CLINICAL SCIENCES

EFFECT OF A LOW-DOSE ORAL CONTRACEPTIVE ON VENOUS ENDOTHELIAL FUNCTION IN HEALTHY YOUNG WOMEN: PRELIMINARY RESULTS

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Giribela CRG, Rubira MC, Melo NR de, Plentz RDM, Angelis K de, Moreno H, Consolim-Colombo FM. Effect of a low-dose oral contraceptive on venous endothelial function in healthy young women: preliminary results. Clinics. 2007;62(2): 151-8.

BACKGROUND: A possible increase in the incidence of venous thromboembolic events has been reported among users of third generation oral contraceptives. The objective of this study was to evaluate the effect of a low dose oral contraceptive (15 μg ethinyl estradiol/60 μg gestodene) on the venous endothelial function of healthy young women.
METHODS: Prospective case control study using the dorsal hand vein technique. Venous endothelial function was evaluated at baseline and after 4 months in the oral contraceptive users group (11 women) and in a control group (9 women). After preconstriction of the vein with phenylephrine, dose-response curves for acetylcholine and sodium nitroprusside were constructed.
RESULTS: In the contraceptive users group, a reduction occurred in the maximum venodilation response to acetylcholine and sodium nitroprusside after 4 months of oral contraceptive use, but this difference was not statistically significant ($P > 0.05$). No significant changes were detected in maximum venodilation responses to acetylcholine and sodium nitroprusside at the 4-month time point in the control group.
CONCLUSION: This study found no significant impairment of endothelium-dependent or independent venodilation in healthy young women following oral contraceptive use. Further studies are necessary using the same methodology in a larger sample over a longer follow-up period.

KEYWORDS: Oral contraceptives. Venous endothelium. Venous thrombosis.

INTRODUCTION

Millions of women of reproductive age around the world use oral contraceptives (OC).[1] However, adverse effects associated with oral contraceptive use, notably the increased risk of a venous thromboembolic event and of cardiovascular diseases, such as strokes and myocardial infarctions, were reported soon after the introduction of this contraceptive method in the early 1960s.[2,3]

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New hormonal formulations and preparations have been developed in an attempt to reduce these adverse effects. First, the estrogen content of oral contraceptives was reduced, and later, newer progestins, such as desogestrel, norgestimate, and gestodene, were developed, giving rise to the third-generation oral contraceptives. In the mid-1990s, however, a few reports suggested a possible increase in the incidence of venous thrombosis among users of oral contraceptives containing these new progestins.[4,5] These publications were followed by several papers either confirming or disputing these findings, and by a number of studies comparing the hemostatic effects of second- and third-generation oral contraceptives.

Although the two generations of oral contraceptives may indeed have slightly different risks, not only with respect to venous thromboembolic event but also stroke and myocardial infarction, the etiology of these differences is unclear. An increase in the incidence of venous thrombosis in oral contraceptives users may, at least partially, be due to impairment of endothelial function.

Endothelial dysfunction is recognized as an initial step in the development of cardiovascular disease and venous thromboembolism. Endothelial cells mediate hemostasis by regulating the balance between procoagulant and anticoagulant factors, proadhesive and antiadhesive forces, vasoconstriction and vasodilation, and a variety of other functions. The vascular endothelium produces a variety of vasoactive substances, including nitric oxide (NO), endothelin-1, and prostacyclin, that influence thrombogenicity and the vasomotor response of underlying smooth muscle cells. Significant alterations in the production of these endothelium-derived factors have been found in venous thrombosis.[6]

Nitric oxide (NO) is an endothelium-derived, vasoactive substance that is generated by enzymatic oxidation of the amino acid L-arginine into L-citrulline by the action of NO-synthase. Among the various functions of NO, 2 are of major importance in the cardiovascular system: its vasodilatory capacity and its inhibitory effect on platelet aggregation and adhesion. L-arginine modulates the thrombotic process and inhibits platelet aggregation.[6] Endogenous NO has been shown to protect against thromboembolism in venules,[7] while L-arginine improves endothelial vasoreactivity and reduces thrombogenicity after thrombolysis in experimental deep venous thrombosis.[8]

There are various techniques available for the evaluation of peripheral endothelial function; however, the noninvasive methods have not yet been adequately standardized despite their widespread use in clinical research.[9] Plethysmography with intra-arterial injections of acetylcholine has been used to measure vessel reactivity due to NO release, but this technique requires intra-arterial puncture, which makes it unsuitable for serial studies. Moreover, this technique evaluates the arterial system, whose dysfunction is related to atherosclerosis and arterial thrombosis, not to venous thromboembolic disease in young women.

