Endothelial Function, But Not Carotid Intima-Media Thickness, Is Affected Early in Menopause and Is Associated with Severity of Hot Flushes

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Context: The effect of early menopause on indices of vascular function has been little studied.

Objective: The objective of the study was to investigate the effect of early menopause on indices of subclinical atherosclerosis and identify predictors of those indices in early menopausal women.

Design, Setting, and Participants: This was a cross-sectional study that included 120 early menopausal women (age range 42–55 yr, <3 yr in menopause) recruited from the menopause outpatient clinic of an academic hospital and 24 age-matched premenopausal women.

Main Outcome Measures: Brachial artery flow-mediated dilation (FMD) and common carotid intima-media thickness (IMT) were studied. Estrogen receptor (ER)-α (rs2234693 T→C and rs9340799 A→G) and ERβ (rs4986938 A→G) polymorphisms were studied in menopausal women.

Results: FMD was significantly lower in early menopausal women compared with controls (5.43 ± 2.53 *vs.* 8.74 ± 3.17%, $P < 0.001$), whereas IMT did not differ between groups ($P > 0.8$). Severity of hot flushes was the most important independent predictor for FMD ($P < 0.001$) in menopausal women. Women with moderate/severe/very severe hot flushes had impaired FMD in contrast to women with no/mild hot flushes or controls. Women with no/mild hot flushes did not differ compared with controls. Age and systolic blood pressure were the main determinants of IMT (both $P = 0.004$). ER polymorphisms were not associated with vascular parameters.

Conclusions: Impairment of endothelial function is present in the early menopausal years, whereas carotid IMT is not affected. Severity of hot flushes is the main determinant of endothelial dysfunction in early menopausal women. The studied ER polymorphisms do not offer important information on vascular health in early menopause. (***J Clin Endocrinol Metab*** **95: 1199–1206, 2010)**

Atherosclerotic cardiovascular diseases (CVD) account for the majority of deaths in women in Western countries. The incidence of CVD increases in women after menopause. Menopause is defined as the permanent cessation of menses after the loss of ovarian function and is characterized by several endocrinological, biological, and clinical features. The most frequent complaint of women entering menopause is hot flushes, episodes of sud-

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Abbreviations: B, β coefficient; BMI, body mass index; CCA, common carotid artery; CI, confidence interval; CVD, cardiovascular disease; DBP, diastolic blood pressure; EF, endothelial function; ER, estrogen receptor; FMD, flow-mediated dilation; HDL-c, high density lipoprotein cholesterol; IMT, intima-media thickness; LDL-c, low density lipoprotein cholesterol; SBP, systolic blood pressure.

den feeling of heat in the face, neck, or chest. The presence of hot flushes has recently been associated with worse cardiovascular profile and adverse vascular changes (1, 2), implying increased risk for atherosclerosis.

Endothelial dysfunction is found in the initial stages of atherosclerosis, long before atherosclerotic lesions are present or clinical events occur (3). Assessment of endothelial function (EF) using brachial artery flow-mediated dilation (FMD) has shown promising results in cardiovascular risk stratification (4, 5). Carotid artery intima-media thickness (IMT) is another widely used surrogate marker of subclinical atherosclerosis with established prognostic value in various populations (6).

Impaired FMD and increased IMT have both been demonstrated in women with natural menopause compared with premenopausal women (7–10), indicating vascular dysfunction associated with menopause. Whether this is the consequence of the loss of endogenous estrogen during menopausal transition or the aging process *per se* remains unclear. Furthermore, menopause-related clinical features such as hot flushes and changes in traditional cardiovascular risk factors as well as genetic factors may also be associated.

