

## Effects of Hormone Therapy on Heart Fat and Coronary Artery Calcification Progression: Secondary Analysis From the KEEPS Trial

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**Background**—Heart fats (epicardial and paracardial adipose tissue [PAT]) are greater after menopause. Endogenous estrogen may regulate these fat depots. We evaluated the differential effects of hormone therapy formulations on heart fat accumulations and their associations with coronary artery calcification (CAC) progression in recently menopausal women from KEEPS (Kronos Early Estrogen Prevention Study).

**Methods and Results**—KEEPS was a multicenter, randomized, placebo-controlled trial of the effects of 0.45 mg/d oral conjugated equine estrogens and 50 µg/d transdermal 17β-estradiol, compared with placebo, on 48-month progression of subclinical atherosclerosis among 727 early menopausal women. CAC progression was defined if baseline CAC score was 0 and 48-month CAC score was >0 or if baseline CAC score was >0 and <100 and annualized change in CAC score was ≥10. Of 727 KEEPS participants, 474 (mean age: 52.7 [SD: 2.6]; 78.1% white) had computed tomography-based heart fat and CAC measures at both baseline and 48 months. Compared with women on placebo, women on oral conjugated equine estrogens were less likely to have any increase in epicardial adipose tissue (odds ratio for oral conjugated equine estrogens versus placebo: 0.62 [95% CI, 0.40–0.97]; *P*=0.03). PAT did not change in any group. Changes in epicardial adipose tissue and PAT did not differ by treatment group. CAC increased in 14% of participants. The assigned treatment modified the association between PAT changes and CAC progression (*P*=0.02) such that PAT increases were associated with CAC increases only in the transdermal 17β-estradiol group.

**Conclusions**—In recently menopausal women, oral conjugated equine estrogens may slow epicardial adipose tissue accumulation, whereas transdermal 17β-estradiol may increase progression of CAC associated with PAT accumulation.

**Clinical Trial Registration**—URL: <http://www.clinicaltrials.gov>. Unique identifier: NCT00154180. (*J Am Heart Assoc.* 2019;8:e012763. DOI: 10.1161/JAHA.119.012763.)

**Key Words:** coronary artery disease • epicardial fat • estrogen • menopause

Heart fat volumes are larger in postmenopausal than premenopausal women.<sup>1</sup> Estrogen may have an important influence on heart fat buildup, as the accumulation of fat outside the pericardium is associated with lower levels and a steeper decline in endogenous estradiol (E2).<sup>1</sup> Relative to the pericardium, 2 heart fat depots can be identified: (1)

epicardial adipose tissue (EAT), which directly covers the heart and is located within the pericardial sac, and (2) paracardial adipose tissue (PAT), which is located anterior to EAT, outside the pericardial sac.<sup>2,3</sup>

Heart fat in EAT and PAT depots may provide a readily detectable, noninvasive, novel risk marker for coronary arterial

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## Clinical Perspective

### What Is New?

- Oral conjugated equine estrogens may slow epicardial adipose tissue accumulation in recently menopausal women.
- Transdermal 17 $\beta$ -estradiol may increase risk associated with paracardial adipose tissue accumulation on progression of coronary artery calcification in recently menopausal women.

### What Are the Clinical Implications?

- Findings underscore the differential contributions of estrogen type and route of administration when assessing risk related to hormone therapy use.
- Hormone therapy remains the best therapeutic option for the relief of debilitating menopausal symptoms.
- Clinicians should continue to individualize hormone therapy prescription using the best available evidence to maximize benefits and minimize risks.

disease in postmenopausal women. Heart fat may contribute to the pathogenesis of coronary arterial disease<sup>4–11</sup> via both paracrine and endocrine release of anti- and proinflammatory adipokines into the coronary arteries and the myocardium.<sup>12</sup> Interestingly, in midlife women, EAT was significantly associated with subclinical atherosclerosis, although this association was not modified by menopausal status or E2 levels. In contrast, a positive association between PAT and subclinical atherosclerosis was stronger in postmenopausal women with lower levels of E2.<sup>13</sup> These findings are in line with the notion that EAT and PAT are distinct depots. EAT and PAT have different embryological origins, blood supplies, and metabolic activities,<sup>2,3</sup> supporting evaluation of heart fat depots separately.

The effect of exogenous estrogen use on cardiovascular disease (CVD) risk remains controversial.<sup>14–18</sup> Current hormone therapy (HT) prescribing practices recommend HT to treat menopausal symptoms in early menopause but not to protect against CVD.<sup>19</sup> Given the potential effects of endogenous estrogens on heart fat accumulations and the ongoing controversy about the CVD risks and benefits of HT in postmenopausal women, it is essential to assess the impact of HT on heart fat accumulations and the linkage of heart fat increases with development of atherosclerosis. Moreover, it is possible that HT may affect cardiometabolic risk differently based on formulation and route of administration.<sup>20</sup> Thus, it is plausible to hypothesize that various formulations of HT will have differential impacts on heart fat accumulations and their association with atherosclerosis development. Furthermore, this impact may be more pronounced for the PAT depot, for which the association with subclinical atherosclerosis was modified by levels of E2 in midlife women.<sup>13</sup>

KEEPS (Kronos Early Estrogen Prevention Study) was a multicenter, randomized, placebo-controlled clinical trial of the effects of oral and transdermal HT, compared with placebo, on 48-month progression of subclinical atherosclerosis among recently menopausal women.<sup>21</sup> The main results from KEEPS showed that 48 months of early use of HT did not affect progression of atherosclerosis despite improving some markers of CVD risk (lipids and insulin resistance).<sup>22</sup> Whether early use of HT has an effect on heart fat accumulation is not known. KEEPS provides an ideal opportunity to examine possible differential effects of 2 routes of HT administration on 2 distinct depots of heart fat accumulation and their associations with the development of atherosclerosis, as measured by coronary artery calcification (CAC).

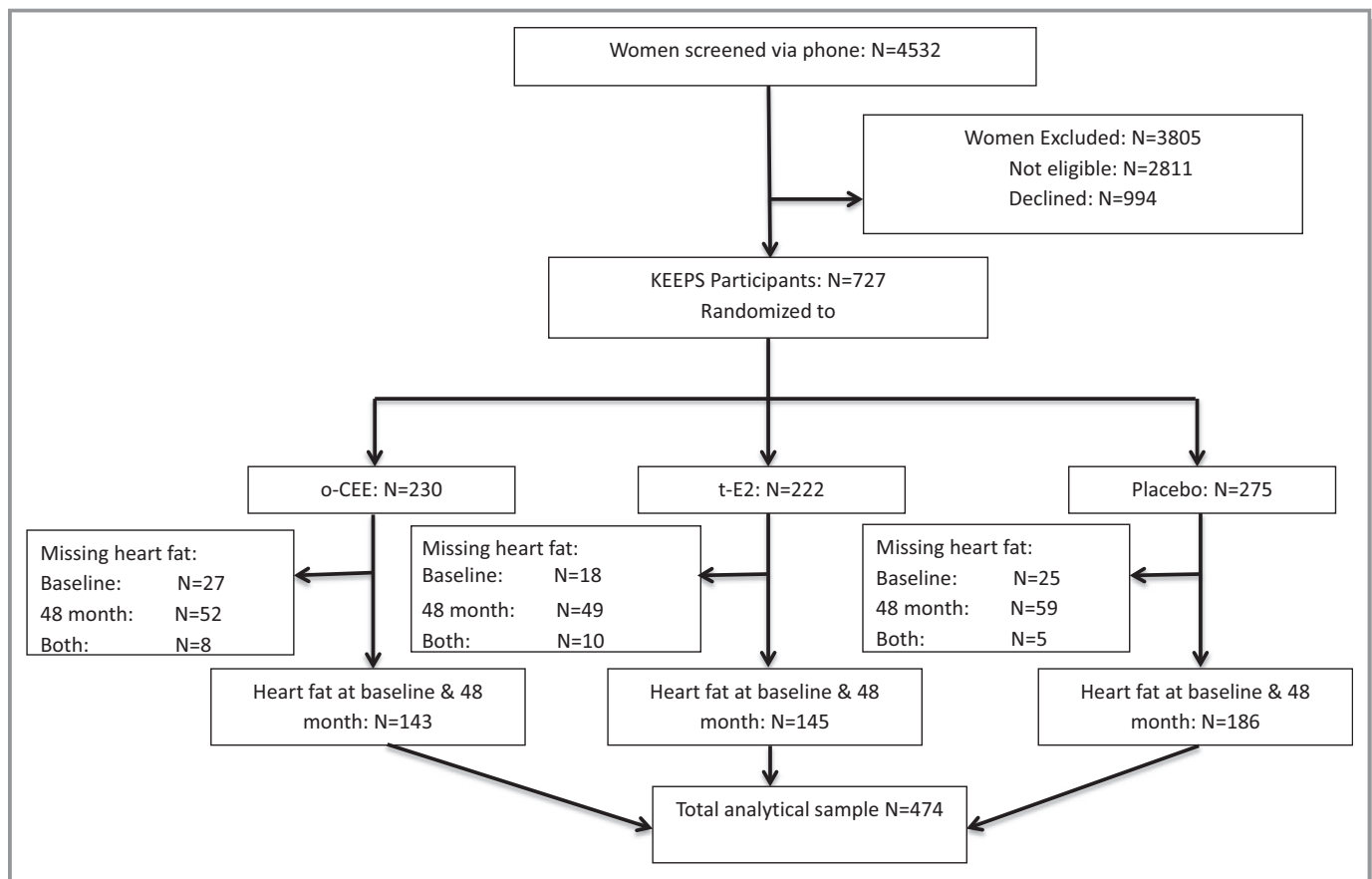
The main aims of this study were (1) to assess the effect of oral and transdermal HT formulations versus placebo on heart fat accumulations over 48 months of treatment and (2) to examine the extent to which HT formulations modify the longitudinal associations of heart fat depots with CAC progression in recently menopausal women.

