Endothelial function in women of the Kronos Early Estrogen Prevention Study

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ABSTRACT

Objective   Endothelial dysfunction occurs early in the atherosclerotic disease process, often preceding clinical symptoms. Use of menopausal hormone treatment (MHT) to reduce cardiovascular risk is controversial. This study evaluated effects of 4 years of MHT on endothelial function in healthy, recently menopausal women.

Methods   Endothelial function was determined by pulse volume digital tonometry providing a reactive hyperemia index (RHI) in a subset of women enrolled in the Kronos Early Estrogen Prevention Study. RHI was measured before and annually after randomization to daily oral conjugated equine estrogen (oCEE, 0.45 mg), weekly transdermal 17β-estradiol (tE2, 50 µg) each with intermittent progesterone (200 mg daily 12 days of the month) or placebo pills and patch.

Results   At baseline, RHI averaged 2.39 ± 0.69 (mean $\pm$ standard deviation; $n = 83$), and over follow-up did not differ significantly among groups: oCEE, 2.26 ± 0.48 ($n = 26$); tE2, 2.26 ± 0.45 ($n = 24$); and placebo, 2.37 ± 0.37 ($n = 33$). Changes in RHI did not correlate with changes in traditional cardiovascular risk factors, but may inversely correlate with carotid intima medial thickness (Spearman correlation coefficient $\rho = -0.268$, $p = 0.012$).

Conclusion   In this 4-year prospective assessment of recently menopausal women, MHT did not significantly alter RHI when compared to placebo.

INTRODUCTION

Sex-specific differences in the pathophysiology, presentation, treatment and clinical outcomes of cardiovascular disease (CVD) are in the early phase of discovery[1-3]. Moreover, common CVD risk predictors may underestimate lifetime risk in both women and men[4, 5]. Identifying women's CVD risk early is critical for proper intervention and prevention of significant adverse outcomes.

Adverse cardiovascular events correlate with loss of endothelial function[6-9]. Thus, changes in endothelial function may reflect early stages of the atherosclerotic disease process. Endothelial function is evaluated in the coronary arteries by measuring the magnitude of arterial dilatation in response to intra-coronary injection of acetylcholine during angiography[6, 8]. In the periphery, endothelial function is monitored by flow-induced dilatation assessed by ultrasound imaging of the brachial artery following either occlusion or arterial infusion of acetylcholine or by digital arterial tonometry recorded as a reactive hyperemia index (RHI) following occlusion of the brachial artery[10]. Flow-induced vasodilatation is affected by risk factors for CVD in asymptomatic individuals[9] and is reduced in the presence of symptomatic disease. The reduction correlates with the severity of coronary artery disease[11, 12]. Likewise, low RHI values correlate with the classic CVD risk factors of increased body mass index (BMI) and low serum high density lipoprotein (HDL) in both men and women with symptomatic coronary artery disease[7, 8] but correlation of RHI

with cardiovascular risk factors in asymptomatic individuals in the general population is equivocal[7, 13].

Menopause represents a period of increasing risk for CVD and declining endothelial function in women[14]. Multiple observational studies have demonstrated that menopausal hormone treatment (MHT) is associated with reduced incidence and all-cause CVD mortality in menopausal women[15-20]. However, in the Women's Health Initiative (WHI), the risk of adverse cardiovascular events increased in women randomized to MHT consisting of conjugated equine estrogen (CEE) in combination with medroxyprogesterone acetate[21]. Endothelial function was not assessed in the WHI. It is important to note that women in the WHI were more than a decade past menopause (average age 63 years old) and not representative of women using MHT for menopausal symptoms, in contrast to the earlier reported observational studies, which showed a beneficial effect of MHT on cardiovascular endpoints[22]. Indeed, results from basic science studies support the hypothesis that the timing of MHT initiation impacts the development of CVD such that initiation of MHT shortly after menopause reduces coronary artery atherosclerosis, whereas delayed initiation of MHT does not[23, 24]. Thus, the timing of initiation of MHT may contribute to its biological consequences[25, 26]. In a study of the long-term (10-year follow-up) effects of MHT, the risk of cardiovascular events, including death, myocardial infarction and heart failure was reduced in recently menopausal women randomized to MHT compared to placebo (hazard ratio 0.48, 95% confidence interval 0.26–0.87; $p = 0.015$) with no increased risk of venous thromboembolism, cancer or stroke[27]. However, MHT is not currently recommended for prevention of CVD. Even for the approved MHT indication of treatment for severe vasomotor symptoms, no specific guidelines regarding formulations and routes of delivery exist. The North American Menopause Society has provided recommendations for a general approach advising 'use of the lowest dose of MHT for the shortest period of time' when necessary for symptom relief[28]. Currently, no information exists on the effects of sustained use of lower doses of MHT delivered by oral or transdermal routes on endothelial function when initiated in recently menopausal women.

This study was designed to assess the effects of two types of MHT (oral and transdermal) compared to placebo on RHI in healthy, recently menopausal women at low risk for cardiovascular disease enrolled in the Kronos Early Estrogen Prevention Study (KEEPS). Digital tonometry to provide the RHI was selected to provide a surrogate for endothelial function because the test is used clinically and, although less sensitive than measuring brachial arterial vasodilatation to acetylcholine, it is a non-invasive technique and thus more appealing for retaining participants in a 4-year study. We hypothesized that MHT administered to women early in menopause would have a positive effect on RHI, consistent with sustained or improved endothelial function. We also evaluated the RHI results within the context of conventional CVD risk factors, and the surrogate imaging endpoints of the main KEEPS trial, carotid intima-medial thickness (CIMT) and coronary artery calcification (CAC). Much controversy has ensued subse-

quent to the WHI reports; MHT is a Class 3 indication (i.e. contraindicated/not recommended) for primary or secondary prevention of cardiovascular disease[29], while the North American Menopause Society, as well as the Global Consensus Statement on Menopausal Hormone Therapy, recommends MHT for women early in menopause with symptoms[28, 30]. These results have the potential to provide needed and clinically relevant information regarding the use of MHT in postmenopausal women.

