Endothelial Function Is Impaired across the Stages of the Menopause Transition in Healthy Women

Kerrie L. Moreau, Kerry L. Hildreth, Amie L. Meditz, Kevin D. Deane, and Wendy M. Kohrt

Divisions of Geriatric Medicine (K.L.M., K.L.H., W.M.K.), Infectious Disease (A.L.M.), and Rheumatology (K.D.D.), Department of Medicine, University of Colorado Anschutz Medical Campus, Aurora, Colorado 80045

Context: The stages of the menopause transition are characterized by changes in ovarian hormones and increased cardiovascular disease (CVD) risk factors and vasomotor symptoms that may adversely affect vascular health.

Objective: We tested the hypothesis that endothelial function, a predictor of CVD, would be reduced across the stages of the menopause transition, independent of CVD risk factors and vasomotor symptoms.

Design, Setting, and Participants: This was a cross-sectional study of 132 healthy women from the general community aged 22–70 yr, categorized as premenopausal (n = 33, 32 ± 6 yr; mean ± SD), early perimenopausal (n = 20, 49 ± 3 yr) or late perimenopausal (n = 22, 50 ± 4 yr), or early (n = 30, 55 ± 3 yr) or late postmenopausal (n = 27, 61 ± 4 yr).

Main Outcome: Endothelial-dependent vasodilation was measured by brachial artery flow-mediated dilation (FMD) using ultrasound.

Results: Brachial artery FMD was significantly different among the groups ($P < 0.001$). It was highest in premenopausal women (9.9 ± 2.1%) with progressive decrements in perimenopausal (early: 8.2 ± 2.5%; late: 6.5 ± 1.9%) and postmenopausal women (early: 5.5 ± 1.9%; late: 4.7 ± 1.7%). Adjustment for risk factors, vasomotor symptoms, and sex hormones did not alter the association ($P < 0.001$). In subgroup analyses of women aged 50–59 yr, brachial artery FMD was lower in late peri- and early and late postmenopausal compared with early perimenopausal women ($P < 0.001$) but was not different between late perimenopausal and either early or late postmenopausal women.

Conclusions: Our findings suggest that a decline in endothelial function begins during the early stages of menopause (perimenopause) and worsens with the loss of ovarian function and prolonged estrogen deficiency. These data add to the accumulating evidence that the perimenopausal window is a critical time period for adverse changes in CVD risk. **(*J Clin Endocrinol Metab* 97: 4692–4700, 2012)**

Even though cardiovascular disease (CVD) death rates have been declining, CVD is still a major public health concern in women (1). The prevalence of CVD increases with age in both women and men but is less prevalent in women than men until midlife. Thereafter the gap narrows until the sixth decade, when the prevalence of CVD no longer differs between the sexes (1). The female advantage in younger women has been attributed to vascular protection by estrogens, which is lost with menopause and resulting estrogen deficiency. Although recent

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Abbreviations: %Δ, Absolute change (percentage); BMI, body mass index; CVD, cardiovascular disease; ET-1, endothelin-1; FMD, flow-mediated dilation; GTN, glyceryl trinitrate; HDL, high-density lipoprotein; HT, hormone therapy; LDL, low-density lipoprotein; mmΔ, absolute change (millimeters); NO, nitric oxide; STRAW, Stages of Reproductive Aging Workshop; WHR, waist to hip ratio.

clinical trial data do not support cardioprotective effects of conjugated equine estrogens when initiated 10–20 yr after the menopause (2, 3), it is plausible based on studies in nonhuman primates that initiating hormone therapy (HT) at the time of menopause is cardioprotective (4).

Vascular aging is a major risk factor for the development of CVD, in that it combines with other known risk factors to create an age-disease interaction (5). Endothelial dysfunction, characterized by reduced endothelial-dependent vasodilation, is a biomarker of aging and a significant predictor of cardiovascular events in women (6). Vascular aging in women is unique in that arteries may also be exposed to adverse changes in traditional CVD risk factors (*i.e.* central adiposity, hypertension, dyslipidemia) and vasomotor symptoms mediated by changes in the hormonal environment across the stages of the menopause transition (7–10). Indeed, the age-related decline in endothelial function appears to be delayed and/or occur at a slower rate in premenopausal women than in men, but catches up during the postmenopausal period, particularly in estrogen-deficient women (11, 12). However, it is unclear how changes in ovarian hormones during the potentially critical perimenopausal years contribute to the age-associated decline in endothelial function. If the menopause transition accelerates vascular aging, this would have important implications for aggressively promoting intervention strategies for CVD prevention in women during the perimenopausal years.

Accordingly, the present study tested the hypothesis that endothelial function is impaired across the stages of the menopause transition in healthy women. Additionally, we determined whether the decline in endothelial function with the menopause transition was related to traditional CVD risk factors and the occurrence of hot flashes.

