Effect of Gender Differences and Estrogen Replacement Therapy on Vascular Reactivity

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The incidence of cardiovascular disease is lower in premenopausal women compared with men; following menopause, the risk of mortality from cardiovascular disease increases in females. Postischemic dilatation of the brachial artery has been used previously as an index of endothelium-mediated vasodilation. Using this index, we examined a group of premenopausal and postmenopausal women, some of whom were on estrogen replacement therapy (ERT). All subjects were normotensive (blood pressure [BP] <140/90 mm Hg) and normoglycemic (blood glucose, <100 mg/dL). Fourteen healthy women (mean age, 27 ± 0.8 years; mean total cholesterol, 174 ± 6.7 mg/dL) and fourteen healthy men (mean age, 26 ± 1.4 years; mean total cholesterol, 181 ± 7.2 mg/dL) were investigated. Nineteen postmenopausal women were also examined; 11 were on ERT (mean age, 55 ± 2.1 years; mean total cholesterol, 213 ± 6.6 mg/dL) and eight were not on ERT (mean age, 60 ± 3.6 years; mean total cholesterol, 222 ± 14.4 mg/dL). Ischemia was induced by inflating a cuff over the forearm to a pressure of 40 mm Hg above systolic for 5 minutes. Doppler ultrasonography (Acuson [Mountain View, CA] 128XP/10c ultrasonograph with a 7.5-MHz linear array transducer) was used to measure the brachial artery diameter before inflation and 15 seconds and 45 to 60 seconds following cuff deflation. Flow-mediated dilatation (FMD%) and hyperemia were defined as the percentage increase over basal diameter and basal flow, respectively. Postischemic median dilatation in men was 4.20% (interquartile range, 2.13% to 5.56%) and 11.48% (interquartile range, 8.70% to 14.29%) in age-matched premenopausal women ($P < .01$). For women on ERT, the postischemic median dilatation was 8.11% (interquartile range, 6.01% to 11.60%), as compared with 2.82% (interquartile range, 1.32% to 3.28%) for women without ERT ($P < .01$). Premenopausal women showed significantly greater dilatation after ischemia than postmenopausal women without ERT ($P < .0001$). Hyperemia was similar in all groups. These findings show that postischemic vasodilation of the brachial artery is greater in premenopausal women versus age-matched men; it is decreased in postmenopausal women, and ERT restores it toward normal. The pathophysiology underlying the diminution in postischemic dilatation may be relevant to atherogenesis and coronary artery disease (CAD).

IT IS KNOWN that cardiovascular disease is more prevalent in males than in females preceding the onset of menopause. However, after menopause, females exhibit an increased risk of coronary artery disease (CAD) nearly equaling male cardiovascular morbidity and mortality rates in the seventh decade of life and later. Whereas premenopausal women have a one in 1,000 risk of CAD, the risk quadruples after menopause. The administration of estrogens to postmenopausal women has been shown to protect this age group from cardiovascular morbidity and mortality. These epidemiological observations suggest that estrogens may exert a protective cardiovascular effect, thereby decreasing the postmenopausal risk for CAD and related vascular diseases.

Numerous studies supporting the beneficial effects of estrogen on the lipid profile, ie, a reduction of low-density lipoprotein (LDL) and an increase of high-density lipoprotein (HDL), and thus vascular health have been published. Estrogen replacement therapy (ERT) has also been shown to have a role in survival statistics for postmenopausal coronary artery bypass grafting procedures, as well as CAD.[1-4] Enhanced fibrinolysis caused by increased circulating levels of estrogen in these patients is one potential mechanism for the increased CAD survivability, although much evidence also points toward an upregulation of the nitric oxide (NO) synthase signaling mechanisms by estrogen.[5-10]

A novel method for noninvasively measuring the reactivity of the arterial vasculature has been developed over the past few years. The use of high-frequency ultrasound allows investigators to accurately visualize and measure small changes in the structure, diameter, and blood flow of a single conduit vessel. The brachial artery is ultrasonographically imaged in a longitudinal section to measure changes in the diameter of the artery and blood flow after an ischemic episode in the forearm. This technique has been studied very well and is accurate and reproducible in the measurement of changes in diameter. Evidence has shown that the effect of postischemic vasodilation measured by ultrasound, plethysmography, and femoral artery probes is an endothelium-dependent NO-mediated event.[11-13] In certain disease states associated with an increased risk of atherosclerosis, a measurable decrease in vascular reactivity has been shown through ultrasound. Some of these conditions include smoking, diabetes mellitus, hypercholesterolemia, and peripheral vascular disease.[14-19]