METHODS

Participants

All women meeting inclusion criteria and randomized to treatment in the Kronos Early Estrogen Prevention Study (KEEPS/ NCT00154180) at Mayo Clinic, Rochester, MN, USA, were invited to participate in this ancillary study.

Women were between the ages of 42 and 59 years and were between 6 months and 3 years from their last menses at the time of enrollment. Participants had a negative history of CVD or related symptoms, were non-diabetic, and without significant hyperlipidemia requiring lipid-lowering therapy. Study participants underwent testing to confirm low CVD risk. Blood chemistries for fasting blood glucose, HDL cholesterol, low density lipoprotein (LDL) cholesterol and triglycerides were within normative ranges. None of the women smoked more than ten cigarettes a day or had untreated hypertension; BMI was < 35 kg/m^2; and CAC scores were < 50 Agatston units. Participants completed questionnaires reporting smoking history (never, past or current) and menopausal symptoms over the last 3 months (insomnia, hot flushes, night sweats and palpitations), ranking them on a numerical scale as none to mild (0–2), or moderate to severe (3–5). This ancillary study was approved by the Institutional Review Board at Mayo Clinic and all participants gave written informed consent for this ancillary study.

Participants were randomized to oral CEE (0.45 mg/day, oCEE), transdermal 17β-estradiol (50 μg/day with patches changed weekly, tE2), each with intermittent progesterone (200 mg/day for the first 12 days of the month), or placebo pills and patch. Details regarding the collection of blood chemistries, carotid ultrasound and coronary calcification have been reported previously[22, 31, 32]. Hormone levels were measured by high-sensitivity liquid chromatography/mass spectroscopy at the clinical core laboratory at Mayo Clinic.

Digital tonometry

A commercially available peripheral tonometer was utilized to detect changes in digital pulse volume during reactive hyperemia (EndoPAT, model 2000; Itamar Medical, Ltd., Caesarea, Israel)[8]. Participants were fasting, and had avoided caffeine and tobacco for at least 2 h prior to the test. Tests were conducted between 07.00 and 12.00.

Testing was performed in a quiet room with participants in the supine position and covered with a light blanket. A blood pressure cuff was placed on the non-dominant upper arm while detection probes were placed on the index finger of each hand. Readings in both fingers were obtained for 10 min. The blood pressure cuff in the non-dominant arm was then inflated to 200 mmHg to occlude flow for 5 min, after which the pressure cuff was deflated and recordings continued for an additional 10 min. Only tests which were indicative of good arterial occlusion and test quality were used for analyses.

The RHI was calculated by dedicated software in the system computer as the average amplitude of the peripheral arterial tonometric signal over a 1-min interval beginning within 90 s after cuff deflation divided by the mean amplitude of the 1–3.5-min period before cuff deflation and then normalized to those values of the dominant arm[8]. RHI was determined prior to MHT randomization (baseline) and annually thereafter for 4 years.

Statistical analysis

Linear regression was used to test for time trends in RHI levels both within and across treatment groups, with repeated measures data accounted for via the generalized estimating equations (GEE) method, assuming an exchangeable working correlation structure. This allowed the inclusion of all participants with at least one RHI measure over follow-up for optimal statistical power. Linear regression with the GEE method was also used to test for treatment effects among the repeated measures of CVD-related serum parameters (for this high-sensitivity C-reactive protein (hs-CRP) and triglycerides were log-transformed) and menopausal symptom scores. Within-subject variability of serial RHI was estimated for each participant based on the square root of residual mean squared error as derived from individual linear regression models (a model regressing RHI on time fit separately to the data of each participant) and based on an intra-subject coefficient of variation (CV), which were then compared across treatment groups using one-way analysis of variance (ANOVA). For brevity, we reported only results of treatment comparisons using a 'per protocol' analysis, in which any follow-up data subsequent to treatment drop-out was excluded. However, these analyses were also performed based on an 'intent-to-treat' approach, and the results did not differ appreciably.

Since the analyses described above demonstrated both high variability and no significant time trends in RHI measures, each participant's set of longitudinal data was transformed into one follow-up value using an average, which was also subtracted from their baseline value to express an average change in RHI over follow-up. Using both the average follow-up and average change measures of RHI, Spearman's rank correlation analysis was performed to evaluate the association of RHI response with follow-up values and changes in traditional CVD risk factors, surrogate measures of CVD, menopausal symptom scores, and estrogen levels. Since this particular analysis was not a comparison of treatments, all observed follow-up data were included. All analyses were carried out with the statistical software package SAS, version 9.3 (SAS Institute, Cary, NC, USA). A *p*-value of less than 0.05 was considered statistically significant.

RESULTS

Baseline assessment

At baseline, 95 women participating in the KEEPS trial at the Mayo Clinic underwent RHI measurement and, of these, 83 participants had at least one follow-up measurement. As per KEEPS inclusion criteria, CVD risk factors placed these women in a low-risk category (Framingham risk score [10-year risk, %]: median = 10 [1%], range = 5–15 [< 1–3%]) for development of a heart attack or coronary disease (Tables 1 and 2). At baseline, RHI averaged 2.39 ($\pm$ 0.69, $n = 83$) and did not differ among treatment assignments ($p = 0.63$; Table 2). At baseline, the percentage of women with RHI values > 2.0 (high), 1.7–2.0 (intermediate) or < 1.7 (low) were 69%, 16% and 15%, respectively.

Longitudinal assessment

Hormone levels

Serum levels of estrone and 17β-estradiol measured at the last follow-up visit were significantly higher in participants on treatment than placebo ($p < 0.001$ for each) and, among those on treatment, estrone was higher in the oCEE group while 17β-estradiol was higher in the tE2 group (Table 2). Levels of sex hormone binding globulin were higher only in the oCEE group ($p < 0.001$) compared to placebo, while testosterone levels did not vary significantly by group ($p = 0.65$).