Dorsal hand veins have also been shown to release endothelial NO when they are precontracted with norepinephrine and then stimulated with an agonist such as acetylcholine or bradykinin.[10–13] The dorsal hand vein technique is a sensitive tool for assessing the in-vivo effects of drugs on the peripheral veins of human subjects. This method is currently being used by various groups of investigators, including ours, to evaluate venous endothelial function in a variety of pathophysiologic conditions.[14–20]

This prospective, case-control study was designed to investigate endothelium-dependent and -independent vasodilation in the venous endothelium of oral contraceptive users compared to a control group of women not currently using any hormonal contraceptive method (intrauterine device users). The objective of the study was to evaluate whether any changes occur in vasodilatory responses following initiation of oral contraceptive use.

This technique has not previously been used in studies of vasomotion in healthy young oral contraceptive users.

MATERIALS AND METHODS

Study population

A prospective case control study was carried out in 2 groups of women aged 18 through 35 years, recruited from the family planning clinic of the Department of Gynecology at the University of São Paulo's teaching hospital.

The sample comprised 11 women who were about to initiate combined oral contraceptive use, and 9 women not currently using any hormonal contraceptive method, comprising the control group. Women in the control group were all current users of an intrauterine device. The contraceptive mechanism of action of the copper intrauterine device involves no hormonal or hemodynamic effects, and the device is a highly effective method of birth control.[21]

Exclusion criteria for both groups comprised the following: age > 35 years, hypercholesterolemia, hypertension, diabetes, impaired renal function, history of smoking, obesity (body mass index > 28), positive pregnancy test, and use of any hormonal contraception in the preceding 12 months.

The study was approved by the Internal Review Board of the institution, and all participants gave their signed, informed consent prior to enrollment.

The venous endothelial function of women in both groups was evaluated using the dorsal hand vein technique (DHVT). In the study group, venous endothelial function was evaluated prior to (baseline) and after 4 months of oral contraceptive use. In the control group, it was evaluated at admission to the study and again 4 months later.

The oral contraceptive selected for use in this study contained a combination of 15 μg ethinylestradiol and 60 μg gestodene. Women in the study group were provided with 4 cycles of the medication and instructed to use the oral contraceptive for 24 days followed by 4 medication-free days, for 4 consecutive months.

Because previous studies have shown a negative correlation between abnormal levels of triglycerides, cholesterol and fractions, and endothelial function, blood was drawn

for evaluation of lipids (triglycerides, total cholesterol, and fractions) and glucose in both groups at the 2 time points of the venous endothelial function evaluation.[17,22–24]

Study Protocol

Dorsal hand vein technique. The dorsal hand vein compliance technique was performed as described previously.[11–14] This technique has the advantage of allowing the infusion of very small amounts of vasoactive substances, thus avoiding potentially confounding systemic hemodynamic effects.[15–20]