Materials and Methods

Study population

One hundred thirty-two healthy women were studied: 33 premenopausal (22–43 yr), 42 perimenopausal (43–56 yr), and 57 postmenopausal (49–70 yr). Menopausal status was determined by self-reported menstrual cycle characteristics. Premenopausal women had regular menstrual cycles with no change in cycle length (21–35 d), confirmed by menstrual cycle calendars and ovulation prediction kits. Perimenopausal women were categorized as either early (n = 20; more than two cycles with cycle length changes of ≥7 d) or late (n = 22; ≥2 but <12 months of amenorrhea) transition, as defined by the Stages of Reproductive Aging Workshop (STRAW) (13). Postmenopausal women had gone 1 yr or longer without menses and were further categorized as early (n = 30; ≤5 yr) or late (n = 27; >5 yr) postmenopause stages according to STRAW criteria (13). Women had not used hormonal contraceptives, HT, or other treatments for menopausal symptoms for at least 6 months and vitamin supplements or antiinflammatory medications for at least 4 wk. Women were included if they were normotensive (resting fasted blood pressure <140/90 mm Hg), nondiabetic, had a fasted glucose less than 110 mg/dl, were sedentary or recreationally active (<3 d/wk vigorous exercise), were nonsmokers, were not taking cardiovascular or lipid-lowering medications, and were healthy as determined by a medical history and physical examination and standard blood chemistries (*i.e.* normal kidney, liver and thyroid function). Women with a history of or active estrogen-dependent neoplasms, cancer, acute liver or gallbladder disease, vaginal bleeding, venous thromboembolism, hysterectomy/oophorectomy, hypertriglyceridemia, and CVD were excluded. All women gave their written informed consent to participate in the study. All procedures were reviewed and approved by the institutional review board.

Measurements

For the vascular assessments, participants were studied after an overnight fast with proper hydration (water drinking only) and abstinence from caffeine. Normal dietary patterns, including sodium intake, were maintained for 2 d immediately before the measurements. Because of irregular cycles in perimenopausal women, premenopausal and perimenopausal (when possible) women were tested 7–10 d after the onset of menses (*i.e.* midfollicular phase) so that vascular comparisons between premenopausal and perimenopausal would be representative across the menstrual cycle. However, late perimenopausal women were tested regardless of menstrual cycle phase after 2 months of amenorrhea. The study took place at the University of Colorado Denver Colorado Clinical and Translational Sciences Institute Clinical and Translational Research Center.

Vascular endothelial-dependent vasodilation

Before vascular assessment, supine blood pressure was measured with a semiautomated device (Dinamap; Johnson & Johnson, New Brunswick, NJ) until at least three stable measures were achieved. Brachial artery flow-mediated dilation (FMD) was measured as described in detail previously (14) and was performed in accordance to current guidelines for assessing FMD in humans (15). Endothelium-independent dilation was determined by measuring brachial artery dilation in response to sublingual nitroglycerine [glyceryl trinitrate (GTN), 0.4 mg] as previously described (16). Because some women declined the procedure and GTN was not given to women with a systolic blood pressure less than 90 mm Hg, this measure was obtained in only 61 women.

Seated blood pressure, body composition, aerobic power, and physical activity

Seated brachial arterial blood pressure was measured in triplicate with a semiautomated device in the morning after an overnight fast, as previously described (17). The total (percent of total mass) and regional (percent of mass in trunk region) body fat were determined using dual-energy x-ray absorptiometry (Hologic Discovery, version 12.6; Hologic Inc., Bedford, MA). Minimal waist and hip circumferences were measured according to previously guidelines, and waist to hip ratio (WHR) was calculated (18). In a subset of women (premenopausal, n = 24; early perimenopausal, n = 20; late perimenopausal, n = 22; early postmenopausal, n = 20; and late postmenopausal, n = 20), the peak oxygen consumption, a measure of the peak aerobic exer-

cise capacity, was determined using an incremental treadmill exercise protocol as described previously (19). Leisure time physical activity was determined by the Modifiable Activity Questionnaire (20).

Metabolic risk factors and sex hormones

Fasting plasma concentrations of glucose, insulin, total cholesterol (Roche Diagnostic Systems, Indianapolis, IN) and high-density lipoprotein (HDL) cholesterol (Diagnostic Chemicals, Ltd., Oxford, CT) were determined using enzymatic/colorimetric methods, and low-density lipoprotein (LDL) cholesterol was determined using the Friedewald equation (21). Serum FSH, estradiol, progesterone, and SHBG were measured using chemiluminescence, estrone using RIA, and total testosterone using a one-step competitive assay. Serum norepinephrine with EGTA/glutathione preservative added was measured using HPLC and plasma endothelin-1 was measured using an enzyme-linked immunoassay. All metabolic risk factors and sex hormones were measured on the vascular testing day. All assays were performed by the University of Colorado Denver Colorado Clinical and Translational Sciences Institute Clinical and Translational Research Center core laboratory. The intra- and interassay coefficients of variation for all assays have been published previously (22, 23).