## Methods

The authors declare that all supporting results are available within the article and its online supplementary files.

## Study Design and Participants

This study is a secondary analysis of data from KEEPS (ClinicalTrials.gov identifier: NCT00154180) and a completed ancillary study of KEEPS on ectopic fat. KEEPS participants were recruited between July 2005 and June 2008 and followed for 48 months. Visits were completed by March 2012. Full KEEPS inclusion and exclusion criteria have been published elsewhere.<sup>21</sup> Briefly, women were eligible if they had an intact uterus, were aged 42 to 58 years, were between 6 and 36 months from their last menses, and had plasma follicle-stimulating hormone levels  $\geq 35$  IU/L, E2 levels  $< 147$  pmol/L, or both. Women reporting a history of clinical CVD, current heavy smoking, body mass index (calculated as  $\text{kg}/\text{m}^2$ )  $\geq 35$ , LDL (low-density lipoprotein) cholesterol  $> 190$  mg/dL or triglycerides  $> 400$  mg/dL, diabetes mellitus, uncontrolled hypertension, or moderate subclinical CVD, defined as a CAC score  $\geq 50$ , were ineligible for randomization. Former or current HT users were screened only after having discontinued therapy for  $\geq 90$  days. A total of 727 women (77% white, 7% black, 3% Asian, 7% Hispanic, and 6% other) met inclusion criteria and were randomized to (1) oral-conjugated equine estrogens (o-CEE; 0.45 mg/d,  $n=230$ , 31.6%), (2) transdermal 17 $\beta$ -estradiol (t-E2; 50  $\mu\text{g}/\text{d}$ ,  $n=222$ , 30.5%), or (3) placebo (inactive pill and patch,  $n=275$ , 37.8%). Women receiving o-



**Figure 1.** CONSORT flow diagram of the KEEPS (Kronos Early Estrogen Prevention Study) heart fat ancillary study. CONSORT indicates Consolidated Standards of Reporting Trials; o-CEE, oral conjugated equine estrogens; t-E2, transdermal  $\beta$ 17-estradiol.

CEE or t-E2 also received oral micronized progesterone 200 mg/d for the first 12 days each month.

An ancillary study designed to measure ectopic fat before (baseline) and 48 months after randomization was conducted in KEEPS. The current analysis excluded all KEEPS participants who did not have heart fat and CAC measured at both baseline and 48 months, leaving 474 participants in the analytical sample (Figure 1). Excluded participants were more likely to have a longer time since menopause, to not be a college graduate, or to be a current smoker. Moreover, they had significantly lower baseline PAT volume than those who were included (Table S1).

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

### Coronary Artery Calcification

CAC was a designated end point of the KEEPS main trial assessed at baseline and at 48 months.<sup>23,24</sup> Images of the coronary arteries were obtained using high-speed axial tomography with rapid acquisition of 30 to 40 contiguous 3-mm-thick images during end diastole using ECG triggering

during a single 30- to 35-second breath hold.<sup>23</sup> CAC score was quantified using the Agatston scoring method<sup>24</sup> by an experienced reader blinded to study group. CAC was defined as a hyperattenuating lesion >130 Hounsfield units, with an area of at least 3 pixels. The total calcification score was the sum of the individual scores for the 4 major epicardial coronary arteries. A mean Agatston score was calculated from the results of 2 sequential scans among all participants for more precise estimates. CAC progression was defined using similar criteria applied in other studies of participants with low CVD risk.<sup>25</sup> CAC was present when baseline CAC score was 0 and 48-month CAC score was >0, when baseline CAC score was >0 and <100 and annualized change in CAC score was  $\geq 10$ , or when baseline CAC score was  $\geq 100$  and annualized percentage change in CAC score was  $\geq 10\%$  (by design, all KEEPS participants had CAC scores <50 at baseline; therefore, none of the participants met this criterion).

### Heart Fat Depots

Heart fat depot volumes were measured as part of a study ancillary to KEEPS (2009–2012) that utilized existing

computed tomography scans before randomization (baseline) and 48 months after randomization. All images were read centrally by experienced readers who were blinded to study group, as described previously.<sup>26</sup> Briefly, 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 volume analysis software, 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 intraclass correlation coefficients between readers (intrareader) were 0.99 each for EAT and 0.86 and 0.96, respectively, for PAT. Similarly, both Spearman and intraclass correlation coefficients between repeated readings (interreader) were 0.98 each for EAT and 0.96 and 0.90, respectively, for PAT.<sup>26</sup>

## Covariates

At baseline and after 48 months of randomization, fasting levels of serum total cholesterol, HDL (high-density lipoprotein) cholesterol, LDL cholesterol, and triglycerides were measured at the Kronos Science Laboratory (KSL) using standard methods. Glucose and insulin levels were also measured through studies ancillary to KEEPS. Total, HDL, and LDL cholesterol and triglycerides were assayed at KSL using Carolina Liquid Chemistries reagent on the Stanbio SIRRUS chemistry analyzer. For total cholesterol, the intra-assay coefficient of variation (CV) was 1.4% to 2.2% and the interassay CV was 4.3% to 5.0%. For HDL cholesterol, the intra-assay CV was 2.7% to 3.1% and the interassay CV was 3.5% to 3.8%. For LDL cholesterol, the intra-assay CV was 1.3% to 1.5% and the interassay CV was 5.3% to 7.1%. For triglycerides, both the intra-assay and interassay CVs were 5.5% to 5.6%.

Glucose was measured at KSL on the Stanbio SIRRUS chemistry analyzer using the Stanbio reagent standard, with an intra-assay CV of 1.7% to 1.3%, and an interassay CV of 2.2% to 2.5%. Insulin assays were conducted at KSL on the Immulite 2000 by solid-phase, chemiluminescent immunometric assay with a method detection limit of 2  $\mu$ IU/mL, intra-assay CV of 2.6% to 2.8%, and interassay CV of 2.8% to 3.3%. The homeostasis model assessment of insulin resistance was calculated as described by Matthews et al.<sup>27</sup>

Demographics, race/ethnicity, income, employment status, education level, history of smoking, medication use, alcohol consumption, and physical activity (metabolic equivalents, calculated as total energy expenditure from recreational physical activity in kcal/wk per kg)<sup>26</sup> were all collected at screening or baseline visits.<sup>22</sup> Physical measures were obtained at baseline and 48 months. Body mass index was calculated from measured weight in kilograms divided by height in square meters. Waist circumference (in cm) was measured at the smallest horizontal circumference using nonstretchable tape. Blood pressure was assessed in the right arm after at least 5 minutes of rest and averaged across 2 readings.

## Statistical Analysis

Comparisons across the 3 treatment groups were performed using ANOVA for normally distributed variables or the Kruskal–Wallis test for skewed variables; the  $\chi^2$  or Fisher exact test was used for categorical variables. Change in EAT and PAT at 48 months was calculated as the difference between baseline and 48-month values. The distribution of EAT and PAT changes were highly skewed, and transformations did not improve the distribution; therefore, nonparametric tests were used to assess within- and between-group differences in 48-month heart fat changes. In addition, logistic regression was used to test whether any increase in each heart fat depot (48-month change  $>0$ ) differed by treatment group. For CAC progression, associations between 48-month changes in each heart fat depot and CAC progression were evaluated using logistic regression. Covariates were selected a priori and based on univariate analysis showing an association with CAC progression at  $P < 0.1$ . For variables that were highly correlated, models were assessed with each one separately, and the models with the best model-fit diagnostics were chosen. Moderation effects of HT use were assessed, and treatment group-specific odds ratios (95% CI) per 1-SD increase in change in heart fat depot were calculated.