Briefly, subjects lay supine in a temperature-controlled room (24 ± 2 °C). One arm was placed on a vacuum cushion (Germa Protec, Germa AB, Kristianstad, Sweden) above heart level, sloping upwards at an angle of 30 degrees from the horizontal plane to allow veins to be completely emptied. A suitable, large superficial hand vein with no apparent tributaries in the immediate area of examination was chosen. A 25-gauge butterfly needle was then inserted into the selected dorsal hand vein, and 0.9% saline was infused for at least 60 min at a constant infusion rate of 0.3 mL/min. A small tripod holding a linear variable differential transformer (LVDT, Model 100 MHR, Schaevitz, Lucas Control System Products, Hampton, VA, USA), was mounted over the selected vein at a distance of 10 mm downstream from the tip of the needle. The linear range of the LVDT core movement was ± 4 mm. The position of the core was recorded on a strip-chart recorder. The diameter of the vein was measured by recording the displacement of the freely movable core of the LVDT, which is linearly related to voltage output for movements of ± 4 mm (ATA 101, Schaevitz Engineering, Pennsauken, NJ, USA) when the pressure in the cuff placed around the upper arm was inflated to 40 mm Hg. Dilation of the vein at baseline between 40 and 60 min of saline infusion was defined as 100%. Due to the low venous tone present under these conditions, venodilator effects can be quantified only in preconstricted vessels. Phenylephrine, a selective a_1-adrenoceptor agonist, was used to preconstrict the hand veins. The dose of phenylephrine able to produce 80% constriction was used (ED_{80}) and for purposes of subsequent calculations, this dose rate and this level of constriction were defined as 0% dilatation. The vasodilatory effects examined in this study were calculated and expressed as a percentage ranging from 0% to 100% dilatation. This same dose of phenylephrine (46-2500 ng/min) was then infused at a constant rate producing stable venoconstriction for the duration of the experiment.

After preconstriction of the vein with phenylephrine, dose response curves for acetylcholine (0.36-3600 ng/min) and sodium nitroprusside (50-1000 ng/min) were constructed, with infusion rates of 5 and 3, respectively, in both the oral contraceptive and control groups.

Systolic and diastolic blood pressure was measured using a mercury sphygmomanometer, and heart rate was measured using the radial artery pulse. All measurements were obtained both before and after each experimental phase.

Statistical analysis. Wilcoxon's nonparametric test for paired samples was used to compare changes in maximum venodilation (Emax) of acetylcholine and sodium nitroprusside in each group. Other biochemical, physical, and hemodynamic parameters were assessed using analysis of variance (ANOVA). Significance level was established at 5% for all statistical analyses. Data are presented as descriptive tables with respective means and standard deviations.

RESULTS

Clinical and laboratory characteristics. The clinical and laboratory data of patients in this study are shown in Table 1. There were no significant differences in weight and body mass index between groups, and there were no changes between admission values and those obtained at the 4-month evaluation for these parameters.

Although there was a significant difference in age between the 2 groups, all patients in the study were under 35 years of age. Because previous studies have shown that the principal deleterious effects of aging on endothelial function occur predominantly after 40 years of age,[25] this difference should have had no impact on the results found in this study with respect to endothelial function.

Table 1 - Clinical and laboratory data of study and control groups

Variable	Group				*P*
	Study Group n = 11		Control Group n = 9		
	Mean	SD	Mean	SD	
Age (years)	24.77	4.11	32.11	7.91	0.030*
Weight (kg)	58.38	6.30	59.67	4.61	0.393
Height (m)	1.59	0.04	1.60	0.06	0.556
BMI (kg/m^2)	23.07	3.11	23.33	2.20	0.512
Glucose (mg/dL)	82.38	8.93	87.11	5.62	0.357
Total Cholesterol (mg/dL)	165.69	29.90	166.11	37.71	0.854
LDL (mg/dL)	93.46	23.29	96.60	12.63	0.939
HDL (mg/dL)	55.08	13.18	53.38	24.32	0.198
Triglycerides (mg/dL)	85.62	25.12	82.44	31.24	0.521
SBP (mm Hg)	108	4.3	111.8	3.35	0.456
DBP (mm Hg)	72.5	3.44	73	2.06	0.632

Values are expressed as mean ± standard deviation. BMI: body mass index, HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP: systolic blood pressure, DBP: diastolic blood pressure (* $P < 0.05$, significant *P* value).

Table 2 - Maximum venous dilation (Emax) with acetylcholine (ACH) and with nitroprusside (NPS) at baseline and after four months, in the study and control groups

Measurement	Group											
	Case						Control					
	Mean	SD	Median	Range	N	p	Mean	SD	Median	Range	n	*P*
ACH (baseline)	68.08	51.23	62.5	195.0	11	0.530	59.33	38.77	54.0	132.0	9	0.767
ACH (4 months)	58.00	34.46	63.5	119.0	11		75.89	60.21	42.0	176.0	9	
NPS (baseline)	164.83	79.42	159.0	237.0	11	0.117	135.56	48.76	124.0	171.0	9	0.500
NPS (4 months)	115.67	45.44	105.5	142.0	11		130.67	56.83	124.0	202.0	9	

Data are expressed as mean values ± standard deviation (SD)

Levels of glucose, triglycerides, total cholesterol, and fractions were normal, according to the guidelines of the National Cholesterol Education Program – Adult Treatment Panel (NCEP-ATP III), in both groups at both time points of the study.[26]

Hemodynamic parameters. Heart rate and systolic and diastolic blood pressure were within the normal range in both groups at baseline. No significant change in these parameters was found after 4 months. Infusion of acetylcholine and sodium nitroprusside during DHVT resulted in no systemic hemodynamic changes (data not shown).