Vasomotor symptoms

As part of the medical history, women were asked whether they had experienced hot flashes in the past year (yes or no). Additionally, in a subsample of women (n = 100), the frequency and severity scores of menopausal symptoms in the preceding 3 months were determined from the nine-item vasosomatic subscale of the Menopausal Symptom List (24).

Statistical analysis

All data elements were examined using descriptive statistics. Parameters with skewed distributions were log transformed and are presented as median and interquartile range. One-way ANOVA was used to assess group differences in characteristics. ANOVA and *post hoc* tests using a Bonferroni correction were used to compare brachial artery FMD, GTN-mediated vasodilation, and brachial artery parameters. Pearson and Spearman rho correlation analyses were used to test for linear bivariate relations of interest (*i.e.* potential modulators of vascular function) with brachial artery FMD- and GTN-mediated vasodilation. Analysis of covariance was used to compare brachial artery FMD- and GTN-mediated vasodilation, with covariates associated with FMD at $P < 0.10$ and prior and duration of HT and hormonal contraceptive use considered as potential confounders. Because menopausal stages and age were highly correlated (r = 0.88), brachial artery FMD was compared in peri- and postmenopausal women aged 50–59 yr to distinguish the independent effect of menopausal stage. $P < 0.05$ was considered significant. All data are reported as mean ± SD unless otherwise stated. Data analysis was performed with IBM SPSS Statistics, version 19.0 (White Plains, NY).

Results

Participant characteristics

A total of 84.8% of the participants were Caucasian. The average reported age of menopause and time since menopause was 52.8 ± 0.7 and 3.0 ± 1.4 yr, respectively, for early postmenopausal and 49.2 ± 0.8 and 11.7 ± 4.3 yr, respectively, for late postmenopausal women. Thirty percent (n = 9) of early postmenopausal were prior HT users with an average duration of 2.7 ± 2.2 yr, whereas 74% (n = 20) of late postmenopausal women had used HT in the past for an average duration of 4.0 ± 2.9 yr. Most of the premenopausal (78.8%) and early (80%) and late (95.5%) perimenopausal women had used hormonal contraceptives in the past for an average duration of 5.2 ± 5.0, 6.5 ± 5.3, and 8.9 ± 7.8 yr, respectively.

Total body and trunk fat, seated systolic blood pressure, endothelin-1 (ET-1), norepinephrine, FSH, glucose, and total and LDL cholesterol concentrations were higher, and maximal aerobic power, estradiol, estrone, progesterone, and testosterone concentrations were lower across the stages of the menopause transition (all $P < 0.001$, Table 1). Additionally, the likelihood of hot flashes were greater across the stages of the menopause transition, with 6% of premenopausal, 50% of early perimenopausal, 74% of late perimenopausal, 70% of early postmenopausal, and 52% of late postmenopausal women reporting hot flashes in the previous year ($P < 0.001$). Similarly, vasomotor frequency and severity scores were greater across the stages of the menopause transition (Table 1; both $P < 0.001$).

Brachial artery FMD and GTN-mediated vasodilation

Brachial artery FMD was reduced across the stages of the menopause transition ($P < 0.001$, Fig. 1). When compared with premenopausal women, brachial artery FMD (%Δ) was lower in early ($P = 0.03$) and late perimenopausal ($P < 0.001$) and early and late postmenopausal (both $P < 0.001$). Similar results were obtained when the data were expressed as absolute (millimeters) change in FMD, with the exception of the comparison between premenopausal and early perimenopausal women ($P = 0.10$, see on-line supplement). Additional differences among the groups are designated in Fig. 1. GTN-mediated vasodilation (both %Δ and mmΔ) was also associated with menopause stages ($P = 0.04$; Fig. 2); however, these associations were driven by a tendency toward lower GTN-mediated vasodilation in the early postmenopausal compared with the early perimenopausal women (%Δ, $P = 0.09$ and mmΔ, $P = 0.06$). Although baseline brachial artery diameter (for FMD measure) was associated with menopause stage, this association was driven by a tendency toward smaller brachial diameters in premenopausal compared with late postmenopausal women ($P = 0.06$) and smaller diameters in late perimenopausal compared with late postmenopausal women ($P = 0.09$). There

