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Changes in carotid artery intima-media thickness 3 years after cessation of menopausal hormone therapy: follow-up from the Kronos Early Estrogen Prevention Study

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Abstract

Objective: Little is known regarding the progression of preclinical atherosclerosis upon cessation of menopausal hormone therapy (MHT). This study evaluated changes in carotid artery intima-media thickness (CIMT) in a subgroup of participants during 4 years and 3 years after the Kronos Early Estrogen Prevention Study (KEEPS).

Methods: Of the women enrolled in KEEPS at Mayo Clinic ($n = 118$), a subset ($n = 76$) agreed to participate in this follow-up study. KEEPS MHT assignments were placebo (PBO), $n = 33$; transdermal 17β-estradiol (tE_2), $n = 23$; and oral conjugated equine estrogens group (oCEE), $n = 20$. CIMT was measured by B-mode ultrasonography. Longitudinal analysis of CIMT was performed using all available data from pre-, on-, and post-treatment periods.

Results: At 7 years, median age of participants was 60.2 years; median time since menopause was 8.5 years. The mean difference in rates of increase was significantly greater over the post- than on-treatment period within the oCEE group (0.010 [0.002-0.017] mm/y), but not within the PBO (0.006 [−0.001 to 0.012] mm/y; $P = 0.072$) or tE_2 (0.002 [−0.005 to 0.010] mm/y; $P = 0.312$) groups. There were, however, no significant treatment differences in the linear trends over those intervals ($P = 0.524$).

Conclusions: Cessation of MHT at the lower doses and formulations used in KEEPS did not appear to alter the trajectory of CIMT over a 3-year follow-up period. CIMT, however, increased in all groups over the entire 7-year timeframe as expected with age and timing of menopause possibly key contributors.

Key Words: 17β-estradiol – Aging – Atherosclerosis – Conjugated equine estrogen – Metabolic Syndrome.

Menopausal hormone therapy (MHT) is recommended for recently postmenopausal women experiencing hot flushes, night sweats, mood swings, and vaginal dryness. Whether use of MHT for these conditions slows progression of cardiovascular disease seems to be influenced by the general health of the woman, especially the presence of hyperlipidemia,[1] the timing of initiation of the treatments,[2] the formulation of the products including mode of delivery and dose,[3,4] and genetic polymorphisms associated with genes of the immune system.[5,6]

The Kronos Early Estrogen Prevention Study (KEEPS) was a multicenter, randomized, double-blinded, placebo controlled trial designed to compare the effects of lower doses of either oral conjugated equine estrogens (oCEE) or transdermal 17β-estradiol (tE_2) on the progression of preclinical atherosclerosis in women who were within 3 years of menopause.[7] After 4 years of therapy, there were no significant differences in carotid artery intima-media thickness (CIMT), a measure of preclinical atherosclerosis, among the oral, transdermal, or placebo-treated groups.[8] It is, however,

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unknown whether use of MHT during the early years of menopause would affect the trajectory for the development of atherosclerosis upon cessation of their use. Therefore, the aim of this study was to evaluate CIMT changes in a subset of KEEPS participants 3 years after cessation of the trial.

METHODS

Participants

All participants had completed the KEEPS trial (NCT00154180) at Mayo Clinic, Rochester, MN. Detailed inclusion and exclusion criteria for KEEPS have been reported.[7] In brief, women were between 42 to 59 years of age, within 5 to 36 months past their last menses, and were in good cardiovascular health at the time of enrollment, in particular, they did not have uncontrolled hypertension, hyperlipidemia, nor diabetes, and coronary arterial calcification >50 Agatston Units. Participants were randomized to either (1) oral CEE (Premarin, 0.45 mg/d); (2) tE_2 (Climara, 50 μg/d skin patch); or (3) placebo pills and patch (PBO). Progesterone was given orally (Prometrium; micronized progesterone, 200 mg/d) for 12 days each month to both active treatment groups. Participants were treated for 4 years and for the present study were evaluated 3 years after their exit date from KEEPS. At Mayo Clinic, 118 women qualified for the KEEPS. All of the KEEPS participants were contacted for the follow-up study. As this was a follow-up study, it was not possible to determine the reasons for a former participant to decline participation in this follow-up study. Those contacted either sent back a form marked as ''decline to participate,'' did not respond to mail or phone contact, or had changes of address with no forwarding information. Of the 118 women enrolled in KEEPS at Mayo Clinic, 76 gave written informed consent to participate in the present study which was approved by the Mayo Clinic Institutional Review Board.