Table 1 Baseline characteristics of women ($n = 95$) participating in the study of reactive hyperemia index (RHI). Data are shown as mean $\pm$ standard deviation or n (%)

Baseline characteristic	Total
Age (years)	53.1 $\pm$ 2.3
Menopausal age (months, at randomization)	19.2 $\pm$ 9.1
Smoking status	
Never	67 (71%)
Current	4 (4%)
Past	24 (25%)
Hypertension medications (any)	4 (4%)
Beta blocker	1 (1%)
Diuretic	1 (1%)
ACE inhibitor	2 (2%)

ACE, angiotensin converting enzyme

Table 2 Reactive hyperemia index (RHI) and cardiovascular risk factors of 83 participants with an RHI measured at baseline and at least one follow-up time point. Data are given as mean ± standard deviation or median (IQR) reported due to heavily skewed distribution

Variable	Placebo (n = 33)		tE2 (n = 24)		oCEE (n = 26)	
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
RHI*	2.49 ± 0.76	2.37 ± 0.37	2.36 ± 0.68	2.26 ± 0.45	2.32 ± 0.70	2.26 ± 0.48
		n = 29		n = 19–20		n = 24
Conventional risk factors						
Body mass index (kg/m²)	27.3 ± 4.1	28.1 ± 4.6	26.2 ± 4.1	26.0 ± 4.5	27.4 ± 4.5	28.0 ± 4.8
Waist circumference (cm)	84.8 ± 12.8	91.1 ± 12.1	84.3 ± 12.2	85.8 ± 11.9	84.4 ± 12.1	88.5 ± 11.6
Total cholesterol (mg/dl)	198.4 ± 30.0	218.1 ± 27.3	206.1 ± 30.3	207.2 ± 33.2	205.3 ± 29.4	218.3 ± 32.6
HDL cholesterol (mg/dl)	67.7 ± 11.3	71.2 ± 10.7	68.8 ± 10.0	73.0 ± 12.3	74.0 ± 17.0	79.0 ± 17.3
LDL cholesterol (mg/dl)	114.9 ± 25.7	117.2 ± 26.9	117.9 ± 30.6	105.2 ± 27.4	117.0 ± 24.0	104.8 ± 30.3
Triglycerides (mg/dl)	68.0 (47.0, 116.0)	86.0 (57.0, 97.0)	79.5 (68.0, 108.0)	81.5 (55.0, 102.5)	69.5 (51.0, 100.0)	102.0 (80.0, 127.0)**
Fasting blood sugar (mg/dl)	80.0 ± 7.1	80.9 ± 6.5	80.8 ± 14.4	79.7 ± 7.0	78.6 ± 9.1	81.8 ± 9.9
Average systolic BP (mmHg)	122.9 ± 13.1	120.0 ± 14.8	122.1 ± 17.0	121.9 ± 15.1	125.1 ± 15.1	123.9 ± 11.8
C-reactive protein (pg/ml)	1.49 (0.47, 3.74)	1.20 (0.35, 4.16)	1.75 (0.69, 2.83)	1.53 (0.68, 3.84)	0.93 (0.35, 3.95)	3.50 (1.32, 6.03)††
Surrogate measures of CVD		n = 29		n = 18/20		n = 23/24
CAC (Agatston score)	1.9 ± 6.6	3.9 ± 13.4	1.1 ± 3.5	3.6 ± 10.1	1.5 ± 6.3	3.6 ± 9.3
CIMT (mm)	0.66 (0.64, 0.71)	0.70 (0.64, 0.73)	0.66 (0.61, 0.76)	0.71 (0.66, 0.78)	0.64 (0.61, 0.73)	0.69 (0.62, 0.77)
Menopausal symptom scores†		n = 32		n = 24		n = 26
Insomnia	1.0 ± 0.8	0.4 ± 0.5	0.9 ± 0.9	0.2 ± 0.3	1.2 ± 0.8	0.2 ± 0.4
Hot flushes	1.3 ± 0.7	0.7 ± 0.7	1.3 ± 0.7	0.1 ± 0.2	1.6 ± 0.8	0.2 ± 0.3
Night sweats	1.0 ± 0.8	0.5 ± 0.5	1.1 ± 1.0	0.1 ± 0.2	1.5 ± 0.8	0.2 ± 0.3
Palpitations	0.5 ± 0.6	0.0 ± 0.1	0.4 ± 0.6	0.1 ± 0.1	0.3 ± 0.5	0.0 ± 0.1
Aggregate score	3.8 ± 1.9	1.6 ± 1.3	3.8 ± 2.4	0.5 ± 0.7	4.6 ± 2.1	0.7 ± 0.9
Hormone levels‡		n = 26		n = 20		n = 24
Estrone (pg/ml)	–	19.4 ± 7.0	–	35.9 ± 15.7	–	62.5 ± 37.1
17β-estradiol (pg/ml)	–	5.9 ± 2.3	–	36.1 ± 30.7	–	12.2 ± 6.6
SHBG (nmol/dl)	–	51.0 ± 26.7	–	55.5 ± 24.3	–	99.3 ± 47.2
Testosterone (ng/dl)	–	23.9 ± 17.0	–	21.3 ± 6.5	–	24.8 ± 11.8

tE2, transdermal 17β-estradiol; oCEE, oral conjugated equine estrogen; CAC, coronary artery calcification; CIMT, carotid intima-medial thickness; SHBG, sex hormone binding globulin; HDL, high density lipoprotein; LDL, low density lipoprotein; BP, blood pressure; CVD, cardiovascular disease;
*, Follow-up RHI represented by average across all follow-up measures of RHI for each subject; †, follow-up severity based on average across all follow-up scores for each menopausal symptom; ‡, hormone levels not available at baseline; follow-up values represented by the end-of-study measures in the majority of participants as available, otherwise levels at the 3-year visit were used; **, changes over follow-up are significantly different from Transdermal group, $p < 0.001$; ††, changes over follow-up are significantly different from Transdermal and Placebo groups, $p = 0.011$ and $p < 0.001$, respectively

Blood chemistries

Of the CVD risk factors assessed, changes in serum levels of triglycerides were significantly higher in the oCEE compared to the tE2 group ($p < 0.001$) but not compared to the placebo group ($p = 0.227$). Changes in hs-CRP in the oCEE group were significantly greater than in both the tE2 and placebo groups over follow-up ($p = 0.011$ and $p < 0.001$, respectively; Table 2).