Estrogen receptors (ER) α and β, found in vascular endothelial and smooth muscle cells, mediate the cardioprotective effects of endogenous estrogen and are involved in vascular function regulation (11). ER polymorphisms, by modifying estrogen action at a tissue level, may contribute to the initiation and progression of the atherosclerotic process and thus be expected to affect early markers of atherosclerosis, especially in women (12). ERα rs2234693 T→C and rs9340799 A→G and ERβ rs4986938 A→G polymorphisms are three of the most extensively studied ER polymorphisms; conflicting results have been reported regarding their relation with both cardiovascular risk factors and clinical events (12–18). Their association with indices of subclinical atherosclerosis has been little studied (19).

The aims of the current study were 1) to compare indices of subclinical atherosclerosis (FMD and IMT) between early menopausal and age-matched premenopausal women, 2) to investigate the role of the ERα (rs2234693 T→C and rs9340799 A→G) and ERβ (rs4986938 A→G) polymorphisms on FMD and IMT in early menopausal women, and 3) to identify independent predictors of FMD and IMT in these women.

Subjects and Methods

Study population

Early menopausal women (n = 120)

Menopausal women were recruited from the menopause outpatient clinic of the Gynecology Department at the University Hospital of Ioannina, Greece. To be eligible for our study, menopausal women had to meet the following inclusion criteria: 1) aged 42–55 yr, 2) cessation of menses at or after age of 40 yr, 3) time from last menstruation at least 6 and but no more than 36 months before study entry (*i.e.* early menopause), 4) good general health, 5) plasma FSH at least 35 mIU/ml and estradiol levels no higher than 25 pg/ml (92 pmol/liter). These women underwent gynecological examination, including transvaginal ultrasound and Papanicolaou smear. On the basis of this examination, the patients had to fulfill the following criteria: no suspicion of malignancy, no endometrial pathology (*i.e.* endometrial thickness <5 or ≥ 5 mm with no endometrial hyperplasia or cancer found on endometrial biopsy), and the presence of normal ovaries. All women participating in the study had normal mammogram at study entry.

Healthy premenopausal control women (n = 24)

Healthy, age-matched, regularly menstruating women (menstrual cycles between 21 and 35 d) who were not taking any medications and were not using hormonal contraception were recruited from the hospital and university staff and served as the control population.

Menopausal women and controls were excluded if they had 1) previous history of thromboembolic disease; 2) clinical signs, symptoms or known history of CVD (coronary artery, cerebrovascular or peripheral arterial disease); 3) hypertension defined as systolic blood pressure (SBP) higher than 155 mm Hg and/or diastolic blood pressure (DBP) higher than 95 mm Hg during the initial examination or administration of anti-hypertensive medications; 4) history of diabetes mellitus or fasting glucose at least 126 mg/dl (7 mmol/liter) or administration of antidiabetic medications; 5) low-density lipoprotein cholesterol (LDL-c) higher than 190 mg/dl (4.9 mmol/liter) or administration of anticholesterolemic medications; 6) serum triglycerides higher than 500 mg/dl (5.65 mmol/liter); 7) body mass index (BMI) lower than 19 and higher than 35 kg/m^2; 8) prior treatment in the past 6 months known to affect vascular function (hormone therapy, phytoestrogen-containing supplements, vitamins, antioxidants, and any cardiovascular medications); 9) excessive alcohol use (>2 U/d); 10) history of chronic diseases such as any cancer, renal failure, liver cirrhosis, and endocrinopathies; or 11) known HIV infection.

Screening was carried out between November 2006 and November 2007. All women were Caucasian. Smoking history was recorded (current, past, never users), but smoking was not an exclusion criterion. Physical activity level was recorded in a three-point scale: 1) no exercise or circumstantial exercise less than once per week, 2) brisk walking for at least 30 min for no more than 5 d/wk, and 3) daily exercise schedule, either brisk walking or more intense exercise. In menopausal women, the presence and severity of menopausal symptoms were evaluated.

Study design

In this cross-sectional study, vascular and biochemical parameters were measured in all early menopausal women and age-matched premenopausal controls. Blood samples were drawn early in the morning after overnight fasting, and vascular studies were performed the next day. In control women, blood sampling was performed during the early follicular phase of their menstrual cycle (d 2–5 of the cycle). Genotyping was performed in all menopausal women.