Endothelium-dependent venodilation assessed by DHVT (Table 2). In the study group, the maximum venous dilation (Emax) with acetylcholine (endothelium–dependent vasodilation) was 68.08% ± 51.23% (mean ± SD) at baseline. After 4 months of oral contraceptive use, Emax decreased to 58.00% ± 34.46%. This change was not significant ($P > 0.05$).

In the control group, there were no significant changes in maximum venous dilation (Emax) with acetylcholine when comparing baseline (59.33% ± 38.76%) and 4-month values (75.89% ± 60 %) ($P > 0.05$).

Endothelium-independent venodilation as assessed by DHVT (Table 2). In the study group, maximum venous dilation (Emax) with sodium nitroprusside (endothelium–independent vasodilation) was 164.83% ± 79.42% (mean ± SD) at baseline. After 4 months of oral contraceptive use, Emax decreased to 115.67% ± 45.44. This decrease in venodilation was not significant ($P > 0.05$). In the control group, no significant changes occurred in maximum venous dilation (Emax) with sodium nitroprusside (endothelium–independent vasodilation) when comparing baseline (135.56% ± 48.76 %) and 4- month values (130.67% ± 56.82 %) ($P > 0.05$).

DISCUSSION

This is the first study evaluating endothelium-dependent and independent responses in the venous system by DHVT in healthy young women following combined oral contraceptive use. Most studies addressing endothelial function and hormone therapy in women have been carried out in an older population, mostly in postmenopausal women using natural estrogens. Moreover, those studies were carried out on the arterial system.

Our results showed a reduction in endothelial dependent and independent venodilation after oral contraceptive use, but without reaching statistical significance, probably due to large intersubject variability in vasodilation responses to acetylcholine and sodium nitroprusside. Intersubject variability in vascular responses, mainly to acetylcholine has also been observed in many studies with DHVT.[10,17,19,20] However, as the variability in our results was larger than in other studies with the same technique, an increase in our sample size is necessary.

Oral contraception is associated with an increased risk of morbid cardiovascular events such as stroke, myocardial infarction, deep vein thrombosis, and venous thromboembolism,[27,28] especially among women who have concomitant risk factors such as smoking.[29,30] Since 1995, several observational studies have reported an increased risk (approximately 2-fold) of nonfatal venous thromboembolism associated with third-generation oral contraceptives.[31,32] Many studies have evaluated the effects of second- and third-generation oral contraceptives on hemostasis in an attempt to explain the apparent differences.[33–36] Estrogen has well-defined effects on hemostasis. It increases fibrinogen levels; coagulation factors VII, VIII, and X; and plasminogen. Its use is associated with lower levels of antithrombin III, protein S, and plasminogen activator inhibitor. Use of oral contraceptives leads to decreased activated protein C resistance (APC resistance). The net effect of these procoagulant and anticoagulant changes is a small increase in coagulation.[28]

It must be emphasized, however, that the endothelium plays a major role as the principal regulator of hemostasis. First, vascular wall integrity is now known to consist of both structural and functional components. There is no

question that physical injury to the vascular wall is a powerful trigger of the coagulation mechanism. However, functional disruption of the intact endothelium represents a far more common catalyst for the prothrombotic state. Secondly, blood flow stasis is a consequence not only of an increase in hydrostatic pressure but also of an imbalance in the endothelial- and vascular smooth muscle cell-derived vasoregulatory factors. Endothelial cells release a number of substances, including endothelial nitric oxide synthase (eNOS), prostacyclin, plasminogen activator factor (PAF), and endothelin-1, that regulate the tone of the blood vessel wall. Because the luminal diameter is a critical determinant of blood flow, the net balance of vasodilatory and vasoconstricting molecules has an important effect on local hemostatic balance. Finally, alterations in the levels of circulating procoagulants and/or anticoagulants are now known to have unique effects on the hemostatic balance of local vascular beds.[6]