TABLE 1. Participant characteristics

Variable	Pre	Early Peri	Late Peri	Early Post	Late Post	*P*
n	33	20	22	30	27	
Age (yr)	32 ± 4	49 ± 3	50 ± 4	55 ± 3	61 ± 4	<0.001
Body mass (kg)	66.4 ± 14.2	69.0 ± 10.8	67.2 ± 13.1	71.5 ± 13.1	69.8 ± 12.3	0.55
BMI (kg/m^2)	24.2 ± 5.8	25.6 ± 3.6	24.2 ± 4.3	27.0 ± 5.0	26.9 ± 4.3	0.062
Total body fat (%)	31 ± 8	34 ± 6	36 ± 7	38 ± 6	40 ± 5	<0.001
Trunk fat (%)	28 ± 9	33 ± 7	35 ± 8	38 ± 8	39 ± 6	<0.001
Waist circumference (cm)	78 ± 9	83 ± 10	82 ± 13	87 ± 14	83 ± 15	0.12
WHR	0.79 ± 0.06	0.80 ± 0.06	0.80 ± 0.06	0.82 ± 0.08	0.81 ± 0.05	0.47
Seated systolic BP (mm Hg)	108 ± 9	114 ± 11	115 ± 13	119 ± 14	121 ± 13	0.001
Seated diastolic BP (mm Hg)	69 ± 7	74 ± 8	72 ± 8	74 ± 10	73 ± 10	0.14
Resting HR (bpm)	66 ± 10	63 ± 8	64 ± 10	63 ± 6	63 ± 7	0.54
Total cholesterol (mg/dl)	154 ± 29	163 ± 27	167 ± 32	192 ± 31	193 ± 30	<0.001
LDL cholesterol (mg/dl)	89 ± 24	94 ± 27	99 ± 31	114 ± 27	119 ± 28	<0.001
HDL cholesterol (mg/dl)	49 ± 12	51 ± 14	50 ± 9	55 ± 15	53 ± 15	0.43
Triglycerides (mg/dl)[a]	68 (55–87)	84 (63–103)	86 (78–107)	83 (68–135)	95 (67–131)	0.06
Fasting insulin (μIU/ml)	7.6 ± 5.1	6.2 ± 4.0	6.6 ± 4.4	9.8 ± 7.6	8.7 ± 7.8	0.21
Fasting glucose (mg/dl)	84 ± 8	85 ± 8	81 ± 8	89 ± 10	87 ± 10	0.03
FSH (μIU/ml)	5.5 ± 3.1	23.4 ± 32.6	67.3 ± 36.6	77.0 ± 29.8	86.4 ± 31.2	<0.001
Estradiol (pg/ml)[a]	64 (38–92)	54 (35–135)	38 (10–113)	11 (10–19)	12 (10–17)	<0.001
Progesterone (ng/ml)[a]	0.6 (0.3–0.9)	0.6 (0.3–1.1)	0.4 (0.3–0.5)	0.3 (0.2–0.5)	0.2 (0.1–0.4)	<0.001
Estrone (ng^{-1}/dl)[a]	46 (35–68)	52 (34–73)	41 (27–72)	25 (10–35)	25 (20–33)	<0.001
SHBG (nmol/liter)	59 ± 26	80 ± 54	70 ± 37	66 ± 39	59 ± 31	0.32
Testosterone (ng/dl)[a,b]	32 (22–46)	22 (17–35)	20 (17–25)	19 (17–28)	17 (17–32)	0.002
ET-1 (pg/ml)	5.4 ± 0.3	5.9 ± 0.3	6.1 ± 0.3	6.5 ± 0.3	6.5 ± 0.3	0.03
Norepinephrine (pg/ml)[c]	198 ± 159	238 ± 82	248 ± 112	284 ± 154	311 ± 137	0.08
LTPA (MET h/wk)[d]	17.5 ± 11.9	19.1 ± 12.5	14.7 ± 11.5	17.1 ± 21.9	11.8 ± 13.1	0.52
VO_{2peak} (ml/kg · min)[e]	33.4 ± 7.3	27.6 ± 4.5	27.4 ± 5.3	24.7 ± 3.1	22.6 ± 4.0	<0.001
Vasosomatic frequency[f]	4.2 ± 3.8	8.3 ± 5.1	15.6 ± 11.2	10.4 ± 5.1	14.6 ± 9.2	<0.001
Vasosomatic severity[f]	4.9 ± 3.2	8.3 ± 5.6	14.8 ± 10.1	10.3 ± 6.0	13.2 ± 7.8	<0.001

Data are mean ± SD, unless otherwise indicated. Conversion factors to SI units: total, HDL, and LDL cholesterol (0.0259); triglycerides (0.0113); insulin (6.945); glucose (0.0555); FSH (1); estradiol (3.671); progesterone (3.18); estrone (37); testosterone (0.0347); and norepinephrine (0.00591). Pre, Premenopausal; Peri, perimenopausal; Post, postmenopausal; BP, blood pressure; HR, heart rate; bpm, beats per minute; LTPA, leisure time physical activity; MET, metabolic equivalent; VO_{2peak}, peak aerobic power.

[a] Data are median (interquartile range).

[b] Sample sizes of 105.

[c] Sample sizes of 102.

[d] Sample sizes of 119.

[e] Sample sizes of 106.

