Journal of the American College of Cardiology
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Published by Elsevier Science Inc.

Vol. 38, No. 7, 2001
ISSN 0735-1097/01/$20.00
PII S0735-1097(01)01658-8

Modulation of Coronary Flow Velocity Reserve by Gender, Menstrual Cycle and Hormone Replacement Therapy

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OBJECTIVES The purpose of this study was twofold: 1) to examine the relationship between menstrual cycle and coronary flow velocity reserve (CFVR) in young healthy women, and 2) to evaluate the effect of hormone replacement therapy by estrogen on CFVR in postmenopausal women, using transthoracic color Doppler echocardiography (TTCDE).

BACKGROUND Although the incidence of cardiovascular disease is lower in women before menopause compared with men, postmenopausal women have an incidence of coronary artery disease similar to that of men of the same age. This is mainly dependent upon estrogen deficiency. However, no clinical report has yet examined the effect of estrogen on CFVR, which is one index of coronary microcirculation.

METHODS We examined 15 male and both 15 premenopausal and 10 postmenopausal female healthy volunteers. We measured coronary flow velocity of the left anterior descending coronary artery at baseline and hyperemic conditions during adenosine triphosphate infusion by TTCDE and determined CFVR. Each premenopausal woman was studied two times (menstrual [M] and follicular [F] phases) in one menstrual cycle. Fifteen men were also studied at a time corresponding to women's menstrual cycle. The postmenopausal women were studied before and two hours after oral administration of conjugated estrogen (CE).

RESULTS Serum 17β-estradiol level in premenopausal women increased in the F phase and decreased to the same levels as in men, as in the M phase and as in postmenopausal women (123 $\pm$ 9 pg/ml vs. 28 $\pm$ 6 pg/ml, 25 $\pm$ 9 pg/ml and 19 $\pm$ 11 pg/ml; $p < 0.0001$, respectively). The CFVR increased in the F phase compared with that in the M phase (4.8 $\pm$ 0.4 vs. 3.7 $\pm$ 0.8, $p < 0.0001$). We found that CFVR in men remained unchanged (3.7 $\pm$ 0.6 vs. 3.8 $\pm$ 0.5). After CE administration, CFVR increased compared with baseline in postmenopausal women (4.1 $\pm$ 0.8 vs. 3.4 $\pm$ 0.8, $p < 0.005$).

CONCLUSIONS In premenopausal women, CFVR determined by TTCDE varied during the menstrual cycle, and in postmenopausal women, CFVR increased after acute estrogen replacement. (J Am Coll Cardiol 2001;38:1879–84) © 2001 by the American College of Cardiology

The incidence of cardiovascular disease is lower in women before menopause compared with men. Epidemiologic studies and large randomized clinical trials consistently find that hormone replacement therapy lowers the risk of coronary heart disease in women (1–3). Estrogen may be supposed to have a cardioprotective effect on women. In addition, it has been reported that treatment with estrogen

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improves exercise time to myocardial ischemia in postmenopausal women with proven coronary heart disease (4,5). A recent report (6) shows that myocardial ischemia was more easily induced in premenopausal women when estrogen concentrations were low during the menstrual cycle. Furthermore, variation in flow-mediated dilation of brachial artery during the menstrual cycle has been reported in human subjects (7). To the best of our knowledge, however, no clinical report has examined the effect of estrogen on coronary flow reserve, which is one index of coronary microcirculation.

Recent development in transthoracic color Doppler echocardiography (TTCDE) enables us to estimate coronary flow reserve by measuring coronary flow velocity reserve (CFVR) in vivo (8,9). Using this technique, the effect of estrogen on coronary microcirculation may be investigated noninvasively. The purpose of this study was to: 1) examine the relationship between menstrual cycle and CFVR in young, healthy women, and 2) using TTDE, evaluate the effect of hormone replacement therapy by estrogen on CFVR in postmenopausal women.

METHODS

Study population. A total of 15 male, and 17 premenopausal and 11 postmenopausal female volunteers were enrolled in this study. The 15 male subjects, 24 to 37 years old, were healthy medical doctors. The 17 premenopausal women, 22 to 38 years old, were healthy nurses and doctors.

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Manuscript received April 13, 2001; revised manuscript received July 18, 2001, accepted August 22, 2001.

