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Evaluation of risk of thrombosis during use of low-dose ethinylestradiol – desogestrel oral contraceptive

C. MASSAFRA (1), P. BUTINI (2), M.A. CAVION (2), S.M. MINUTO (2) and G. COLOMBINI (2)

(1) Department of Obstetrics and Gynecology, University of Siena, Siena, Italy
(2) Department of Haematology, USL 30, Siena, Italy

Abstract

Thirty healthy young women, non-smokers and of normal weight, used a combined oral contraceptive consisting of 20 μg ethinylestradiol and 150 μg desogestrel for 9 cycles. Before and during the 3rd, 6th and 9th cycles of contraceptive use, the following parameters were measured: triglycerides, total cholesterol, HDL-cholesterol, apolipoprotein A and B, prothrombin time, partial thromboplastin time, fibrinogen, antithrombin III, protein C, plasminogen, antiplasmin, tissue plasminogen activator, platelet count, platelet aggregation, β-thromboglobulin and platelet factor 4. The ratios of total cholesterol/HDL-cholesterol and apolipoprotein A/B remained constant or showed only a slight increase. The clotting/fibrinolytic balance showed a similar trend. There was however, an inconstant but significant increase in antithrombin III and protein C. Platelet count and platelet function parameters were unmodified. Hence the contraceptive induced no substantial changes in lipid balance or blood clotting, at least during the study period.

Introduction

Cardiovascular changes leading to thrombosis, myocardial infarction and stroke during oral contraceptive (OC) use have a multifactorial etiopathogenesis essentially linked to alterations of lipoprotein metabolism and clotting-fibrinolytic balance [1–3].

Blood clotting is modified by estrogens during OC use, and the changes observed are associated with increased liver synthesis of factors favoring clotting. This is compensated by higher consumption and decreased levels of antithrombin III (AT III), a glycoprotein responsible for about 70% of total antithrombin activity [4].

By bringing about an increase in plasminogen (PL), estrogens are likewise responsible for an increase in fibrinolytic activity leading to an apparent balance [4].

At similar doses, progestogens affect these phenomena to a different extent [5].

As far as lipid balance is concerned, estrogens directly stimulate liver synthesis of triglycerides (Trg) and HDL-cholesterol (HDL-C) [6,7]. The 19-nor-derived progestogens have the opposite effect, causing an increase in LDL-cholesterol (LDL-C) and thus altering the HDL/LDL-cholesterol ratio [8] with possible atherosclerotic degeneration of artery walls [1,2,9].

The progressive reduction in the estrogen component of OCs has lessened their side effects [10]. In the meantime, new progestogen molecules like desogestrel (DOG [11] and gestodene [12] have been synthesized; their high affinity for progesterone receptors, lack of estrogen activity and negligible residual androgen activity have greatly reduced their negative metabolic effects [4].

The aim of the present study has been to verify the effect of prolonged administration of a new monophasic combined OC, containing 20 μg ethinylestradiol (EE) and 150 μg DOG, in a group of young women not at risk for cardiovascular complications. Variations in lipid parameters, clotting and fibrinolytic processes, platelet count and function were investigated.

Materials and methods

Thirty healthy female volunteers began OC use. They were eumenorrheic non-smokers, 18–25 years of age (mean age 21.7 years) and of normal weight. All followed a mediterranean-type diet. None of the subjects had ever been on OCs before. Three subjects had given birth once, and two had suffered miscarriages. For nine cycles they used the monophase combined OC, 20 μg EE–150 μg DOG.

In the early follicular phase of the cycle immediately preceding OC use, a blood sample was taken to measure Trg, total C (t-C), HDL-C, apolipoprotein A (Apo-A) and B (Apo-B), fibrinogen (F), fibrinopeptide A (FPA), prothrombin time (PT) and partial thromboplastin time (PTT). The activities of plasminogen (PL), antiplasmin (APL), tissue plasminogen activator (t-PA), AT III and protein C were also assayed. Additional parameters measured were: platelet count, platelet aggregation in vitro, plasma β-TG and PF4. All assays were repeated in a further three blood samples taken between 17th and 21st day of the 3rd, 6th and 9th cycles of OC use.

Blood samples (43 cc) were taken in the morning under basal conditions; each sample consisted of 7–8 cc taken with and 35 cc without hemostasis using three sterile syringes and a single 38/39 mm RR-Cono Luer Lock no. 1 needle. A small quantity was used immediately for the platelet aggregation test and platelet count. Another aliquot was centrifuged and Quick's time, PTT, immuno-F, Trg, t-C, HDL-C, Apo-A and B were immediately determined in the citrated plasma. The remaining citrated plasma was frozen for subsequent determination of AT III, PL, APL, t-PA and protein C. FPA, β-TG and PF4 were determined retrospectively for all samples in a single matrix assay at the end of the study period.

