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Heart Fat and Carotid Artery Atherosclerosis Progression in Recently Menopausal Women: Impact of Menopausal Hormone Therapy. The KEEPS Trial

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Abstract

Objective: Heart fat deposition has been linked to atherosclerosis, and both accelerate after menopause. Hormone therapy (HT) may differentially slow heart fat deposition and progression of atherosclerosis, depending on the specific HT agent or its route of administration. Our objective was to evaluate the effects of different HT agents, oral and transdermal, on associations between heart fat accumulation and atherosclerosis progression, measured by carotid intima-media thickness (CIMT), in recently menopausal women from the KEEPS trial.

Methods: KEEPS was a randomized, placebo-controlled trial of the effects of 0.45 mg/d oral conjugated equine estrogens (o-CEE) or 50 mcg/d transdermal 17β-estradiol (t-E2), compared to placebo, on 48-months progression of CIMT. Epicardial (EAT) and paracardial adipose tissue (PAT) volumes were quantified by CT.

Results: 467 Women [mean age(SD): 52.7(2.5); 78.2% White; 30% on o-CEE, 30.8% t-E2, 39.2% placebo] with heart fat volumes and CIMT at baseline and 48-months were included. EAT and PAT changes were not associated with CIMT progression; however, the assigned treatment significantly modified the association between PAT (but not EAT) change and CIMT progression. In the o-CEE group, adjusted CIMT progression was 12.66μm (95% CI: 1.80, 23.52) lower than in t-E2 group (P=0.02), and 10.09μm (95% CI 0.79, 19.39) lower than in placebo group (P=0.03), per 1-SD increase in PAT.

Conclusion: Compared to t-E2, o-CEE appears to slow down the adverse effect of increasing PAT on progression of atherosclerosis. Whether this beneficial association is specific to CEE or to the oral-route of CEE administration is unclear and should be assessed further.

Introduction

Midlife women experience accelerated progression of atherosclerosis and adverse changes in body fat redistribution as they traverse menopause.[1] Significant increases in carotid intima-media thickness (CIMT), a well-established measure of subclinical atherosclerosis,[2] have been reported after the menopause compared with the premenopausal state, independent of aging.[3] Moreover, in addition to having greater abdominal visceral fat,[4–6] postmenopausal women have greater fat deposition around the heart than premenopausal women.[7] Strong evidence supports a contribution of heart fat to coronary artery disease pathogenesis[8,9] at least in part through variations in local release of paracrine and endocrine pro- and anti-inflammatory cytokines.[10] Interestingly, greater heart fat has also been linked to atherosclerosis distant from the heart, in the carotid arteries, in form of thicker CIMT, in various populations at increased cardiovascular risk.[11–18] There are no data in this regard, however in clinically healthy postmenopausal women.

Estrogen may play a role both in atherosclerosis progression and in heart fat deposition in midlife women.[19,7] Interestingly, clinical trials of exogenous estrogens showed significant associations of hormone therapy (HT) use with CIMT and heart fat accumulation, considered separately. In recently menopausal women, oral 17β-estradiol was associated with less progression of CIMT than placebo.[20] Additionally, in an ancillary study of the Kronos Early Estrogen Prevention Study (KEEPS), a multicenter, randomized, placebo-controlled clinical trial of the effects of oral-conjugated equine estrogens (o-CEE) and transdermal 17β-estradiol (t-E2) on 48-month progression of CIMT, recently menopausal women assigned to o-CEE were less likely to have any increase in the heart fat located within the pericardial sac than those assigned to t-E2 or placebo.[21]

Relative to the pericardial sac, two heart fat depots can be identified: 1) epicardial adipose tissue (EAT), which directly covers the myocardium and is located within the pericardial sac, and 2) paracardial adipose tissue (PAT), which is located outside the pericardial sac. EAT and PAT are distinct fat depots with different embryological origins, blood supplies, and metabolic activities.[22, 23] Previous studies suggested PAT as a potential menopause-specific risk factor for coronary atherosclerosis.[7,24] Higher PAT, but not EAT, volume has been independently linked to lower levels of endogenous estradiol in midlife women at different stages of the menopause transition. Moreover, higher PAT volume has been associated with greater risk of coronary atherosclerosis in midlife women and this association was stronger in women with a lower level of endogenous estradiol.[7,24] Interestingly, an analysis from the KEEPS trial ancillary study on heart fat showed differing impacts of exogenous estrogen use on EAT and PAT associations with coronary atherosclerosis, depending on the type of estrogen used and/or its route of administration.[21] In recently menopausal women from KEEPS, women on t-E2 showed a significant progression of coronary artery calcification (CAC) associated with PAT accumulation over 48-months of follow-up. Interestingly, similar progression was not seen in women on o-CEE.[21]