Clinical assessments

All participants underwent a brief medical examination which included body morphometrics, fasting blood clinical chemistries, and evaluation of CIMT by B-mode ultra sonography as described previously.[7,9,10] CIMT was measured before randomization (baseline), annually at 1, 2, 3, and 4 years during the study, and at 3 years after cessation of study treatment.[7] All ultrasound images were read by the same observer who was blinded to the previous MHT assignment during KEEPS. Mean coefficient of variation of measures of CIMT at baseline ranged from 0.0% to 7.7%. Plasma hormone levels were measured at the clinical core laboratories at Mayo Clinic by high-sensitivity liquid chromatography/mass spectroscopy.

Statistical analysis

Demographic and clinical data were described with quartiles (median [50th percentile], lower quartile [25th percentile], and upper quartile [75th percentile]), or with absolute numbers and percentages. CIMT values are presented as mean and SD (or as 95% CI of the mean change). To describe longitudinal trends, data were presented in tabular form at baseline (pretreatment visit), at 4 years (end-of-treatment visit), and at 7 years (3-y post-treatment visit). As CIMT was measured repeatedly during treatment, a 4-year estimate informed by all the measurements made up until that time point was calculated by assuming a linear time trend. Additional details of the longitudinal analysis of CIMT are provided below. For variables other than CIMT, within-person change from baseline to 4 years (on-treatment period), from 4 to 7 years (post-treatment period), and from baseline to 7 years (extended follow-up period) were tested for significance within treatment groups using the Wilcoxon signed rank test. For between-group comparisons, baseline differences were assessed with Kruskal–Wallis tests, whereas the baseline-adjusted difference in changes at the two follow-up times was assessed with analysis of covariance (ANCOVA) models. To ensure adequate comparisons of on-treatment and post-treatment trends within the same study population, only participants from the original KEEPS trial who completed the 7-year follow-up visit were included in the analysis.

Primary analysis for the comparison of serial CIMT measurements across treatment groups was based on two-stage analysis, in which repeated measures on the same patient were reduced to a single measure of linear trend. This analysis was performed by regressing CIMT values against measurement times in each individual person and using each person's predicted response at the last time point. These predicted values were then compared across treatment groups using an analysis of covariance (ANCOVA) model that adjusted for baseline-measured CIMT. Estimated absolute increase in CIMT over time was computed as the difference between the predicted response and baseline measurement; annual increase in CIMT was calculated as the absolute increase divided by total number of years. For comparison, a sensitivity analysis was performed with use of generalized linear modeling to include all repeated measurements of CIMT, with the correlation pattern over time within person taken into account using a continuous autoregressive correlation structure. The model included the raw follow-up measurements of CIMT as the responses, and baseline CIMT, treatment group, study time (as a linear continuous variable), and the interaction between treatment group and time as independent variables. Difference of treatments from this sensitivity analysis, as indicated by the group by time interaction, was checked for consistency with findings of the primary two-stage analysis. In both analyses, separate models were fitted for each of the three intervals of interest, with linear contrasts constructed to estimate the mean within-person change in CIMT at 4 and 7 years, as well as the baseline-adjusted difference in changes at these times between treatment groups. We also examined the adequacy of a 2-slope repeated measures model that could delineate on- and post-treatment trends and facilitate testing of treatment contrasts at both time points in one unified analysis.

As an exploratory analysis to address the question whether the rate of change of CIMT depends on age (or time), we

tested for within-person curvilinear change in CIMT over the 7-year study period by refitting each woman's CIMT trajectory using a quadratic function of time. We then computed the proportion of women for whom a quadratic time effect was significant at the $\alpha = 0.05$ level, and among those, compared the frequency of a positive versus negative coefficient of time^2 to indicate the tendency of quadratic increases or decreases in CIMT. Under the null hypothesis, we would expect by chance a low number of participants (5%) showing evidence of curvature, and of those equal proportions with positive (accelerating) and negative (decaying) curvature. A formal statistical test was performed using the binomial test, both for the abundance of significant quadratic coefficients, and of the equality between positive and negative coefficients.

To assess the sensitivity of the analysis to selection bias in women who agreed to participate in the present study, we repeated the longitudinal analysis of on-treatment trends using the entire KEEPS population at the Mayo site with available CIMT data. As an additional analysis, comparisons of KEEPS women who did and did not participate in the present study were examined on the basis of baseline and end-of-trial measures that may have influenced the selection of participants. All data analyses were conducted with SAS statistical software, version 9.4 (SAS Institute, Cary, NC).

