

Estradiol Supplementation Suppresses Hyperventilation-Induced Attacks in Postmenopausal Women With Variant Angina

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OBJECTIVES	We sought to examine whether estradiol (E ₂) supplementation suppresses anginal attacks in women with variant angina.
BACKGROUND	Estrogen is known to improve endothelial function. Coronary spasm plays an important role in the pathogenesis of not only variant angina but also ischemic heart disease in general, and endothelial dysfunction seems to be involved in the pathogenesis of coronary spasm.
METHODS	Fifteen postmenopausal women with variant angina (mean age 54.2 years) were given a hyperventilation (HV) test, a provocation test for coronary spasm, in the early morning of day 1 (baseline), day 3 (after 2-day transdermal E ₂ supplementation, 4 mg) and day 5 (after 2-day placebo administration). We measured the flow-mediated (endothelium-dependent) dilation (FMD) of the brachial artery with the ultrasound technique before each HV test.
RESULTS	The anginal attacks with ST segment elevation were induced by HV in all patients on days 1 and 5. However, no attacks were induced on day 3. Supplementation with E ₂ augmented FMD ($3.5 \pm 0.6^*$, 8.9 ± 0.7 and $4.0 \pm 0.5^*$ on days 1, 3 and 5, respectively; * $p < 0.01$ vs. day 3). The serum E ₂ levels on days 1, 3 and 5 were $22.7 \pm 2.8^*$, 96.2 ± 9.2 and $30.7 \pm 7.1^*$ pg/ml, respectively (* $p < 0.01$ vs. day 3).
CONCLUSIONS	The present results demonstrated for the first time, to our knowledge, that E ₂ supplementation suppresses the HV-induced attacks in women with variant angina, in part because of the improvement of endothelial function. (J Am Coll Cardiol 2001;37:735-40) © 2001 by the American College of Cardiology

Coronary spasm plays an important role in the pathogenesis of not only variant angina but also ischemic heart disease in general (1). The incidence of ischemic heart disease is relatively uncommon in premenopausal women, but shows a sharp rise after natural or surgical menopause (2). The decline of endogenous ovarian hormones is commonly assumed to be a major component of this phenomenon (2-4).

Estrogen improves lipid profiles (3,4), stimulates endothelium-derived nitric oxide (NO) and prostacyclin production/release, scavenges superoxide and decreases endothelin-1 activity (5). These effects serve to slow the development and to limit the adverse effects of atherosclerosis, in part by amelioration of vascular endothelial dysfunction (6). As endothelial dysfunction is reported to be one of the reasons for coronary spasm (7-9), estrogen may further be effective for suppression of coronary spasm itself. The hyperventilation (HV) test was previously reported to be an effective noninvasive provocation test for coronary spasm in patients with variant angina (7,10). The present study was designed to examine whether estrogen replacement suppresses the HV-induced attacks in women with variant angina.

METHODS

Study patients. In the present study, variant angina was defined as recurrent attacks of chest oppression occurring spontaneously at rest, with ST segment elevation on the electrocardiogram (ECG), rapidly relieved by nitroglycerin. Fifteen postmenopausal women (mean age 54.2 years old) admitted to our institution from April 1997 to April 2000 met these criteria and were enrolled in the study. All of them had proven coronary spasm by chest oppression and ST segment elevation after intracoronary injection of acetylcholine (ACh) (11,12). No patient showed fixed coronary stenosis after administration of nitroglycerin, as assessed by cardiac catheterization. No patient had diabetes, a history of myocardial infarction or evidence of impaired left ventricular function at rest, as assessed by echocardiography and cardiac catheterization.

All antianginal drugs, except sublingual nitroglycerin, were stopped at least seven days before the study. The clinical details of the patients were kept unknown to all investigators participating in data collection and analysis. All patients gave written, informed consent, and the study was conducted in accordance with the guidelines approved by the Ethics Committee at our institution.