RHI measurements

There was no significant change in RHI over the 4-year follow-up period within any of the treatment groups or in the full combined set of participants. The variability in longitudinal RHI measurements within participants was high in each treatment group (within-subject standard deviations and CV%, averaged: 0.41 and 18.3% in the tE2 group, 0.49 and 20.5% in the oCEE group, and 0.50 and 20.5% in the placebo group) and did not differ significantly across groups (Figure 1, $p = 0.31$).

Percentages of women with RHI values in the high (> 2.0), intermediate ($1.7 < 2.0$) and low ranges (< 1.7) are displayed in Figure 2 across study visits and by treatment group. A total of 41 (43%) women had at least one low (< 1.7) reading over the study period.

The time trend of these RHI categories did not differ significantly across the three treatment groups, neither with time defined on a linear scale ($p = 0.075$) or as a contrast between baseline and all follow-up levels pooled together ($p = 0.74$). Likewise, when analyzing serially measured RHI as values instead of categories, the response over time was not significantly different across treatment groups (linear time, $p = 0.56$; contrast of baseline vs. follow-up time, $p = 0.90$; Table 2 and Figure 3).

Based on a linear GEE model testing for a significant change in RHI with the three treatment groups pooled together, the standard error of the coefficient for time, defined as a contrast of all follow-up measures versus baseline value, was 0.0682. In terms of a longitudinal effect, this implies that a change in RHI of $2.8 \times 0.0682 = 0.19$ units (i.e. a 0.19 unit shift between baseline and pooled follow-up values) is detectable with 80% power. Alternatively, using a more efficient analysis that assumes the change in RHI over time follows a linear trend, a separate model was fitted with a linear effect for time (years from baseline visit). Based on a standard error of 0.0184, the detectable difference is $2.8 \times 0.0184 \times 1 = 0.10$
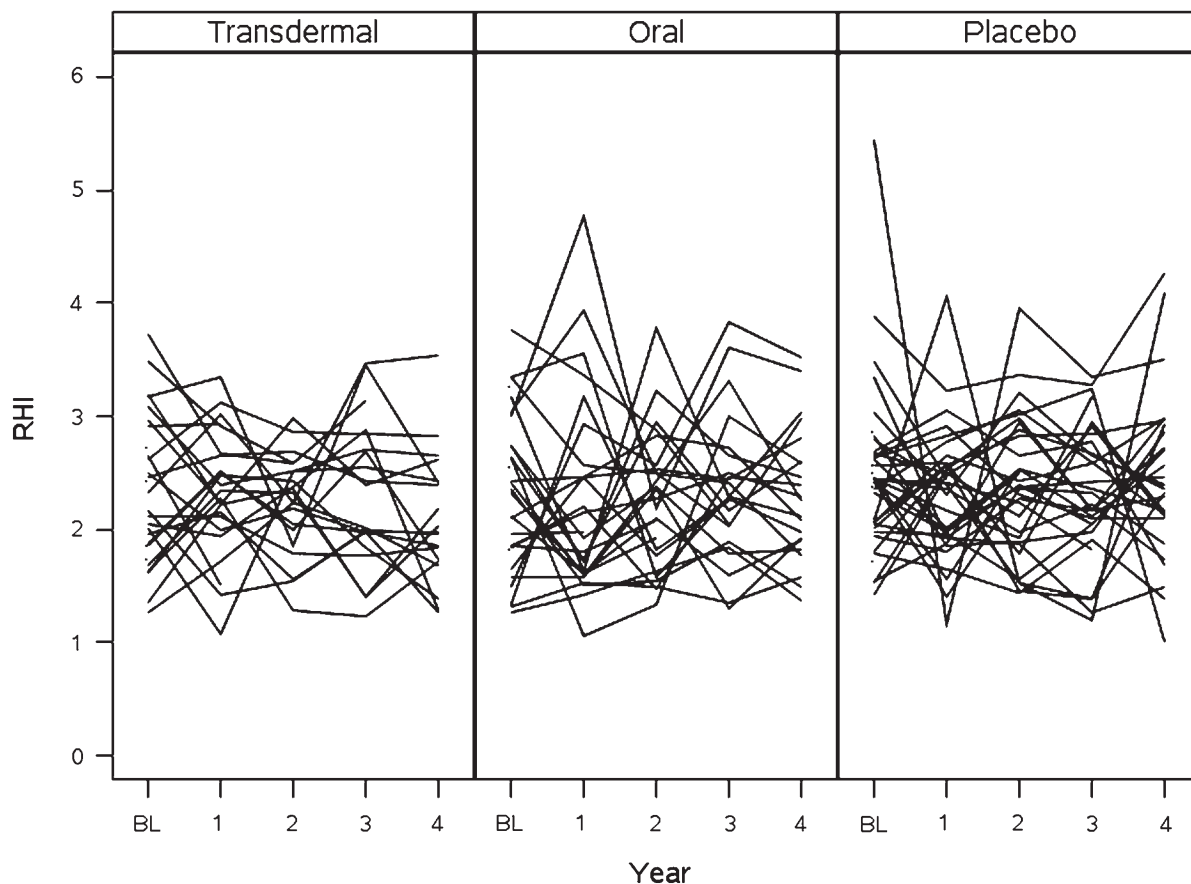