No previous study has assessed effect modification of different HT types/routes of administration, started soon after the menopause, on the association between heart fat depots and CIMT progression, although both increase after the menopause.[2,7] Using data from the KEEPS trial of the effects of o-CEE and t-E2 on 48-month progression of CIMT among recently menopausal women,[25] we examined possible differential effects of o-CEE and t-E2 on the associations of EAT and PAT accumulation with progression of CIMT. We hypothesized that HT use would differentially modify the association between heart fat accumulation and CIMT progression based on the agent and/or route of administration; such that women assigned to o-CEE would experience less or no CIMT progression associated with heart fat accumulation compared to those on t-E2 or placebo and that this effect would be more pronounced for the PAT depot than for the EAT depot. Understanding the contribution of HT use on how heart fat might impact CIMT in recently menopausal women is crucial since HT is commonly prescribed to treat debilitating menopause-related symptoms[26] during a time of accelerated risk of atherosclerosis progression and heart fat deposition.[1] It is possible that HT might help reducing the adverse impact of heart fat on atherosclerosis progression in recently menopausal women.

Methods

Study design and participants:

Detailed methods of the KEEPS trial have been published before.[25] Briefly, KEEPS participants were enrolled between July/2005-June/2008 and followed for 48-months. Visits were completed by March/2012. Women with an intact uterus, between 6–36 months from their last menstrual period and aged 42–58 years who had plasma estradiol levels < 147 pmol/L, and follicle-stimulating hormone levels 35 IU/L, or both were eligible. Women reporting a history of clinical CVD, current heavy smoking, a body mass index (BMI) 35 kg/m^2, low-density lipoprotein-cholesterol (LDL-C) >190 mg/dL or triglycerides >400

mg/dL, diabetes, uncontrolled hypertension, or with moderate subclinical CVD, defined as a CAC score 50 units, were ineligible for randomization. Former/current HT users were screened only after having discontinued therapy for 90 days. A total of 727 women (77% White, 7% African-American, 3% Asian, 7% Hispanic, and 6% others) met inclusion criteria and were randomized to: 1) o-CEE; 0.45mg/day, n=230(31.6%), 2) t-E2; 50μg/day, n=222(30.5%), or 3) placebo (inactive pill and patch), n=275(37.8%). Women receiving o-CEE or t-E2 also received oral micronized progesterone, 200mg/day for first 12 days/month and others received progesterone placebo.

This study is a secondary analysis of data from KEEPS and a completed ancillary study to KEEPS on heart fat measured before (baseline) and 48-months after randomization. The current analysis excluded all KEEPS participants who did not have heart fat and CIMT measured at both baseline and 48-months, leaving 467 participants in the analytical sample, Figure 1. Excluded participants were more likely to be college graduates and they had significantly higher baseline leptin and CIMT, and lower baseline PAT volume than those who were included, Supplemental Table 1.

The institutional review board at each participating site approved the trial and all participants provided informed consent to participate in the trial.

Carotid intima-media thickness:

CIMT was the primary end-point of the KEEPS main trial.[27] CIMT of the far wall of the distal common carotid artery was determined as the average of 70 to 100 standardized measurements between the intima–lumen and media–adventitia interfaces by automated computerized edge detection with a software package developed at the University of Southern California Atherosclerosis Research Unit Core Imaging and Reading Center. All measures of carotid wall thickness were done blinded of the treatment allocation. Two baseline measurements of CIMT were done at isolated visits (about 3 days to 6 weeks apart), and the results were averaged to provide an estimate of baseline CIMT. The mean coefficient of variation between baseline scans was 0.6% (SD, 0.7 [range, 0.0% to 7.7%]). For the current analysis CIMT measured at the same time of heart fat measures were utilized (baseline and 48-month visit).