RESULTS

The 76 women from the KEEPS trial at the Mayo Clinic who participated in the 3-year post-treatment follow-up visit were included in the analysis. All participants were white and all were nonsmokers. Median age of the women at baseline visit was 53.3 years, with a median time since menopause of 1.6 years; at the final follow-up visit 3 years after completion of KEEPS, the median age of participants was 60.2 years, whereas the median time past menopause was 8.5 years (Table 1). Neither of these demographics nor any of the clinical parameters differed significantly among treatment groups at baseline. At the end of treatment, triglycerides and hs-CRP levels increased more in the oCEE group compared with the other groups. A decrease in HDL cholesterol was observed among the groups after 4 years of treatment, but at 3 years after trial completion, HDL returned to pretreatment levels. Conversely, baseline LDL values were sustained at 4 years but then decreased after treatment cessation. Serum levels of sex steroids differed among groups at 4 years, with higher levels of E_2 in women randomized to the tE_2; after treatment cessation, the values decreased to baseline levels at year 7 (Table 2). After the trial, some women elected to use MHT, but the percentages of women self-selecting to use MHT did not differ by prior treatment assignment (Table 3), and only a smaller number of these woman reported use of MHT at the time of the 7-year examination. Use of other medications that may have impacted progression of CIMT varied among previous treatment groups and may be confounders (Table 3): use of antihypertensive medication was greater in the previous PBO group; whereas the use of lipid lowering medications trended to be higher in the previous treated groups compared to PBO. Compared with 36 women in the KEEPS trial who did not contribute to the present study, these 76 participants represented a higher percentage of placebo-allocated women, and had lower systolic blood pressure and higher HDL cholesterol at end-of-trial (Supplemental Table 1, http://links.lww.com/MENO/A345).

Although CIMT increased in the PBO and tE_2 groups and did not change significantly in the oCEE group after 4 years of treatment (Table 4), no significant difference in the linear trend across treatments was observed ($P = 0.067$). All three groups had similar increases over the 3 years after treatment cessation (Fig. 1), and there were no significant differences in linear change from 4 to 7 years or from baseline to 7 years among groups (Table 4). When expressed as percent change from baseline CIMT, the average increase across groups was 4.1% at year 4 and 9.1% at year 7, with a 5.4% increase from year 4 to 7. Compared with the on-treatment period, the rate of increase during the post-treatment period was greater both for the overall group (mean [95% CI] difference, 0.006 [0.002-0.009] mm/y; $P = 0.002$) and those treated with oCEE (0.010 [0.002-0.017] mm/y; $P = 0.014$). There were no significant differences in annual CIMT increases by period (post- vs. on-treatment) in the PBO (0.006 [−0.001 to 0.012] mm/y; $P = 0.072$) or tE_2 (0.002 [−0.005 to 0.010] mm/y; $P = 0.312$) groups. The sensitivity analysis performed using repeated measures modeling yielded similar results (data not shown), and a model for 7-year trends allowing 2 time-varying slopes resulted in the same conclusions. An additional sensitivity analysis with inclusion of all 113 women in the KEEPS trial (at the Mayo site) for whom CIMT data was available, yielded comparable results except a larger 4-year increase was noted in the oCEE group (Supplemental Table 2, http://links.lww.com/MENO/A345 and Figure 1, http://links.lww.com/MENO/A345).

In a multivariable model, neither age ($P = 0.39$) nor menopausal age ($P = 0.13$) at the time of randomization was associated with the rate of increase in CIMT. Additional analyses were performed to assess whether change in CIMT correlated with change in metabolic syndrome components but these also revealed no significant associations.

To explore the possibility of a nonconstant rate of change in CIMT, the first step of the primary two-stage analysis was repeated by fitting a quadratic function to time to estimate each participant's time-response curve. A significant quadratic trend was detected in 25 (33.8%) of the 74 participants with at least 4 CIMT measurements, which well exceeds the number expected by chance under the null hypothesis (5% or ~4; binomial test $P < 0.001$). Furthermore, in this subset with a significant quadratic term, the regression coefficient was positive in 20 (80%) participants, indicating progressively increasing CIMT values over time. That the pattern of positive curvature was significantly more likely than a quadratic trend of negative curvature (binomial test $P = 0.003$) is further evidence of an accelerated pattern of change in these women over the study timeframe (Fig. 2).