Abbreviations and Acronyms

ANOVA = analysis of variance
ATP = adenosine triphosphate
CE = conjugated estrogen
CFVR = coronary flow velocity reserve
F = follicular
HDL = high-density lipoprotein
LAD = left anterior descending coronary artery
LDL = low-density lipoprotein
M = menstrual
MDV = mean diastolic velocities
NO = nitric oxide
TTDE = transthoracic color Doppler echocardiography

They had regular menstrual cycles (26 to 36 days) for more than three months before this study. None had a history of pregnancy. The postmenopausal women, 56 to 76 years old, were also healthy volunteers, all with plasma estradiol levels <50 pg/ml and cessation of menses for at least a year. All subjects were asymptomatic, normotensive, nondiabetic and nonsmokers. They had no significant medical history. No subjects had taken any cholesterol-lowering agent, estrogen therapy or antioxidant vitamin supplements for the preceding two months. Each subject gave written informed consent before the enrollment in this study after thorough explanation of the study design and protocol.

Study design. Each premenopausal woman was studied twice in one menstrual cycle. The measurement was done once in the menstrual (M) phase and the follicular (F) phase. To estimate their menstrual cycles, they checked their body temperature every morning during this study. Fifteen men were also studied twice (at the beginning of the study and at two weeks), corresponding to women's menstrual phase, to evaluate the "cycle" effect. The postmenopausal women were studied before and two hours after oral administration of 1.25 mg of conjugated estrogen (CE).

Laboratory assays. Blood sampling was performed on the morning of the ultrasound examination after a 14-h overnight fasting (including caffeine) to measure serum concentrations of 17β-estradiol, total cholesterol, triglycerides and high-density lipoprotein (HDL) cholesterol. Serum 17β-estradiol levels were measured by radioimmunoassay. Blood (5 ml) was drawn from the individuals immediately before each echocardiographic study and was centrifuged at 1,000 *g* for 10 min. Following centrifugation, serum was kept at −80°C until assay. Total cholesterol and triglycerides in the serum were quantified by automated enzymatic techniques. Serum HDL cholesterol was quantified after dextran sulfate precipitation of other lipoproteins. All specimens were measured within 48 h after the blood sampling.

Coronary flow studies. Echocardiography was performed with the Acuson Sequoia 512 (Mountainview, California) with a frequency of 7.0 MHz. In color Doppler flow mapping, velocity range was set in the range of ±12 cm/s. The color gain was adjusted to provide the optimal images. The ultrasound beam was transmitted toward the heart to visualize coronary blood flow in the left anterior descending coronary artery (LAD) by color Doppler echocardiography. First, the left ventricle was imaged in the long-axis cross section, and the ultrasound beam was inclined laterally. Next, coronary blood flow in the distal portion of the LAD was searched under the guidance of color Doppler flow mapping. With a sample volume (2.5 or 3.0 mm wide) positioned on the color signal in the LAD, Doppler spectral tracings of flow velocity in the LAD were recorded by fast Fourier transformation analysis. Although we tried to align ultrasound beam direction to distal LAD flow as parallel as possible, angle correction was needed in each examination because of incident Doppler angle (mean angle 42°; range 31° to 58°).

We first recorded baseline spectral Doppler signals in the distal portion of the LAD over five cardiac cycles at end-expiration by TTDE. Intravenous adenosine triphosphate (ATP) was administered (140 $\mu g \cdot kg^{-1} \cdot min^{-1}$ IV) for 2 min to record spectral Doppler signals during hyperemic conditions. All subjects had a continuous heart rate and electrocardiographic monitoring. Blood pressure was recorded at baseline and every minute during ATP infusion.

Each study was analyzed by two experienced investigators who were blinded to the other subject data. Measurements were performed off-line by tracing the contour of the spectral Doppler signal using the computer incorporated in the ultrasound system. Mean diastolic velocities (MDVs) were measured at baseline, and peak hyperemic conditions were measured from the Doppler signal recordings. Measurements were averaged over three cardiac cycles. The CFVR was defined as the ratio of hyperemic to basal MDV.

Statistical analysis. All the data were expressed as mean ± SD. Echocardiographic and hemodynamic variables during ATP infusion in both pre- and postmenopausal women and in men were evaluated by two-way repeated-measures analysis of variance (ANOVA), testing for estrogen effect, ATP effect, and interaction. The Fisher protested least-significant difference test was used for the post hoc test. The CFVR values for M and F phases in premenopausal women, before and after CE intake in postmenopausal women, and twice in men were compared by paired *t* test. Laboratory data were also analyzed by paired *t* test. For all analysis, $p < 0.05$ was considered significant. Interobserver and intraobserver variabilities were assessed for velocity measurement in 20 recordings in 10 randomly selected patients. Interobserver variability was calculated as the SD of the differences between the measurements of two independent observers who were unaware of the other patient data, and this was expressed as a percent of the average value. Intraobserver variability was calculated as the SD of the differences between the first and second determination (three-week interval) for a single observer, and this was expressed as a percent of the average value.