There is evidence that synthetic sex steroids may have different effects on endothelial cells from those of endogenous steroids, which are suppressed during the use of oral contraceptives.[37–39] In contrast to the effects of physiologic estrogens, which have been shown to preserve endothelial function, one of the possible mechanisms linked to this increase in venous thromboembolism and cardiovascular disease in oral contraceptive users may be endothelial dysfunction.

There are several methods, both experimental and clinical, of evaluating endothelial function. Most involve the arterial system, the dysfunction of which is associated with the development of atherosclerosis, hypertension, and arterial thrombosis. Some clinical studies rely on circulating levels of activation markers as an indicator of endothelial cell dysfunction. Mutunga et al, 2001, for example, have developed a method of detecting circulating endothelial cells (EC) that provides direct evidence of EC shedding in human sepsis.[40] However, these methods do not provide any information about the site of disease involvement. Moreover, high levels of activation markers do not indicate whether endothelial cell dysfunction is a cause or consequence of the underlying disorder. This type of evaluation is complicated by the fact that many stimuli such as exercise and infections can change their release.[6,9]

The DHVT is a less invasive method and allows direct assessment of the influence of many pathophysiological conditions related to venous endothelial dysfunction, such as thromboembolic disease. Despite the limitations of being performed in the superior limb, which leads to an underestimation of the role of hydrostatic pressure in physiopathology of thrombosis, the DHVT is currently the only method available for assessing venous endothelial function in humans. Methods performed in the inferior limbs, like the occlusion plethysmographic technique, although assessing the limb volume change in response to venous filling, measure whole limb compliance and do not discriminate between the venous and soft tissue elements involved in limb compliance. Another important limitation of the occlusion plethysmographic method is that there is no direct measure of venous pressure and venous endothelial function after direct pharmacological stimuli.[41]

A few studies have recently been published on the effects of OC on endothelial function. However, because a wide variety of different techniques were used to evaluate endothelial function in those studies, such as imaging techniques (magnetic resonance, Doppler), in-vitro cultures, serum markers of endothelial dysfunction, and brachial artery plethysmography, a comparison of the results is not possible.[42–47] Similarly, they cannot be compared with the results of our study, because we used an in-vivo, direct evaluation of venous endothelial function.

For example, in one of those studies, Stefan et al,[44] analyzed endothelial function using forearm plethysmography in women taking OC, and compared them to a control group in a cross sectional study. These investigators found no significant differences in endothelium-dependent or endothelium-independent vasodilatation responses between the 2 groups. In contrast, NG-monomethyl-L-arginine infusion induced a significant decrease in blood flow in women using OC compared to the control group (–26% ± 3% vs –14% ± 5%; $P = 0.009$). The authors concluded that although NO bioavailability remained unaffected in a group of premenopausal women receiving oral contraceptives, basal NO production and release appeared to be enhanced by oral contraceptive use.[44] Virdis et al [45] also studied the effect of a third-generation oral contraceptive on endothelial function using forearm arterial plethysmography in healthy young women. Endothelial function remained unchanged after 6 months of oral contraceptive use (30 μg ethinylestradiol + 75 μg gestodene daily) despite the unfavorable changes in the lipid profile that occurred during the study. The authors speculated that the adverse effects of increased lipid levels were counterbalanced by the assumed beneficial influences of the oestrogen component. Their assumption implies a neutral effect of the gestodene component. However, data on the influence of progestogens on endothelial function are inconsistent. In some studies, the favorable effect of estrogen on endothelial function was unchanged by progestogens.[46] Other investigators, such as Sorensen et al,[47] evaluated the effects of progestogens on endothelial function using cardiovascular magnetic resonance and found that progestogens reduced or offset the estrogen-mediated response.