[f] Sample sizes of 100.

were no differences in baseline GTN brachial diameter or peak shear rate among the groups (Supplemental Table 1).

Correlates of brachial artery FMD

Higher seated systolic blood pressure, total body and trunk fat, body mass index (BMI), total and LDL cholesterol, ET-1, norepinephrine and FSH concentrations, and lower estradiol, estrone, and progesterone concentrations and peak aerobic capacity were associated with a lower brachial artery FMD (Table 2). Higher vasosomatic symptom frequency, but not severity scores, tended to be associated with a lower brachial artery FMD. The association of menopausal stage with brachial artery FMD remained highly significant ($P < 0.001$) after adjustment for these correlates, brachial artery baseline diameter, and hot flash reporting the previous year. Age was a significant correlate of brachial artery FMD, but analyses were not age adjusted because age and menopausal stage were highly correlated (r = 0.88). However, menopausal stage was more strongly associated with brachial artery FMD (%Δ, r = −0.70, and mmΔ, r = −0.68, both $P < 0.001$) than age (%Δ, r = −0.64, and mmΔ, r = −0.61, both $P < 0.001$). Adjusting for the prior and duration of HT and hormonal contraceptives did not alter the association with brachial artery FMD (all $P > 0.20$).

Subsample analysis of women aged 50–59 yr

Similar to the larger cohort, total body fat and FSH were higher ($P = 0.006$) and estradiol and estrone concentrations were lower across the stages of the menopause transition (Table 3, $P < 0.001$). There were no other differences in clinical characteristics across the groups. Brachial artery FMD was reduced in late perimenopausal and early and late postmenopausal compared with early peri-

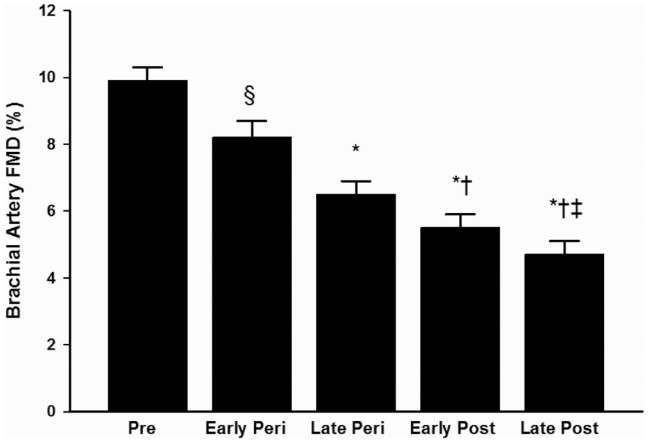

FIG. 1. Brachial artery FMD in premenopausal (Pre), early (Early Peri) and late perimenopausal (Late Peri), and early (Early Post) and late postmenopausal (Late Post) women. *, $P < 0.001$ and §, $P = 0.03$ *vs.* premenopausal women; †, $P < 0.001$ *vs.* early perimenopausal; ‡, $P < 0.001$ *vs.* late perimenopausal.

menopausal women ($P < 0.001$) but was not different between late perimenopausal and either early or late postmenopausal women (Fig. 3). Adjusting for age, total body fat, brachial diameter, and sex hormones did not influence the results ($P < 0.001$).

Discussion

The menopause transition is characterized by menstrual cycle irregularities, ovarian hormone changes, and increased risk for the development of CVD. To our knowledge, the present study was the first to examine vascular endothelial function across the stages of the menopause transition. The primary findings indicate that the stages of the menopause transition are associated with impaired endothelial function, independent of traditional CVD risk factors and hot flashes. The impairment in endothelial function is more evident during the late than early perimenopausal transition when controlling for age and becomes worse with prolonged estrogen deficiency during the postmenopausal years.

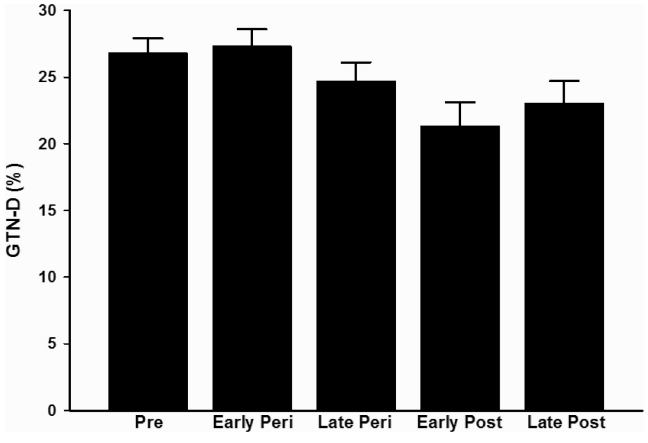

FIG. 2. GTN-mediated vasodilation in premenopausal (Pre), early (Early Peri) and late perimenopausal (Late Peri), and early (Early Post) and late postmenopausal (Late Post) women.