Table 1. Clinical Characteristics of Subjects

	Women			Men	
	Premenopausal				
	M Phase	F Phase	Postmenopausal	Initial	Follow-Up at Two Weeks
Age (yr)	27 ± 5		64 ± 7	27 ± 4	
Hormone (pg/ml)					
17β-estradiol	25 ± 9	123 ± 9*	19 ± 11	28 ± 6	26 ± 8
Lipids (mg/dl)					
Total cholesterol	152 ± 21	150 ± 28	139 ± 17	148 ± 27	143 ± 22
Triglycerides	70 ± 36	51 ± 17	63 ± 14	66 ± 11	66 ± 8.0
HDL cholesterol	59 ± 11	59 ± 12	60 ± 11	59 ± 11	57 ± 12

*$p < 0.0001$ for the comparison with the value for the menstrual (M) phase.
F = follicular; HDL = high-density lipoprotein.

RESULTS

Under the guidance of color Doppler flow mapping, adequate spectral Doppler recordings of coronary flow in the distal portion of the LAD for the assessment of CFVR were obtained in 40 (15 men, and 15 premenopausal and 10 postmenopausal women) of 43 study patients (93%). No patient noted atrioventricular block, chest pain, flushing, or palpitations during ATP infusion in the TTDE studies. Table 1 shows the clinical characteristics of subjects, including serum lipid profile and serum 17β-estradiol level. During drug administration, heart rate and systolic blood pressure increased significantly ($p < 0.02$, all groups). Diastolic blood pressure decreased significantly ($p < 0.01$, all groups). However, the two-way ANOVA showed no interaction in terms of heart rate, systolic blood pressure and diastolic blood pressure between M and F phases, before and after CE intake, and initial and follow-up at two weeks in men during ATP infusion. There was no significant difference of age between the men and the premenopausal women groups.

Effects of gender, menstrual cycle and menopause on serum 17β-estradiol levels. Serum 17β-estradiol levels of premenopausal women were proved to be appropriate to each menstrual phase with the consecutive estimation of their previous menstrual cycles, morning body temperature and actual menstruation during this study. Serum 17β-estradiol levels in premenopausal women increased in the F phase. Serum 17β-estradiol levels in men and in postmenopausal women were also within normal ranges. In men, serum 17β-estradiol levels were measured twice a month at a time corresponding to the women's menstrual phase, and serum hormone levels remained unchanged (Fig. 1).

Effects of gender and menstrual cycle on CFVR. Table 2 shows hemodynamic data, coronary flow velocity and CFVR data in the study population. In premenopausal women, no significant difference was seen in hemodynamics between the M phase and F phase. No significant difference in MDV at baseline was observed in premenopausal women between the M and F phases. However, the two-way ANOVA showed significant estrogen effect and interaction in MDV over ATP ($p = 0.003$ and $p = 0.0001$, respectively). In premenopausal women, MDV during ATP infusion was significantly greater in the F phase compared with that in the M phase (Table 2). Also, CFVR in the F phase was greater than that in the M phase (Table 2, Fig. 2, left). In men, CFVR remained unchanged (Table 2, Fig. 2, right).

Effects of estradiol administration on CFVR. Hemodynamic levels after CE intake did not differ from levels before CE intake in postmenopausal women (Table 2). The MDV at baseline was comparable in postmenopausal women before and after administration of CE (Table 2). Two-way

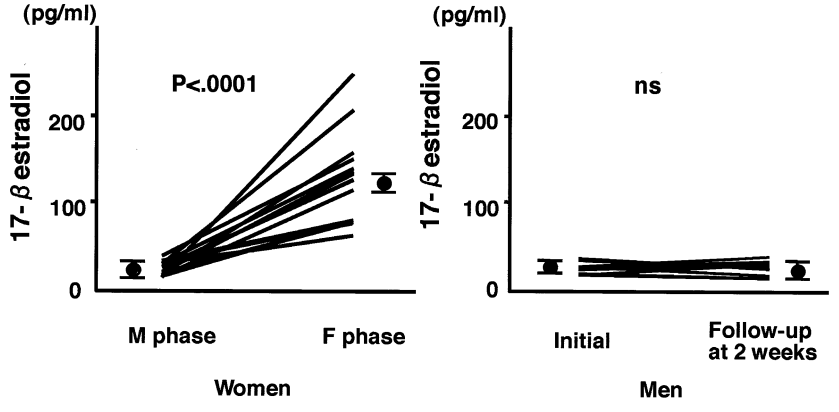

Figure 1. Individual change in serum 17β-estradiol concentration in the menstrual (M) and follicular (F) phases in premenopausal women **(left)** and in initial and second studies at two weeks in men **(right)**.