However, those studies evaluated the arterial endothelium, the dysfunction of which is associated with the development of atherosclerosis, arterial thrombosis, and hypertension. Since endothelial cells play an important role in hemostasis, we elected to use DHVT to evaluate the venous system instead of the arterial system to investigate the influence of an OC on venous endothelial function. Our goal was to investigate whether venous endothelium dysfunction is one of the possible mechanisms of the increased incidence of venous thromboembolic event in healthy young oral contraceptive users. Our results show no significant difference in endothelium-dependent or endothelium-independent venodilation following oral contraceptive use.

Although NO-dependent vasodilatation is an important component of endothelial function related to increased venous thromboembolism and cardiovascular risk in oral contraceptive users, it is not the only one, and it may not be the only mechanism by which oral contraceptive use increases the risk of venous thromboembolism. The intact endothelium also exerts antithrombotic and antiinflammatory effects that were not evaluated and were not detected in the assessment of NO-dependent vasodilatation.

CONCLUSION

Although the sample size was limited, this is the first study in which the association between oral contraceptive use and venous endothelial function has been evaluated in vivo. Our results indicate no significant impairment of endothelium-dependent or independent venodilation in healthy young women following initiation of oral contraceptive use. Since there was a large intersubject variation in the results obtained with the technique used in this study, further studies using the same method should be carried out in a larger group of patients over a longer follow-up period.

RESUMO

Giribela CRG, Rubira MC, Melo NR de, Plentz RDM, Angelis K de, Moreno H, Consolim-Colombo FM. Efeito de um anticoncepcional hormonal oral de baixa dose na função endotelial venosa em mulheres jovens saudáveis: resultados preliminares. Clinics. 2007;62(2):151-8.

Um aumento no risco de tromboembolismo venoso têm sido descrito em usuárias de anticoncepcionais hormonais oral de terceira geração.

OBJETIVO: Avaliar o efeito de um anticoncepcional combinado hormonal oral de baixa dose (15 µg etinil estradiol/ 60 µg gestodeno) na função endotelial venosa de mulheres jovens saudáveis.

MÉTODOS: Realizou-se um estudo caso-controle prospectivo em vinte mulheres jovens saudáveis que foram avaliadas pela técnica da complascência venosa. A função endotelial venosa foi avaliada em um momento basal e após 4 meses no grupo das usuárias de anticoncepcional oral (11 mulheres) e em um grupo controle (9 mulheres). Foram construídas curvas dose resposta para acetilcolina e nitroprussiato de sódio após a pré-constrição da veia com fenilefrina.

RESULTADOS: No grupo de usuárias de anticoncepcional combinado hormonal oral houve diminuição da venodilatação máxima em resposta a acetilcolina e nitroprussiato de sódio, porém esta mudança não foi estatisticamente significante (p> 0,05). No grupo controle não foram detectadas mudanças significantes na venodilatação máxima, em resposta a acetilcolina e nitroprussiato de sódio no intervalo de 4 meses.

CONCLUSÃO: Este estudo não observou redução significante da venodilatação endotélio dependente e independente após os uso de anticoncepcional combinado hormonal oral. Mais estudos são necessários utilizando a mesma metodologia em uma amostra maior e com maior tempo de seguimento.

UNITERMOS: Anticoncepcional Combinado Hormonal Oral/ Endotélio Venoso/ Trombose Venosa

REFERENCES

1. Burkman RT. Current perspectives on OCs. Dialogues in Contraception. 2001;6:15-7.

2. Jordan WM. Pulmonary embolism. Lancet. 1961;1146-47.

3. Lorentz IT. Parietal lesion and "Enavid". Br Med J. 1962;2:1191.

4. Spitzer WO, Lewis MA, Heinemann LA, Thorogood M, MacRae KD. Third generation oral contraceptives and risk of venous thromboembolic disorders: an international case-control study. Transnational Research Group on Oral Contraceptives and the Health of Young Women. BMJ. 1996;312:83-8.

5. World Health Organization Collaborative Study of Cardiovascular Disease and Steroid Contraception. Effect of different progestagens in low oestrogen oral contraceptives on venous thromboembolic disease. Lancet. 1995;346:1582-8.

