# Modulation of Plasma Endothelin Levels by the Menstrual Cycle

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**Blood pressure varies during the menstrual cycle, but the reason for this is unclear. Administration of (synthetic) sex hormones can influence the level of vasoactive substances such as endothelin (ET). However, it is not known whether short-term variations in sex hormone levels in physiological situations affect ET levels. We assessed the effects of the menstrual cycle on plasma ET-1 in 8 healthy premenopausal women not using oral contraceptives (OCs) and 8 premenopausal women using OCs. ET-1 levels were measured in all subjects on days 1 to 3 (menstrual phase), 9 to 12 (follicular phase), and 20 to 23 (luteal phase) of the menstrual cycle. ET-1 levels remained constant in OC users (2.4 ± 0.4, 2.6 ± 0.4, and 2.4 ± 0.4 pg/mL on days 1 to 3, 9 to 12, and 20 to 23 of the pill cycle). In contrast, ET-1 levels in non–OC users decreased in all women during the follicular and luteal phase of the menstrual cycle compared with the menstrual (low-estrogenic) phase (3.6 ± 0.5, 2.8 ± 0.5, and 2.9 ± 0.3 pg/mL for the menstrual, follicular, and luteal phase, respectively, $P < .01$ for menstrual *v* follicular and $P < .01$ for menstrual *v* luteal). The differences between OC users and nonusers were significant in the menstrual phase of the cycle ($P < .01$). We conclude that ET levels fluctuate during the menstrual cycle. Previously reported effects of the menstrual cycle on blood pressure may be partly explained by the effects of sex hormones on the level of vasoactive mediators. This fluctuation is not present in OC users. Studies on hemodynamic parameters in premenopausal women should account for hormonal variations in the various phases of the menstrual cycle.**



ENDOTHELIN (ET) is a potent vasoconstrictor synthesized by the vascular endothelium that may be involved in the pathogenesis of hypertension[1] and atherosclerosis.[2] Three distinct subtypes of ET (ET-1, ET-2, and ET-3) have been determined thus far.[3] Of these subtypes, ET-1 has the strongest effect on vessel contraction and ET-3 the weakest.

We previously reported that ET levels are higher in men than in age-matched women and that ET decreases during pregnancy when estrogen levels are high. We also found that ET levels decrease in male transsexual patients during estrogen administration.[4,5] Others have reported that ET decreases in postmenopausal women during estrogen replacement therapy.[6] Thus, ET levels appear to be influenced by sex hormones. This may be one of the mechanisms by which estrogens confer protective effects in atherogenesis, and one of the reasons for the much lower incidence of atherosclerosis in premenopausal women versus age-matched men. However, the above-mentioned studies were performed with relatively high doses of (synthetic) hormones or with older (postmenopausal) subjects. Thus, the effects on ET of fluctuations of sex hormone levels in physiologic situations are unknown.

We decided to assess whether the short-term fluctuations in hormone levels that occur during a normal menstrual cycle affect ET levels. There is some evidence suggesting that vascular function is affected by the menstrual cycle. For example, Hashimoto et al[7] demonstrated that endothelium-derived vasodilatation varies during the menstrual cycle, with increased vasodilatation during the follicular and luteal (high-estrogenic) phases of the cycle. It has also been reported that blood pressure is modulated by the menstrual cycle,[8-10] though this finding has been challenged.[11-12]

Thus, it has been shown that administration of estrogens is associated with a decrease in ET levels and that ET levels decrease during pregnancy. Further, there is evidence suggesting that hemodynamic parameters are affected by the menstrual cycle. During a normal menstrual cycle lasting 28 days, estrogen levels are lowest during the menstrual phase (days 0 to 4) and highest during the follicular phase, reaching peak values between days 8 to 13 of the menstrual cycle. Estrogen levels decrease slightly in the midluteal phase (days 18 to 24) and sharply in the last days before menstruation.[13] Levels of progesterone are low during the menstrual and follicular phases of the cycle, increase sharply after ovulation, and remain high until the end of the luteal phase.[13]

To assess whether plasma levels of ET are modulated by the menstrual cycle, we measured ET levels in premenopausal women in various phases of their menstrual cycle. For comparison, we measured ET levels in women using monophasic oral contraceptives (OCs), ie, women without the hormone fluctuations associated with a normal menstrual cycle.