We conducted inverse probability weighting sensitivity analyses<sup>28</sup> to account for missing data on heart fat depots and CAC at 48 months ( $n=160$ ). With this method, completed cases are weighted by the inverse of their probability of being a completed case (Tables S1–S7). SAS v9.4 (SAS Institute) and STATA (release 15; StataCorp) were used for statistical analyses.

## Results

Participants' baseline variables and CAC progression are presented in Table 1. No significant differences were observed in baseline EAT and PAT volumes among the treatment groups (Table 2).

**Table 1.** Baseline Variables and CAC Progression for Overall Analytical Sample and by Treatment Group

Variable	Total N=474	o-CEE n=143 (30.2%)	t-E2 n=145 (30.6%)	Placebo n=186 (39.2%)	P Value*
Age at baseline, y, mean (SD)	52.7 (2.6)	52.8 (2.8)	52.8 (2.6)	52.5 (2.4)	0.23
Age at menopause, y, mean (SD)	50.9 (2.6)	51.0 (2.9)	50.9 (2.6)	50.8 (2.3)	0.36
Time since menopause, y, mean (SD)	1.8 (0.8)	1.8 (0.8)	1.8 (0.7)	1.7 (0.8)	0.33
White race, n (%)	370 (78.1)	112 (78.3)	111 (76.6)	147 (79.0)	0.86
Education, n (%)					0.06
Declined to answer	5 (1.1)	0 (0.0)	2 (1.4)	3 (1.6)	
High school graduate or less	29 (6.1)	11 (7.7)	3 (2.1)	15 (8.1)	
Some college	80 (16.9)	28 (19.6)	20 (13.8)	32 (17.2)	
College graduate	360 (75.9)	104 (72.7)	120 (82.8)	136 (73.1)	
Employed, n (%)	391 (82.5)	122 (85.3)	120 (82.8)	149 (80.1)	0.47
Income, n (%)					0.56
<\$60 000	84 (17.7)	27 (18.9)	29 (20.0)	28 (15.1)	
\$60 000 to <\$100 000	65 (13.7)	17 (11.9)	19 (13.1)	29 (15.6)	
>\$100 000	85 (17.9)	23 (16.1)	22 (15.2)	40 (21.5)	
Unknown	240 (50.6)	76 (53.1)	75 (51.7)	89 (47.8)	
Physical activity level, MET-h/wk, median (IQR)	16.7 (7.0, 28.6)	17.5 (7.4, 32.7)	14.6 (5.3, 24.6)	17.2 (7.5, 28.0)	0.16
Alcohol consumption, n (%)	361 (76.2)	120 (83.9)	103 (71.0)	138 (74.2)	0.03
Smoking status, n (%)					0.77
Never	380 (80.2)	119 (83.2)	115 (79.3)	146 (78.5)	
Past	70 (14.8)	19 (13.3)	21 (14.5)	30 (16.1)	
Current	24 (5.1)	5 (3.5)	9 (6.2)	10 (5.4)	
Ever use HT, n (%)	100 (21.1)	36 (25.2)	29 (20.0)	35 (18.8)	0.35
Antihypertensive medication use, n (%)					0.50
Never	392 (82.7)	115 (80.4)	119 (82.1)	158 (84.9)	
Past	26 (5.5)	11 (7.7)	9 (6.2)	6 (3.2)	
Current	56 (11.8)	17 (11.9)	17 (11.7)	22 (11.8)	
BMI, kg/m <sup>2</sup> , mean (SD)	26.0 (4.3)	26.1 (4.2)	25.7 (4.4)	26.3 (4.3)	0.55
Waist circumference, cm, mean (SD)	84.4 (11.7)	84.3 (11.2)	83.4 (11.9)	85.1 (11.9)	0.48
Systolic blood pressure, mm Hg, mean (SD)	118.4 (15.1)	119.3 (14.7)	116.6 (16.0)	119.1 (14.7)	0.99
HDL cholesterol, mg/dL, mean (SD)	72.2 (15.0)	72.8 (15.1)	74.5 (16.6)	70.0 (13.4)	0.06
LDL cholesterol, mg/dL, mean (SD)	111.4 (27.5)	111.2 (26.7)	111.1 (28.9)	111.8 (27.0)	0.84
Triglycerides, mg/dL, median (IQR)	69.5 (50.0, 105.5)	69.0 (50.0, 105.0)	66.0 (47.0, 101.0)	73.5 (55.0, 110.0)	0.16
Fasting glucose, mg/dL, median (IQR)	78.5 (74.0, 85.5)	79.0 (74.0, 84.0)	78.0 (74.0, 84.0)	79.0 (73.0, 87.0)	0.66
Insulin, $\mu$ U/mL, median (IQR)	4.2 (2.0, 7.4)	3.9 (1.0, 6.9)	3.7 (1.0, 6.7)	4.7 (2.1, 8.3)	0.28
HOMA, median (IQR)	0.8 (0.3, 1.5)	0.8 (0.3, 1.3)	0.8 (0.2, 1.4)	0.9 (0.4, 1.7)	0.24
CAC Agatston score, median (IQR)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.35
Any CAC (>0), n (%)	55 (11.6)	12 (8.4)	20 (13.8)	23 (12.4)	0.33
CAC progression, n (%)	66 (13.9)	20 (14.0)	19 (13.1)	27 (14.5)	0.93

BMI indicates body mass index; CAC, coronary artery calcification; HDL, high-density lipoprotein; HOMA, homeostasis model assessment of insulin resistance index; HT, hormone therapy; IQR, interquartile range; LDL, low-density lipoprotein; MET, metabolic equivalents; o-CEE, oral conjugated equine estrogen; t-E2, transdermal 17 $\beta$ -estradiol.

\* $\chi^2$  test was used for categorical variables, and ANOVA or the Kruskal–Wallis test was used for continuous variables, as appropriate.



**Table 2.** Medians of EAT and PAT 48-Month Changes Overall and by Treatment Group

	EAT Volume, cm <sup>3</sup> , Median (IQR)				PAT Volume, cm <sup>2</sup> , Median (IQR)			
	Baseline	48 mo	Change	P Value*	Baseline	48 mo	Change	P Value*
All	36.8 (25.6, 54.5)	37.6 (26.3, 50.5)	1.3 (−4.9, 5.7)	0.004	14.5 (10.2, 21.3)	14.1 (10.0, 20.8)	0.5 (−3.5, 3.9)	0.13
o-CEE	40.6 (28.6, 59.0)	39.9 (28.8, 52.9)	−0.1 (−5.9, 6.1)	>0.99	14.6 (11.1, 22.1)	15.1 (10.2, 22.5)	0.9 (−3.9, 4.5)	0.18
t-E2	35.9 (25.0, 48.3)	35.7 (25.3, 46.6)	1.7 (−4.1, 4.9)	0.07	13.3 (8.6, 20.8)	13.3 (9.4, 18.9)	0.4 (−3.2, 3.6)	0.32
Placebo	36.1 (25.6, 51.7)	37.9 (25.2, 51.9)	2.1 (−4.6, 6.4)	0.003	14.9 (10.5, 22.1)	14.4 (10.5, 20.0)	0.2 (−3.7, 4.0)	0.83
P value†	0.07	0.07	0.40		0.12	0.13	0.89	

EAT indicates epicardial adipose tissue; IQR, interquartile range; o-CEE, oral conjugated equine estrogen; PAT, paracardial adipose tissue; t-E2, transdermal 17β-estradiol.

\*Sign test.

†Kruskal–Wallis test was used to compare the medians.

PAT and EAT changes did not differ significantly across treatment groups. EAT did not increase in the o-CEE group, increased marginally in the t-E2 group, and increased significantly in the placebo group. PAT did not change in any group (Table 2). When analyzing any increase in EAT or PAT (change >0) as related to treatment groups, women on o-CEE had significantly lower risk of developing any increase in EAT compared with placebo and also tended to have lower risk than the t-E2 group (Table 3).

CAC progressed in 14% of the study participants. Women who had CAC progression were more likely to be less educated, to have a longer time since menopause, to have higher triglycerides, insulin, and insulin resistance indexes compared with women who did not show CAC progression (Table S2).