Assays

The parameters were measured using commercially available kits based on colorimetry for Trg and t-C (Chem 1 Trig, Tecnico Bayern, Milan), precipitation and colorimetry for HDL-C (Peg 6000, Roche Biochemia, Milan), specific antisera for Apo-A and B (Huma Apolipoprotein A and B, Boehring, Milan), RIA for β-TG (Beta IM 88, Amersham, Milan), PF4 (PF4 Abbot, Aprilia, Rome) and PFA (Byk Gulden, Milan), a clotting method for F (F test, Valdacci, Pisa), Coatests for Pl, AP and AT III activities and t-PA (Orthodiagnostics, Milan), colorimetry for protein C (Diachrom protein C, Wellcome, Pomezia, Rome). PT (Thromorel, Inst. Boehring, Scoppito, L'Aquila) and PTT (Actin FS, Lab. Don Baxter, Trieste) were expressed as ratio indices, or as the ratio between the patient value and that of a laboratory control. Platelet aggregation was measured with the Adeplat T kit (Mascia Brunelli, Milan) and platelet count with a Tecnico model H1 counter (Milan).

Statistical analysis

The results were analyzed by Student's *t*-test for paired data. For each parameter, the mean value at the 3rd, 6th and 9th cycles was expressed as percentage variation with respect to the mean obtained before OC use.

Results

All the parameters measured during contraceptive use varied within the normal range for the methods adopted. Figures 1 and 2 show the pattern of Trg (% variation of the means: +35, 45, 52%), t-C (+3, 4, 4%), HDL-C (+5, 4, 7%), apo A (+8, 13, 16%), and apo-B (+5, 8, 9%). Figure 3 shows the pattern of PT and PTT, the mean values of which remained unchanged throughout the study period; the trends of F (+16, 18, 16%) and FPA (+14, 17, 21%) are also shown. Figure 4 shows the percentage variations in AT III (+4, 1, 5%) and protein C (+6, 1, 1%). Figure 5 indicates the trends of factors related to fibrinolytic activity; PL (+16, 23, 17%) and APL (+13, 19, 16%) showed similar percentage increases in each of the three blood samples, whereas t-PA varied randomly (−3, +5, −3%). Table 1 shows the changes in platelet count and function.

Discussion

The increase in Trg, HDL-C and Apo-A and B indicates the overall prevalence of the estrogen effect of EE, despite the dose of only 20 μg [13], over the progestogen and residual androgen effects of DOG [11,14] in the present combined OC formulation.

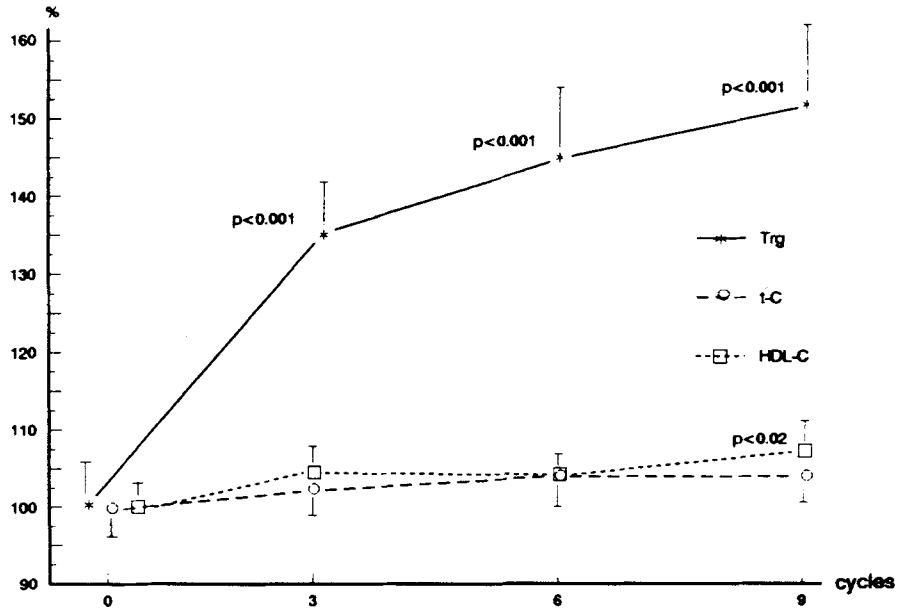

Figure 1. Percentage variations from the mean (±SE) of triglycerides, total cholesterol and HDL-cholesterol during use of the combined oral contraceptive EE–DOG