## SUBJECTS AND METHODS

Plasma ET-1 levels were measured in 8 healthy premenopausal women (aged 30 ± 5 years) on days 1 to 3 (menstrual phase), days 9 to 12 (follicular phase), and days 20 to 23 (luteal phase) of the menstrual cycle. Plasma was separated within 1 hour and stored at −70°C until analysis. All women had a regular menstrual cycle (duration, 27 to 30 days), and none used OCs or other hormones in the 6 months prior to inclusion in the study. ET-1 levels were measured using a specific ET-1 assay (Amersham, Little Chalfont, UK) after extraction on a $C_{18}$ Sep-Pak column as previously described.[4] The sensitivity of this assay was 0.3 pg/mL; intraassay and interassay coefficients of variation were 4% and 9%, respectively. Cross-reactivity with ET-2 was 100%, and cross-reactivity with ET-3 and with "big" ET was less than 1%.

Immunoreactive ET (irET) levels were measured by radioimmunoassay (Nichols Institute, formerly ITS, Wijchen, The Netherlands) after extraction as described for ET-1. The sensitivity of this assay is 2 pg/mL, with intraassay and interassay coefficients of variation of 10.5% and 10.3%, respectively. Cross-reactivity with ET-2 is 52%, with ET-3 96%, and with "big" ET 7%.

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*Submitted June 2, 1999; accepted October 11, 1999.*

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*0026-0495/00/4905-0001$10.00/0*

Levels of ET-1 and irET were also measured in 8 women using monophasic OCs on days 1 to 3, 9 to 12, and 20 to 23 of a 28-day pill cycle. Five women used Marvelon (Organon, Oss, The Netherlands), containing 0.03 mg ethinylestradiol and 0.15 mg desogestrel; 3 women used Microgynon 30 (Schering, Weesp, The Netherlands), containing 0.03 mg ethinylestradiol and 0.15 mg levonorgestrel. ET-1 and irET levels were measured using the same assays with the same coefficients of variation described before.

Informed consent was obtained from all subjects, and the study was approved by the hospital ethics committee. All patients were within 10% of their ideal body weight (Metropolitan Life Insurance Tables 1959). None had a personal or family history of diabetes or hypertension or evidence of cardiovascular disease on routine examination (medical history and physical examination).

### *Statistical Analysis*

Results are expressed as the mean ± SD. ANOVA for repeated measures was used for comparison of plasma ET levels within each group. Student's 2-tailed unpaired $t$ test was used for comparison between groups. Statistical significance was accepted for a $P$ level less than .05.

## RESULTS

ET-1 levels in women not using OCs are shown in Fig 1. ET-1 was highest during the menstrual phase of the cycle (3.6 ± 0.5 pg/mL) and decreased in all women in the follicular phase (2.8 ± 0.5 pg/mL, $P < .01$). During the luteal phase, ET-1 levels increased slightly (2.9 ± 0.3 pg/mL, $P$ = NS $v$ follicular phase) but remained lower versus the menstrual phase ($P < .01$). Similar results were obtained for irET levels measured with the Nichols kit (3.8 ± 0.5, 2.6 ± 0.5, and 2.8 ± 0.5 pg/mL during the menstrual, follicular, and luteal phase of the cycle, respectively, $P < .05$ for menstrual $v$ follicular and luteal).

No such fluctuation in ET-1 levels was observed in women using OCs (2.4 ± 0.4, 2.6 ± 0.4, and 2.4 ± 0.4 pg/mL on days 1 to 3, 9 to 12, and 20 to 23, respectively; Fig 2). Similar results were found when irET levels were measured (data not shown). The difference between fluctuations of ET-1 levels in OC users and nonusers (expressed as a percentage of the initial value) was statistically significant. A decrease in ET-1 levels during the follicular phase of the cycle was observed in all women not using OCs; the average decrease was 22% versus the menstrual phase. In contrast, ET-1 levels increased by an average of 8% in women using OCs during the same period ($P < .01$).

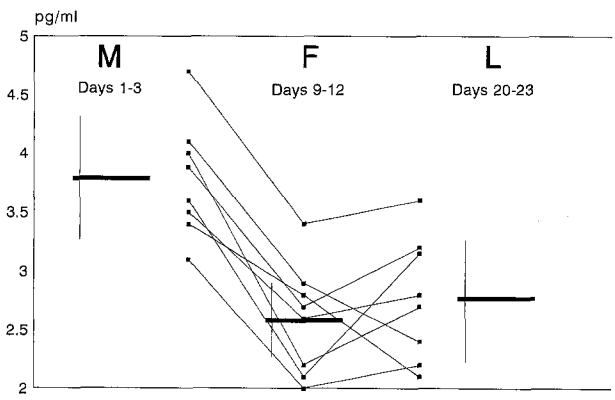


**Fig 1. ET levels in premenopausal women not using OCs. ET levels fluctuated during the menstrual cycle, with higher levels during the menstrual phase $v$ the follicular phase ($P < .01$) or luteal phase ($P < .01$).**