Coronary angiography. Before study entry, coronary angiography was performed in all patients by using the Judkin's technique. Coronary spasm was provoked by intracoronary injection of ACh into the right and left coronary arteries separately, as described in our previous reports

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Abbreviations and Acronyms

ACh	=	acetylcholine
ECG	=	electrocardiogram
E ₂	=	estradiol
FMD	=	flow-mediated (endothelium-dependent) dilation
HV	=	hyperventilation
NO	=	nitric oxide

(11,12). The appearance of total or subtotal occlusion of a major coronary artery associated with ST segment deviation on the ECG was considered to be a manifestation of coronary spasm (11,12). After intracoronary injection of nitroglycerin, the coronary angiograms were filmed in multiple projections. The stenotic lesion was quantitatively analyzed using Cardio 500 (Kontron Instruments, Echting, Germany) (8,9). Significant coronary stenosis was defined as >50% lumen diameter narrowing.

Study protocol. All patients were treated with an estradiol-17-beta (E₂) patch (Estraderm TTS, Novartis, Basel, Switzerland; 4 mg) for two days and a placebo patch corresponding to E₂ for another two days. Because anginal attacks are most easily induced in the early morning (7,10), the HV test, a provocation test for coronary spasm, was performed at 7:00 AM during the fasting state on day 1 (baseline), day 3 (after E₂ supplementation) and day 5 (after placebo administration). The study protocol is shown in Figure 1A.

On each study day, the patients underwent the following procedures. They were asked to hyperventilate vigorously for 6 min (10). Three ECG leads (V₃, V₅ and aVF) were continuously monitored on an oscilloscope (Case IV, Marquette, Milwaukee) from 20 min before to 15 min after HV. Twelve-lead ECGs were recorded every minute during and up to 10 min after HV. Blood pressure was measured with a cuff sphygmomanometer at appropriate intervals. If chest pain or ischemic ST segment changes on the ECG appeared during HV, the test was terminated immediately. The change on the ECG was defined as positive with ST segment elevation >0.1 mV, as compared with the baseline level, at 80 ms after the onset of the J point. The HV test was considered positive if ischemic ST segment changes were induced by HV.

Vascular studies. Endothelial function was evaluated by flow-mediated (endothelium-dependent) dilation (FMD)

of the brachial artery, as measured with the ultrasound technique. The measurement was performed before every HV test, as shown in Figure 1, A and B. The validity of this method has been demonstrated in our previous studies and by others (13-18). In brief, the diameter of the brachial artery was measured from B-mode ultrasound images using a 7.5-MHz linear array transducer (SSH-160A, Toshiba, Tokyo, Japan), and flow velocity was measured using a pulsed Doppler signal. The brachial artery images were obtained in the antecubital fossa in a longitudinal fashion. This location on the brachium was marked, and all subsequent images were obtained at the same location. Depth and gain settings were optimized at the beginning of the measurement and were kept constant throughout the recording period. The optimal location of the transducer and the machine settings were recorded for each subject to ensure that the vascular reactivity measurements were performed under the same conditions at each study.

Each subject lay quietly for 10 min before the first scan. After baseline measurements of the diameter of and flow velocity in the brachial artery, a blood pressure cuff placed around the forearm was inflated to 250 to 300 mm Hg and was released after 5 min. Measurements of the arterial diameter and flow velocity were continuously taken during cuff inflation and after cuff deflation. After the HV test, sublingual nitroglycerin (0.3 mg) was administered to all subjects, and 5 min later the nitroglycerin-induced dilation was measured (Fig. 1B).

The ultrasound images were recorded on a super-VHS video cassette recorder (BR-S601M, Victor, Tokyo, Japan), and the arterial diameter was measured at a fixed distance from an anatomic marker with ultrasonic calipers by two independent observers who had no knowledge of which treatment the patients had been receiving. The measurements were taken from the anterior to the posterior interface between the media and adventitia ("m" line) at end diastole, incident with the R wave on a continuously recorded ECG (13-19). The diameters at four cardiac cycles were analyzed for each scan and were averaged. The peak diameter for reactive hyperemia was measured for 45 to 90 s after cuff deflation (13-17). Blood flow was calculated by multiplying the velocity-time integral of the Doppler flow signal by heart rate and the vessel cross-sectional area. Increase in blood flow was calculated by dividing the maximal blood flow within the first 15 s after cuff deflation by the blood flow at baseline (13-17).