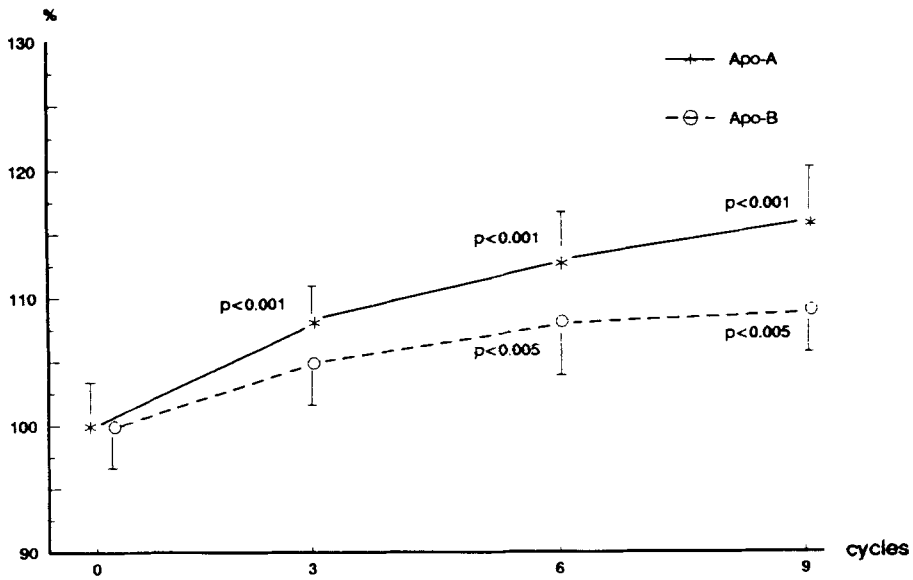

Figure 2. Percentage variations from the mean (±SE) of apolipoprotein-A and B during use of the combined oral contraceptive EE–DOG

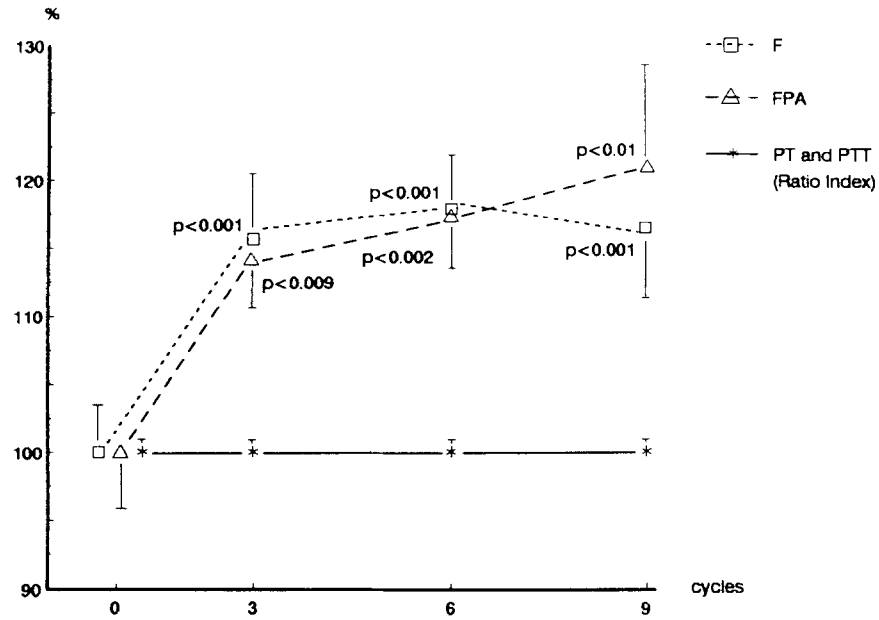

Figure 3. Percentage variations from the mean (±SE) of fibrinogen, fibrinopeptide A, prothrombin time and partial thromboplastin time during use of the combined oral contraceptive EE–DOG