TABLE 2. Correlations of CVD risk factors, sex hormone concentrations, vasoconstrictors, and hot flashes with brachial artery FMD

Variables	Brachial artery FMD	
	r	*P*
Seated systolic BP	−0.38	<0.001
BMI	−0.19	0.03
Total body fat	−0.40	<0.001
Trunk body fat	−0.37	<0.001
Waist circumference	−0.11	0.23
Total cholesterol	−0.33	<0.001
LDL cholesterol	−0.29	0.001
Glucose	−0.12	0.17
FSH	−0.57	<0.001
Estradiol	0.46	<0.001
Progesterone	0.29	0.001
Estrone	0.35	<0.001
SHBG	0.01	0.91
Testosterone	0.09	0.36
ET-1	−0.20	0.02
Norepinephrine	−0.30	0.002
VO_{2peak}	0.35	<0.001
Vasosomatic frequency	−0.17	0.08
Vasosomatic severity	−0.12	0.24

BP, Blood pressure; VO_{2peak}, peak aerobic power.

The loss of normal endothelial function is believed to be a critical step in the initiation and progression of atherosclerosis (5). Although endothelial dysfunction is a biomarker of vascular aging, in women, the age-related decline in endothelial function is evident only after menopause, suggesting that estrogen may play a key role in maintaining a healthy, functional endothelium (11, 12). However, it was unclear how changes in ovarian hormones during the perimenopausal years influenced endothelial function. In the present study, endothelial function was impaired across the stages of the menopause transition, providing the first evidence that endothelial protection may be lost with declines in ovarian function. We found that the impairment in endothelial function was more pronounced during the late perimenopausal transition as indicated by a greater impairment in brachial artery FMD (from premenopausal levels) in late perimenopausal compared with early perimenopausal women of similar age (~35 *vs.* ~17% impairment, respectively). Moreover, a lower brachial artery FMD was strongly related to higher FSH and lower estradiol concentrations that characterize the menopausal transition. Our findings suggest that ovarian hormone levels in the early perimenopausal period may be sufficient to provide some level of endothelial protection and that declines in ovarian function and estrogen levels in the late perimenopausal transition initiate the rapid deterioration in endothelial function that

TABLE 3. Participant characteristics of perimenopausal and postmenopausal women aged 50–59 yr

Variable	Early Peri	Late Peri	Early Post	Late Post	*P*
n	9	12	27	9	
Age (yr)	52 ± 2	52 ± 2	55 ± 2	57 ± 2	<0.001
Body mass (kg)	63.6 ± 6.3	68.2 ± 15.7	71.3 ± 13.2	72.5 ± 14.1	0.42
BMI (kg/m^2)	23.7 ± 2.6	24.8 ± 5.0	26.7 ± 4.7	28.4 ± 5.4	0.12
Total body fat (%)	30 ± 5	36 ± 7	38 ± 6	40 ± 5	0.006
Waist circumference (cm)	78 ± 6	82 ± 15	86 ± 14	86 ± 13	0.46
WHR	0.80 ± 0.07	0.80 ± 0.08	0.82 ± 0.09	0.80 ± 0.05	0.81
Systolic BP (mm Hg)	111 ± 12	120 ± 14	118 ± 14	118 ± 16	0.53
Diastolic BP (mm Hg)	72 ± 3	76 ± 3	73 ± 2	74 ± 3	0.82
Total cholesterol (mg/dl)	166 ± 24	173 ± 41	192 ± 32	194 ± 30	0.11
LDL cholesterol (mg/dl)	101 ± 27	106 ± 38	113 ± 28	119 ± 26	0.54
HDL cholesterol (mg/dl)	48 ± 6	50 ± 7	55 ± 16	54 ± 13	0.52
Triglycerides (mg/dl)[a]	81 (68–99)	94 (56–112)	85 (69–149)	101 (78–129)	0.55
Fasting insulin (μIU/ml)	4.9 ± 4.1	5.1 ± 2.8	10.1 ± 7.8	9.9 ± 10.8	0.11
Fasting glucose (mg/dl)	85 ± 4	80 ± 10	88 ± 10	87 ± 9	0.09
FSH (μIU/ml)	15.3 ± 24.6	80.5 ± 34.5	77.2 ± 29.8	89.7 ± 24.4	<0.001
Estradiol (pg/ml)[a]	98 (43–137)	28 (10–92)	13 (10–19)	15 (10–19)	<0.001
Progesterone (ng/ml)[a]	0.6 (0.3–0.6)	0.4 (0.3–0.6)	0.3 (0.1–0.5)	0.3 (0.1–0.4)	0.06
Estrone (ng/dl)	54 ± 10	43 ± 9	28 ± 6	31 ± 10	0.002
SHBG (nmol/liter)	86 ± 52	72 ± 41	65 ± 41	67 ± 32	0.60
Testosterone (ng/dl)[b]	22 (17–37)	20 (17–24)	18 (17–30)	25 (17–53)	0.27
ET-1 (pg/ml)	5.8 ± 1.4	6.4 ± 1.9	6.4 ± 1.6	6.0 ± 1.2	0.72
Norepinephrine (pg/ml)[b]	271 ± 60	264 ± 123	291 ± 155	273 ± 134	0.95
LTPA (MET h/wk)[a,b]	17.4 (5.0–30.5)	12.9 (5.8–22.7)	9.5 (5.2–20.0)	6.3 (3.1–9.2)	0.12
VO_{2peak} (ml/kg · min)[b]	28.1 ± 5.0	27.8 ± 6.8	24.7 ± 3.3	22.8 ± 5.1	0.09