Heart fat depots:

Heart fat depot volumes were quantified as part of an ancillary study to KEEPS (2009–2012) that utilized existing CT scans before randomization (baseline) and 48-months after randomization. All images were read centrally by experienced readers who were blinded to study group.[28] In brief, total heart fat volume (EAT plus PAT) was determined from 15 mm above to 30 mm below the superior extent of the left main coronary artery. This region of the heart was selected because it includes the epicardial fat surrounding the proximal coronary arteries. The anterior border of the heart fat volume was the chest wall, and the posterior borders were the aorta and the bronchus. Using the volume analysis software (GE Healthcare), fat was distinguished from other heart tissue by a threshold of −190 to −30 Hounsfield units. EAT was measured by manually tracing out the pericardium every 2 to 3 slices below the start point and then using the software to automatically trace out the

segments in between these selected slices. PAT was measured by subtracting EAT volume from total heart fat volume. Reproducibility measurements of EAT and PAT were performed on 20 randomly selected scans from another study that used a similar protocol. Both Spearman and intra-class correlation coefficients between readers (intra-reader) were 0.99 each for EAT and 0.86 and 0.96, respectively, for PAT. Similarly, both Spearman and intra-class correlation coefficients between repeated readings (inter-reader) were 0.98 each for EAT and 0.96 and 0.90, respectively, for PAT.[28]

Covariates:

Demographics, race/ethnicity, income, employment status, education levels, history of smoking, medication use, alcohol drinking, and physical activity (Metabolic Equivalents (METs); calculated as total energy expenditure from recreational physical activity in kcal/week/kg)[28] were all collected at screening/baseline visits. Physical measures were obtained at baseline and 48-months. BMI was calculated from measured weight (kilogram)/height2(meter). Waist circumference(cm) was measured at the smallest horizontal circumference using a non-stretchable tape. Blood pressure was assessed after at least 5 min of rest in right arm and averaged across two readings.

Fasting levels of serum total cholesterol, HDL-C, LDL-C and triglycerides (TG) were measured at Kronos Science Laboratories (KSL) using Carolina Liquid Chemistry Reagent (Carolina Liquid Chemistries Corporation, Brea California) on the Stanbio Sirrus Chemistry Analyzer, at both baseline and the 48-month visit. For total cholesterol, the intra-assay coefficient of variation (CV) was 1.4%–2.2% and inter-assay CV was 4.3–5.0%. For HDL-C the intra-assay CV was 2.7%–3.1% and interassay CV was 3.5–3.8%. For LDL-C the intra-assay CV was 1.3%–1.5% and the inter-assay CV was 5.3–7.1%. For TG the intra-assay CV was 5.5–5.6% and the inter-assay CV was also 5.5–5.6%. Glucose and insulin levels were also measured through ancillary studies to KEEPS at KSL. Glucose was measured on the Stanbio Sirrus Chemistry Analyzer using the Stanbio Reagent standard (Stanbio Laboratory, Boerne, TX) with an intra-assay CV of 1.7–1.3%, and inter-assay CV of 2.2–2.5%. Insulin assays were conducted on the Immulite 2000 by solid-phase, chemiluminescent immunometric assay (Siemens HealthCare Diagnostics, Tarrytown, NY) with a MDL of 2 μIU/ml, intra-assay CV of 2.6–2.8% and inter-assay CV of 2.8 3.3%. The Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as described before.[29]

Statistical analysis:

Participants' demographics and clinical characteristics at baseline and after 48-months were summarized and compared between those included and excluded using chi-square or t-tests as appropriate. Changes in EAT, PAT and CIMT were calculated as the difference between baseline and 48-months values. Geometric means (SE) of the 48-months CIMT progression by treatment group were estimated from a linear model of 48-months change in CIMT as a function of treatment groups. Associations of 48-months CIMT progression with baseline and 48-months changes in each heart fat depot were evaluated using linear regression to guide model building. Covariates were selected *a priori* or if they were associated with CIMT progression and/or heart fat depots with a P value <0.1, Supplemental Table 2. For variables that were highly correlated, models were assessed with each one separately and the

models with the best model fit diagnostics were chosen. Effect modification of assigned HT was assessed by testing the interaction between HT use groups and change in each heart fat depots. Beta-coefficients were presented per 1 SD increase in change in heart fat depot. Because the distributions of EAT and PAT were skewed, all analysis were repeated with the log-transformed EAT and PAT variables to improve the approximate normality of these distributions. Results did not change. For ease of interpretation, results using the original scale were presented in the final manuscript. SAS, version 9.4 (Cary, North Carolina) was used for statistical analyses.

Results:

At baseline, participants had been menopausal for an average of 1.8 years and the majority of them were White (78.2%). Other demographics and clinical characteristics are summarized in Table 1, including baseline and 48-months levels of heart fat depots and CIMT.