The study was performed at the Michaelidion Cardiac Center, University of Ioannina, Greece, and was approved by the Ethics Committee of the Michaelidion Cardiac Center, University of Ioannina, Greece. The study complied with the Declaration of Helsinki, and all participants provided written informed consent.

Evaluation of menopausal symptoms

All menopausal women completed a questionnaire based on the Menopause Rating Scale, a menopause-specific health-related quality of life scale (20). The following symptoms or complaints were evaluated: hot flushes and sweating (vasomotor symptoms), heart discomfort, sleep problems, depressive mood, irritability, anxiety, physical and mental exhaustion, sexual problems, bladder problems, vaginal dryness, and joint/ muscular discomfort. Each of these symptoms could get zero (no complaint) or up to four (very severe symptoms) scoring points depending on the severity of the complaints perceived by women. Especially for vasomotor symptoms, women were asked to classify them as follows: mild, if they lasted less than 5 min and resolved spontaneously without disturbing daily life; moderate, if they lasted less than 15 min and disturbed life only during the day; severe, if they lasted up to 20 min, disturbing both daily life and sleep; and very severe if they lasted up to 45 min, disrupting daily life and sleep (21). In our study, all menopausal women were asked to score the symptoms they had during the last 2 wk before baseline assessment.

Biochemical assays

Microparticle enzyme immunoassays were used for estradiol and FSH measurement (AxSYM estradiol and AxSYM FSH, respectively; Abbott Laboratories, Abbott Park, IL). Serum levels of total and high-density lipoprotein cholesterol (HDL-c), triglycerides, and glucose were determined with standard automated methods on the Olympus AU640 Clinical Chemistry Analyzer (Olympus Diagnostica GmbH, Hamburg, Germany). LDL-c was calculated using the Friedewald formula: LDL-c = total cholesterol − HDL-c − (triglycerides/5).

Vascular studies

All studies were performed by the same operator, who was unaware of the gonadal status of the women. Optimal imaging of the brachial and common carotid arteries (CCA) was obtained using an Echo-Doppler ultrasound (Ultrasound ATL; HDI 5000, Bothell, WA) and a 5- to 12-MHz transducer.

Brachial artery ultrasound protocol

Endothelial function was assessed in all women by measurement of FMD in the right brachial artery in response to hand hyperemia, based on previously described methodology (22), according to published guidelines (23). Images were acquired at baseline and every 30 sec, from the first to the third minute after deflation of a wrist cuff inflated to 300 mm Hg for 4 min for measurement of FMD. Brachial artery blood flow was measured by continuous-wave Doppler at baseline and 15 sec after cuff release. FMD was calculated as the maximum percent increase in arterial diameter during the first 3 min of hyperemia compared with the diameter at rest.

Carotid artery ultrasound protocol

CCA IMT measurement was performed in all women using a standardized protocol published previously (24). Three consecutive longitudinal images of each CCA 1–2 cm proximal to the bifurcation were acquired. Measurements were always made at the far wall of the artery. The mean value of IMT for right and left CCA was obtained by averaging the three measurements at each artery. Finally, the mean and maximum IMT (IMTmean and IMTmax) of the CCA were determined.

Off-line analysis and measurement of brachial artery end-diastolic diameter and IMT were performed by another blinded operator by means of the software QLAB (Philips Ultrasound, Bothell, WA) with manual and automatic (for brachial and carotid artery, respectively) determination of the relative vascular wall margins. Measurements were made at end-diastole coincident with the R-wave on electrocardiogram.

Reproducibility

The intra-observer variability of repeated measurements of the same image for brachial artery diameter and IMT were 0.021 ± 0.038 and 0.008 ± 0.008 mm, respectively. In studies performed on 2 separate days (7–10 d apart) in 10 subjects by a single operator, the within-subject coefficient of variation of the brachial artery diameter, endothelium-dependent vasodilation, and IMT were 0.7, 6.9, and 1.5%, respectively.