Table 2. Hemodynamics and Coronary Flow Velocity Measurements

	Women				Men	
	Premenopausal		Postmenopausal			
	M Phase	F Phase	Before CE	After CE	Initial	Follow-Up at Two Weeks
HR (beats/min)						
Baseline	67 ± 6	66 ± 6	67 ± 6	69 ± 6	66 ± 5	68 ± 6
ATP infusion	72 ± 6	71 ± 5	72 ± 7	72 ± 6	73 ± 6	74 ± 6
Systolic BP (mm Hg)						
Baseline	118 ± 11	119 ± 10	129 ± 9	130 ± 9	123 ± 9	123 ± 9
ATP infusion	114 ± 11	114 ± 10	124 ± 8	123 ± 8	117 ± 9	116 ± 10
Diastolic BP (mm Hg)						
Baseline	69 ± 8	69 ± 9	74 ± 7	75 ± 8	65 ± 9	68 ± 8
ATP infusion	65 ± 8	65 ± 8	67 ± 7	68 ± 8	59 ± 9	62 ± 9
MDV (cm/s)						
Baseline	23 ± 5	23 ± 4	25 ± 6	24 ± 6	24 ± 5	24 ± 4
ATP infusion	85 ± 23	114 ± 19†	82 ± 21	102 ± 28	89 ± 16	89 ± 13
CFVR	3.7 ± 0.8	4.8 ± 0.4†	3.4 ± 0.8	4.1 ± 0.8*	3.7 ± 0.6	3.8 ± 0.5

*$p < 0.0001$ for the comparison with the value for Before CE. †$p < 0.0001$ for the comparison with the value for the menstrual (M) phase.
ATP = adenosine triphosphate; BP = blood pressure; CE = conjugated estrogen; CFVR = coronary flow velocity reserve; F = follicular; HR = heart rate; MDV = mean diastolic velocity.

ANOVA showed significant interaction in MDV over ATP ($p = 0.04$). Also, CFVR significantly increased after CE intake in postmenopausal women (Fig. 3, Table 2).

OBSERVER VARIABILITIES. Interobserver and intraobserver variabilities for the measurement of Doppler velocity recordings were 4.8% and 4.1%, respectively.

DISCUSSION

We have demonstrated that: 1) CFVR by TTDE varied during the menstrual cycle depending on serum 17β-estradiol levels in premenopausal women, and 2) acute estrogen replacement increased CFVR in postmenopausal women.

The CFVR assessment by TTDE. Using TTDE, we assessed CFVR, which has been alternatively used as coronary flow reserve (8,9). Coronary flow reserve has been reported to be reflective coronary microcirculation abnormality in patients with hypercholesterolemia (10), diabetes (11,12) and hypertension (13,14). In these studies, coronary flow reserve was measured by positron emission tomography, Doppler catheter or guide wire and transesophageal Doppler echocardiography. Therefore, no coronary flow reserve data of young subjects have been demonstrated prior to this study. The TTDE we used in the present study is completely noninvasive and a relatively inexpensive examination. Furthermore, this technique provides CFVR measurement at a high success rate with a high-frequency transducer and a reduced velocity range in color Doppler echocardiography. Thus, this noninvasive technique was suitable for evaluation of serial change in CFVR in the present study subjects.

Effect of estrogen on coronary circulation. This is the first report showing a relationship between CFVR and estrogen level in young, healthy human subjects. We found that CFVR varied depending on gender and on menstrual cycle in female subjects and that the variation was associated with the increase in serum 17β-estradiol concentration during the menstrual cycle. We also found that CFVR in male subjects was comparable to that in premenopausal women in the M phase and in postmenopausal women, and that estrogen supplementation increased CFVR in post-