TABLE 1. *Demographic and clinical characteristics of KEEPS participants at baseline, end-of-trial (year 4), and post-trial (year 7) study visits*

Characteristic	*N*	Placebo ($n = 33$)	Transdermal E_2 ($n = 23$)	Oral CEE ($n = 20$)
Age, y				
BL visit	76	53 (52, 54)	53 (52, 55)	54 (52, 55)
4-y visit	76	57 (56, 58)	57 (56, 59)	58 (56, 59)
7-y visit	76	60 (59, 61)	60 (59, 61)	61 (59, 62)
Time past menopause, y				
BL visit	76	1.1 (0.9, 1.8)	1.5 (1.2, 2.4)	2.0 (1.5, 2.4)
4-y visit	76	5.1 (4.9, 5.8)	5.5 (5.2, 6.4)	6.0 (5.5, 6.4)
7-y visit	76	8.0 (7.8, 8.9)	8.5 (7.9, 9.4)	9.1 (8.4, 9.4)
Body mass index, kg/m^2				
BL visit	76	26 (25, 30)	26 (22, 31)	27 (25, 32)
4-y visit	72	27 (26, 32)[a]	26 (22, 30)	28 (24, 32)
7-y visit	76	27 (26, 32)[a]	27 (22, 32)[a]	28 (24, 31)
Waist circumference, cm				
BL visit	75	85 (76, 91)	81 (72, 93)	83 (75, 92)
4-y visit	64	90 (84, 99)[a]	84 (73, 93)	86 (84, 94)
7-y visit	76	94 (84, 99)	84 (78, 97)	88 (82, 97)
Systolic blood pressure, mm Hg				
BL visit	76	122 (115, 128)	114 (104, 121)	121 (111, 130)
4-y visit	68	118 (108, 129)	115 (109, 125)	119 (111, 125)
7-y visit	76	128 (116, 137)	127 (114, 133)	123 (115, 138)
Diastolic blood pressure, mm Hg				
BL visit	76	76 (70, 81)	73 (66, 77)	78 (70, 83)
4-y visit	68	75 (66, 82)	71 (68, 78)	75 (73, 80)
7-y visit	76	80 (71, 83)	76 (73, 83)	77 (70, 84)
Total cholesterol, mg/dL				
BL visit	76	217 (200, 232)	223 (202, 248)	201 (187, 231)
4-y visit	69	213 (186, 232)	212 (188, 236)	214 (206, 231)
7-y visit	75	204 (185, 224)[a]	204 (183, 228)	203 (193, 228)
HDL cholesterol, mg/dL				
BL visit	76	58 (50, 65)	64 (53, 72)	60 (51, 72)
4-y visit	69	55 (46, 64)[a]	60 (53, 65)[a]	54 (45, 61)
7-y visit	75	58 (51, 67)[a]	68 (56, 75)[a]	63 (47, 75)
LDL cholesterol, mg/dL				
BL visit	76	132 (99, 148)	137 (106, 162)	128 (118, 162)
4-y visit	69	144 (113, 156)	131 (115, 154)	137 (124, 149)
7-y visit	75	119 (110, 144)[a]	117 (91, 140)[a]	120 (106, 135)
Triglycerides, mg/dL				
BL visit	76	77 (64, 110)	83 (66, 115)	85 (57, 114)
4-y visit[b]	69	99 (72, 110)[a]	94 (65, 112)	115 (86, 145)[a]
7-y visit	75	97 (75, 115)	83 (69, 109)	104 (78, 130)
Fasting blood glucose, mg/dL				
BL visit	76	92 (88, 96)	95 (88, 99)	88 (82, 96)
4-y visit	0	NA	NA	NA
7-y visit	75	94 (90, 101)	93 (89, 100)	94 (91, 100)[a]
hs-CRP, mg/dL				
BL visit	76	1.0 (0.5, 2.3)	1.7 (0.5, 3.4)	1.3 (0.6, 2.9)
4-y visit[b]	75	1.3 (0.4, 3.6)	1.5 (0.5, 3.3)	3.5 (2.0, 7.6)[a]
7-y visit	75	1.4 (0.5, 2.9)	1.4 (0.7, 2.4)	1.9 (0.7, 4.8)[a]

Values presented as median (25th, 75th percentiles); N is the total number of nonmissing measurements.
BL, baseline measurements prior to the initiation of treatment; CEE, conjugated equine estrogens group; E_2, 17b-estradiol; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein.
[a]$P < 0.05$ from Wilcoxon signed-rank test for assessment of within-person change by treatment group, indicating a significant difference in the visit measurement compared to the preceding visit.
[b]$P < 0.05$ from ANCOVA for assessment of between-group difference in the change from baseline, indicating that at least 2 of the 3 groups differed in regards to the visit measurement after controlling for the baseline measurement.