After 48-months of follow-up, the mean (SE) of the overall 48-months CIMT progression was 33 (2.0) μm. The 48-months CIMT progression did not differ by treatment group as reported before;[27] the geometric mean (SE) of 48-months CIMT progression in o-CEE vs. t-E2 vs. Placebo was 31.9 (3.7) vs. 35.1 (3.6) vs. 33 (3.2) (P=0.82).

48-months changes in CIMT were not associated with either baseline or changes in EAT or PAT in unadjusted or fully adjusted analysis, Table 2. However, the assigned treatment significantly modified the association between changes in PAT, but not EAT, and CIMT progression in unadjusted (P=0.009) and fully adjusted (P=0.04) models, Table 3. In fully adjusted model (Model 3, Table 3) in o-CEE group, the estimated CIMT progression per 1-SD increase in PAT was 12.66 μm (95% CI: 1.80, 23.52) lower than the estimated effect in the t-E2 group, P=0.02, and was 10.09 μm (95% CI: 0.79, 19.39) lower than estimated effect in the placebo group, P=0.03.

Discussion:

To the best of our knowledge, this is the first study to assess associations between heart fat accumulation and CIMT progression in clinically healthy recent menopausal women on different HT regimens. We did not find significant overall associations between either baseline or 48-months changes in EAT or PAT with CIMT progression. However, the associations between changes in PAT, but not EAT, and CIMT progression at 48-months varied by HT regimen. Women assigned to o-CEE had less CIMT progression than women assigned to t-E2 or placebo for each 1-SD unit increase in PAT. Consistent with previous work from the Study of Women's Health Across the Nation (SWAN),[7,24] our findings suggest a stronger contribution of estrogen to the pathophysiological consequences of PAT depot than EAT depot in midlife women.

Several studies have assessed associations between heart fat, particularly EAT depot, and CIMT. Findings consistently showed higher EAT to be associated with thicker CIMT in several population settings including obese children and adults,[30] obese hypertensive patients,[31] and patients with insulin resistance,[16] metabolic syndrome,[32] or type 2 diabetes.

[18] In this analysis from the KEEPS trial neither EAT nor PAT at baseline nor at 48-months was associated with CIMT progression. This inconsistency could be related to the different study populations and designs utilized. In contrast to our study population of a relatively healthy recent menopausal women, all previous studies evaluated populations with cardiometabolic health issues known to impact adipose tissue volume and function.

We previously reported a significant association between PAT, but not EAT, and endogenous estradiol level in midlife women.[7] Further, we found that associations between PAT volume and coronary artery calcification vary by endogenous level of estradiol.[24] Most recently, we reported that associations between PAT, but not EAT, progression and coronary artery calcification development also vary by exogenous estrogen use in recently menopausal women.[21] Taken together, these accumulating findings along with current findings provide evidence for a direct impact of estrogen on PAT and the relationship of PAT with progression of atherosclerosis after the menopause.

EAT is in direct contact with the myocardium and coronary arteries, and its bimolecular, biochemical and physiological properties, as related to CVD pathogenesis, have been extensively evaluated. In contrast, PAT which does not have that direct contact,[33] a few studies, however, have showed PAT to be more closely linked to intra-abdominal (visceral) adiposity and to related metabolic risk factors than EAT,[33] suggesting PAT to have a systemic (rather than local) impact on atherosclerosis progression. The mechanisms by which exogenous HT might impact the association between PAT and CIMT have not been well studied. PAT might impact CIMT remotely through adipokine release into the systemic circulation, which in turn could induce expression of cell-adhesion molecules in arterial endothelial cells.[34] Alternatively, PAT might impact CIMT progression indirectly, since both are strongly associated with visceral adiposity, which in turn is strongly associated with insulin resistance, which appears to alter many aspects of atherogenesis, including macrophage recruitment and function[35] and vascular smooth muscle proliferation.[36] Thus, it is possible that the use of o-CEE may reduce the apparent role of PAT on carotid atherogenesis (and thus, on CIMT) via reducing adipokine release or reducing insulin resistance, or possibly via other mechanisms.