Overall, changes in EAT and PAT were not significantly associated with CAC progression (Table S3). Assigned treatment significantly modified the association between PAT changes (Figure 2B), but not EAT changes (although a similar trend was observed; Figure 2A), and CAC progression in models that were both minimally adjusted (model 1) and fully adjusted (model 2;  $P \leq 0.02$ ; Table S4). PAT changes were associated with greater CAC progression risk only in the t-E2

group (Figure 2B). Further adjustment for time since menopause and index of homeostasis model assessment of insulin resistance provided similar results (data not shown). The results from the sensitivity analyses that accounted for missing data with inverse probability weighting were consistent with results from the main analyses, which excluded missing data (Tables S5–S7).

## Discussion

The current results provide the first evidence of potential differential impacts of exogenous estrogen preparations on heart fat accumulations and their associations with CAC development in recently menopausal women. We did not find a significant difference in PAT or EAT changes across treatment groups. However, early menopausal women on o-CEE demonstrated no change in EAT volume over 48 months relative to women randomized to placebo, whose EAT increased over time. PAT did not change in any group. In addition, we found that the use of t-E2, but not o-CEE, augmented the positive association between increases in PAT and CAC progression.

Heart fat is associated with coronary arterial disease risk factors, CVD events, and all-cause mortality.<sup>4–11</sup> Compared with premenopausal women, postmenopausal women have higher volumes of fat in heart fat depots.<sup>1</sup> Greater PAT volume is significantly associated with atherosclerosis risk,<sup>13</sup> and such associations are more evident at lower levels of E2,<sup>1,13</sup> suggesting a possible impact of estrogen on heart fat accumulation and raising the question of whether exogenous estrogen use could affect heart fat depots. Our study suggests that this is the case and that the specific exogenous estrogen preparations and/or routes of administration may contribute to or protect against heart fat deposition and CVD risk. The findings also underscore the likelihood that EAT and PAT are metabolically distinct adipose tissue depots that should be evaluated separately in assessing their contributions to CVD risk.<sup>2,3</sup>

**Table 3.** Any Increase in Heart Fat Depots Over 48 Months by Treatment Group

Treatment	EAT*		PAT†	
	OR (95% CI)	P Value	OR (95% CI)	P Value
		0.10		0.66
o-CEE	0.62 (0.40, 0.97)	0.03	1.22 (0.79, 1.88)	0.38
t-E2	0.87 (0.56, 1.35)	0.54	1.12 (0.72, 1.72)	0.62
Placebo	...	...	...	...

EAT indicates epicardial adipose tissue; o-CEE, oral conjugated equine estrogen; OR, odds ratio; PAT, paracardial adipose tissue; t-E2: transdermal 17β-estradiol.

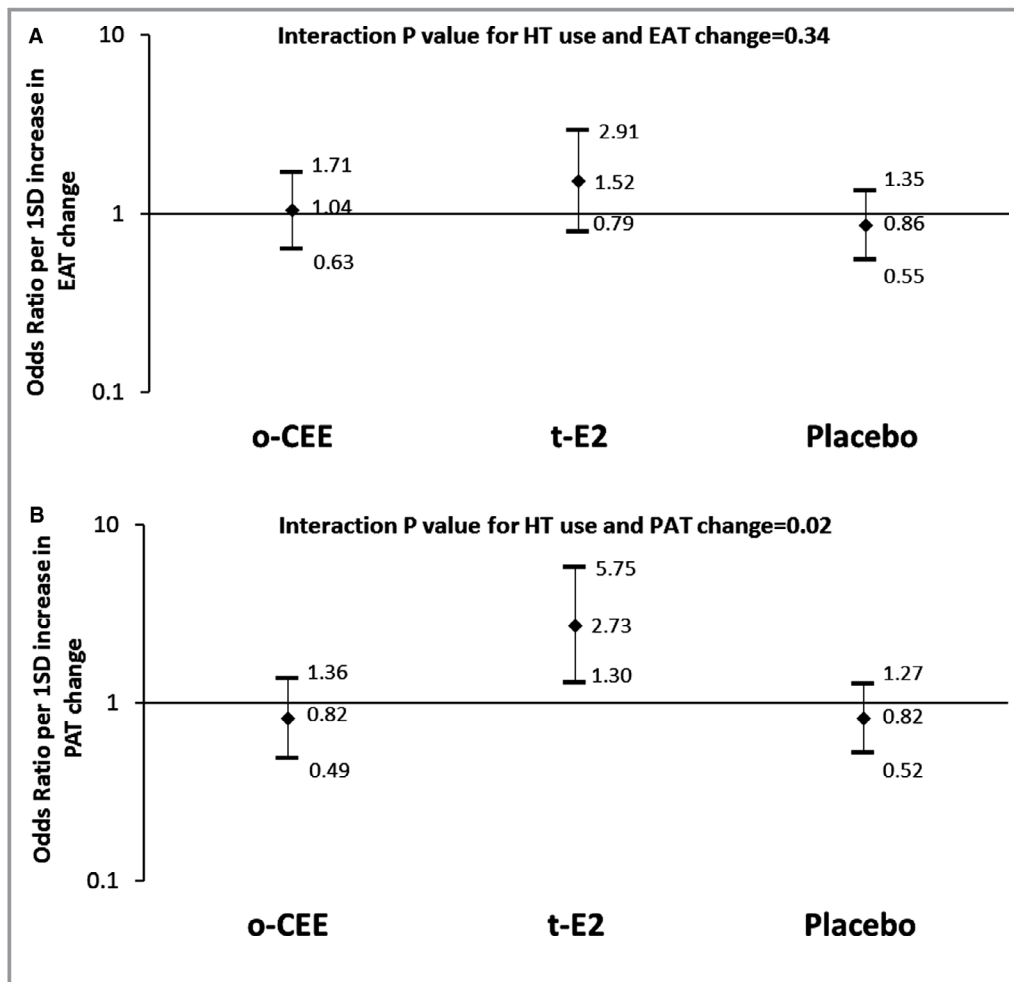
\*EAT: o-CEE vs t-E2, OR: 0.72 (95% CI, 0.45, 1.14);  $P=0.05$ .

†PAT: o-CEE vs t-E2, OR: 1.09 (95% CI, 0.69, 1.74);  $P=0.48$ .

We are not aware of any other clinical trial of postmenopausal women that assessed the effect of exogenous estrogen on heart fat accumulations. However, several small scale clinical trials evaluated effects of different HT preparations on other fat depots and adiposity measures. Results of these studies were not consistent.<sup>29–37</sup> The available data suggest that exogenous estrogen likely plays a complex role in adipose tissue accumulation that could vary by the type and route of administration of estrogen used, by the type of fat depot, and possibly by age, menopausal duration, or other population characteristics. Most recently, different HT preparations and routes of administration were found to be associated with different levels of risk for venous thromboembolism in 2 nested case–control studies that utilized large databases in the United Kingdom.<sup>38</sup> This new evidence

suggests that HT use produces different effects based on type, combination, dose, and route of administration of the preparations used.

In line with these results, we found differential effects of o-CEE and t-E2 on heart fat depots and their associations with CAC. Other exogenous estrogen preparations such as oral micronized 17 $\beta$ -estradiol, ethinyl estradiol, and estrone might produce other effects. Interestingly, the transdermal estrogen used in this trial contains only 17 $\beta$ -estradiol, whereas o-CEE is a complex of at least 10 natural estrogens including estrone, 17 $\beta$ -estradiol, and ring B unsaturated estrogens (equilin and equilenin) with almost 50% as estrone and  $\leq$ 1% as 17 $\beta$ -estradiol.<sup>39</sup> Ring B unsaturated estrogens are believed to be stronger antioxidants than 17 $\beta$ -estradiol and estrone, and some ring B unsaturated estrogens could act selectively in



**Figure 2.** Effect modification of assigned HT use on the association between the change in EAT (A) or PAT (B) and CAC progression. Odds ratio (95% CI) represents the increase in the risk of coronary artery calcification progression per 1-SD increase in the change of heart fat. Models adjusted for age, race and study site, education, smoking, physical activity, alcohol consumption, lipids, systolic blood pressure, waist circumference, antihypertensive medication and treatment, and baseline heart fat volume. EAT indicates epicardial adipose tissue; HT, hormone therapy; o-CEE, oral conjugated equine estrogens; PAT, paracardial adipose tissue; t-E2, transdermal  $\beta$ 17-estradiol.