DISCUSSION

The results of this study suggest that there is not a rebound effect on progression of CIMT after cessation of lower doses of either oCEE or tE_2 for 4 years. These results might be expected given that the hormonal treatments would exert activational effects (ie, reversible effects) on cells of the vascular wall (endothelium, vascular smooth muscle, and adventitial fibroblasts) as well as circulating blood elements (platelets and leukocytes) all of which are implicated in development of vascular lesions.[11] The temporal relationships among the various activational effects, however, have not been studied in detail relative to cell type and ongoing structural changes in various vascular beds. The results of this study provide a reference point for such studies and should provide some useful information to women deciding about timing of cessation of MHT.

Although the KEEPS was designed to investigate how initiation of MHT within 3 years of menopause affects progression of subclinical atherosclerosis and cardiovascular risk, our study of postmenopausal women is not designed such

TABLE 2. *Concentration of serum hormone in KEEPS participants at baseline, end-of-treatment (year 4), and post-treatment (year 7) study visits.*

Variable	*N*	Placebo ($n = 33$)	Transdermal E_2 ($n = 23$)	Oral CEE ($n = 20$)
Follicle-stimulating hormone, IU/L				
BL visit	76	74 (61, 85)	89 (69, 122)	76 (64, 104)
4-y visit	0	NA	NA	NA
7-y visit	71	75 (54, 86)	76 (54, 97)[a]	78 (56, 91)
Estrone, pg/mL				
BL visit	0	NA	NA	NA
4-y visit[b]	71	19 (14, 23)	35 (26, 41)	56 (25, 93)
7-y visit	75	20 (14, 29)[a]	22 (17, 30)[a]	21 (16, 30)[a]
17β-estradiol, pg/mL				
BL visit[c]	76	NA	NA	NA
4-y visit[b]	71	5.3 (4.1, 6.9)	27.5 (6.9, 44.0)	11.0 (5.6, 17.0)
7-y visit	75	5.0 (2.7, 9.6)	6.2 (3.3, 12.0)[a]	5.1 (3.3, 9.7)[a]
Testosterone, total, ng/dL				
BL visit	0	NA	NA	NA
4-y visit	71	19 (17, 22)	25 (17, 30)	20 (18, 32)
7 y visit	67	15 (11, 20)[a]	17 (12, 21)[a]	16 (11, 28)[a]
Sex hormone binding globulin, nmol/L				
BL visit	76	49 (33, 72)	60 (41, 76)	56 (42, 84)
4-y visit[d]	75	45 (29, 70)	65 (42, 83)	106 (73, 153)[a]
7-y visit	75	48 (30, 58)[a]	51 (38, 81)[a]	53 (39, 78)[a]
Thyroid stimulating hormone, mIU/mL				
BL visit	76	2.0 (1.2, 2.9)	2.0 (1.3, 3.2)	1.8 (1.0, 2.6)
4-y visit	0	NA	NA	NA
7-y visit	75	2.5 (1.7, 3.6)[a]	3.7 (2.0, 4.6)[a]	2.6 (1.7, 3.6)[a]
Insulin, mcIU/mL				
BL visit	76	4.9 (2.7, 10.8)	4.5 (1.0, 6.1)	5.7 (3.1, 7.8)
4-y visit	75	5.8 (2.6, 9.9)	2.7 (1.0, 7.1)	4.8 (2.3, 6.4)
7-y visit	75	5.2 (3.9, 8.4)	3.7 (3.0, 6.1)	5.6 (3.5, 7.0)