Data are mean ± SD, unless otherwise indicated. Peri, Perimenopausal; Post, postmenopausal; BP, Blood pressure; LTPA, leisure time physical activity; MET, metabolic equivalent; VO_{2peak}, peak aerobic power.

[a] Data are median (interquartile range).

[b] Sample size of 45.

worsens with prolonged estrogen deficiency. These findings are consistent with previous observations demonstrating that menstrual cycle irregularity and hypoestrogenemia related to the loss of ovarian function are associated with endothelial dysfunction and increased CVD risk (25, 26).

Because menopausal stage was highly correlated with age in the present study, it is plausible that the decline in endothelial function was mediated by aging. However, the correlation with brachial artery FMD was stronger with menopausal stage than age. Additionally, when we examined endothelial function in a subgroup of women aged 50–59 yr, brachial artery FMD was lower in late perimenopausal and postmenopausal women compared with early perimenopausal women. Although these findings suggest that declining ovarian function rather than age mediates impaired endothelial function in healthy middle-aged women, within the current cross-sectional study, we cannot completely discern the effects of declining ovarian function from that of age.

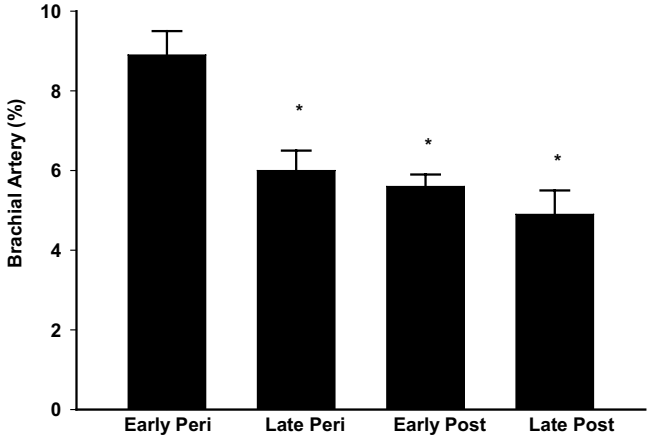

FIG. 3. Brachial artery FMD in early (Early Peri) and late perimenopausal (Late Peri) and early (Early Post) and late postmenopausal (Late Post) and postmenopausal women aged 50–59 yr. *, $P < 0.001$ *vs.* early perimenopausal.

In the present study, we examined whether CVD risk factors were related to the endothelial dysfunction across the stages of the menopause transition. Both prospective and longitudinal studies have shown that the menopausal transition is associated with changes in CVD risk factors known to adversely affect endothelial function, including adiposity, blood lipids, and lipoproteins (9, 10, 27). In the prospective Healthy Women Study, LDL cholesterol, triglycerides, and BMI showed greater increases during the perimenopausal transition than after menopause, whereas increases in systolic blood pressure and fasted glucose were greater after menopause (27). Similarly, the Study of Women's Health Across the Nation (SWAN) showed increases in total and LDL cholesterol that were greater 1 yr

before the final menstrual period compared with increases observed during the early perimenopausal or postmenopausal period, suggesting that the late perimenopausal transition may be the most critical time period for adverse changes in lipids (9). In the present study, adiposity (total and trunk fat), systolic blood pressure and total and LDL cholesterol were higher across the stages of the menopause transition and were inversely correlated with brachial artery FMD. However, adjusting for these risk factors did not alter the association between menopause stage and endothelial dysfunction. Moreover, in the subgroup analyses of women aged 50–59 yr in whom brachial artery FMD was lower in late perimenopausal and postmenopausal compared with early perimenopausal women, there were no differences in CVD risk factors across the groups.