Figure 1   Representation of actual reactive hyperemia index (RHI) values over time by treatment group. Each line represents an individual. BL, baseline
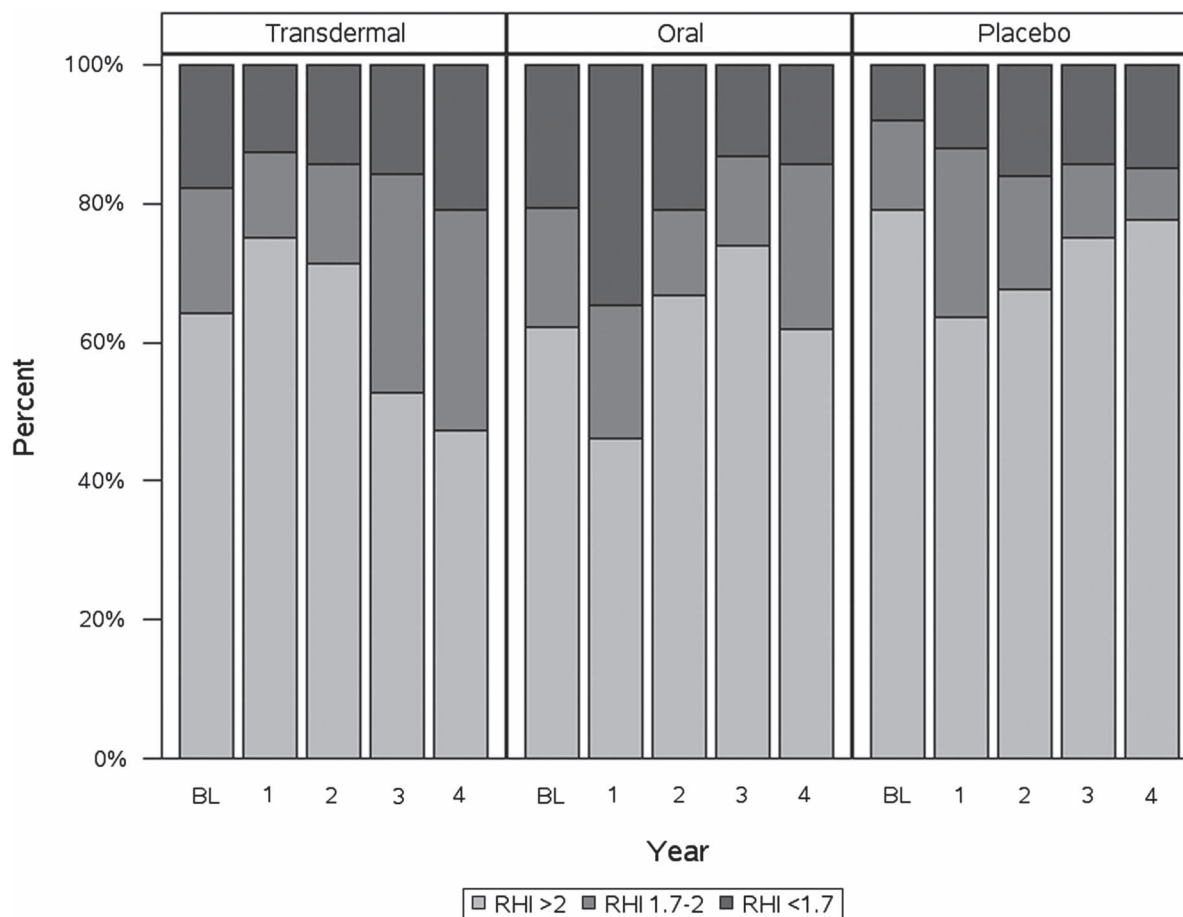
Figure 2 Percentage of women with reactive hyperemia index (RHI) values in the high (RHI > 2), low (RHI < 1.7) and intermediate (RHI 1.7–2) ranges by treatment group over the 4 years of study. Transdermal, $n = 24$; Oral, $n = 26$; Placebo, $n = 33$. BL, baseline

units per 1 follow-up year, or 0.13 units per 2.5 years for a more comparable estimate to the original approach (since the average time among the 1-, 2-, 3- and 4-year follow-up visits is 2.5 years).

Menopausal symptom scores

Changes in the aggregate menopausal symptom scores were different among groups ($p = 0.004$). Reductions in self-reported severity of hot flushes ($p < 0.001$) and night sweats ($p = 0.002$) were significantly greater in the two treatment groups compared to the placebo group (Figure 4).

RHI measurement correlations

Consistent with the finding of no significant change in RHI over time, changes in RHI (averaged over follow-up) did not significantly correlate with changes in CVD risk factors nor with end-of-study estrogen levels (Table 3). However, there was a nominally significant inverse correlation between the change in RHI values and the change in CIMT relative to

baseline (Spearman correlation coefficient $\rho = -0.268$, $p = 0.012$; Figure 5). There was no correlation with change in RHI values with changes in CAC scores. However, the change in average severity of night sweats (relative to baseline) had a nominally significant inverse correlation with change in average RHI (Spearman $\rho = -0.252$, $p = 0.016$; Figure 6).