Our study suggests a distinct effect modification of HT on the relationship between heart fat depots and atherosclerosis progression based on the type of estrogen and/or route of administration. In KEEPS, the orally administered estrogen was conjugated equine estrogens while the transdermal estrogen was 17β-estradiol. The differential combination of estrogens and related properties in each of the two HT agents used in KEEPS may have had different impacts on how PAT affects CIMT.[37] It is also plausible that the differential impact of HT regimens on association between PAT and CIMT reported in our study could be related to the first-pass effects in the liver, present with the oral administration but absent with the transdermal route.[38] In addition to direct hepatic effects, it is possible that first-pass effects might include differential impact on metabolites of estrogens which may vary in their affinity to estrogen receptor α (ERα) in adipose tissue. ERα plays a critical role in maintaining adipose tissue function and preventing inflammatory damage,[39] the latter being an important factor in atherosclerosis progression. Interestingly, KEEPS women on o-CEE did not show any change in EAT over 48-months whereas women on t-E2 showed non-

significant marginal increases.[21] Similar patterns were observed for 48-month changes in BMI and waist circumference in another KEEPS analysis.[40] Although no statistically significant differences were observed in those changes across treatment groups, women on o-CEE reported smaller increases in BMI compared to those on t-E2 and placebo. Interestingly, in the Danish Osteoporosis Prevention Study in early menopausal women, women on oral estradiol showed less gain in fat mass compared to placebo.[41] Perhaps, the oral route of HT administration has a stronger impact on adipose tissue accumulation and function than does transdermal HT. Because HT type and route varied together in KEEPS (neither oral E2 nor transdermal CEE were used) we were not able to disentangle differences relevant to HT type vs. route of administration. Future studies should aim to address this question, since EAT and PAT differ in many ways which may include key mechanisms of impact on carotid atherosclerosis.[22, 23]

Our study has several limitations and strengths. This is the first study to assess whether use of different HT regimens impact associations between heart fat accumulations and CIMT progression in clinically healthy recent menopausal women, a population at high risk of both fat redistribution and atherosclerosis progression.[1] The unique design of KEEPS, randomly assigning women to 2 HT regimens is both a strength and a weakness. Although KEEPS studied two common HT regimens, the fact that both the agent and the route differed between the two active treatment arms means that we cannot conclude which difference might account for differences in treatment outcomes. Irrespective of this, some limitations include the lack of generalizability to populations other than those similar to KEEPS' population, and the potential bias inherited due to those excluded from current analysis having thicker CIMT but lower PAT volume at baseline. However, analysis adjusted for baseline volume of PAT did not change the overall conclusions.

Our study adds to the accumulating evidence that the impact of HT on clinically-relevant outcomes such as postmenopausal increases in EAT, PAT and atherosclerosis may vary based on the specific HT regimen.[42] Moreover, the current findings support the notion that EAT and PAT are distinct heart fat depots, with PAT being more sensitive to menopause and HT use. Our findings suggest a complex role of HT on the association between heart fat and CIMT in recently menopausal women, and call for more research to help clinicians individualize HT prescription to maximize benefit and reduce related risk.

Computed tomography and magnetic resonance imaging have been used in clinical studies to quantify heart fat due to high spatial resolution and the opportunity of volumetric assessment. However, heart fat can also be measured using standard two-dimensional echocardiography supporting feasibility of assessing heart fat along with other echocardiographic parameters associated with CVD risk in clinical practice. Echocardiographic heart fat measure correlates with metabolic syndrome, insulin resistance, coronary artery disease, and subclinical atherosclerosis, and has shown good reproducibility.[23, 43]

Conclusions

In summary, the associations between changes in PAT, but not EAT, and CIMT progression at 48-months varied by HT regimen. Results suggests that o-CEE may slow down the adverse impacts of heart fat accumulation outside the pericardial sac on CIMT in recently menopausal women as compared to t-E2. Whether this beneficial impact on CIMT is due to use of CEE or the oral route of administration is unclear and should be assessed in future studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