6. Gross PL, Aird WC. The Endothelium and Thrombosis. Semin Thromb Hemost. 2000;26:463-78.

7. Broeders MAW, Tangelder GJ, Slaaf DW, Reneman RS, Mirjam GA, Egbrink OD. Endogenous nitric oxide protects against thromboembolism in venules but not in arterioles. Arterioscler Thromb Vasc Biol. 1998;18:139-45.

8. Lin PH, Johnson K, Pullium JK, Bush L, Conklin BS, Chen C, et al. L-Arginine improves endothelial vasoreactivity and reduces thrombogenicity after thrombolysis in experimental deep venous thrombosis. J Vasc Surg. 2003;38:1396-403.

9. Kuvin J, Patel A, Karas R. Need for standardization of non-invasive assessment of vascular endothelial function. Am Heart J. 2001;141:327-8.

10. Vallance P, Collier J, Moncada S. Nitric oxide syntheside from L-arginine mediates endothelium dependent vasodilation in human veins in vivo. Cardiovasc Res. 1989;23:1053-7.

11. Aellig WH. Use of a linear variable differential transformer to measure compliance of human hand veins in situ [proceedings]. Br J Clin Pharmacol. 1979;8:395.

12. Aellig WH. Clinical pharmacology, physiology and pathophysiology of superficial veins—1. Br J Clin Pharmacol. 1994;38:181-96

13. Aellig WH. Clinical pharmacology, physiology and pathophysiology of superficial veins—2. Br J Clin Pharmacol. 1994;38:289-305.

14. Grossmann M, Abiose A, Tangphao O, Blaschke TF, Hoffman BB. Morphine induced venodilation in humans. Clin Pharmacol Ther. 1996;60:554-60.

15. Grossmann M, Dobrev D, Kirch W. Amiodarone causes endothelium-dependent vasodilation in human hand veins in vivo. Clin Pharmacol Ther. 1998;64:302-11.

16. Grossmann M, Dobrev D, Himmel HM, Kirch W. Local venous response to N-desethylamiodarone in humans. Clin Pharmacol Ther. 2000;67:22-31.

17. Grossmann M, Matthias A, Dobrev D, Siffert W, Kirch W. Heterogeneity in hand veins responses to acetylcholine is not associated with polymorphisms in the G-protein $[beta]_3$-subunit (C825T) and endothelial nitric oxide synthase (G894T) genes but with serum low density lipoprotein cholesterol. Pharmacogenetics. 2001;11:307-16.

18. Grossmann M, Dobrev D, Himmel H, Ravens U, Kirch W. Hypertension Ascorbic Acid–Induced Modulation of Venous Tone in Humans. Hypertension. 2001;37:949-54.

19. Schindler C, Grossmann M, Dobrev D, Francke K, Ravens U, Kirch W. Reproducibility of dorsal hand vein responses to phenylephrine and prostaglandin F2 alpha using the dorsal hand vein compliance method. J Clin Pharmacol. 2003;43:228-36.

20. Sousa MG, Yugar-Toledo JC, Rubira, MC. H. Ascorbic acid improves impaired venous and arterial endothelium-dependent function. Acta Pharmacol Sinica. 2005;26:447-52.

21. Nelson AL. Intrauterine device practice guidelines: medical conditions. Contraception. 1998; 58(3 suppl): 59S-63S.

22. Lewis TV, Dart AM, Chin-Dusting JP. Endothelium-dependent relaxation by acetylcholine is impaired in hypertriglyceridemic humans with normal levels of plasma LDL cholesterol. J Am Coll Cardiol. 1999;33:805-12.

23. Stepniakowski KT, Lu G, Davda RK, Egan BM. Fatty acids augment endothelium-dependent dilation in hand veins by a cyclooxygenase-dependent mechanism. Hypertension. 1997;30:1634-9.

24. Bedarida GV, Bushell E, Haefeli WE, Blaschke TF, Hoffman BB. Responsiveness to bradykinin in veins of hypercholesterolemic humans. Circulation. 1993;88:2754-61.

25. Stefano T, Virdis A, Ghiadoni L, Versari D, Salvetti A. Endothelium, aging and hypertension. Curr Hypertesion Reports. 2006;8(1):84-9.

26. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA. 2001;285:2486-97.

27. Jick H, Jick SS, Gurewich V, Myers MW, Vasilakis C. Risk of idiopathic cardiovascular death and nonfatal venous thromboembolism in women using oral contraceptives with differing progestagen components. Lancet. 1995;346:1589-93.