DISCUSSION

These observations are the first to provide longitudinal assessment of endothelial function using the non-invasive digital tonometry technique in recently menopausal women with a low-risk cardiovascular profile and the impact of MHT on those measurements. There are several important conclusions from this study. First, using this technique and contrary to the hypothesis, it was not possible to detect an effect of MHT on endothelial function by RHI. This neutral outcome could reflect two possibilities: (1) efficacy of treatment or (2) the sensitivity of the measurement. First, functional efficacy of treatment is confirmed by increases in
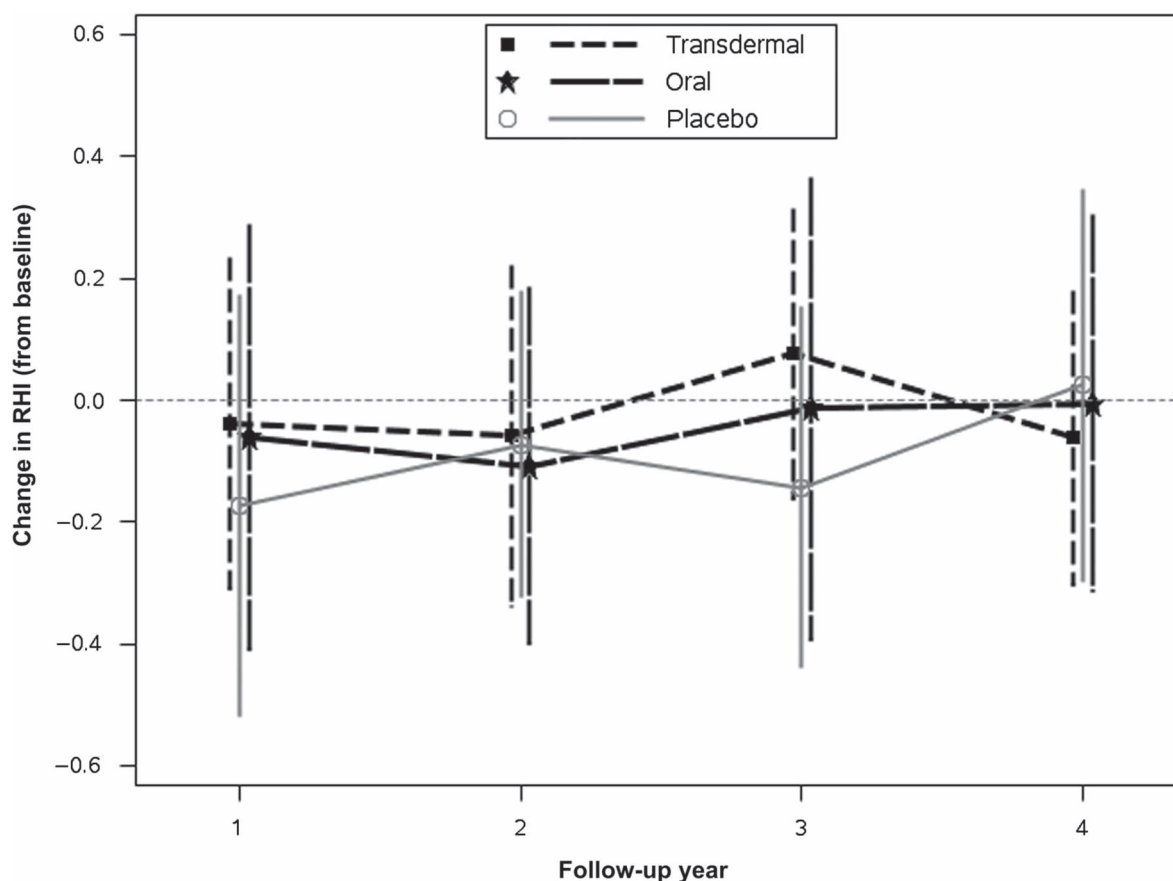
Figure 3 Change in reactive hyperemia index (RHI) values from baseline in each treatment group over the course of the study. In order to reduce the effect of variability, an average RHI value was calculated for each woman for those years for which there were follow-up measurements and those were averaged for each year. Data are shown as the mean ± standard deviation for the change within each time point by treatment group

serum levels of both estrone and 17β-estradiol in the MHT groups, maintenance of bone density[33] and relief of menopausal symptoms, especially vasomotor symptoms (hot flushes) and night sweats (Figure 4). However, the threshold for treatment effects on some endothelial functions may not have been reached. Serum levels of 17β-estradiol in participants of the present study averaged 36 pg/ml, whereas, in studies measuring endothelial function by reactive hyperemia by ultrasound[34] and changes in serum levels of nitric oxide[35], serum 17β-estradiol averaged 144 pg/ml and 76 pg/ml, respectively. Although serum levels of hormone may not reflect the concentrations at the tissue level where conversion of estrone to 17β-estradiol may occur, the possibility remains that the doses of hormone used in KEEPS were below the threshold to sustain some endothelial function postmenopause. Another possibility is that progesterone may counter effects of estrogen on endothelial function[36], but this seems unlikely as only measurements at 36 months were made during the active progesterone treatment and these values were not different from those obtained during the estrogen-only phase of treatment[37].