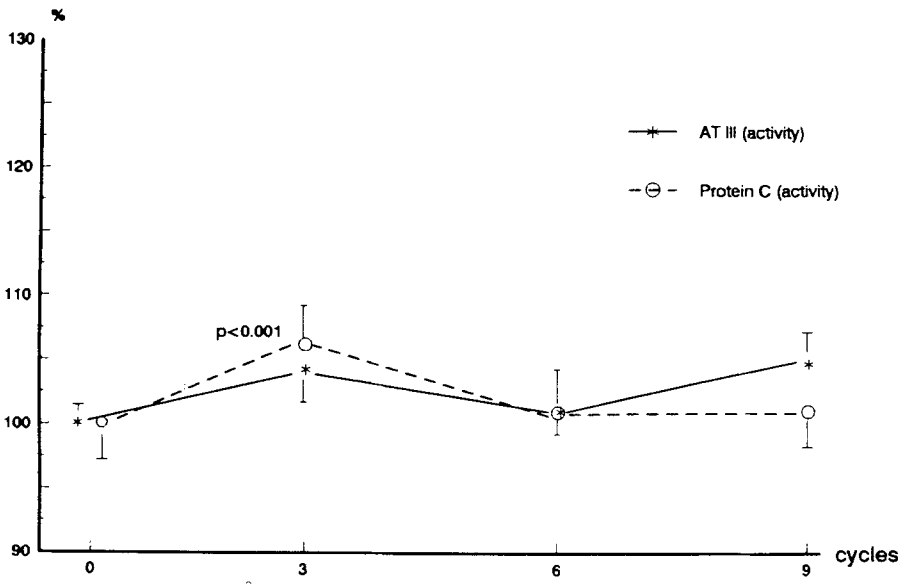

Figure 4. Percentage variations from the mean (±SE) of antithrombin III and protein C during use of the combined oral contraceptive EE–DOG

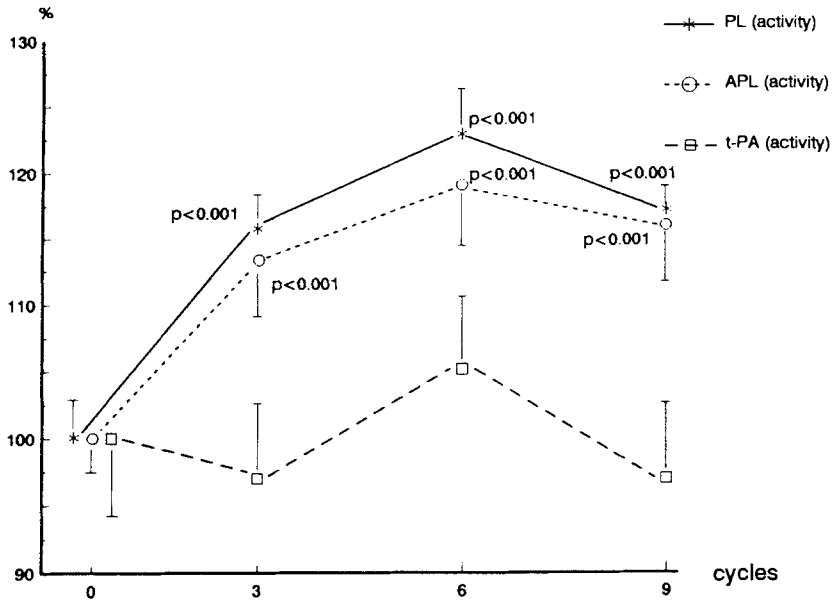

Figure 5. Percentage variations from the mean (±SE) of plasminogen activity, antiplasmin activity and tissue plasminogen activator during use of the combined oral contraceptive EE–DOG

Taking the basal index of HDL-C/t-C to be one, the index increased to 1.01, 1.00 and 1.02 in the 3rd, 6th and 9th cycles of OC use. The Apo-A/Apo-B ratios were 1.02, 1.04 and 1.06 showing a slight progressive dominance of Apo-A. The HDL-C/t-C and Apo-A/B ratios, significant indices of cardiovascular risk, were therefore stable or slightly higher than basal values, which is a desirable feature for new generation combined contraceptive pills [7].

Table 1. Variations in platelet count, aggregation and factors during use of the combined oral contraceptive EE–DOG; variations not significant

	Cycle			
Parameter	*0*	*3rd*	*6th*	*9th*
Platelet count	100	102	100	100
Platelet aggregation	100	101	104	103
β-TG	100	105	105	97
PF4	100	97	97	98

It is now accepted that an increase in Trg is clinically important only in the presence of low levels of HDL-C, HDL and especially HDL2 playing a central role in the prevention of atheromatous degeneration [7]. HDL not only stimulates the synthesis of prostacyclins, strong inhibitors of platelet aggregation, but also directly protects the endothelial cells of the artery walls by receptor antagonism with LDL-C [9].

Quick's time and PTT were unchanged; this is important since they are an indirect measure of liver synthesis factors affecting the extrinsic, intrinsic and common clotting phases.