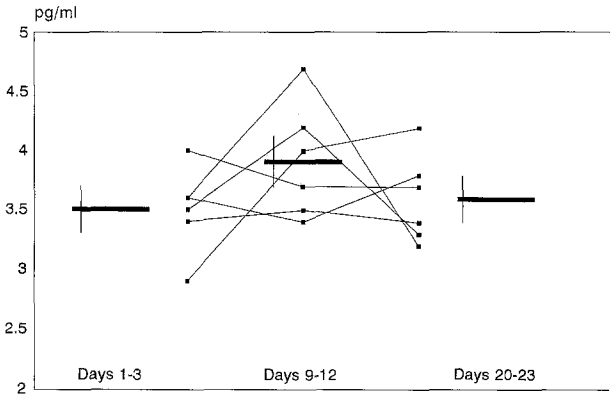


**Fig 2. ET levels in women using OCs, measured 3 times during a full pill cycle (days 1-21, pill days; days 22-28, pill-free days). No significant changes occurred during the pill cycle.**

## DISCUSSION

We report that the levels of ET, a potent vasoconstrictor involved in the regulation of vascular tone, fluctuate during the menstrual cycle, with higher levels during the menstrual phase when estrogen levels are low. Although we previously found lower ET levels in the hyperestrogenic state of pregnancy,[4] to our knowledge, this is the first report suggesting that short-term physiological variations in estrogen levels affect ET levels.

It has been shown that estrogen receptors are present in the arterial wall and the receptor concentration varies with the hormonal status.[14] Various experiments have shown that vascular reactivity and the level of several vasoactive substances are affected by the menstrual cycle.[15] Other studies have demonstrated that estradiol has vasodilatory effects.[15] The possible mechanisms of estradiol-induced vasodilatation include an increased synthesis of vasodilators such as nitric oxide (NO) and prostacyclin and a modulation of adrenergic responsiveness.[15] Rosselli et al[16] reported that nitrate, the stable end product of NO, increases during the follicular phase of the menstrual cycle in conjunction with 17β-estradiol levels. There is evidence suggesting that NO is a regulator of ET production.[17] Therefore, the fluctuations in ET levels observed in our study may have been mediated by NO, although we cannot exclude direct effects of estrogens on ET secretion and/or clearance. The observation that administration of (high-dose) estrogens is associated with a decrease in both ET and big ET, which share a common pathway with regard to synthesis and secretion but not with regard to clearance,[5] argues in favor of an effect of estrogens on ET synthesis and/or secretion.

We did not assess blood pressure during the various phases of the menstrual cycle in our subjects. Previous studies on this matter have shown that blood pressure is affected by the menstrual cycle, although some of the data have been conflicting.[8-12,18] Differences in methodology (1-time measurements $v$ 1-hour or 24-hour measurements) and in the selection of days for measurements to be made (ranging from 2 to 7 periods per month) may explain these differences. In a meticulous study by Dunne et al,[9] women were studied in 7 separate time intervals

during a 24-hour period, using an automatic blood pressure device. These investigators reported an increase in systolic and diastolic blood pressure near the onset of menstruation in both normotensive and hypertensive women; no other significant changes were observed during the cycle. Our observation that ET levels are highest during the menstrual phase raises the possibility that these fluctuations in blood pressure and ET levels are related.[9]

There is evidence that natural progesterone (in contrast to synthetic progestagens) may have a blood pressure–lowering effect.[19] However, we did not observe a decrease in ET levels in the luteal phase compared with the follicular phase. Others have reported that NO levels appear to decrease in the postovulatory (high-progesterone) phase of the cycle. Thus, if progesterone does indeed have an additional blood pressure–lowering effect, it does not appear to be mediated by endothelial mediators such as ET or NO.

Levels of ET-1 remained more or less constant in OC users. Thus, ET-1 levels fluctuate in women with a normal menstrual cycle, but not in women in whom the normal cycle is suppressed by OCs. Overall, ET-1 levels were slightly lower in OC users, possibly reflecting their high-estrogenic status induced by the use of synthetic estrogens.

In conclusion, we have found that levels of ET fluctuate during the menstrual cycle, suggesting that small variations in estrogen levels over a relatively short period of 10 days may affect the secretion of vasoactive mediators by the endothelium. The effects of sex hormones on these vasoactive mediators, in part, may explain the reported effects of the menstrual cycle on blood pressure and vasoreactivity in premenopausal women.

Finally, our findings underscore the premise that studies in premenopausal women should account for hormonal variations during the menstrual cycle. This is true not only for studies on vasoactive mediators, as demonstrated by this and previous studies, but also for other areas of investigation. For example, it has recently been shown that lipoprotein and apolipoprotein levels vary during the menstrual cycle.[20] Therefore, studies in premenopausal women should account for the menstrual cycle and, if feasible, should perform their observations in the same phase of the cycle to avoid confounding effects. Our study shows that this certainly applies to studies dealing with ET-1.

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