Values presented as median (25th, 75th percentiles); *N* is the total number of nonmissing measurements.
BL, baseline measurements prior to the initiation of treatment; CEE, conjugated equine estrogens group; E_2, 17b-estradiol; NA, values not available.
[a] $P < 0.05$ from Wilcoxon signed-rank test for assessment of within-person change by treatment group, indicating a significant difference in the visit measurement compared to the preceding visit.
[b] $P < 0.05$ from Kruskal–Wallis test for assessment of between-group difference in the baseline measurement (if no baseline values were available, the comparison was made on the measurement at the 4-y visit).
[c] Measurements of baseline hormones were performed on a different platform than those at 4 and 7 years and therefore not included for comparisons.
[d] $P < 0.05$ from ANCOVA for assessment of between-group difference in the change from baseline, indicating that at least two of the three groups differed in regards to the visit measurement after controlling for the baseline measurement (if no baseline values were available, change from 4 to 7 years was assessed for difference between groups by treating the 4-year measurement as the baseline covariate in the ANCOVA model).

that we can analyze the impact of menopause sensitivity. Nor can we necessarily disentangle the collinear effects of age and time since menopause. Despite nonsignificant effects of baseline age and time since menopause on the rate of change in CIMT in our primary analysis, we explored a more sensitive analysis of these relationships and found evidence of curvature in the CIMT trajectories. Consistent with this finding, a larger magnitude of CIMT increase was observed during the 3-year period after completion of the trial, a relationship that did not seem to be modulated by the MHT used in KEEPS. The association of CIMT with aging processes is consistent with observations in other studies.[1,12]

The changes in HDL and LDL in this subset of women while on and after use of MHT were unexpected and somewhat inconsistent with the changes found in the full cohort of women completing KEEPS.[8] These differences most likely reflect the subset of women who participated in this follow-up study, the use of lipid lowering medications, and perhaps differences in assays used to assess the lipids for the entire cohort at the end of the study compared to the follow-up time point. How these differences may affect CIMT independent of age, immunological factors, metabolic syndrome or genetic polymorphisms cannot be determined given the sample size limitations of the cohort.[6]

Limitations

The number of women in this study was small and the duration of menopausal hormone treatment (only 4 y) and follow-up (3 y) was relatively short.[13]

TABLE 3. *Self-reported use of medications in women participating in the follow-up study*

Variable	PL ($n = 33$) (%)	tE_2 ($n = 23$) (%)	oCEE ($n = 20$) (%)	*P*
Self-selected use of MHT	7 (21.2%)	7 (30.4%)	6 (30.0%)	0.676
Antihypertensive medication	10 (30.3%)	1 (4.3%)	3 (15.0%)	0.043
Lipid-lowering medication	1 (3.0%)	5 (21.7%)	2 (10.0%)	0.080

MHT, menopausal hormone therapy; oCEE, oral conjugated equine estrogens group; PL, placebo; tE_2, transdermal 17b-estradiol.

TABLE 4. *Change in CIMT by treatment assignment and time*

Measurement	*N*	Overall	Placebo ($n = 33$[a])	Transdermal E_2 ($n = 23$)	Oral CEE ($n = 20$)	P[b]
CIMT from baseline to 4 y	74					0.067
Baseline value (mean ± SD), mm		0.671 ± 0.075	0.670 ± 0.074	0.672 ± 0.077	0.672 ± 0.078	
Follow-up value (mean ± SD), mm		0.699 ± 0.093	0.705 ± 0.095	0.706 ± 0.097	0.680 ± 0.087	
Absolute change (95% CI), mm		0.028 (0.018-0.038)[c]	0.035 (0.020-0.050)[c]	0.035 (0.017-0.052)[c]	0.008 (−0.011-0.027)	
Annual change (95% CI), mm		0.007 (0.004-0.009)[c]	0.009 (0.005-0.013)[c]	0.009 (0.004-0.013)[c]	0.002 (−0.003-0.007)	
CIMT from 4 to 7 y	74					0.524
Year 4 value (mean ± SD), mm		0.699 ± 0.093	0.705 ± 0.095	0.706 ± 0.097	0.680 ± 0.087	
Follow-up value (mean ± SD), mm		0.736 ± 0.095	0.748 ± 0.096	0.739 ± 0.099	0.715 ± 0.093	
Absolute change (95% CI), mm		0.038 (0.029-0.046)[c]	0.043 (0.030-0.057)[c]	0.033 (0.018-0.048)[c]	0.034 (0.017-0.050)[c]	
Annual change (95% CI), mm		0.013 (0.010-0.015)[c,d]	0.014 (0.010-0.019)[c]	0.011 (0.006-0.016)[c]	0.012 (0.006-0.017)[c,d]	
CIMT from baseline to 7 y	76					0.085
Baseline value (mean ± SD), mm		0.672 ± 0.075	0.671 ± 0.073	0.672 ± 0.077	0.672 ± 0.078	
Follow-up value (mean ± SD), mm		0.733 ± 0.099	0.744 ± 0.100	0.738 ± 0.102	0.708 ± 0.093	
Absolute change (95% CI), mm		0.061 (0.048-0.075)[c]	0.073 (0.053-0.093)[c]	0.066 (0.042-0.090)[c]	0.036 (0.011-0.062)[c]	
Annual change (95% CI), mm		0.009 (0.007-0.011)[c]	0.010 (0.008-0.013)[c]	0.009 (0.006-0.013)[c]	0.005 (0.002-0.009)[c]	