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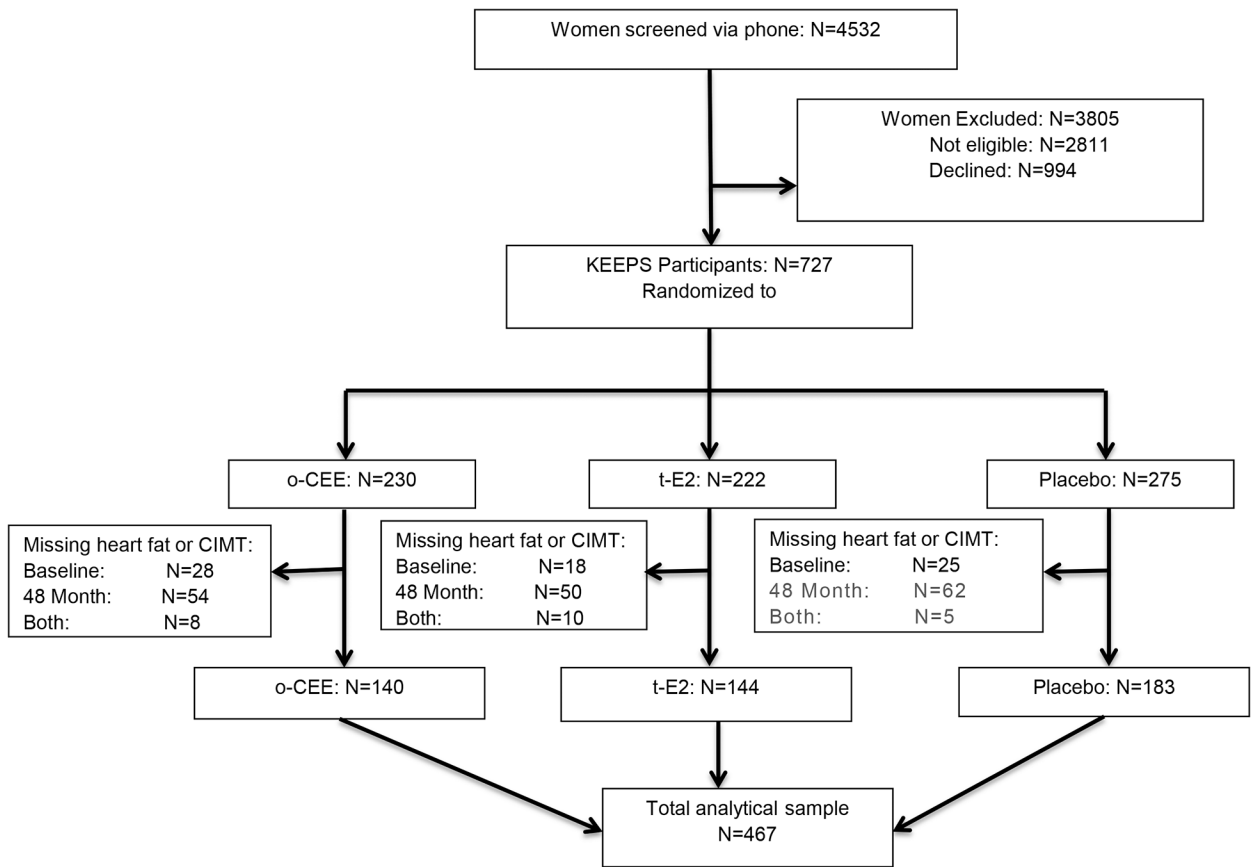
Figure 1.
CONSORT Flow Diagram of the Analytical Sample from the KEEPS-Heart FAT Ancillary Study
Abbreviations: o-CEE: oral conjugated equine estrogen; t-E2: transdermal

Table 1.

Demographics and clinical characteristics of KEEPS participants evaluated in this study