Within the past 5 years, both ultrasound and plethysmography have revolutionized the way investigators are able to study important vascular parameters. Coronary vasomotor function and arterial compliance have both been shown to increase with ERT in the postmenopausal years. Increases in peripheral blood flow have also been reportedly linked to ERT use in the same age group.[20-22]

It was thus our intention to initiate a study using the ultrasound technique to examine the effect of circulating estrogen on vascular reactivity in various groups of individuals. We hypothesized that the study of younger age groups will show a higher relative vascular reactivity in females versus

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males. We also expected to show that vascular reactivity will be lower in postmenopausal women, and the prolonged use of exogenous estrogens after menopause (ERT) will restore endothelium-dependent vasodilation as measured by this technique.

SUBJECTS AND METHODS

Subjects

We began the study with an initial examination of male-female vascular reactivity differences in the younger age group. Fourteen men and 14 age-matched women aged 20 to 31 years were examined for the postischemic vasodilatory response of the brachial artery. All women were in the follicular phase of the menstrual cycle. We then studied 19 age-matched healthy postmenopausal women aged 49 to 75 years with the same methods; 11 were on ERT and eight were not. Nine women on ERT were taking Premarin (Wyeth-Ayerst, Philadelphia, PA) at a dose of 0.625 to 1.25 mg daily. Two women on ERT were taking a stable dose of Prempro (0.625 mg Premarin and 2.5 mg progesterone; Wyeth-Ayerst). All 11 women were on ERT for at least 3 years, while those without ERT had an average postmenopausal duration of 10 years without ERT. All subjects were healthy caucasians, nonsmokers, normoglycemic, and normotensive (blood pressure [BP]) <140/90 mm Hg) with no history of CAD or other vascular disease. The total cholesterol level was less than 250 mg/dL, and the body weight was not more than 30% over their ideal body weight (Tables 1 and 2). Other than ERT, no subjects were taking prescription or over-the-counter medications known to directly affect vascular responsivity, including angiotensin-converting enzyme inhibitors or cholesterol-reducing agents.

Methods

All vascular imaging studies were conducted in an environmentally controlled laboratory with a constant temperature of 21°C. Participants were made comfortable in the supine position, at which point a sphygmomanometer cuff was placed on the forearm and a three-lead electrocardiogram (ECG) was set in the normal fashion. An Acuson (Mountain View, CA) 128XP/10c high-resolution ultrasonograph with a 7.5-MHz linear array transducer was used. The Doppler signal was placed at a 70° angle to the arterial lumen with the gate set at 1.5 mm in the center of the artery. The transducer with room temperature ultrasound gel was held stationary at a constant distance from the skin. The transducer was placed over a fixed point parallel to the brachial artery with the help of a custom-built stand to reduce investigator motion error and local pressure. A longitudinal view of the brachial artery proximal to the antecubital space was continuously recorded on super-VHS videocassettes. Arterial flow was confirmed by color Doppler, and calipers were placed at the center of the longitudinal image on the split screen to measure the diameter of the artery and blood flow velocity represented as a triphasic waveform. These initial baseline measurements allowed patients a 10-minute rest before proceeding with any experimental intervention.

Table 1. Characteristics of Younger Age Group

Characteristic	Men	Premenopausal Women	*P*
No. of subjects	14	14	
Age (yr)	26 ± 1.4	27 ± 0.8	NS
BP (mm Hg)			
SBP	122 ± 1.8	114 ± 2.3	<.05
DBP	80 ± 1.6	72 ± 1.5	<.05
Lipid profile			
LDL (mg/dL)	112 ± 7.4	100 ± 6.7	NS
HDL (mg/dL)	41 ± 2.1	51 ± 2.8	<.05
TG (mg/dL)	138 ± 15.8	94 ± 7.3	<.05
BMI (kg/m²)	26 ± 2.0	22 ± 0.9	<.05
Glucose (mg/dL)	81 ± 4.4	73 ± 2.3	NS
Multiple linear regression analysis*†			
Baseline diameter			.23
SBP			.33
DBP			.31
BMI			.50
TG			.05
HDL			.06

NOTE. Data are the mean ± SE and were analyzed by *t* test.
*Dependent variable, absolute diameter change.
†After forward stepwise regression yielded no independent variables.

