of gestodene and desogestrel on this enzyme system were the cause for differences in the pharmacokinetics of EE$_2$, as hypothesized by Kuhl and coworkers (18), one should expect lower urinary
excretion values for 6ß-hydroxycortisol and/or a decrease in the urinary 6ß-hydroxycortisol/cortisol ratio in the presence of elevated EE₂ serum levels. This study, however, fails to provide evidence that the two progestogens under discussion differ to any major extent in their influence on the hepatic microsomal mixed function oxidases in vivo.

In summary, it is clear from this study, as well as from those of Dibbelt et al.(7), Kuhnz et al.(5) and others, that there are no differences between the gestodene- and desogestrel- containing contraceptive formulations with respect to the metabolism and pharmako-kinetics of EE₂. This conclusion has been emphasized also in a recent consensus statement (20).

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