The interobserver and intraobserver variabilities for the repeated measurements of the rest arterial diameter were 0.05 ± 0.02 and 0.02 ± 0.02 mm, respectively. In a preliminary study, when these procedures were performed at the same time on two separate days in 20 volunteers, the average intrasubject test-retest difference for the measurements of the arterial diameter during reactive hyperemia was 0.05 ± 0.04 mm (13,15).

Blood sampling and assay. Blood samples for evaluation of serum levels of E₂ were obtained before each HV test and

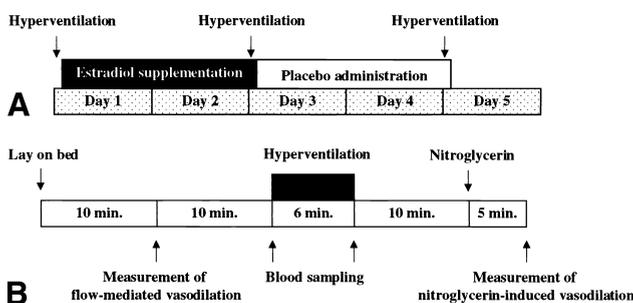


Figure 1. Schematic representation of the experimental protocol.

Table 1. Clinical Characteristics of the Subjects

Subject No.	Age (yrs)	Smoking Status	Total Cholesterol (mg/dl)	HDL Cholesterol (mg/dl)	LDL Cholesterol (mg/dl)	TG (mg/dl)	Spasm Artery	ST Segment Elevation Site During Hyperventilation Test (lead)
1	57	No	209	46	141	108	RCA	II, III, aVF
2	51	Yes	214	40	158	79	LAD	V ₁₋₅
3	56	No	176	60	95	106	LAD, LCx	V ₁₋₄
4	59	Yes	217	56	131	151	RCA	II, III, aVF
5	52	Yes	222	52	139	156	RCA	II, III, aVF
6	49	Yes	193	47	121	125	LAD	V ₂₋₆
7	45	Yes	146	50	75	105	LAD	V ₂₋₆
8	56	Yes	237	83	122	160	LAD, RCA	V ₂₋₆
9	55	Yes	201	52	122	136	RCA	II, III, aVF
10	57	Yes	185	46	120	95	LAD	V ₂₋₆
11	59	Yes	224	53	149	112	RCA	II, III, aVF
12	51	Yes	221	44	152	126	RCA, LAD	II, III, aVF
13	49	No	212	69	117	132	RCA, LAD, LCx	II, III, aVF, V ₂₋₆
14	60	No	255	57	166	161	RCA	II, III, aVF
15	58	Yes	232	54	149	146	RCA	II, III, aVF

HDL = high density lipoprotein; LAD = left anterior descending coronary artery; LCx = left circumflex coronary artery; LDL = low density lipoprotein; RCA = right coronary artery; TG = triglycerides.

were measured by a specific immunoradiometric assay (Diagnostic Products Corporation, Los Angeles, California) (13,14). Arterial blood samples for pH and gas analysis were obtained from the femoral artery before and at the end of each HV test and were measured with an autoanalyzer (ABL-2, Radiometer Medical, Copenhagen, Denmark).

Statistical analysis. The data were analyzed by analysis of variance. When statistically significant effects were found, the Newman-Keuls test was used to isolate the differences between groups. Hemodynamic variables, arterial blood pH and blood gases before and after HV were compared by using the two-tailed paired *t* test. Statistical significance was set at *p* < 0.05. All data are expressed as the mean value ± SE.

RESULTS

Coronary angiography. Coronary angiography was performed in all subjects. Intracoronary injection of ACh could provoke coronary spasm with ST segment elevation and chest symptoms in all subjects (Table 1). After intracoronary injection of nitroglycerin, fixed coronary artery stenosis was not documented in any subject.

Anginal attacks induced by HV. Most patients experienced mild light-headedness and numbness of the hands and feet, which began 2 to 3 min after the beginning and continued to the end of the HV test. On day 1 (baseline), anginal attacks occurred after HV in all patients. On day 3 (after E₂ supplementation), no anginal attacks occurred either during or after HV. On day 5 (after placebo administration), the anginal attacks occurred again after HV in all patients. All 30 attacks recorded on days 1 and 5 were associated with ST segment elevation. The attacks occurred 1 to 4 min after the end of HV and spontaneously disappeared within a few minutes. Every patient had anginal pain during the attack. A representative case (subject no. 1) is shown in Figure 2.