Table 2. Characteristics of Postmenopausal Group

Characteristic	With ERT	Without ERT	*P*
No. of subjects	11	8	
Age (yr)	55 ± 2.1	60 ± 3.6	NS
BP (mm Hg)			
SBP	131 ± 3.4	121 ± 4.1	NS
DBP	81 ± 2.5	74 ± 2.2	NS
Lipid profile			
LDL (mg/dL)	123 ± 7.1	138 ± 12.5	NS
HDL (mg/dL)	62 ± 5.0	61 ± 5.5	NS
TG (mg/dL)	148 ± 26.3	118 ± 26.4	NS
BMI (kg/m²)	25 ± 1.4	25 ± 1.5	NS
Glucose (mg/dL)	75 ± 3.0	75 ± 4.8	NS

NOTE. Data are the mean ± SE and were analyzed by *t* test.

Once baseline measurements were achieved in the resting state, the cuff was inflated to 40 mm Hg above the measured systolic BP to ensure occlusion of the arterial supply to the forearm. A lack of systolic flow was validated by color Doppler scanning of the radial artery with a second transducer. After 5 minutes, the cuff was rapidly deflated and postischemic diameter and flow measurements were taken at 15 seconds and again at 45 to 60 seconds (the time window of maximal reactive diameter change). Measurements were continued for 5 to 10 minutes until a return to the baseline arterial diameter. The videocassette recordings were then evaluated by a blinded observer to assess the dilatory responses of the brachial artery. Diameter measurements were made with the ultrasonograph video playback function and taken between the intima/media margins of opposing arterial walls on the longitudinal scan. All measurements were made in millimeters and taken at the peak of the R-wave from the synchronous ECG recording. Interobserver variability in our laboratory had a calculated value of 2% to 3%. Postischemic dilatation of the brachial artery (FMD%) was expressed as a percent change over baseline diameter. Blood flow was calculated in milliliters per minute using the formula, $\pi d^2/4 \times TAV \times 60$, where d is the diameter of the vessel and TAV is the velocity-time integral of the Doppler flow signal (corrected for angle). Flow velocity used in this calculation was taken from the center of the artery and may be overestimated, but relative flow values before and after cuff deflation are accurate.[23] Hyperemia was expressed as the percent increase in basal arterial blood flow 15 seconds postdeflation. A subgroup of seven males were given nitroglycerin after baseline was achieved again and were observed by the same methods as previously described.

The Institutional Review Board of Millard Fillmore Hospital at the State University of New York at Buffalo approved all protocols of this study. All participants received a clear explanation of the study, and informed consent was obtained for the ultrasound study and blood tests.

Statistics

All statistics were calculated using the Sigmastat (San Rafael, CA) statistical package with a level of significance (*P*) set at .05 or less.

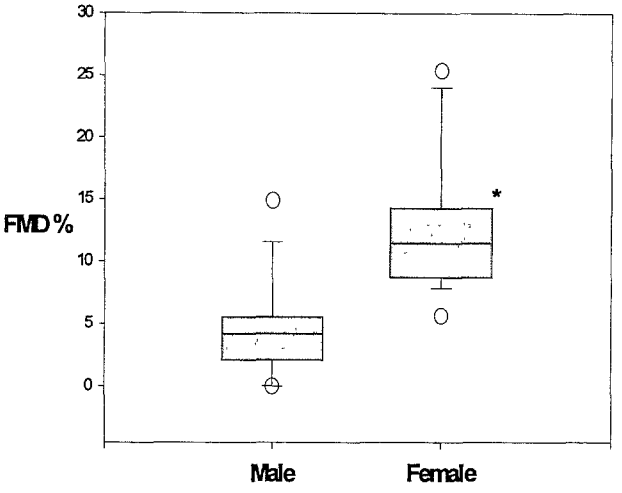

Fig 1. FMD% for young age group by sex. Data are the median (center lines) with interquartile range (grey zones). T-bars represent 95th percentile range for respective data. Circles represent max/min values for data set. *$P < .005$ calculated by Mann-Whitney rank-sum test.