Variables	Total N=467	o-CEE N=140 (30.0%)	t-E2 N=144 (30.8%)	Placebo N=183 (39.2%)	P-value [a]
		At baseline			
Age, mean (SD), Yr	52.7 (2.5)	52.9 (2.8)	52.8 (2.5)	52.4 (2.4)	0.21
Menopausal age, mean (SD), Yr	50.9 (2.5)	51.1 (2.8)	51.0 (2.5)	50.7 (2.3)	0.38
Time since menopause, mean (SD), Yr	1.8 (0.8)	1.8 (0.8)	1.8 (0.7)	1.7 (0.8)	0.36
White Race, N (%)	365 (78.2)	110 (78.6)	110 (76.4)	145 (79.2)	0.82
Education, N (%)					0.05
Declined to answer	5 (1.1)	0 (0.0)	2 (1.4)	3 (1.6)	
High school graduate or less	29 (6.2)	11 (7.9)	3 (2.1)	15 (8.2)	
Some college	77 (16.5)	27 (19.3)	19 (13.2)	31 (16.9)	
College graduate	356 (76.2)	102 (72.9)	120 (83.3)	134 (73.2)	
Employed, N (%)	386 (82.7)	120 (85.7)	120 (83.3)	146 (79.8)	0.37
Income, N (%)					0.51
<$60K	81 (17.3)	26 (18.6)	28 (19.4)	27 (14.8)	
$60K–<$100K	64 (13.7)	16 (11.4)	19 (13.2)	29 (15.8)	
>$100K	85 (18.2)	23 (16.4)	22 (15.3)	40 (21.9)	
Unknown	237 (50.7)	75 (53.6)	75 (52.1)	87 (47.5)	
Physical Activity Level, Median(Q1, Q3), MET-hr/wk	16.7 (7.0, 28.4)	17.2 (7.5, 31.7)	15.1 (5.3, 24.7)	17.2 (7.5, 28.0)	0.46
Alcohol consumption, N (%)	356 (76.2)	117 (83.6)	102 (70.8)	137 (74.9)	0.04
Smoking Status, N (%)					0.79
Never	373 (79.9)	116 (82.9)	114 (79.2)	143 (78.1)	
Past	70 (15.0)	19 (13.6)	21 (14.6)	30 (16.4)	
Current	24 (5.1)	5 (3.6)	9 (6.3)	10 (5.5)	
Ever use hormone therapy, N (%)	99 (21.2)	35 (25.0)	29 (20.1)	35 (19.1)	0.41
Anti-Hypertensive medication use, N (%)					0.48
Never	386 (82.7)	112 (80.0)	118 (81.9)	156 (85.2)	
Past	26 (5.6)	11 (7.9)	9 (6.3)	6 (3.3)	
Current	55 (11.8)	17 (12.1)	17 (11.8)	21 (11.5)	

Variables	Total N=467	o-CEE N=140 (30.0%)	t-E2 N=144 (30.8%)	Placebo N=183 (39.2%)	P-value [a]
BMI, mean (SD), Kg/m²	26.1 (4.3)	26.1 (4.2)	25.7 (4.4)	26.4 (4.2)	0.35
C-reactive protein, median (Q1, Q3), mg/dL	1.0 (0.4, 2.3)	0.8 (0.4, 2.1)	1.0 (0.4, 2.3)	1.1 (0.5, 2.4)	0.25
Leptin, median (Q1, Q3), ng/ml	18.4 (11.0, 30.7)	17.4 (10.6, 28.4)	17.8 (9.2, 30.4)	18.8 (12.5, 32.2)	0.23
High molecular weight adiponectin, median (Q1, Q3), µg/mL	6.6 (4.5, 9.7)	7.3 (4.6,11.4)	7.0 (5.1, 9.5)	5.8(3.8, 8.5)	0.005
Systolic blood pressure, mean (SD), mmHg	118.4 (15.0)	119.7 (14.6)	116.8(15.8)	119.2 (14.7)	0.22
Diastolic blood pressure, mean (SD), mmHg	75.2 (9.3)	75.9 (8.5)	74.1 (9.8)	75.6 (9.3)	0.20
HDL cholesterol, mean (SD), mg/dL	72.3 (15.1)	72.9 (15.1)	74.5 (16.7)	70.0 (13.4)	0.02
LDL cholesterol, mean (SD), mg/dL	111.5 (27.6)	111.7 (26.8)	111.2 (29.0)	111.6 (27.2)	0.98
Triglycerides, median (Q1, Q3), mg/dL	70.0 (51.0, 106.0)	69.5 (51.0, 111.0)	66.0 (46.5, 103.0)	74.0 (55.0, 112.0)	0.16
Fasting glucose, median (Q1, Q3), mg/dL	79.0 (74.0, 86.0)	78.5 (74.0, 84.0)	78.0 (74.0, 84.0)	79.0 (73.0, 87.0)	0.55
Insulin, median (Q1, Q3), µIU/mL	4.2 (2.0, 7.4)	4.0 (2.2, 7.0)	3.7 (1.0, 6.7)	4.7 (2.2, 8.3)	0.26
HOMA, median (Q1, Q3)	0.82 (0.37, 1.48)	0.78 (0.41, 1.36)	0.76 (0.22, 1.42)	0.95 (0.40, 1.72)	0.22
EAT volume, median (Q1, Q3), cm³	36.7 (25.6, 54.6)	38.7 (28.6, 59.3)	36.0 (24.9, 48.4)	36.4 (25.6, 54.6)	0.10
PAT volume, median (Q1, Q3), cm³	14.5 (10.3, 21.5)	14.6 (11.2, 22.0)	13.4 (8.7, 20.8)	14.9 (10.6, 22.4)	0.12
CIMT, mean (SD), µm	714.5 (89.2)	720.7 (92.0)	710.5 (83.3)	712.9 (91.8)	0.60
At 48-Months					
EAT volume, median (Q1, Q3), cm³	37.6 (26.3, 50.2)	39.8 (28.9, 53.1)	35.6 (25.2, 46.6)	38.0 (25.2, 51.9)	0.07
PAT volume, median (Q1, Q3), cm³	14.1(10.1, 21.1)	15.6 (10.6, 22.6)	13.3 (9.2, 19.2)	14.4 (10.6, 20.0)	0.11
CIMT, mean (SD), µm	747.9 (94.4)	752.6 (93.7)	745.60 (93.3)	746.2 (96.2)	0.78