Arterial blood pH and gases before and after HV. After HV, arterial pH increased from 7.39 ± 0.05 to 7.56 ± 0.08 (*p* < 0.01) on day 1 (baseline), from 7.40 ± 0.08 to 7.57 ± 0.10 (*p* < 0.01) on day 3 (after E₂ supplementation) and from 7.40 ± 0.06 to 7.59 ± 0.10 (*p* < 0.01) on day 5 (after placebo administration). Arterial blood partial carbon dioxide pressure decreased from 40 ± 6 to 26 ± 7 mm Hg (*p* < 0.01) on day 1, from 41 ± 5 to 24 ± 8 mm Hg (*p* < 0.01) on day 3 and from 41 ± 6 to 25 ± 6 mm Hg (*p* < 0.01) on day 5. Arterial blood oxygen partial pressure increased from 92 ± 13 to 114 ± 20 mm Hg (*p* < 0.01) on day 1, from 93 ± 12 to 112 ± 25 mm Hg (*p* < 0.01) on day 3 and from 92 ± 14 to 115 ± 21 mm Hg (*p* < 0.01) on day 5 after HV. There were no significant differences in these variables among the three study days either before or after HV (Table 2).

Effects of E₂ on FMD. Supplementation with E₂ did not elicit any changes in heart rate, mean blood pressure, basal arterial diameter, basal blood flow or percent increase in blood flow during reactive hyperemia (Table 2). Significant improvement was observed in FMD after E₂ supplementation (3.5 ± 0.6%*, 8.9 ± 0.7% and 4.0 ± 0.5%* on days 1, 3 and 5, respectively (**p* < 0.01 vs. day 3)). After placebo administration, FMD declined to a level comparable to that at baseline. The serum E₂ levels increased after E₂ supplementation and decreased to the baseline levels after placebo administration (22.7 ± 2.8*, 96.2 ± 9.2 and 30.7 ± 7.1* pg/ml on days 1, 3 and 5, respectively (**p* < 0.01 vs. day 3)). There were no significant changes in the nitroglycerin-induced vasodilation throughout the study (Table 2).

DISCUSSION

The present study clearly showed that E₂ supplementation completely suppresses the HV-induced attacks in postmenopausal women with variant angina. On day 1 (baseline) and day 5 (after placebo administration), the anginal attacks were induced by HV in all patients. Nevertheless, no