Analyses were performed with Student's t tests for paired and unpaired data, the Mann-Whitney rank-sum test for nonparametric data, or one-way ANOVA. All statistics are reported as the mean ± SE unless otherwise noted. The power of all statistical tests was set at 0.80. Sigmaplot was used for all graphical displays of the data.

RESULTS

The men had a mean resting arterial diameter of 4.16 ± 0.15 mm, dilating to a mean of 4.35 ± 0.14 mm after cuff deflation (P = NS), while age-matched women had a mean resting diameter of 2.98 ± 0.09 mm that dilated to a mean of 3.36 ± 0.12 mm ($P < .05$). The mean change in absolute diameter for men and women was 0.19 ± 0.05 and 0.38 ± 0.05 mm, respectively ($P < .05$). The median postischemic percent dilatation was 4.20% (2.13% to 5.56%) in men and 11.48% (8.70% to 14.29%) in age-matched women ($P < .0001$) (Fig 1). There was a significant difference in baseline measurements in males versus age-matched females ($P < .05$) (Table 3). Once baseline was achieved again, seven of these males were given nitroglycerin. It was observed that while the mean FMD% of this group was 4.09 ± 1.22, similar to the larger group, the response to nitroglycerin yielded a mean percentage change in arterial diameter of 20.01% ± 4.37% ($P < .05$).

Although some of the group characteristics such as BP, HDL, triglycerides, and BMI differed significantly between men and women, forward stepwise regression analysis (F to enter, 4.000) indicated that there were no independent variables affecting the absolute diameter change. In addition, multiple linear regression analysis of the same data showed that the laboratory values were not correlated with a change in absolute diameter after arterial reperfusion (Table 1). Furthermore, the aforementioned laboratory studies were all within normal limits for both young men and women, and there is no evidence to support the proposition that such differences within the normal range would affect postischemic arterial dilation.

Table 3. Vessel Diameter Data for Young Age Group

Arterial Diameter (mm)	Men	Premenopausal Women	P
Baseline	4.16 ± 0 15	2 98 ± 0.09	<.05
Reactive	4.35 ± 0.14	3.36 ± 0.12*	<.05
Change	0.19 ± 0.05	0.38 ± 0.05	< 05

NOTE. Data are the mean ± SE and were analyzed by t test for paired and unpaired data.

*Significant difference between baseline and reactive diameters within a group.

Table 4. Vessel Diameter Data for Postmenopausal Group

Arterial Diameter (mm)	With ERT	Without ERT	P
Baseline	3.55 ± 0.15	3.56 ± 0.10	NS
Reactive	3.88 ± 0.17	3.64 ± 0.11	NS
Change	0.33 ± 0.06	0.09 ± 0.02	<.05

NOTE. Data are the mean ± SE and were analyzed by t test for paired and unpaired data. There was no significant difference between baseline and reactive diameters within either group.

Postmenopausal women on ERT had a mean resting arterial diameter of 3.55 ± 0.15 mm, dilating to a mean of 3.88 ± 0.17 mm (P = NS), and women without ERT had a similar mean baseline diameter of 3.56 ± 0.10 mm, but had an increase to a mean value of 3.64 ± 0.11 mm following deflation (P = NS). The mean change in the absolute diameter for women with and without ERT was 0.33 ± 0.06 and 0.09 ± 0.02 mm, respectively ($P < .05$) (Table 4). Women treated with ERT had a median postischemic percent dilatation of 8.11% (6.01% to 11.60%), while women without ERT had a median dilatation of 2.82% (1.32% to 3.28%; $P < .01$) (Fig 2). There was no significant difference between the baseline diameter in both postmenopausal groups, although premenopausal baseline measurements differed significantly from both postmenopausal values ($P < .002$). None of the group characteristics of postmenopausal women showed any significant difference.

Young men had a mean hyperemic response of 337% ± 39%, while age-matched women had a value of 372% ± 70%.