target tissues.<sup>39,40</sup> The stronger antioxidant property of ring B unsaturated estrogens in o-CEE likely contributes to the observed favorable impact of o-CEE on EAT accumulation in this study. Weak antioxidant mechanism together with overexpression of oxidative stress could contribute to adiposity-related complications.<sup>41</sup>

Growing evidence supports vital roles of estrogen signaling in the development and function of adipose tissue.<sup>42–45</sup> Most of estrogen's effects are mediated by estrogen receptor  $\alpha$  (ER $\alpha$ ) and ER $\beta$ ,<sup>41</sup> both of which are expressed in human mature white adipocytes with a relative abundance of ER $\alpha$ .<sup>46,47</sup> ER $\alpha$  plays a critical role in maintaining adipose tissue function and preventing inflammatory damage.<sup>48</sup> However, functionality of ER $\alpha$  depends on circulating estrogen levels. In the absence of adequate estrogen levels, adipocyte ER $\alpha$  may contribute to adipose tissue expansion and adipose tissue macrophage infiltration.<sup>48</sup> When the ligand is lacking, ER $\alpha$  may bind to other estrogen response elements, adipocyte function-specific factors, or other signaling factors that could produce detrimental metabolic effects or prompt a differential signaling program.<sup>48</sup> It is possible that the use of t-E2 in this study did not raise circulating estrogens to an adequate level to prevent the adverse metabolic activity of heart fat accumulation, explaining the negative impact of t-E2 use on the association between CAC progression and PAT accumulation in our study. Interestingly, we observed a similar, but not significant, effect modification of t-E2 on the association between EAT accumulation and CAC progression. In addition, we reported a marginal increase in EAT after 48 months of t-E2 use.

Despite the neutral effect of HT use on CAC progression among recently menopausal women reported in the original KEEPS trial,<sup>22</sup> we found that HT use significantly modifies the association between PAT increase and CAC progression in the same target population. The nonsignificant effect of HT use observed in the overall KEEPS trial was attributed to insufficient power to compare progression of CAC among treatment groups. In particular, KEEPS included only relatively healthy women with CAC scores <50 at baseline; therefore, it was challenging to detect modest progression in CAC with 48 months of follow-up.<sup>22</sup> This criterion may have limited our ability to detect main associations between heart fat depots and CAC progression, as in other studies.<sup>4–11</sup>

This study has several inherent strengths and weaknesses. The ability to study a sample of well-characterized, community-dwelling women randomized to 2 different estrogen preparations is a paramount strength. However, some limitations are imposed by the design of KEEPS, as described earlier. Furthermore, the current findings are specific to the KEEPS population, and the durations, types, and doses of HT used cannot be readily generalized. Finally, participants excluded because of missing heart fat data at any of the 2 time points had lower PAT volumes at baseline

than those included, and this may have reduced variability in PAT compared with EAT. However, sensitivity analyses accounting for missing data suggested similar findings. Regardless, this study is the first to assess the effects of 2 different routes of administration and estrogen preparations on heart fat accumulation, an evolving menopause-specific risk factor.<sup>1,13</sup>

## Clinical Implications and Conclusions

Our results are in line with the recent North American Menopause Society position statement on HT, which underscores the differential contributions of estrogen type, dose, duration of use, route of administration, timing of initiation, and whether a progestogen is used when assessing risk related to HT use.<sup>49</sup> We reported differential effects of HT use on heart fat depots in recently menopausal women. Use of o-CEE may slow EAT accumulation, whereas t-E2 appears to augment the association between PAT accumulation and CAC progression. HT remains the best therapeutic option for the relief of debilitating menopausal symptoms.<sup>49</sup> Clinicians should continue to individualize HT prescription using best available evidence to maximize benefits and minimize risks.

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## Author Contributions

El Khoudary, Venugopal, and Black have full access to all of the analyzed data in the study and take responsibility for the integrity of the data. El Khoudary, Qian, and Venugopal take responsibility for the accuracy of the data analysis. Concept and design: El Khoudary, Brooks, Manson, Santoro, Black, Harman, and Budoff. Acquisition, analysis, or interpretation of data: all authors. Drafting of the article: El Khoudary, Critical revision of the article for important intellectual content: all authors, Statistical analysis: Zhao and Venugopal, Final approval of the version to be published: all authors. Heart fat ancillary study funding: El Khoudary.



## Institutional Review Board Numbers for KEEPS Institutions

- Central KEEPS (Kronos Early Estrogen Prevention Study) and Phoenix KEEPS (institutional review board protocol by the Western Institutional Review Board (WIRB)): study no. 1058663 and WIRB PRO no. 20040792; KEEPS (main study and cognitive substudy) no. 10-02980 and Mammographic Density And Breast Health Ancillary Study no. 11-05383.
- Brigham and Women's Hospital (partners): 2004-P-002144 BWH.
- Mayo Clinic: 2241-04.
- Columbia: AAAA-8062.
- Yale: 0409027022.
- University of Utah: 13257.
- Einstein/Montefiore: 04-08-213.
- University of Wisconsin: H-2005-0059.
- University of California, San Francisco: KEEPS (main study and cognitive substudy) no. 10-02980.
- University of Washington: 26702; Veterans Administration Puget Sound Health Care System: 01048.

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## References

1. El Khoudary SR, Shields KJ, Janssen I, Hanley C, Budoff MJ, Barinas-Mitchell E, Everson-Rose SA, Powell LH, Matthews KA. Cardiovascular fat, menopause, and sex hormones in women: the SWAN cardiovascular fat ancillary study. *J Clin Endocrinol Metab*. 2015;100:3304–3312.
2. Iacobellis G, Willens HJ. Echocardiographic epicardial fat: a review of research and clinical applications. *J Am Soc Echocardiogr*. 2009; 22:1311–1319. quiz 1417–8.
3. Kaushik M, Reddy YM. Distinction of “fat around the heart”. *J Am Coll Cardiol*. 2011;5:1640; author reply 1640-1.