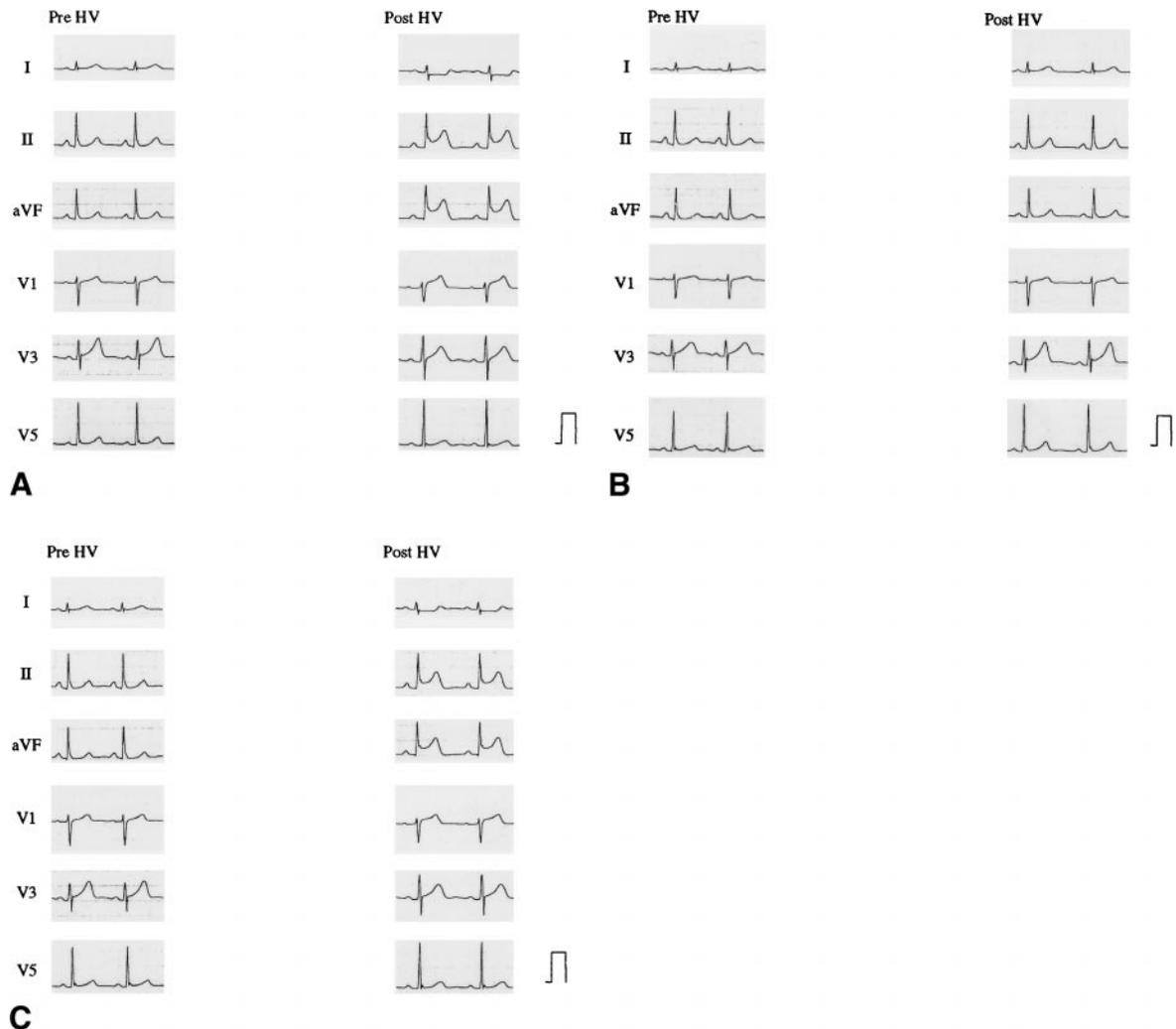


Figure 2. A representative case (case 1). At baseline (day 1), the hyperventilation (HV) test induced anginal attacks with ST segment elevation in leads II, III and aVF and depression in leads I and aVL (**A**), but not after estradiol (E_2) supplementation (day 3) (**B**). After placebo administration (day 5), the HV test again induced anginal attacks with ST segment elevation in leads II, III and aVF and depression in leads I and aVL (**C**).

attacks occurred on day 3 (after E_2 supplementation), although the degree of HV was comparable among the three study days, as assessed by arterial blood pH and gases.

We previously reported that the HV-induced attacks in patients with variant angina pectoris were due to coronary spasm (7,10). In all patients enrolled in the present study,

Table 2. Hemodynamic Variables and Arterial Blood pH and Gases

	Day 1		Day 3		Day 5	
	Before HV	After HV	Before HV	After HV	Before HV	After HV
Heart rate (beats/min)	62.1 ± 2.1	63.0 ± 2.0	63.1 ± 1.9	62.4 ± 1.8	61.5 ± 2.6	62.5 ± 2.1
Mean BP (mm Hg)	105.2 ± 2.9	104.9 ± 3.0	106.9 ± 3.1	105.9 ± 3.5	106.1 ± 2.8	105.4 ± 3.2
Basal arterial diameter (mm)	3.31 ± 0.12	—	3.25 ± 0.22	—	3.32 ± 0.26	—
Basal blood flow (ml/min)	278.3 ± 31.2	—	275.8 ± 29.6	—	278.6 ± 29.6	—
Increase in flow during reactive hyperemia (%)	256.3 ± 36.3	—	261.2 ± 35.2	—	261.8 ± 34.5	—
Flow-mediated vasodilation (%)	3.5 ± 0.6*	—	8.9 ± 0.7	—	4.0 ± 0.5*	—
Nitroglycerin-induced vasodilation (%)	—	19.8 ± 2.1	—	20.4 ± 1.5	—	19.8 ± 2.5
Serum estradiol level (pg/ml)	22.7 ± 2.8*	—	96.2 ± 9.2	—	30.7 ± 7.1*	—
pH	7.39 ± 0.05	7.56 ± 0.08	7.40 ± 0.08	7.57 ± 0.10	7.40 ± 0.06	7.59 ± 0.10
PCO ₂ (mm Hg)	40 ± 6	26 ± 7	41 ± 5	24 ± 8	41 ± 6	25 ± 6
PO ₂ (mm Hg)	92 ± 13	114 ± 20	93 ± 12	112 ± 25	92 ± 14	115 ± 21