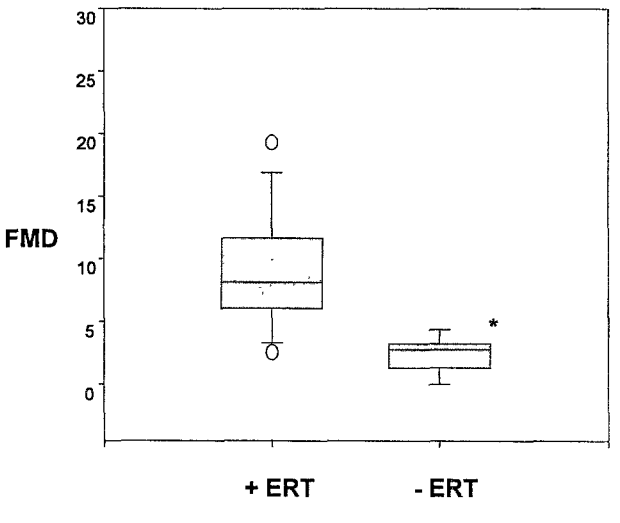

Fig 2. FMD% for postmenopausal women by ERT status. Data are the median (center lines) with interquartile range (grey zones). T-bars represent 95th percentile range for respective data. Circles represent max/min values for data set. *$P < .005$ calculated by Mann-Whitney rank-sum test.

Table 5. Hyperemia (%) by Paired Groups

Men	Premenopausal Women	Postmenopausal Women		P
		With ERT	Without ERT	
337 ± 39	372 ± 70			NS
		232 ± 31	318 ± 106	NS
	372 ± 70		318 ± 106	NS
	372 ± 70	232 ± 31		NS
337 ± 39			318 ± 106	NS
337 ± 39		232 ± 31		NS

NOTE. Data are the mean ± SE and were analyzed by *t* test.

Women without ERT had a similar response of 318% ± 106%, and those with ERT responded with a mean value of 232% ± 31%. Flow measurements (hyperemia) were not significantly different between any group, indicating that the shear stress placed on the vessel, the stimulus for dilatation of the artery, was similar in all groups (Table 5).

With relation to the younger age group, men had a significantly higher baseline diameter compared with age-matched women ($P < .05$). They also exhibited a significantly reduced median percent dilatory response to ischemia than the women. The postmenopausal groups had initial mean baseline measurements that were almost identical. However, the median postischemic dilatory response and the mean change in absolute diameter in women with ERT were significantly higher than the values in the untreated group. The postischemic dilatation was greater in premenopausal women versus postmenopausal women without ERT ($P < .0001$). There was no significant difference in dilation between premenopausal and postmenopausal women with ERT (Figs 3 and 4). The vasodilatory response was similar between postmenopausal women without ERT and younger men (Fig 5). Lastly, the response was significantly less in men versus postmenopausal women on ERT (Table 6 and Fig 6).

DISCUSSION

Our data clearly demonstrate for the first time that postischemic vasodilation is increased in premenopausal women compared with age-matched men. We have also shown that the vasodilatory response to ischemia in postmenopausal women treated with ERT is markedly greater than the response in postmenopausal women without ERT.

These data suggest that estrogen may have dramatic vascular effects, the absence of which could be related to atherogenic disease states, hypertension, and CAD risk in the postmenopausal woman. The endothelium-dependent vasodilatory response of the brachial artery was much greater in the groups who probably had higher circulating levels of estrogen: namely premenopausal women and postmenopausal women on treatment with ERT. Women who opted not to use ERT had dramatically lower levels of vascular reactivity closely similar to the pattern of younger men. It would seem that with