CEE, conjugated equine estrogens group; CIMT, carotid artery intima-medial thickness.
[a]Two of the 33 participants in the placebo group were excluded from the first two time point comparisons due to lack of follow-up measurements that precluded a 4 year CIMT estimate (these two participants did, however, have a 7 year value which made them eligible for the final comparison).
[b]*P*-value from ANCOVA model to assess change in CIMT across treatments after adjustment for baseline CIMT; for this two-stage derived variable analysis was performed using a slope from fitting each participant's data with a linear regression model.
[c]Within-person change from baseline to 4 or 7 years, and from 4 to 7 years, were all significant, except for the change at 4 years in the Oral CEE group.
[d]Wilcoxon signed-rank test demonstrated that annual change during the 4 to 7 year interval was significantly greater than that during baseline to 4 years.

Also, by limiting our analysis to the subset of participants who completed the 7-year visit (to study on- and post-treatment trends in the same individuals), the effect of treatment randomization may have been compromised. Accordingly, we investigated the sensitivity of our results to potential confounding and selection bias by repeating the longitudinal analysis of on-treatment trends using all KEEPS women (at the Mayo site) for whom CIMT data were available. This analysis yielded results comparable to those of the primary analysis, although the 4-year increase in CIMT in the oCEE group was greater and more consistent with that of the other treatments. Although our primary results may have underestimated this group's response to treatment, the lack of 7-year CIMT measurements from nonparticipants limits our ability to address the implications of post-treatment trends. Additional analysis of potential selection bias revealed lower systolic blood pressure and higher HDL cholesterol measurements during KEEPS among participants in the present study compared with nonparticipants. Therefore, we cannot rule out the possibility of a healthier subset in the present study, even if clinical descriptors were otherwise similar.

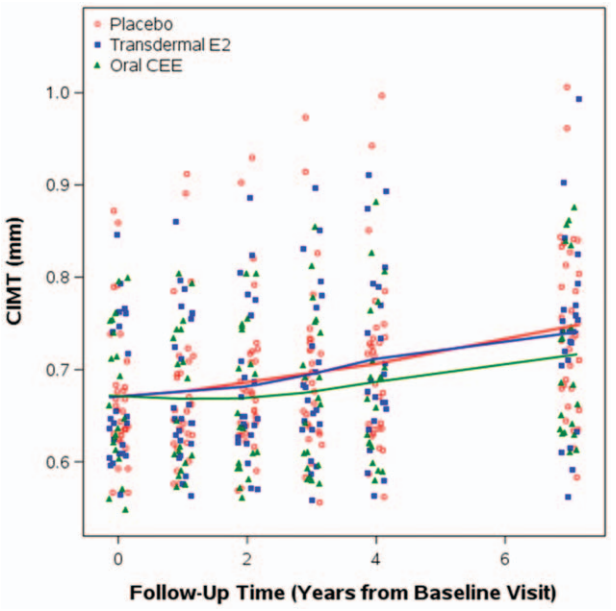

FIG. 1. Increases in carotid artery intima-media thickness (CIMT) of Mayo KEEPS participants from values at the time of randomization to treatment (year 0) through the 4 years of the trial and at 3 years after cessation of treatment. Each point represents a measurement per individual per visit, with repeated measurements over all study visits. Curves depict time trends based on the LOWESS method, which estimate, by nonparametric analysis, the relationship between time and CIMT by group without assuming linearity. CEE, conjugated equine estrogen; E_2, 17β-estradiol.