Abbreviations: o-CEE: oral conjugated equine estrogen; t-E2: transdermal 17β-estradiol; BMI: body mass index; HDL: high density lipoprotein; LDL: low density lipoprotein; MET: metabolic equivalents; EAT: epicardial adipose tissue; PAT: paracardial adipose tissue; CIMT: carotid-intima media thickness; HOMA: Homeostasis Model Assessment insulin resistance index;

Table 2.

Association of 48-months changes in CIMT with baseline and 48-months changes in heart fat depots

	Unadjusted model		Fully adjusted model	
	β (95% CI)[a]	P-value	β (95% CI)[a]	P-value
Baseline heart fat depots				
EAT	3.01(−0.94, 6.97)	0.14	2.37 (−2.24, 6.97)	0.31
PAT	3.47 (−0.49, 7.44)	0.09	2.37 (−2.40, 7.14)	0.33
48-months heart fat depots				
EAT	−2.56 (−6.51,1.40)	0.21	−1.71 (−5.82,2.40)	0.41
PAT	−3.59 (−7.56,0.37)	0.08	−2.95 (−7.15,1.25)	0.17

[a]Estimates are per 1 SD increase in heart fat depot (SD for baseline EAT=26.78 cm^3; EAT change=15.47 cm^3; for baseline PAT=12.47 cm^3; PAT change=7.79 cm^3)

Full model includes treatment, age, race (white v/s non-white), location of study site (east, west and middle), employment, LDL-C, BMI, alcohol, smoking, systolic blood pressure, diastolic blood pressure, anti-hypertensive medication, log-triglycerides, log-HOMA, log-C-reactive protein, log-leptin, log-high molecular weight adiponectin

Table 3.

Effect modifications of treatment on the association between 48-months changes in each heart fat depot and 48-months changes in CIMT

	48-months CIMT			
	EAT		PAT	
	48-months β (95% CI)	P-value[a]	48-months β (95% CI)	P-value[a]
Model 1				
Per 1 SD increase in 48-months heart fat[b]				
o-CEE	-----	-----	-----	-----
t-E2	7.23 (−3.03,17.48)	0.17	14.28 (3.67,24.90)	0.009
Placebo	5.01 (−4.14,14.17)	0.28	12.11 (3.06,21.16)	0.009
P-value for interaction				
48-months heart fat depot * treatment group	0.34		0.009	
Model 2				
Per 1 SD increase in 48-months heart fat[b]				
o-CEE	-----	-----	-----	-----
t-E2	6.77 (−3.67,17.20)	0.20	12.54 (1.75,23.34)	0.02
Placebo	4.61 (−4.75,13.97)	0.33	10.00 (0.75,19.25)	0.03
P-value for interaction				
48-months heart fat depot * treatment group	0.41		0.04	
Model 3				
Per 1 SD increase in 48-months heart fat[b]				
o-CEE	----	-----	----	-----
t-E2	6.83 (−3.62,17.29)	0.20	12.66 (1.80, 23.52)	0.02
Placebo	4.64 (−4.73,14.02)	0.33	10.09 (0.79, 19.39)	0.03
P-value for interaction				
48-months heart fat depot * treatment group	0.40		0.04	

[a] P value of each treatment group compared to o-CEE

[b] Estimates per 1 SD increase in heart fat depot change (SD for EAT change=15.47; for PAT change=7.79)

Model1. Unadjusted

Model 2. includes age, race (white v/s non-white), location of study site (east, west and middle), employment, LDL-C, BMI, alcohol, smoking, systolic blood pressure, diastolic blood pressure, anti-hypertensive medication, log-triglycerides, log-HOMA, log-C-reactive protein, log-Leptin, log-high molecular weight adiponectin

Mode 3. Model 2 plus log transformed baseline heart fat depot volume as appropriate