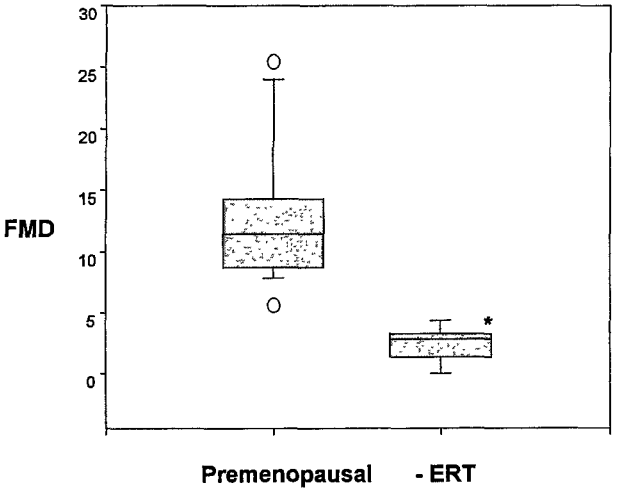

Fig 3. FMD% for premenopausal women and postmenopausal women without ERT. Data are the median (center lines) with interquartile range (grey zones). T-bars represent 95th percentile range for respective data. Circles represent max/min values for data set. *$P <$ .005 calculated by Mann-Whitney rank-sum test.

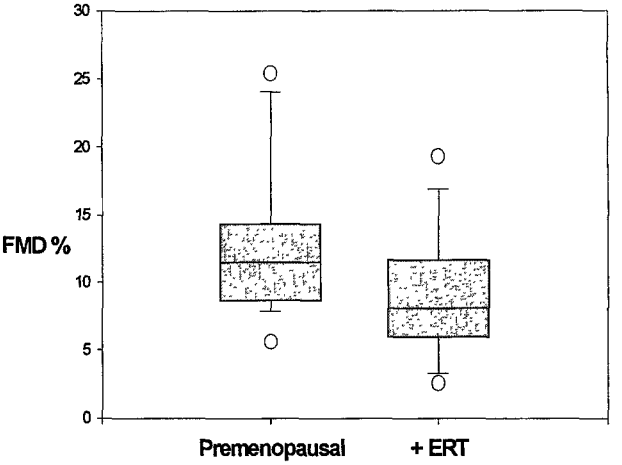

Fig 4. FMD% for premenopausal women and postmenopausal women with ERT. Data are the median (center lines) with interquartile range (grey zones). T-bars represent 95th percentile range for respective data. Circles represent max/min values for data set. The data were not significantly different by Mann-Whitney rank-sum test.

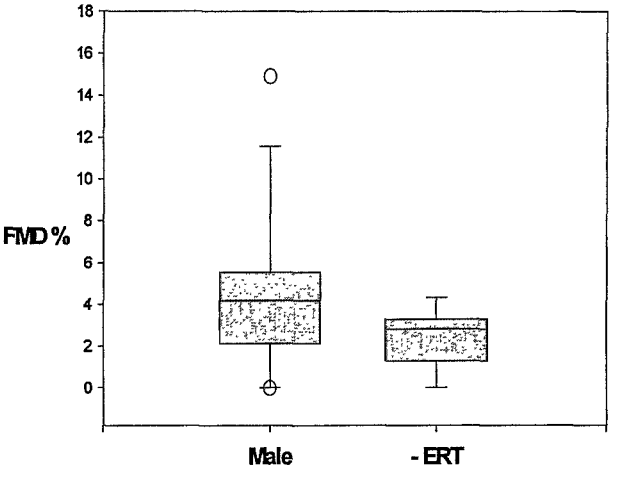

Fig 5. FMD% for men and postmenopausal women without ERT. Data are the median (center lines) with interquartile range (grey zones). T-bars represent 95th percentile range for respective data. Circles represent max/min values for data set. The data were not significantly different by Mann-Whitney rank-sum test.

Table 6. FMD% by Paired Groups

Men	Premenopausal Women	Postmenopausal Women		P
		With ERT	Without ERT	
4 20 (2.13-5.56)	11.48 (8.70-14.29)			<.005
		8.11 (6.01-11.60)	2.83 (1.32-3.28)	<.005
	11.48 (8.70-14.29)		2.83 (1.32-3.28)	<.005
	11.48 (8.70-14.29)	8.11 (6.01-11.60)		NS
4.20 (2.15-5.56)			2.83 (1.32-3.28)	NS
4.20 (2.15-5.56)		8.11 (6.01-11.60)		<.005

NOTE. Data are the median (interquartile range) and were analyzed by Mann-Whitney rank-sum test.

menopause, the endothelium-dependent response to ischemia in women diminishes to a level resembling that of younger men. This would explain the epidemiological trend for CAD incidence in older women, increasing in the sixth decade of life and equaling that of men. The data show that the use of ERT after menopause preserves the endothelium-dependent vasodilatory response and thus probably protects against certain vascular incidents throughout the body.