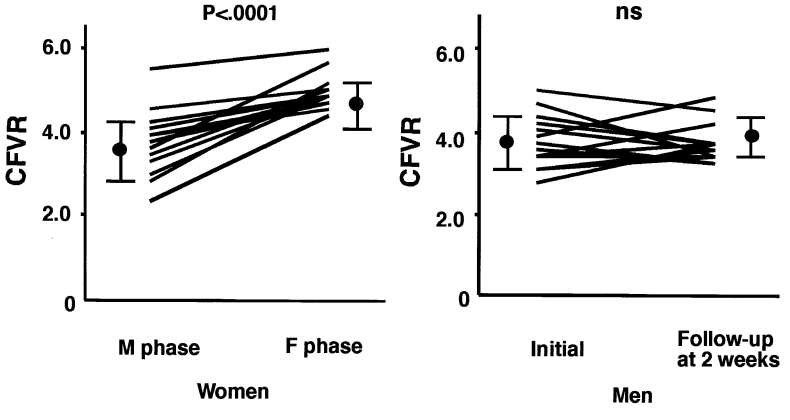

Figure 2. Individual change in coronary flow velocity reserve (CFVR) in the menstrual (M) and follicular (F) phases in premenopausal women **(left)** and in initial and second studies at two weeks in men **(right)**.

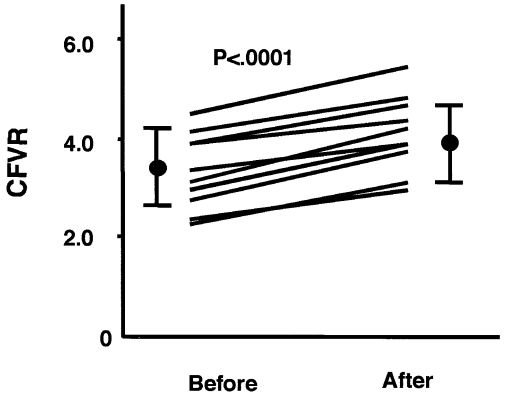

Figure 3. Individual change in coronary flow velocity reserve (CFVR) at baseline and after conjugated estrogen administration.

menopausal women. These results suggest that estrogen may be associated with an increase in CFVR and that estrogen may have a good effect on coronary microcirculation.

Possible mechanism of cardioprotective effect of estrogen. Several mechanisms have been proposed to explain the cardioprotective effect of estrogen on postmenopausal women. Some studies have shown that lower risk of coronary heart disease is attributable to the favorable effects of estrogen on lipid and lipoprotein levels (15,16). Other mechanisms of potential benefit include antioxidant effects. Antioxidant effects of estrogen, inhibiting low-density lipoprotein (LDL) oxidation (17), result in enhancing nitric oxide (NO) availability (18–20). Oxidized LDL interferes with receptor-mediated stimulation and signal transduction in the release of NO (19,20).

In addition, estrogen has been shown to increase transcription and activity of NO synthase in endothelial cell culture studies (21). Stefano et al. (22) have shown that physiologic doses of estrogen immediately stimulate NO release from human endothelial cells through activation of a cell-surface estrogen receptor that is coupled to increases in intracellular calcium. This rapid action of estrogen might be one mechanism of increase in CFVR after acute hormone replacement in the present study. However, further investigations will be necessary to clarify precisely the mechanism of estrogen's effect on CFVR.

Study limitations. There are several limitations in the present study. First, the study measured coronary flow velocity change in epicardial coronary artery, not coronary flow volume, although assessment of coronary flow reserve is ideal for estimation of coronary microcirculation. However, change in coronary flow velocity during drug-induced hyperemia has been alternatively used for assessment of coronary flow reserve because coronary flow velocity correlates well with coronary flow reserve (23). The CFVR in LAD could be reflective of coronary microcirculation.

Second, long-term effect of estrogen on CFVR has not been evaluated in postmenopausal women, although we showed acute effect of estrogen on CFVR. Further studies are necessary to evaluate the long-term effect of estrogen on CFVR in postmenopausal women.

A combination of estrogen and progesterone is more frequently used owing to the risk of uterine cancer with unopposed estrogen therapy. However, a randomized trial showed that treatment with combined hormone replacement therapy did not reduce the rate of coronary heart disease events in postmenopausal women with established coronary disease (24). It was also reported that combined hormone replacement therapy in postmenopausal women did not improve brachial artery flow-mediated vasodilation (25). Further examination is needed to evaluate the effect of combined hormone replacement therapy on coronary circulation in healthy postmenopausal women.

Conclusions. We draw the following conclusions from this study: 1) in premenopausal women, CFVR determined by TTDE varied during the menstrual cycle depending on serum 17β-estradiol level; and 2) the CFVR increased after acute estrogen replacement in postmenopausal women.

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