Genotyping

Genomic DNA was extracted from peripheral blood lymphocytes according to the standard salt extraction procedure. PCR-restriction fragment length polymorphisms analysis was used as the screening method for polymorphisms rs2234693 (also known as *Pvu*II 454-397T→C) and rs9340799 (also known as *Xba*I 454-351A→G) of ERα and rs4986938 (also known as *Alu*I G1730A) of ERβ (25). Specifically, the following primers pairs were used: for variants *Pvu*II and *Xba*I, forward 5′-CTG CCA CCC TAT CTG TAT CTT TTC CTA TTC TCC-3′ and reverse 5′-TCT TTC TCT GCC ACC CTG GCG TCG ATT ATC TGA-3′, and for variant Alu I, forward 5′-GAC CTG CTG CTG GAG ATG CT-3′ and reverse 5′-AAT GAG GGA CCA CAC AGC A-3′. Subsequently, restriction assays were employed using the restriction endonucleases *Pvu*II and *Xba*I on PCR product of ERα and *Alu*I on PCR product of ERβ according to manufacturer instructions (New England Biolabs Inc., Ipswich, MA). The digested PCR products were separated on 2% agarose, and the restriction patterns were visualized by ethidium bromide. All samples were run in duplicate and random samples were sequenced to assure quality control. In addition, all runs included positive controls for each of the three genotypes and blanks.

Statistical analysis

Continuous data are presented as mean ± SD. Kolmogorov-Smirnov Z test was used to determine the normal distribution of all continuous variables; age, serum estradiol, glucose, and time since last menstruation were not normally distributed. Independent-samples t test and χ^2 test were used to compare continuous and categorical variables, respectively, between premenopausal and menopausal women. For not normally distributed variables, the Mann-Whitney U test was used. Difference in FMD between premenopausal and menopausal women was adjusted for confounding factors using univariate general linear model analysis.

Pearson and Spearman correlation coefficients were estimated between baseline FMD and IMT and all relevant demographic, clinical, biochemical, and genetic data in menopausal women. Subsequently, multiple regression analysis was imple-

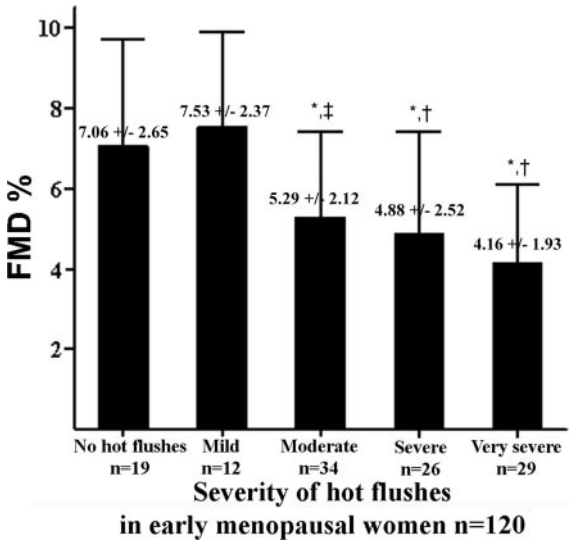

FIG. 1. FMD in early menopausal women by level of hot flush severity. The bar graph shows FMD values in the five subgroups of early menopausal women based on the level of hot flushes severity. No significant differences were observed among menopausal women with moderate, severe, and very severe hot flushes as well as between menopausal women with no and mild hot flushes Data are expressed as mean ± SD. *, $P < 0.05$ compared with menopausal women with mild hot flushes; †, $P < 0.05$ compared with menopausal women with no hot flushes; ‡, $P = 0.077$ compared with menopausal women with no hot flushes.

mented to determine possible independent predictors of vascular parameters. Multivariate models were constructed on the basis of significant univariate associations at the $P < 0.10$ level. Not normally distributed variables were logarithmically transformed to be used in these analyses.