The increase in F synthesis is to be expected during OC use. The increase in AT III activity, significant only in the 3rd and 9th cycles, is probably a result of the variation in this parameter. Protein C activity also increased but was significant only in the 3rd cycle. The increase in the two proteins may be a compensation phenomenon for the greater availability of thrombin substrate.

As far as changes in AT III during low-dose OC use are concerned, the findings reported in the literature vary from slight increases or decreases to no variation [3,4,16–18]. As observed by Kloosterboer [3], these differences probably depend on the assay methods used, immunological assays [17,18], generally indicating a decrease in the parameter considered, and functional assays (undoubtedly the more reliable) [19] no change or a slight increase. It is therefore difficult to draw conclusions on this aspect, particularly as the increased tendency for thrombosis suggested by a decrease in AT III is one of the greatest risk factors associated with OC use. On the contrary, there is more agreement on the modifications in protein C levels [4,16].

In this study the complete lack of variations in PT and PTT and the positive trend of AT III and protein C indicate that there were no increases in clotting factors likely to affect plasma phase results. The increase in FPA, which is normally interpreted as a sign of thrombin system activation, should be assessed with caution because of the extreme sensitivity of the method. Since pre-analytical factors like sample collection, time between collection and storage, and centrifugation may affect the results, this assay is being critically revised in the hematology sector. The increase is in line with that of F and may be derived from protein catabolism of the increased quantities put into circulation by EE; in fact, the clotting pathway is not the main physiological degradation pathway of this molecule.

The results indicate that fibrinolytic balance is maintained. The increase in PL is matched by that in APL. Platelet count and aggregation do not change significantly, nor do plasma levels of β-TG and PF4 which are the most important platelet secretion factors with antiprostacyclin and antiheparin activity.

In the subjects studied, use of the combined OC formulation did not lead to any substantial modification of lipid and clotting situations, and hemostatic equilibrium was practically undisturbed, at least in the 9 cycles of the study. The variations recorded in certain parameters were within normal limits and are of no clinical importance, despite the fact that they reached statistical significance. These findings are in agreement with the reports of other authors [7] and are important in view of the general nature of the research published in the last few years on low-dose OCs.

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Resumé

Trente jeunes femmes en bonne santé, non fumeuses et de poids normal, ont utilisé durant 9 cycles un contraceptif oral combiné de 20 μg d'éthinyloestradiol et 150 μg de désogestrel. Avant et pendant les troisième, sixième et neuvième cycles de l'utilisation de ce produit, on a mesuré les paramètres suivants : triglycérides, cholestérol total, cholestérol-HDL, apolipoprotéines A et B, temps de prothrombine, temps

de thromboplastine, fibrinogène, antithrombine III, protéine C, plasminogène, antiplasmine, plasmonigène-proactivateur, numération des plaquettes, agrégation des plaquettes, thromboglobuline-β et facteur 4 des plaquettes. Les rapports cholestérol total/cholestérol-HDL et apolipoprotéines A/B sont restés constants ou ont légèrement augmenté. On a observé la même tendance pour l'équilibre coagulation/fibrinolyse. On a cependant constaté une hausse inconstante mais significative de l'antithrombine III et des protéines C. La numération des plaquettes et les paramètres de la fonction plaquettaire sont restés inchangés. Le contraceptif n'a donc provoqué aucun changement important de l'équilibre des lipides ou de la coagulation sanguine, pour le moins durant la période d'étude.

Resumen

Treinta mujeres jóvenes, en buen estado de salud, no fumadoras y de peso normal, utilizaron durante 9 ciclos un anticonceptivo oral combinado de 20 μg de etinilestradiol y 150 μg de desogestrel. Antes y durante los ciclos tercero, sexto y noveno de utilización de este anticonceptivo, se midieron los siguientes parámetros: triglicéridos, colesterol total, colesterol HDL, apolipoproteína A y B, tiempo de protrombina, tiempo de tromboplastina parcial, fibrinógeno, antitrombina III, proteína C, plasminógeno, antiplasmina, activador plasminógeno tisular, recuento de plaquetas, agregación de plaquetas, β-tromboglobulina y factor 4 de las plaquetas. Las relaciones colesterol total/colesterol HDL y apolipoproteína A/B permanecieron constantes o bien mostraron un ligero aumento. Se observó la misma tendencia en el equilibrio coagulación/fibrinólisis. Sin embargo, se observó un aumento no constante pero significativo de la antitrombina III y la proteína C. El recuento de plaquetas y los parámetros de función de las plaquetas no sufrieron modificaciones. En consecuencia, el anticonceptivo no provocó ningún cambio importante en el equilibrio de los lípidos o en la coagulación de la sangre, al menos durante el período de estudio.