We did not directly measure circulating estrogen levels because Prempro and Premarin both contain various forms of estrogen, thus making an absolute quantification of these substances impractical. There are no current studies that identify a specific effect of the progesterone component on endothelial tissues; this issue requires further study. The two women who used this combination-type ERT had dilatory values similar to those obtained on Premarin alone. A previous well-controlled crossover study demonstrated that a 9-week course of postmenopausal estrogen elicited a significant difference in vascular reactivity compared with age-matched placebo groups.[24] Another study has shown that ERT improved endothelium-dependent vasodilation within 36 hours of supplementation in women, but not in men.[25] However, until now, there are insufficient data in the literature to show that this vascular response to estrogen continues to have long-term effects on postischemic vasodilation in the years following initiation of the therapy. There is no study to date showing gender-dependent differences in younger age groups.

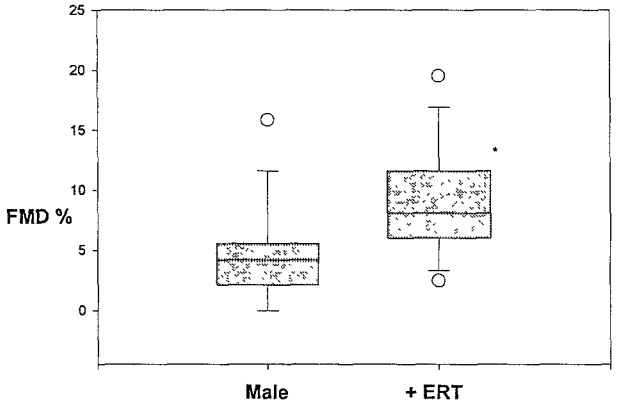

Fig 6. FMD% for men and postmenopausal women with ERT. Data are the median (center lines) with interquartile range (grey zones). T-bars represent 95th percentile range for respective data. Circles represent max/min values for data set. *$P < .005$ calculated by Mann-Whitney rank-sum test.

Although men had a significantly higher baseline diameter than premenopausal women, they did not dilate significantly with respect to both the absolute change and the percent change over baseline, whereas the age-matched female group dilated significantly in both respects. A subgroup of these men dilated significantly when given nitroglycerin, therefore indicating that although the baseline diameter was high, they retained the ability to dilate significantly. Two women opted to take nitroglycerin, and they also showed a significant increase in diameter exceeding the FMD%. Additionally, the absolute change in diameter for this group of women was significant and was twice the value for the men. We conclude that there is a significant difference in vasodilatory function between the sexes at a younger age.

The effects of estrogen that we have demonstrated through this ultrasound technique could be mediated via the endothelium-dependent NO–cyclic guanosine monophosphate pathway. Other effects of estrogen, which include beneficial effects on the lipid profile, could also reduce the risk of atherogenesis by decreasing LDL and elevating HDL serum levels. However, it has been reported that only up to half of the benefits of ERT in clinical outcome are due to changes in lipid concentrations.[26] Postischemic vasodilation is believed to be dependent on endothelial function, including the secretory products of the endothelium: the balance between endothelial vasodilators such as NO and prostacyclin (PGI_2) and endothelial vasoconstrictors such as endothelin-1. The balance between these factors may allow for an increased or decreased response to ischemic episodes based on the relative production of these compounds by the endothelium. Multiple animal studies have also implicated estrogen as an antioxidant protecting NO from degradation, as well as a prostaglandin promoter, thus all possibly leading to similar vasoprotective phenomena.[27-32]

In conclusion, we have demonstrated that premenopausal women have greater postischemic vasodilation of the brachial artery than age-matched men and postmenopausal women without ERT. In addition, postmenopausal women with ERT have markedly greater postischemic vasodilation than postmenopausal women without ERT. These data show that estrogens improve this endothelium-mediated function. This may be relevant to the pathogenesis of atherosclerosis postmenopausally and to its prevention by ERT.

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