4. Rosito GA, Massaro JM, Hoffmann U, Ruberg FL, Mahabadi AA, Vasan RS, O'Donnell CJ, Fox CS. Pericardial fat, visceral abdominal fat, cardiovascular disease risk factors, and vascular calcification in a community-based sample: the Framingham Heart Study. *Circulation*. 2008;117:605–613.
5. Mahabadi AA, Lehmann N, Kälsch H, Robens T, Bauer M, Dykun I, Budde T, Moebus S, Jöckel KH, Erbel R, Möhlenkamp S. Association of epicardial adipose tissue with progression of coronary artery calcification is more pronounced in the early phase of atherosclerosis: results from the Heinz Nixdorf Recall Study. *JACC Cardiovasc Imaging*. 2014;7:909–916.
6. Mahabadi AA, Massaro JM, Rosito GA, Levy D, Murabito JM, Wolf PA, O'Donnell CJ, Fox CS, Hoffmann U. Association of pericardial fat, intrathoracic fat, and visceral abdominal fat with cardiovascular disease burden: the Framingham Heart Study. *Eur Heart J*. 2009;30:850–856.
7. Ding J, Hsu FC, Harris TB, Liu Y, Kritchevsky SB, Szklo M, Ouyang P, Espeland MA, Lohman KK, Criqui MH, Allison M, Bluemke DA, Carr JJ. The association of pericardial fat with incident coronary heart disease: the Multi-Ethnic Study of Atherosclerosis (MESA). *Am J Clin Nutr*. 2009;90:499–504.
8. Mahabadi AA, Berg MH, Lehmann N, Kälsch H, Bauer M, Kara K, Dragano N, Moebus S, Jöckel KH, Erbel R, Möhlenkamp S. Association of epicardial fat with cardiovascular risk factors and incident myocardial infarction in the general population: the Heinz Nixdorf Recall Study. *J Am Coll Cardiol*. 2013;61:1388–1395.
9. Larsen BA, Laughlin GA, Saad SD, Barrett-Connor E, Allison MA, Wassel CL. Pericardial fat is associated with all-cause mortality but not incident CVD: the Rancho Bernardo Study. *Atherosclerosis*. 2015;239:470–475.
10. Cetin M, Cakici M, Polat M, Suner A, Zencir C, Ardic I. Relation of epicardial fat thickness with carotid intima-media thickness in patients with type 2 diabetes mellitus. *Int J Endocrinol*. 2013;2013:769175.
11. Abazid RM, Smettei OA, Kattea MO, Sayed S, Saqqah H, Widyam AM, Opolski MP. Relation between epicardial fat and subclinical atherosclerosis in asymptomatic individuals. *J Thorac Imaging*. 2017;32:378–382.
12. Iacobellis G, Gao YJ, Sharma AM. Do cardiac and perivascular adipose tissue play a role in atherosclerosis? *Curr Diab Rep*. 2008;8:20–24.
13. El Khoudary SR, Shields KJ, Janssen I, Budoff MJ, Everson-Rose SA, Powell LH, Matthews KA. Postmenopausal women with greater paracardial fat have more coronary artery calcification than premenopausal women: the SWAN cardiovascular fat ancillary study. *J Am Heart Assoc*. 2017;6:e004545 DOI: 10.1161/JAHA.116.004545.
14. Nkonde-Price C, Bender JR. Menopause and the heart. *Endocrinol Metab Clin North Am*. 2015;44:559–564.
15. Hulley S, Grady D, Bush T, Furberg C, Herrington D, Riggs B, Vittinghoff E. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group. *JAMA*. 1998;280:605–613.
16. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM, Ockene J; Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. *JAMA*. 2002;288:321–333.
17. Naftolin F, Taylor HS, Karas R, Brinton E, Newman I, Clarkson TB, Mendelsohn M, Lobo RA, Judelson DR, Nachtigall LE, Heward CB, Hecht H, Jaff MR, Harman SM; Women's Health Initiative. The Women's Health Initiative could not have detected cardioprotective effects of starting hormone therapy during the menopausal transition. *Fertil Steril*. 2004;81:1498–1501.
18. Manson JE, Chlebowski RT, Stefanick ML, Aragaki AK, Rossouw JE, Prentice RL, Anderson G, Howard BV, Thomson CA, LaCroix AZ, Wactawski-Wende J, Jackson RD, Limacher M, Margolis KL, Wassertheil-Smoller S, Beresford SA, Cauley JA, Eaton CB, Gass M, Hsia J, Johnson KC, Kooperberg C, Kuller LH, Lewis CE, Liu S, Martin LW, Ockene JK, O'Sullivan MJ, Powell LH, Simon MS, Van Horn L, Vitamins MZ, Wallace RB. Menopausal hormone therapy and health outcomes during the intervention and extended poststopping phases of the Women's Health Initiative randomized trials. *JAMA*. 2013;310:1353–1368.
19. Stuenkel CA, Gass ML, Manson JE, Lobo RA, Pal L, Rebar RW, Hall JE. A decade after the Women's Health Initiative—the experts do agree. *J Clin Endocrinol Metab*. 2012;97:2617–2618.
20. Goodman MP. Are all estrogens created equal? A review of oral vs. transdermal therapy. *J Womens Health (Larchmt)*. 2012;21:161–169.
21. Harman SM, Brinton EA, Cedars M, Lobo R, Manson JE, Merriam GR, Miller VM, Naftolin F, Santoro N. KEEPS: the Kronos Early Estrogen Prevention Study. *Climacteric*. 2005;8:3–12.
22. Harman SM, Black DM, Naftolin F, Brinton EA, Budoff MJ, Cedars MI, Hopkins PN, Lobo RA, Manson JE, Merriam GR, Miller VM, Neal-Perry G, Santoro N, Taylor HS, Vittinghoff E, Yan M, Hodis HN. Arterial imaging outcomes and cardiovascular risk factors in recently menopausal women: a randomized trial. *Ann Intern Med*. 2014;161:249–260.
23. Nasir K, Raggi P, Rumberger JA, Braunstein JB, Post WS, Budoff MJ, Blumenthal RS. Coronary artery calcium volume scores on electron beam tomography in 12,936 asymptomatic adults. *Am J Cardiol*. 2004;93:1146–1149.
24. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol*. 1990;15:827–832.
25. Berry JD, Liu K, Folsom AR, Lewis CE, Carr JJ, Polak JF, Shea S, Sidney S, O'Leary DH, Chan C, Lloyd-Jones DM. Prevalence and progression of subclinical atherosclerosis in younger adults with low short-term but high lifetime estimated risk for cardiovascular disease: the coronary artery risk development in young adults study and Multi-Ethnic Study of Atherosclerosis. *Circulation*. 2009;119:382–389.
26. Huang G, Wang D, Zeb I, Budoff MJ, Harman SM, Miller V, Brinton EA, El Khoudary SR, Manson JE, Sowers MR, Hodis HN, Merriam GR, Cedars MI, Taylor HS, Naftolin F, Lobo RA, Santoro N, Wildman RP. Intra-thoracic fat, cardiometabolic risk factors, and subclinical cardiovascular disease in healthy, recently menopausal women screened for the Kronos Early Estrogen Prevention Study (KEEPS). *Atherosclerosis*. 2012;221:198–205.
27. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28:412–419.
28. Seaman SR, White IR. Review of inverse probability weighting for dealing with missing data. *Stat Methods Med Res*. 2013;22:278–295.
29. Kristensen K, Pedersen SB, Vestergaard P, Mosekilde L, Richelsen B. Hormone replacement therapy affects body composition and leptin differently in obese and non-obese postmenopausal women. *J Endocrinol*. 1999;163:55–62.
30. Gambacciani M, Ciaponi M, Cappagli B, Piaggini L, De Simone L, Orlandi R, Genazzani ARJ. Body weight, body fat distribution, and hormonal replacement therapy in early postmenopausal women. *J Clin Endocrinol Metab*. 1997;82:414–417.
31. Reubinoff BE, Wurtman J, Rojansky N, Adler D, Stein P, Schenker JG, Brzezinski A. Effects of hormone replacement therapy on weight, body composition, fat distribution, and food intake in early postmenopausal women: a prospective study. *Fertil Steril*. 1995;64:963–968.
32. Mattiasson I, Rendell M, Törnquist C, Jeppsson S, Hulthén UL. Effects of estrogen replacement therapy on abdominal fat compartments as related to glucose and lipid metabolism in early postmenopausal women. *Horm Metab Res*. 2002;34:583–588.
33. Sites CK, L'Hommedieu GD, Toth MJ, Brochu M, Cooper BC, Fairhurst PA. The effect of hormone replacement therapy on body composition, body fat distribution, and insulin sensitivity in menopausal women: a randomized, double-blind, placebo-controlled trial. *J Clin Endocrinol Metab*. 2005;90:2701–2707.
34. Aloia JF, Vaswani A, Russo L, Sheehan M, Flaster E. The influence of menopause and hormonal replacement therapy on body cell mass and body fat mass. *Am J Obstet Gynecol*. 1995;172:896–900.
35. Yuksel H. Effects of postmenopausal hormone replacement therapy on body fat composition. *Gynecol Endocrinol*. 2007;23:99–104.
36. dos Reis CM, de Melo NR, Meirelles ES, Vezozzo DP, Halpern A. Body composition, visceral fat distribution and fat oxidation in postmenopausal women using oral or transdermal oestrogen. *Maturitas*. 2003;46:59–68.
37. Hänggi W, Lippuner K, Jaeger P, Birkhäuser MH, Horber FF. Differential impact of conventional oral or transdermal hormone replacement therapy or tibolone on body composition in postmenopausal women. *Clin Endocrinol (Oxf)*. 1998;48:691–699.
38. Vinogradova Y, Coupland C, Hippisley-Cox J. Use of hormone replacement therapy and risk of venous thromboembolism: nested case-control studies using the QResearch and CPRD database. *BMJ*. 2019;364:k4810.
39. Bhavnani BR, Stanczyk FZ. Pharmacology of conjugated equine estrogens: efficacy, safety and mechanism of action. *J Steroid Biochem Mol Biol*. 2014;142:16–29.
40. Washburn SA, Honoré EK, Cline JM, Helman M, Wagner JD, Adelman SJ, Clarkson TB. Effects of 17 alpha-dihydroequilenin sulfate on atherosclerotic male and female rhesus monkeys. *Am J Obstet Gynecol*. 1996;175:341–349; discussion 349–51.
41. Marseglia L, Manti S, D'Angelo G, Nicotera A, Parisi E, Di Rosa G, Gitto E, Arrigo T. Oxidative stress in obesity: a critical component in human diseases. *Int J Mol Sci*. 2014;16:378–400.
42. Gao H, Dahlman-Wright K. Implications of estrogen receptor alpha and estrogen receptor beta for adipose tissue functions and cardiometabolic complications. *Horm Mol Biol Clin Investig*. 2013;15:81–90.
43. Barros RP, Gustafsson JA. Estrogen receptors and the metabolic network. *Cell Metab*. 2011;14:289–299.