Because of an indication of a threshold for FMD at no/mild *vs.* moderate/severe/very severe hot flushes (Fig. 1), severity of hot flushes was used as a dichotomous variable in multivariate models, and menopausal women were divided in two subgroups: no/mild *vs.* clinically important hot flushes. Continuous and categorical variables in these two subgroups were compared using independent-samples *t* test and χ^2 test, respectively. For not normally distributed variables, the Mann-Whitney *U* test was used. ANOVA and the Bonferroni test for *post hoc* comparisons were used to compare FMD values among premenopausal and the two subgroups of menopausal women and among different ERα and -β genotype groups. For not normally distributed variables, the Kruskal-Wallis test was used. χ^2 analysis was performed to investigate whether the observed genotype frequencies were in Hardy-Weinberg equilibrium. *P* values were always two-sided, and a value of <0.05 was considered significant. Statistical analysis was performed using SPSS 15.0 for Windows.

Results

Table 1 summarizes baseline characteristics of the study population. Median time from last menstruation was 10 months (range 6–36 months). Menopausal women had significantly higher DBP ($P = 0.03$) and higher FSH and lower estradiol (both $P < 0.001$) levels compared with controls. FMD was significantly lower in menopausal women compared with controls ($P < 0.001$). Menopausal status remained the only significant determinant of FMD in the two groups after adjusting for DBP [β coefficient (B) = 2.90; 95% confidence interval (CI) = 0.76–5.06; $P = 0.009$]. IMT did not differ between the two groups (Table 1).

After univariate analysis, the factors associated with FMD ($P < 0.1$) were brachial artery diameter, HDL-c, and severity of hot flushes; these were used in the multivariate model of FMD in menopausal women. Severity of hot flushes (B = −2.13; 95% CI = −3.13 to −1.12; $P <$

TABLE 1. Characteristics of the study participants

	Premenopausal women (n = 24)	Early menopausal women (n = 120)	*P*
Age (yr)	49 ± 3	50 ± 4	0.1
Current smokers, n (%)	4 (16.7)	21 (17.5)	0.7
Physical activity level, n (%)			
Low	13 (54.2)	62 (51.7)	
Medium	8 (33.3)	49 (40.8)	
High	3 (12.5)	9 (7.5)	0.6
BMI (kg/m^2)	27 ± 4	28 ± 5	0.2
SBP (mm Hg)	119 ± 10	124 ± 15	0.09
DBP (mm Hg)	76 ± 8	80 ± 8	0.02
Glucose (mg/dl)	94 ± 14	92 ± 11	0.3
FSH (mIU/ml)	11 ± 7	69 ± 25	0.001
Estradiol (pg/ml)	85 ± 77	16 ± 6	0.001
Total cholesterol (mg/dl)	212 ± 31	228 ± 35	0.07
HDL-c (mg/dl)	62 ± 12	62 ± 10	0.8
LDL-c (mg/dl)	131 ± 27	143 ± 27	0.09
Triglycerides (mg/dl)	97 ± 35	106 ± 19	0.4
Baseline brachial artery diameter (mm)	3.63 ± 0.31	3.71 ± 0.44	0.2
FMD (%)	8.74 ± 3.17	5.43 ± 2.53	0.001
IMTmean (mm)	0.56 ± 0.06	0.56 ± 0.06	0.9
IMTmax (mm)	0.58 ± 0.06	0.58 ± 0.07	0.8

Data are shown as mean ± SD unless stated otherwise. Conversion factors to SI units are 0.0555 for glucose, 1 for FSH, 3.671 for estradiol, 0.0259 for total cholesterol and HDL-c and LDL-c, and 0.0113 for triglycerides.