We also examined whether the endothelial dysfunction across the stages of the menopause transition was related to the presence of hot flashes. Bechlioulis *et al.* (8) reported that early (<3 yr) postmenopausal women reporting moderate to severe hot flashes had reduced brachial artery FMD compared with age-matched early postmenopausal women reporting no or mild hot flashes, with the latter group not different from premenopausal controls. Similarly, in the Study of Women's Health Across the Nation Heart Study, women with hot flashes had reduced brachial artery FMD compared with women without hot flashes, independent of CVD risk factors, menopausal status, and estradiol concentrations (7). In the present study, although menopause stage, particularly late perimenopausal, was associated with a greater reporting of hot flashes in the previous year and with higher vasosomatic frequency and severity scores, we found no association between hot flashes and brachial artery FMD. Adjustment for the vasosomatic frequency scores and hot flashes did not alter the association between menopausal stage and endothelial dysfunction. These discordant findings may be related to differences in the health status of the population studied and measurement of brachial artery FMD (*i.e.* cuff occlusion duration) and/or hot flashes from the previous studies (7, 8). Because self-report measures of hot flashes may underestimate the frequency and/or severity, it is possible that an association may have been observed if we used objective measures of hot flashes (*i.e.* sternal skin conductance).

We can only speculate as to the mechanisms underlying endothelial dysfunction across the stages of the menopause transition. Reduced endothelial-dependent vasodilation is characterized by reduced nitric oxide (NO) production and by increased ET-1 activity and norepinephrine release (28, 29). Because estradiol preserves endothelial function in part by enhancing NO release (30) and by modulating the vascular effects of ET-1 and norepinephrine (31, 32), it is plausible that the endothelial dysfunction observed across the stages of the menopause transition is a consequence of reduced NO bioavailability and increased ET-1 and norepinephrine bioactivity. In the present study, ET-1 and norepinephrine concentrations were elevated across the stages of the menopause transition and were inversely correlated with brachial artery FMD. However, adjustment for ET-1 and norepinephrine did not alter the association between menopause stage and brachial artery FMD. The norepinephrine findings are in contrast to those reported by Kaplon *et al.* (33), who found that elevated plasma norepinephrine was an independent predictor of endothelial function with aging in women. The reason for this finding could be related to different populations of women. The present study included women across all the menopausal stages, whereas Kaplon *et al.* included only premenopausal and postmenopausal women. Nonetheless, because plasma concentrations do not necessarily reflect the differences at the local vascular level or vascular responsiveness to these factors, we cannot rule out that these or other vasoactive factors were modulated across the stages of the menopause transition. Other potential mechanisms include oxidative stress, tetrahydrobiopterin deficiency (a critical cofactor for NO production), and impaired estrogen receptor-α and endothelial nitric oxide synthase intracellular signaling (14, 34).

There were some limitations of the present study. First, the cross-sectional design precludes any conclusions about causality. Perimenopausal and postmenopausal status was determined by self-reported menstrual cycle characteristics; thus, group classifications may have been inaccurate. However, sex hormone concentrations at each menopausal stage were consistent with data from observational and longitudinal investigations of the menopause transition (35–37). Second, our findings can only be generalized to apparently healthy, mostly Caucasian, nonsmoking, normotensive, and sedentary/recreationally active women. Third, this study was done before the release of the STRAW+10 menopausal staging system (38). We also applied the previous STRAW criteria to women with a BMI greater than 30 kg/m^2, which is not recommended (13). However, excluding women with BMI greater than 30 kg/m^2 (n = 22) did not alter our findings (data not shown). Finally, the Pathobiological Determinants of Atherosclerosis in Youth Study found the presence of subclinical atherosclerosis in the abdominal aorta and coronary arteries in women 35 yr of age (39), and although our population was screened for overt disease, it is plausible that some women had subclinical, progressing atherosclerosis.

Clinical significance and conclusions

Endothelial dysfunction is a critical factor in the etiology of CVD and a significant predictor of cardiovascular events in postmenopausal women (6). As women age, they endure an additional insult in that their arteries are exposed to adverse changes in CVD risk factors (*i.e.* blood pressure, lipids, adiposity) during a time of profound changes in the hormonal environment. Because the menopause transition may be a triggering event that leads to increased vascular vulnerability as women age, a better understanding of the mechanisms mediating endothelial dysfunction in women as they lose the protective effects of estrogen on the vasculature is needed. Although recent clinical trials do not support cardioprotective benefits of HT in older postmenopausal women (2, 3), it is plausible that the timing of HT during a critical window may offer cardioprotection. The present study's findings add to the accumulating evidence that the most critical time period for adverse changes in CVD risk may be the late perimenopausal transition (9, 10, 40). Future investigations should examine whether maintaining estrogen concentrations and/or implementing lifestyle or therapeutic prevention strategies in the early stages of the menopause transition can preserve or attenuate the decline in endothelial function and decrease CV events in women.

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Address all correspondence and requests for reprints to: Kerrie L. Moreau, Ph.D., Associate Professor of Medicine, Division of Geriatric Medicine, University of Colorado Denver, Building L15, Room 8111, 12631 East 17th Avenue, P.O. Box 6511, Aurora, Colorado 80045. E-mail: kerrie.moreau@ucdenver.edu.

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