44. Cooke PS, Naaz A. Role of estrogens in adipocyte development and function. *Exp Biol Med (Maywood)*. 2004;229:1127–1135.
45. Pallottini V, Bulzomi P, Galluzzo P, Martini C, Marino M. Estrogen regulation of adipose tissue functions: involvement of estrogen receptor isoforms. *Infect Disord Drug Targets*. 2008;8:52–60.
46. Dieudonne MN, Leneuve MC, Giudicelli Y, Pecquery R. Evidence for functional estrogen receptors alpha and beta in human adipose cells: regional specificities and regulation by estrogens. *Am J Physiol Cell Physiol*. 2004;286:C655–C661.
47. Rodriguez-Cuenca S, Monjo M, Proenza AM, Roca P. Depot differences in steroid receptor expression in adipose tissue: possible role of the local steroid milieu. *Am J Physiol Endocrinol Metab*. 2005;288:E200–E207.
48. Davis KE, Neinast MD, Sun K, Skiles WM, Bills JD, Zehr JA, Zeve D, Hahner LD, Cox DW, Gent LM, Xu Y, Wang ZV, Khan SA, Clegg DJ. The sexually dimorphic role of adipose and adipocyte estrogen receptors in modulating adipose tissue expansion, inflammation, and fibrosis. *Mol Metab*. 2013;2:227–242.
49. The 2017 hormone therapy position statement of the North American Menopause Society. *Menopause*. 2018;25:1362–1387.

# SUPPLEMENTAL MATERIAL

**Table S1. Baseline variables and CAC progression for women included and excluded from the analytical sample.**

<b>Variables*</b>	<b>Included N=474</b>	<b>Excluded N=253</b>	<b>P value †</b>
<b>Age at baseline, mean (SD), Yr</b>	52.7 (2.6)	52.7 (2.5)	0.70
<b>Age at menopause, mean (SD), Yr</b>	50.9 (2.6)	50.8 (2.6)	0.79
<b>Time since menopause, mean (SD), Yr</b>	1.8 (0.8)	1.9 (0.8)	0.03
<b>White Race, N (%)</b>	370 (78.1)	187 (73.9)	0.21
<b>Education, N (%)</b>			0.01
Declined to Answer	5 (1.1)	5 (2.0)	
High School graduate or less	29 (6.1)	29 (11.5)	
Some college	80 (16.9)	52 (20.6)	
College graduate	360 (75.9)	167 (66.0)	
<b>Employed, N (%)</b>	391 (82.5)	201 (79.4)	0.31
<b>Income, N (%)</b>			0.47
<\$60K	84 (17.7)	55 (21.7)	
\$60K-<\$100K	65 (13.7)	36 (14.2)	
>\$100K	85 (17.9)	37 (14.6)	



Unknown	240 (50.6)	125 (49.4)	
<b>Physical activity level, median(Q1, Q3), MET-hr/wk</b>	16.7 (7.0, 28.6)	17.5 (7.5, 28.3)	0.84
<b>Alcohol consumption, N (%)</b>	361 (76.2)	176 (69.6)	0.05
<b>Smoking Status, N (%)</b>			0.03
Never	380 (80.2)	191 (75.5)	
Past	70 (14.8)	36 (14.2)	
Current	24 (5.1)	26 (10.3)	
<b>Ever use hormone therapy, N (%)</b>	100 (21.1)	52 (20.6)	0.86
<b>Anti-Hypertensive medication use, N (%)</b>			0.29
Never	392 (82.7)	220 (87.0)	
Past	26 (5.5)	12 (4.7)	
Current	56 (11.8)	21 (8.3)	
<b>BMI, mean (SD), Kg/m<sup>2</sup></b>	26.0 (4.3)	26.5 (4.4)	0.20
<b>Waist circumference, mean (SD), cm</b>	84.4 (11.70)	84.3 (11.7)	0.92
<b>Systolic blood pressure, mean (SD), mmHg</b>	118.4 (15.1)	119.6 (14.5)	0.29
<b>HDL cholesterol, mean (SD), mg/dL</b>	72.2 (15.0)	71.7 (13.8)	0.68
<b>LDL cholesterol, mean (SD), mg/dL</b>	111.4 (27.5)	110.1 (28.4)	0.56

<b>Fasting glucose, median (Q1, Q3), mg/dL</b>	78.5 (74.0, 85.5)	78.0 (73.0, 84.0)	0.32
<b>Insulin, median (Q1, Q3), <math>\mu</math>U/mL</b>	4.2 (2.0, 7.4)	4.4 (2.10, 7.2)	0.75
<b>HOMA, median (Q1, Q3)</b>	0.8 (0.3, 1.5)	0.8 (0.4 1.4)	0.88
<b>EAT volume, median (Q1, Q3), cm<sup>3</sup></b>	36.8 (25.6, 54.5)	38.9 (27.7, 53.0)	0.50
<b>PAT volume, median (Q1, Q3), cm<sup>3</sup></b>	14.5 (10.2, 21.3)	6.5 (3.2, 11.8)	<0.001
<b>CAC Agatston score, median (Q1, Q2)</b>	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.24
<b>Any CAC (CAC&gt;0), N (%)</b>	55 (11.6)	37 (14.6)	0.24
<b>CAC progression, N (%)</b>	66 (13.9)	13 (13.5)	0.92
<b>Treatment groups, N (%)</b>			0.44
o-CEE	143 (30.2)	87 (34.4)	
t-E2	145 (30.6)	77 (30.4)	
Placebo	186 (39.2)	89 (35.2)	

CAC: coronary artery calcification; MET: metabolic equivalents; BMI: body mass index; HDL: high density lipoprotein; LDL: low density lipoprotein; HOMA: Homeostasis Model Assessment insulin resistance index EAT: epicardial adipose tissue; PAT: paracardial adipose tissue; o-CEE: oral conjugated equine estrogen; t-E2: transdermal 17 $\beta$ -estradiol;

\* Baseline levels presented unless otherwise specified

† Chi-square test for categorical variables; ANOVA or Wilcoxon Mann Whitney tests for continuous variables as appropriate

**Table S2. Baseline Study variables by 48-month CAC progression.**

Variables *	48-month CAC progression		P value †
	Yes N=66 (13.9%)	No N=408 (86.1%)	
Age at baseline, mean (SD), Yr	52.76 (2.73)	52.63 (2.55)	0.70
Age at menopause, mean (SD), Yr	50.80 (2.70)	50.89 (2.55)	0.79
Time since menopause, mean (SD), Yr	1.95 (0.82)	1.73 (0.77)	0.03
White Race, N (%)	54 (81.8)	316 (77.5)	0.43
Education, N (%)			0.01
Declined to Answer	0 (0.0)	5 (1.2)	
High School graduate or less	10 (15.2)	19 (4.7)	
Some college	14 (21.2)	66 (16.2)	
College graduate	42 (63.6)	318 (77.9)	
Employed, N (%)	51 (77.3)	340 (83.3)	0.23
Income, N (%)			0.78
<\$60K	13 (19.7)	71 (17.4)	
\$60K-<\$100K	10 (15.2)	55 (13.5)	
>\$100K	9 (13.6)	76 (18.6)	

Unknown	34 (51.5)	206 (50.5)	
<b>Physical activity level, median(Q1, Q3), MET-hr/wk</b>	16.2 (7.3, 29.8)	16.7 (7.0, 28.5)	0.62
<b>Alcohol consumption, N (%)</b>	48 (72.7)	313 (76.7)	0.48
<b>Smoking Status, N (%)</b>			0.24
Never	52 (78.8)	328 (80.4)	
Past	8 (12.1)	62 (15.2)	
Current	6 (9.1)	18 (4.4)	
<b>Ever use hormone therapy, N (%)</b>	18 (27.3)	82 (20.1)	0.19
<b>Anti-Hypertensive medication use, N (%)</b>	N=66	N=408	0.62
Never	52 (78.8)	340 (83.3)	
Past	5 (7.6)	21 (5.1)	
Current	9 (13.6)	47 (11.5)	
<b>BMI, mean (SD), Kg/m<sup>2</sup></b>	26.3 (4.3)	26.0 (4.3)	0.55
<b>Waist circumference, mean (SD), cm</b>	85.4 (11.1)	84.2 (11.8)	0.43
<b>Systolic blood pressure, mean (SD), mmHg</b>	118.9 (14.2)	118.3 (15.3)	0.77
<b>HDL cholesterol, mean (SD), mg/dL</b>	70.7 (17.0)	72.4 (14.7)	0.39