The doses of hormones used in the KEEPS reflected the clinical recommendations after the Women's Health Initiative for using lower doses of hormones for the shortest period of time at the time.[14] It is unclear as to what the optimal dose of hormone might be for various outcomes (vasomotor, sleep quality, sexual dysfunction, bone, and CIMT). The doses of MHT used in KEEPS were effective in alleviating hot flushes, improving sleep and sexual function, and reducing bone loss in participants randomized to active treatment[15-18] but were not effective in altering CIMT during the study.[8] Evaluation of progression of cardiovascular disease with other formulations of estrogen (oral E_2)[2] or similar formulations of higher dosage[19] as used in KEEPS for longer treatment periods or longer follow-up are warranted.

CONCLUSIONS

Cessation of lower dosages of MHT such as those used for 4 years in the KEEPS study did not accelerate changes in CIMT over a 3-year period. Larger studies of longer duration

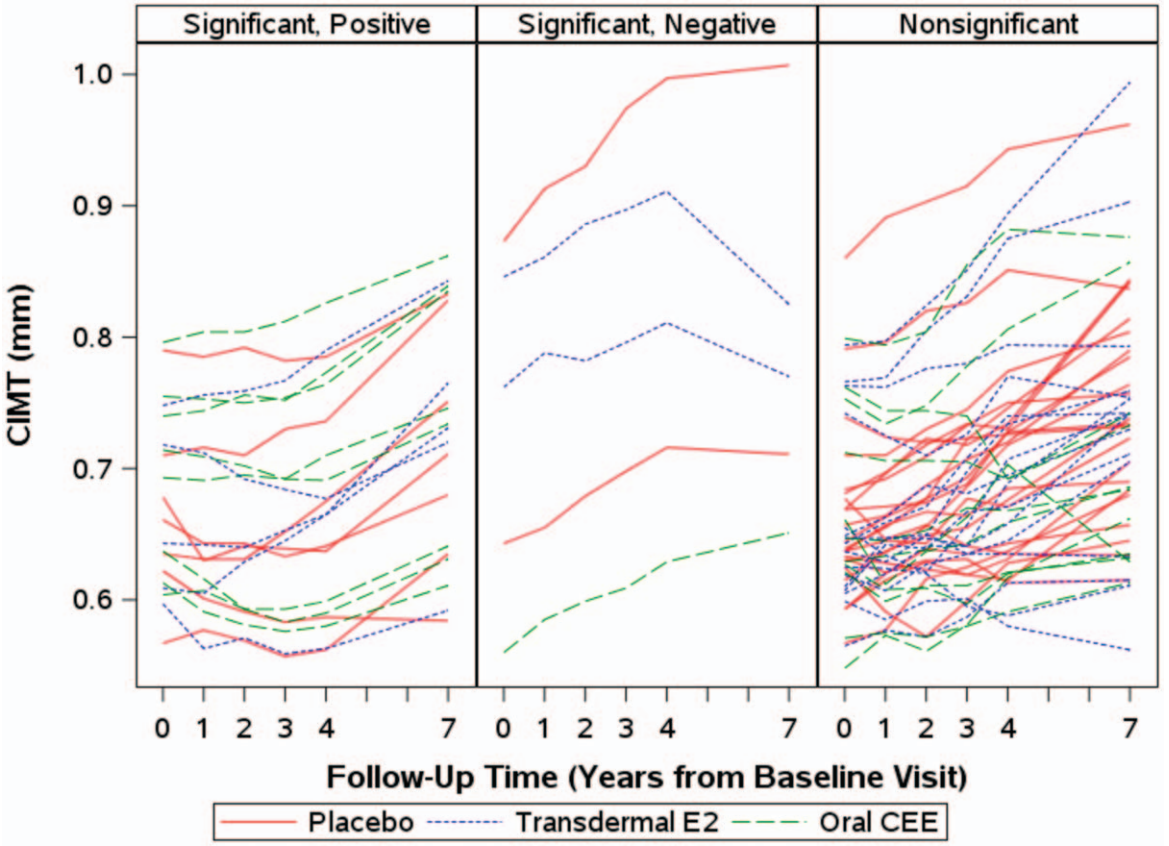

FIG. 2. Changes in carotid artery intima-media thickness (CIMT) of KEEPS participants from values at the time of randomization to treatment (0 mo) through the 48 months of the trial and at 3 years after cessation of treatment (84 mo) stratified by presence and direction of a quadratic trend (ie, possible acceleration in change). Each line represents data from an individual participant by quadratic equation over time.

of MHT on CIMT in healthy women and longer follow-up for women once they stop using MHT are warranted to better understand the temporal sequence of activational effects of MHT on vascular function.

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