The second possibility is that the study may have been biased against observing effects of MHT on endothelial function due to the variability in the RHI method. Variability in RHI was unexpectedly high and serves as a caution in the utility of digital tonometry to detect subtle changes in risk factor profile or treatment effects as would be needed to add value to existing cardiovascular risk algorithms. Changes in blood flow to the finger as a measure of arterial endothelial function may be less sensitive than measurement of changes of brachial artery diameter by ultrasound due to arterial–venous anastomoses in the finger and the influence of adrenergic neurotransmission on vasomotor tone in the digits[10]. Indeed, the inverse association between changes in reported night sweats and changes in RHI scores suggests that measurement of reactive hyperemia by digital tonometry may reflect, to some extent, contributions of the autonomic nervous system[10], which may also account for the variability among groups. In addition, accumulation of metabolites may follow the 5 minutes of occlusion of the brachial artery as needed for the operation of the machine. These metabolites will also modulate local vasomotor tone that would alter the response to changes in shear stress of
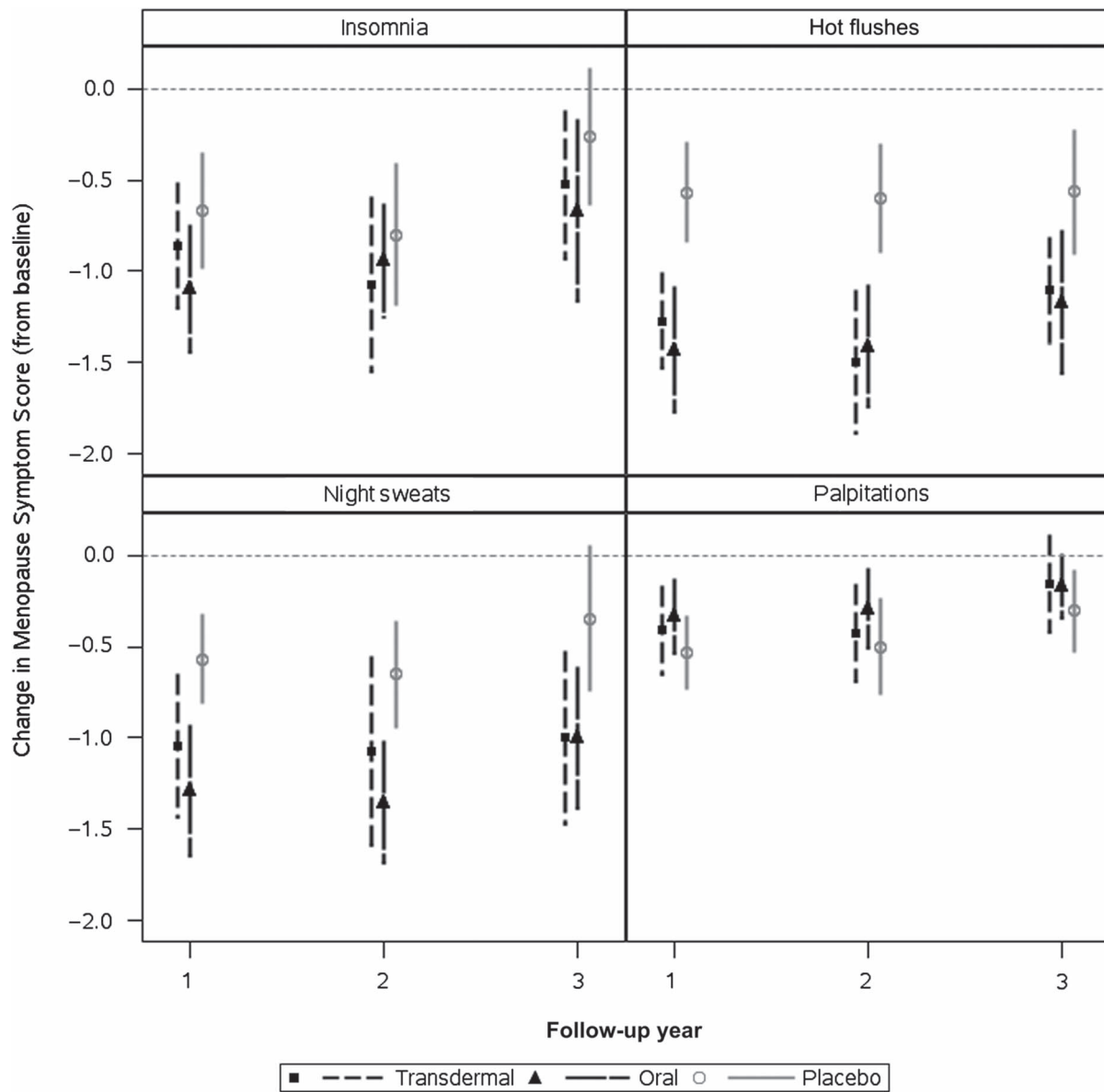
Figure 4 Changes in menopausal symptom scores by self-report. Decreases in scores represent decrease in severity of symptoms. Data are shown as mean $\pm$ standard deviation of change in symptom score at each time point from baseline. $n = 33$, 24, and 26 for placebo, transdermal and oral treatments, respectively

the blood across the endothelial surface once blood flow is restored (for review, see reference 38).

Although two studies comparing reactive hyperemia by brachial artery ultrasound to digital arterial tonometry found a correlation between the two measurements[39, 40], both studies were biased toward males with coronary artery disease and included participants using medication for heart disease. Thus, a single measure of RHI may provide value in identifying symptomatic, non-occlusive coronary disease but not for discerning early disease processes.

However, longitudinal changes in RHI may reflect some functional alterations within the vascular wall as decreases in

RHI, independent of treatment, correlated with increases in CIMT (Figure 5), suggesting a functional relationship between endothelial (vascular) responsiveness to occlusive hyperemia and vascular remodeling. While the magnitude of this correlation was fairly weak and the level of significance unadjusted for multiple testing, an *ad hoc* analysis revealed a significant reduction in RHI within a portion of these women deemed to have appreciable objective atherosclerotic change. In particular, choosing a margin of ≥ 0.05 mm as signifying a 'real' increase in CIMT based on the range of negative changes in Figure 2 (a crude approximation of the measurement error since this measure should not decrease over time), this

Table 3 Reactive hyperemia index (RHI) correlation analyses with cardiovascular risk factor parameters and hormone levels

		Spearman ρ*	
Variable	n	Follow-up RHI[†]	Change in RHI[†]
Conventional risk factors			
Body mass index (kg/m²)	76	−0.021	−0.117
Waist circumference (cm)	74	−0.040	−0.132
Total cholesterol (mg/dl)	88	−0.025	0.087
HDL cholesterol (mg/dl)	88	0.001	0.129
LDL cholesterol (mg/dl)	88	0.057	−0.032
Triglycerides (mg/dl)	88	−0.059	−0.007
Fasting blood sugar (mg/dl)	88	0.125	0.044
Average systolic BP (mmHg)	76	0.149	−0.133
C-reactive protein (pg/ml)	88	−0.076	0.035
Surrogate measures of CVD			
CAC (Agatston score)	84	−0.072	−0.148
CIMT (mm)	87	0.046	−0.268**
Menopausal symptom scores[‡]			
Insomnia	91	0.095	0.001
Hot flushes	91	0.109	0.020
Night sweats	91	0.156	−0.252**
Palpations	91	0.185	0.109
Global score	91	0.188	−0.016
Hormone levels[††]			
Estrone (pg/ml)	85	−0.105	
17β-estradiol (pg/ml)	85	−0.100	
SHBG (nmol/dl)	85	−0.017	
Testosterone (ng/dl)	85	0.050	