TABLE 2. Characteristics of early menopausal women by reported hot flushes

	Hot flushes		
	No or mild (n = 31)	Moderate/severe/very severe (n = 89)	*P*
Age (yr)	51 ± 4	49 ± 4	0.1
Current smokers, n (%)	7 (22.6)	14 (15.7)	0.4
Physical activity level, n (%)			
Low	16 (51.6)	45 (50.6)	
Medium	14 (45.2)	35 (39.3)	
High	1 (3.2)	9 (10.1)	0.2
BMI (kg/m^2)	27 ± 3	27 ± 4	0.8
SBP (mm Hg)	123 ± 14	125 ± 15	0.6
DBP (mm Hg)	79 ± 8	81 ± 8	0.4
Glucose (mg/dl)	94 ± 13	92 ± 11	0.4
FSH (mIU/ml)	63 ± 24	72 ± 26	0.1
Estradiol (pg/ml)	14 ± 5	17 ± 6	0.02
Total cholesterol (mg/dl)	228 ± 31	224 ± 35	0.5
HDL-c (mg/dl)	66 ± 12	58 ± 12	0.02
LDL-c (mg/dl)	143 ± 23	143 ± 31	0.8
Triglycerides (mg/dl)	106 ± 44	106 ± 53	0.7
Baseline brachial artery diameter (mm)	3.71 ± 0.48	3.72 ± 0.43	0.9
FMDmax (%)	7.24 ± 2.51	4.80 ± 2.22	0.001
IMTmean (mm)	0.56 ± 0.06	0.56 ± 0.06	0.9
IMTmax (mm)	0.59 ± 0.06	0.58 ± 0.07	0.9

Data are shown as mean ± SD unless stated otherwise. See Table 1 for conversion factors to SI units.

0.001) was the most important independent predictor of FMD, accounting for 75% of the variance of FMD in our model ($R^2 = 0.22$; $P < 0.001$), whereas HDL-c was another predictor of FMD (B = 0.05; 95% CI = 0.02–0.08; $P = 0.005$). Women with clinically important hot flushes had significantly lower FMD compared with either menopausal women with no/mild hot flushes (Table 2) or controls (Fig. 2). No significant difference was observed in FMD between menopausal women with no/mild hot flushes and controls ($P = 0.2$) (Fig. 2). Vascular parameters did not show any significant association with other menopausal symptoms reported in Menopause Rating Scale questionnaire (data not shown) and were not included in the regression analysis.

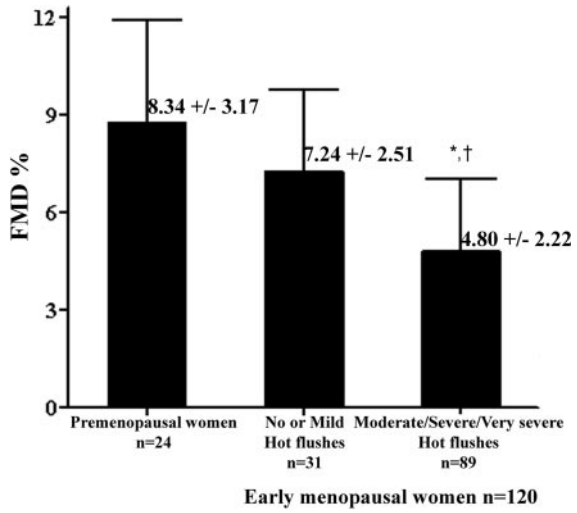

FIG. 2. FMD in early menopausal women and age-matched controls. The bar graph shows FMD values in premenopausal women and two subgroups of early menopausal women (women with no/mild hot flushes *vs.* women with clinically important hot flushes). Data are expressed as mean ± SD. *, $P < 0.001$ compared with premenopausal women; †, $P = 0.001$ compared with menopausal women with no or mild hot flushes.

The sole predictor of IMTmean was SBP (B = 0.001; 95% CI = 0.000–0.002; $P = 0.004$), whereas age (B = 0.23; 95% CI = 0.08–0.38; $P = 0.004$) was the only predictor of IMTmax in our population (variables included in multivariate analysis were age, SBP, total cholesterol, and smoking). Both prediction models could explain only about 7% of the variance of IMT in our population.