<b>LDL cholesterol, mean (SD), mg/dL</b>	113.5 (26.4)	111.1 (27.6)	0.51
<b>Triglycerides, median (Q1, Q3), mg/dL</b>	80.0 (64.0, 131.0)	68.0 (49.0, 103.0)	0.01
<b>Fasting glucose, median (Q1, Q3), mg/dL</b>	83.0 (73.0, 89.0)	78.0 (74.0, 85.0)	0.11
<b>Insulin, median (Q1, Q3), <math>\mu</math>U/mL</b>	5.1 (3.0, 9.7)	3.9 (1.0, 7.0)	0.01
<b>HOMA, median (Q1, Q3)</b>	1.1 (0.6, 1.9)	0.8 (0.2, 1.4)	0.01
<b>EAT volume, median (Q1, Q3), cm<sup>3</sup></b>	41.6 (29.5, 60.3)	36.5 (25.4, 51.7)	0.20
<b>PAT volume, median (Q1, Q3), cm<sup>3</sup></b>	16.7 (10.6, 21.3)	14.3 (10.2, 21.5)	0.23
<b>Treatment groups, N (%)</b>			0.93
o-CEE	20 (30.3)	123 (30.1)	
t-E2	19 (28.8)	126 (30.9)	
Placebo	27 (40.9)	159 (39.0)	

CAC: coronary artery calcification; MET: metabolic equivalents; BMI: body mass index; HDL: high density lipoprotein; LDL: low density lipoprotein; HOMA: Homeostasis Model Assessment insulin resistance index EAT: epicardial adipose tissue; PAT: paracardial adipose tissue; o-CEE: oral conjugated equine estrogen; t-E2: transdermal 17 $\beta$ -estradiol;

\* Baseline levels presented unless otherwise specified

†Chi-square test: for categorical variables, ANOVA or Wilcoxon Mann Whitney tests for continuous variables as appropriate



**Table S3. Multivariable logistic regression results of the association between the change in heart fat depots and CAC progression.**

Change in heart fat depots	Model 1 *		Model 2 †	
	OR (95% CIs) ‡	P value	OR (95% CIs) ‡	P value
<b>EAT change</b>	1.08(0.83,1.42)	0.57	1.04(0.75,1.45)	0.80
<b>PAT change</b>	1.11(0.85,1.45)	0.44	1.04(0.77,1.42)	0.79

CAC: coronary artery calcification; MET: metabolic equivalents; BMI: body mass index; LDL-C: low density lipoprotein cholesterol; EAT: epicardial adipose tissue; PAT: paracardial adipose tissue

\* Model 1: Adjusted for age, race (white vs. non-white) and study site (east, west and middle)

† Model 2: Model 1 + smoke, METs, education, triglycerides, LDL-C, anti-hypertensive medication, alcohol consumption, SBP, WC, treatment and baseline heart fat (EAT in model for EAT change and Pat in model for PAT change).

‡ OR represents the relative increase in the odds of CAC per 1 SD increase in the change of heart fat volume

**Table S4. Multivariable logistic regression results of the association between the change in heart fat depots and the CAC progression, by treatment.**

Change in heart fat depots	Treatment	Model 1 *			Model 2 †		
		OR (95% CIs) ‡	P value		OR (95% CIs) ‡	P value	
EAT change	<b>o-CEE</b>	1.05(0.68,1.63)	0.69	ref	1.04(0.63,1.70)	0.56	ref
	<b>t-E2</b>	1.54(0.81,2.91)	0.20	0.34	1.52(0.79,2.91)	0.14	0.34
	<b>Placebo</b>	0.94(0.63,1.39)	ref	0.69	0.86(0.55,1.35)	ref	0.56
<b>EAT change*treatment P values</b>		0.43			0.34		
PAT change	<b>o-CEE</b>	0.96(0.62,1.48)	0.79	ref	0.82(0.50,1.36)	0.99	ref
	<b>t-E2</b>	2.89(1.4,5.98)	0.005	0.01	2.73(1.29,5.75)	0.007	0.008
	<b>Placebo</b>	0.88(0.6,1.3)	ref	0.79	0.82(0.52,1.27)	ref	0.99
<b>PAT change*treatment P values</b>		0.01			0.02		

CAC: coronary artery calcification; MET: metabolic equivalents; BMI: body mass index; LDL-C: low density lipoprotein cholesterol;

EAT: epicardial adipose tissue; PAT: paracardial adipose tissue

\* Model 1: Adjusted for age, race (white vs. non-white) and study site (east, west and middle)

† Model 2: Model 1 + smoke, METs, education, triglycerides, LDL-C, anti-hypertensive medication, alcohol consumption, SBP, WC, treatment and baseline heart fat (EAT in model for EAT change and Pat in model for PAT change).

‡ OR represents the relative increase in the odds of CAC progression per 1 SD increase in the change of heart fat

**Table S5. IPW sensitivity analysis for odds ratio (95% CI) of any increase in heart fat depots over 48 months by treatment group.**

Treatment	Any increase after 48 month			
	EAT *		PAT †	
	OR(95% CI)	P value	OR(95% CI)	P value
			0.06	
<b>o-CEE</b>	0.62(0.40, 0.97)	0.03	1.26(0.81, 1.97)	0.30
<b>t-E2</b>	0.99(0.64, 1.55)	0.99	1.20(0.77, 1.86)	0.42
<b>Placebo</b>	----	---	----	---

EAT: epicardial adipose tissue; PAT: paracardial adipose tissue; o-CEE: oral conjugated

equine estrogen; t-E2: transdermal 17 $\beta$ -estradiol

\* EAT: o-CEE vs. t-E2; OR(95% CI): 0.62(0.39, 0.99), P=0.02

† PAT: o-CEE vs. t-E2; OR(95% CI): 1.05(0.66, 1.68), P=0.49

**Table S6. IPW sensitivity analysis of the association between the change in heart fat depots and CAC progression from multivariable logistic regression.**

Change in heart fat depots	Model 1 *		Model 2 †	
	OR (95% CIs) ‡	P value	OR (95% CIs) ‡	P value
<b>EAT change</b>	1.11 (0.83,1.49)	0.48	1.06 (0.75,1.50)	0.73
<b>PAT change</b>	1.09 (0.82,1.44)	0.55	1.01 (0.73,1.40)	0.95

CAC: coronary artery calcification; MET: metabolic equivalents; BMI: body mass index; LDL-C: low density lipoprotein cholesterol;

EAT: epicardial adipose tissue; PAT: paracardial adipose tissue

\* Model 1: Adjusted for age, race (white vs. non-white) and study site (east, west and middle)

† Model 2: Model 1 + smoke, METs, education, triglycerides, LDL-C, anti-hypertensive medication, alcohol consumption, SBP, WC, treatment and baseline heart fat (EAT in model for EAT change and Pat in model for PAT change).

‡ OR represents the relative increase in the odds of CAC progression per 1 SD increase in the change of heart fat volume

**Table S7. IPW sensitivity analysis of the association between the change in heart fat depots and the CAC progression from multivariable logistic regression, by treatment.**

Change in heart fat depots	Treatment	Model 1 *			Model 2 †		
		OR (95% CIs) ‡	P value		OR (95% CIs) ‡	P value	
EAT change	<b>o-CEE</b>	1.01 (0.63,1.64)	0.99	ref	0.98 (0.58,1.68)	0.86	ref
	<b>t-E2</b>	1.48 (0.78,2.83)	0.33	0.35	1.49 (0.77,2.87)	0.23	0.32
	<b>Placebo</b>	1.01 (0.66, 1.55)	ref	0.99	0.92 (0.57,1.49)	ref	0.86
<b>EAT change*treatment P values</b>		0.58			0.47		
PAT change	<b>o-CEE</b>	0.91 (0.56,1.48)	0.81	ref	0.74 (0.44,1.27)	0.89	Ref
	<b>t-E2</b>	2.91 (1.39,6.10)	0.004	0.009	2.68 (1.26,5.74)	0.007	0.006
	<b>Placebo</b>	0.85 (0.57,1.26)	ref	0.81	0.78 (0.48,1.26)	ref	0.89
<b>PAT change*treatment P values</b>		0.01			0.01		

CAC: coronary artery calcification; MET: metabolic equivalents; BMI: body mass index; LDL-C: low density lipoprotein cholesterol;

EAT: epicardial adipose tissue; PAT: paracardial adipose tissue

\* Model 1: Adjusted for age, race (white vs. non-white) and study site (east, west and middle)

† Model 2: Model 1 + smoke, METs, education, triglycerides, LDL-C, anti-hypertensive medication, alcohol consumption, SBP, WC, treatment and baseline heart fat (EAT in model for EAT change and Pat in model for PAT change).

‡ OR represents the relative increase in the odds of CAC progression per 1 SD increase in the change of heart fat