HDL, high density lipoprotein; LDL, low density lipoprotein; BP, blood pressure; CVD, cardiovascular disease; CAC, coronary artery calcification; CIMT, carotid intima-medial thickness; SHBG, sex hormone binding globulin
[*], Results in table measure the correlation between follow-up values of RHI and follow-up values of the corresponding variable, or between changes in RHI over follow-up and changes in the corresponding variable over follow-up; [†], follow-up RHI represented by average across all follow-up measures of RHI for each participant, with change in RHI the average difference between follow-up measures and baseline value; [‡], follow-up severity based on average across all follow-up scores for each menopausal symptom; [††], hormone levels not available at baseline; follow-up values represented by the end-of-study measures in the majority of participants as available, otherwise levels at the 3-year visit were used; [**], correlation was significant at the 0.05 level

subgroup ($n = 29$) showed an average reduction in RHI of 0.24 units ($p = 0.011$). Therefore, a functional relationship between endothelial function and arterial remodeling may exist and changes in RHI may become larger at time points greater than 4 years past menopause with subsequent influences of aging[7]. Additional follow-up of women as they age is needed to address this important question.
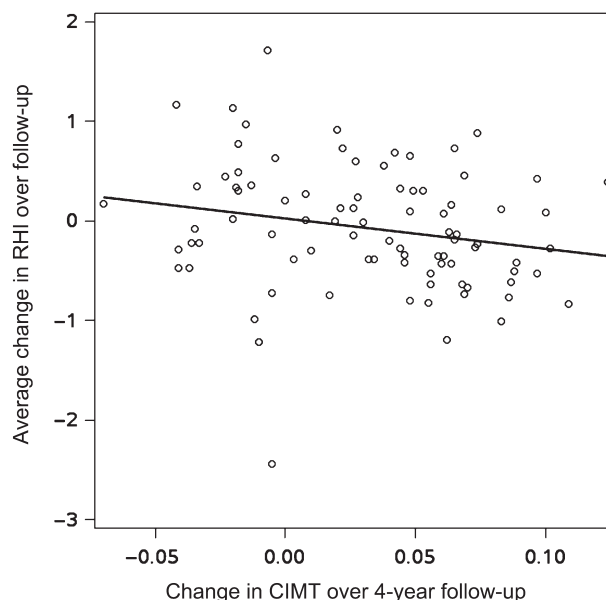

Figure 5 Changes in carotid intima-medial thickness (CIMT) over 4-year follow-up by average change in reactive hyperemia index (RHI). Each point represents an individual

Limitations

RHI values were found to demonstrate high variability within each group over time, an observation corroborated in other studies[10]. This variability may reflect both the volatile and changing endothelial and autonomic function in women transitioning through menopause as well as vasodilatory effects
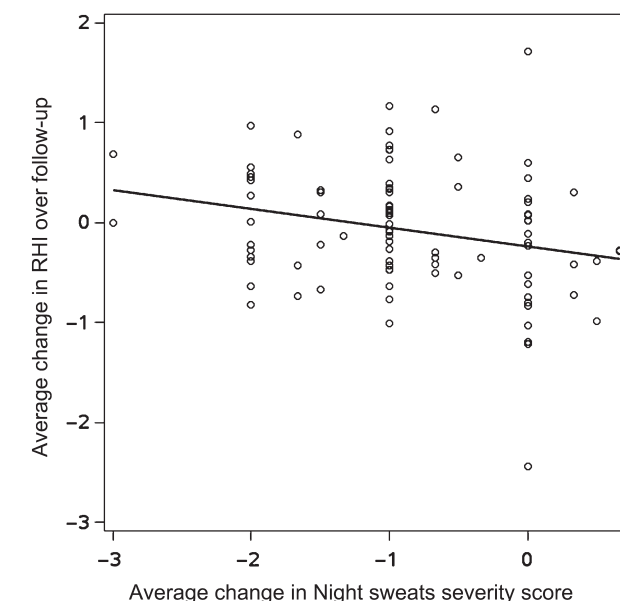

Figure 6 Change in night sweats severity score over follow-up by average change in reactive hyperemia index (RHI). Each point represents an individual

of local metabolites which accumulate during the cuff occlusion. Alternatively, it may reflect the inherent variability of the test itself. RHI variability may partially be explained by differences in brachial artery diameter as a negative association has been described between brachial artery diameter and RHI. Accounting for brachial artery diameter eliminated gender differences in RHI measurements[41]. Additionally, the study was not powered to demonstrate hormonal effects on endothelial function by this test based on the observed variability which was similar among groups.

CONCLUSION

This study is the first to provide longitudinal data on digital tonometry and its variability among recently menopausal women who are healthy and asymptomatic of cardiovascular disease and who are using two different formulations and delivery modalities of MHT. These MHT regimes and dosages are relevant to current clinical practice and did not adversely affect endothelial function, as measured by digital tonometry, in healthy, recently menopausal women. These observations provide further support to the North American Menopause Society and the Global Consensus Statement on Menopausal Hormone Therapy recommendation to utilize MHT in early symptomatic postmenopausal women. Our observations do not provide any evidence of a role for MHT in primary or secondary prevention of cardiovascular disease. RHI did not show significant association with other markers of cardiovascular disease, suggesting that RHI may not add value in cardiovascular screening for women with low cardiovascular risk profiles.

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