Genotype analysis

The mean values and observed frequencies of clinical, metabolic, and vascular parameters in menopausal women in the various groups of genotypes are presented in Supplemental Table 1 (published as supplemental data on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). The genotypes' distribution met the requirements of the Hardy-Weinberg equilibrium. ERα rs2234693 and rs9340799 polymorphisms were in linkage disequilibrium ($P < 0.001$).

No significant differences in vascular parameters were found among the various groups of genotypes of studied polymorphisms (Supplemental Table 1), and thus genotypes were not included in the regression analysis described above ($P > 0.1$ for all in the univariate analysis). Presence of the ERα rs9340799 GG and rs2234693 CC genotypes were associated with higher triglycerides levels

($P = 0.007$ and $P = 0.05$, respectively). After *post hoc* analysis, only the association of ERα rs9340799 A/G polymorphism with triglycerides remained significant (133 ± 62 for GG *vs.* 97 ± 35 and 106 ± 44 mg/dl for AA and AG genotypes, respectively). ERβ rs4986938 polymorphism showed a statistically significant relation ($P = 0.008$) to glucose levels; *i.e.* AG genotype carriers had higher fasting glucose levels compared with other genotype carriers (95 ± 13 for AG *vs.* 90 ± 11 and 88 ± 7 mg/dl for AA and GG genotypes, respectively). A trend for higher SBP and DBP ($P = 0.08$ for both) was also found when the allele rs4986938 G was present (Supplemental Table 1).

Discussion

The findings of the current study indicate that early menopausal women (*i.e.* aged <55 yr and <3 yr in menopause) had significant endothelial dysfunction, as shown by reduced FMD, compared with age-matched premenopausal women. Severity of hot flushes was found to be the most important independent predictor of endothelial dysfunction in these women. IMT was similar between premenopausal and early menopausal women and was related to classical cardiovascular risk factors such as age and SBP. Studied polymorphisms of ER genes were not associated with either FMD or IMT in early menopausal women.

Our study provides clear evidence that endothelial dysfunction occurs early in natural menopause and is not due to aging. Previous studies reporting endothelial dysfunction in menopausal women enrolled significantly younger premenopausal women (aged 30–35 yr) to serve as controls (7, 9), and therefore, it was not clear whether endothelial dysfunction could be attributed to menopause or aging *per se*. In the current study, FMD was found to be reduced (by ~40%) in early menopausal compared with age-matched premenopausal women. The impairment of EF in these early menopausal women is consistent with the decline in EF observed in women early after ovariectomy and in women with premature ovarian failure (22, 26, 27).

The current study associated endothelial dysfunction in early menopausal women with hot flushes, the most prominent vasomotor symptom and the most common complaint of women entering menopause, reported by almost 80% of them (28). Hot flushes have long been considered as a symptom that mainly affects the quality of life of menopausal women without any other clinical significance. This premise, however, has been challenged recently (1, 2, 29). The presence of hot flushes has been associated with worse cardiovascular risk profile; higher cholesterol levels, DBP and SBP, and BMI were found in symptomatic compared with asymptomatic menopausal women (1). Menopausal women reporting hot flushes were recently found to have impaired FMD and greater aortic calcification compared with menopausal women of similar age without vasomotor symptoms (2). Our study further showed that clinically important hot flushes was the most important independent predictor of endothelial dysfunction in early menopausal women. It was also demonstrated that women with clinically important hot flushes had impaired EF in contrast to women with no/mild hot flushes or premenopausal controls, whereas women with no/mild hot flushes did not differ compared with controls.

HDL-c levels were also significantly lower in women with moderate/severe/very severe hot flushes compared with women with no/mild hot flushes. Besides this association of HDL-c levels and hot flushes' severity, HDL-c was found to be an independent predictor of EF in our population; however, its prognostic value was inferior compared with the value of hot flush severity. The association of higher HDL-c levels with greater FMD in early menopausal women is in accordance with previously reported findings (30). An inverse correlation between circulating levels of HDL-c and cardiovascular risk is well established (31).