*p < 0.01 vs. day 3. Data are presented as the mean value ± SE.

BP = blood pressure; HV = hyperventilation test; PCO₂ = partial carbon dioxide pressure; PO₂ = partial oxygen pressure.

the anginal attack was induced by intracoronary injection of ACh, and coronary spasm was observed on the coronary angiogram. Taken together, the present results suggest that E₂ supplementation suppressed the HV-induced attacks by relieving coronary spasm.

Probable mechanism. Estrogen has been demonstrated to increase NO synthase activity in the vascular endothelium (5). In the present study, the magnitude of FMD of the brachial artery during reactive hyperemia after transient occlusion, which is mainly endothelium-derived NO dependent (20), increased after E₂ supplementation and decreased to baseline levels after placebo administration. Nitroglycerin-induced, endothelium-independent vasodilation remained unchanged. That is to say, E₂ supplementation improved the endothelial function of the brachial artery. As estrogens have been reported to improve endothelial function in humans, as reported in our previous studies (13,14) and by others (5), the present results are in agreement with the previous studies.

Endothelium-dependent vasodilation has been shown to play a crucial role in the regulation of vascular tone (6,21-24). We have previously reported that patients with coronary spasm have a disturbance in endothelial function of the coronary arteries, as well as a hypercontractile response of vascular smooth muscle (7-9,25). The subjects included in this study had a disturbance of endothelium-regulated coronary artery vasomotor function, for all of them showed coronary spasm with ACh, which causes vasodilation when the endothelium is functioning normally (21-23). We have provided evidence for endothelial dysfunction not only in the coronary arteries, but also in the brachial arteries in the patients with coronary spasm (15). Because there is a close linkage between endothelial function of the brachial artery and that of the coronary arteries (15,18), this modulation of endothelium-dependent dilation in the brachial artery observed in the present study most likely takes place in the coronary arteries as well.

Administration of E₂ is reported to modulate the coronary artery responses induced by ACh in women with coronary heart disease (26). Estrogen is also known to directly relax vascular smooth muscle by antagonizing calcium channels (27). Therefore, it can be presumed that estrogen supplementation affects endothelial and smooth muscle function and contributes to suppression of HV-induced anginal attacks.

Reactive hyperemia after temporary interruption of the blood flow may result from an interplay between physical (myogenic) and local metabolic factors, including prostaglandins and adenosine (28,29). Increased wall shear stress owing to an increase in blood flow results in the production of endothelium-derived vasodilators, including NO (21,24). We have demonstrated that after transient arterial occlusion, NO plays a major role in the duration of hyperemia or flow debt repayment, but not in peak reactive hyperemia (30). In fact, local administration of the NO synthase inhibitor N^G-monomethyl-L-arginine did not affect the peak increase in blood flow during reactive hyperemia in

human peripheral conduit arteries (20). This may explain why the comparable increases were observed in peak blood flow on days 1, 3 and 5, despite the differences in FMD.

Study limitations. In the present study, a double dose of E₂ (4 mg/day for 2 days) was administered to the patients for a short period, instead of common hormone replacement therapy. It was not confirmed that E₂ improved coronary endothelial function in the postmenopausal women with variant angina. Further study is needed to examine whether long-term supplementation of smaller dose of E₂ is also effective in suppressing the anginal attacks, and whether E₂ improves coronary endothelial function in postmenopausal women with variant angina.

Conclusions. The present study demonstrated for the first time, to our knowledge, that estrogen supplementation is highly effective in suppressing HV-induced attacks of variant angina or coronary spasm in postmenopausal women.

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