28. World Health Organization Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception. Venous thromboembolic disease and combined oral contraceptives: results of international multicentre case-control study. Lancet.1995;346:1575-82.

29. Jick H, Jick SS, Myers MW. Risk of acute myocardial infarction and low-dose combined oral contraceptives. Lancet. 1996;347:627-8.

30. Lerner DJ, Kannel WB. Patterns of coronary heart disease morbidity and mortality in the sexes: a 26-year follow-up of the Framingham population. Am Heart J. 1986;111:383-90.

31. Farley TM, Meirik O, Collins J. Cardiovascular disease and combined oral contraceptives: reviewing the evidence and balancing risks. Hum Reprod Update. 1999;5:721-35.

32. Hannaford PC, Owen-Smith V. Using epidemiological data to guide clinical practice: review of studies on cardiovascular disease and use of combined oral contraceptives. BMJ. 1998;316:984-7.

33. Conard J. Biological coagulation findings in third-generation oral contraceptive pills. Hum Reprod Update. 1999;5:672-680.

34. Tans G, Curvers J, Middeldorp S. A randomized cross-over study on the effects of levonorgestrel- and desogestrel-containing oral contraceptives on the anticoagulant pathways. Thromb Haemost. 2000;84:15-21.

35. Winkler UH. Hemostatic effects of third- and second-generation oral contraceptives: absence of a causal mechanism for a difference in risk of venous thromboembolism. Contraception. 2000;62:11S-20S.

36. Rosing J, Middeldorp S, Curvers J. Low-dose oral contraceptives and acquired resistance to activated protein C: a randomised cross-over study. Lancet.1999;354:2036-40.

37. Peek MJ, Markham R, Fraser IS. The effects of natural and synthetic sex steroids on human decidual endothelial cell proliferation. Hum Reprod. 1995; 10:2238-43.

38. Lima SM, Aldrighi JM, Consolim-Colombo FM. Acute administration of 17 beta-estradiol improves endothelium-dependent vasodilation in postmenopausal women. Maturitas. 2005;50:266-74.

39. Subakir SB, Hadisaputra W, Siregar B, Irawati. Reduced endothelial cell migratory signal production by endometrial explants from women using Norplant contraception. Hum Reprod. 1995; 10:2579-83

40. Mutunga M, Fulton B, Bullock R, Batchelor A, Gascoigne A, Gillespie JI, Baudouin SV. Circulating endothelial cells in patients with septic shock. Am J Respir Crit Care Med. 2001;163:195-200.

41. Risk MR, Lirofonis V, Armentano RL, Freeman R. A biphasic model of limb venous compliance: a comparison with linear and exponential models. J Appl Physiol. 2003;95:1207-15.

42. Hoetzer GL, Stauffer BL, Greiner JJ, Casas Y, Smith DT, and Souza CA. Influence of oral contraceptive use on t-PA release in healthy premenopausal women. Am J. Physiol Endocriol Metabol. 2003;284: E90-E95,

43. Herkert O, Kuhl H, Busse R, Schini-Kerth VB. The progestin Levonorgestrel induces endothelium- independent relaxation of rabbit jugular vein via inhibition of calcium entry and protein kinase C: role of cyclic AMP. Br J of Pharmacol. 2000;130:1911-8

44. John S, Jacobi J, Schlaich MP, Delles C, Schmieder RE. Effects of oral contraceptives on vascular endothelium in premenopausal women. Am J Obstet Gynecol. 2000;183:28-33.

45. Virdis A, Pinto S, Versari D. Effect of oral contraceptives on endothelial function in the peripheral microcirculation of healthy women. J Hypertens. 2003;21:2275-80.

46. Suzy YH, Pang B, Stojanovska L, Sudhir K, Komesaroff PA. Progesterone does not influence vascular function in postmenopausal women. J Hypertens. 2003;21:1145-9.

47. Sorensen MB, Collins P, Ong PJL. Long-term use of contraceptive depot medroxyprogesterone acetate in young women impairs arterial endothelial function assessed by cardiovascular magnetic response. Circulation 2002;106:1646-51.