Women with moderate/severe/very severe hot flushes had slightly but significantly higher estradiol levels compared with women with no/mild hot flushes. However, estradiol levels were not found to be related to FMD in early menopausal women ($P > 0.1$), whereas the absolute difference between the two subgroups (~3 pg/ml) is probably very small to account for the difference in FMD.

The current study demonstrated no difference in IMT between early menopausal and age-matched premenopausal women, implying that menopausal transition did not play a role in subclinical carotid atherosclerosis. Previous studies have shown that IMT is increased in women who are postmenopausal for more than 3–5 yr compared with age-matched controls (8, 10). Our study further showed that IMT in early menopausal women was associated with increasing SBP and age, a finding also reported by others (8). It is probable that the prolonged loss of endogenous estrogen, observed in advanced rather than early menopause, may contribute to carotid atherosclerosis as assessed by increased IMT, whereas aging may also play a role.

Discrepant results on two different indices of atherosclerosis were reported in our study; FMD was reduced, whereas IMT did not differ in early menopausal compared with age-matched premenopausal women. This finding indicates that menopausal transition may be related to endothelial dysfunction but not to structural atherosclerotic changes such as increased IMT that are reported to

appear later (>3 yr in menopause) (8, 10). Endothelial dysfunction can be detected before structural atherosclerotic changes to the vessel wall appear on angiography or ultrasound (3), and it has recently been reported to predict the progression of preclinical carotid arterial disease (32). FMD testing has thus been suggested as an integrated measure to identify individuals in the preclinical setting who may be at greater risk for developing structural atherosclerotic disease (33) and possibly later overt clinical vascular disease. According to our findings, clinically important hot flushes (*i.e.* moderate/severe/very severe), a symptom easy to recognize, could possibly serve as a warning signal to identify a group of women in the early stages of menopause who demonstrate endothelial dysfunction and may be at increased risk for developing CVD later in life. These women may benefit from more intense follow-up and more aggressive preventive strategies to reduce cardiovascular risk.

Finally, no significant association was found between the studied ERα and -β gene polymorphisms and FMD or IMT in menopausal women. In accordance with this, no association of FMD with the specific ER gene polymorphisms has been reported previously in elderly men and women (19). An effect of these ER polymorphisms would probably be expected to be more prominent in our population of relatively young and healthy early menopausal women, in whom aging, cardiovascular risk factor accumulation, and long duration of estrogen loss should not yet have an impact on vascular function. Indeed, another ERβ gene polymorphism has been recently related to IMT in young adults (34). In our study, a significant association of ERα rs9340799 polymorphism was shown only with triglyceride levels; the small difference among genotypes is not expected to have an important clinical relevance. ERβ rs4986938 AG genotype was related to higher glucose levels, a finding difficult to interpret because no effect of either A or G allele on serum glucose was found.

Study limitations

This was a cross-sectional, observational study that could not assess the causal nature of associations. Self-reporting of severity of menopausal symptoms is a subjective measure. However, the operator who performed the assessment of vascular function was blinded both to the menopausal status of women and the severity of their symptoms. More detailed measures to assess severity of hot flushes could be examined in future research. Smoking was not an exclusion criterion but was well matched between early menopausal and control women and should not influence our findings. Our model could explain only 22% of the variance in FMD in our population, suggesting that other contributing factors should also be examined in future studies. Lastly, the associations observed between genetic polymorphisms and clinical phenotypes in our study require further investigation in larger population groups.

In conclusion, early menopausal women had significant endothelial dysfunction compared with age-matched premenopausal women. The presence of clinically important hot flushes was the most important independent predictor of endothelial dysfunction in these women. Subclinical carotid atherosclerosis did not differ between early menopausal and age-matched premenopausal women and did not relate to vasomotor symptoms. The studied ERα and -β gene polymorphisms did not appear to play a significant role on endothelial dysfunction or subclinical structural atherosclerosis in this group of women. Additional studies in larger groups of women in the early stages of menopause are needed to confirm the clinical and prognostic implications of these observations.

Acknowledgments

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