

# Postmenopausal Women With Greater Paracardial Fat Have More Coronary Artery Calcification Than Premenopausal Women: The Study of Women's Health Across the Nation (SWAN) Cardiovascular Fat Ancillary Study

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**Background**—Volumes of paracardial adipose tissue (PAT) and epicardial adipose tissue (EAT) are greater after menopause. Interestingly, PAT but not EAT is associated with estradiol decline, suggesting a potential role of menopause in PAT accumulation. We assessed whether volumes of heart fat depot (EAT and PAT) were associated with coronary artery calcification (CAC) in women at midlife and whether these associations were modified by menopausal status and estradiol levels.

**Methods and Results**—EAT and PAT volumes and CAC were measured using electron beam computed tomography scans. CAC was evaluated as (1) the presence of CAC (CAC Agatston score  $\geq 10$ ) and (2) the extent of any CAC (log CAC Agatston score  $>0$ ). The study included 478 women aged 50.9 years (58% pre- or early perimenopausal, 10% late perimenopausal, and 32% postmenopausal). EAT was significantly associated with CAC measures, and these associations were not modified by menopausal status or estradiol. In contrast, associations between PAT and CAC measures were modified by menopausal status (interaction- $P \leq 0.01$ ). Independent of study covariates including other adiposity measures, each 1-SD unit increase in log PAT was associated with 102% higher risk of CAC presence ( $P=0.04$ ) and an 80% increase in CAC extent ( $P=0.008$ ) in postmenopausal women compared with pre- or early perimenopausal women. Additional adjustment for estradiol and hormone therapy attenuated these differences. Moreover, the association between PAT and CAC extent was stronger in women with lower estradiol levels (interaction  $P=0.004$ ).

**Conclusions**—The findings suggest that PAT is a potential menopause-specific coronary artery disease risk marker, supporting the need to monitor and target this fat depot for intervention in women at midlife. (*J Am Heart Assoc.* 2017;6:e004545. DOI: 10.1161/JAHA.116.004545.)

**Key Words:** calcification • epicardial fat • menopause • paracardial fat

The incidence of coronary artery disease (CAD), which is the leading cause of death in women, increases after menopause.<sup>1,2</sup> As women transition through menopause, they undergo adverse alterations in body fat composition,<sup>3–7</sup> lipids, and lipoproteins<sup>8</sup> and vascular remodeling<sup>9</sup> that could increase their CAD risk. The menopausal transition, independent of aging, is believed to be associated with changes in body fat deposition rather than increases in weight.<sup>3–7,10,11</sup>

The fat surrounding the heart (heart fat) is hypothesized to be more detrimental for cardiovascular risk than other fat depots because of its close anatomic location.<sup>12</sup> Increasing evidence supports a role of heart fat in the pathogenesis of CAD,<sup>13,14</sup> cardiovascular disease (CVD) events,<sup>15–17</sup> and all-cause mortality.<sup>18</sup> Based on fat location in the pericardium, 2 distinct heart fat depots can be quantified: (1) epicardial adipose tissue (EAT), which is the fat that directly covers the

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heart and is located between the outer wall of the myocardium and the visceral layer of the pericardium, and (2) paracardial adipose tissue (PAT), which is located anterior to EAT, outside the parietal layer of the pericardium.<sup>19</sup> Although EAT and PAT have different anatomic locations and endocrine features, the terms EAT and PAT have been used interchangeably in the literature because they were thought to be identical fat depots. We recently showed that postmenopausal women have greater volumes of EAT and PAT than premenopausal women, independent of age, race, obesity, and other potential risk factors.<sup>20</sup> Interestingly, greater volumes of PAT, but not EAT, were significantly associated with lower levels and declines of estradiol (E2) in women at midlife,<sup>20</sup> suggesting a potential role of menopause and menopause-related alterations in sex hormones in PAT accumulation. In addition, these findings support the notions that EAT and PAT depots have distinct endocrine features and should not be evaluated as a single fat depot.

To the best of our knowledge, no previous study has evaluated whether heart fat depots (EAT and PAT separately) are significantly associated with greater risk of subclinical CVD in women at different stages of the menopausal transition or assessed the effect modification of menopausal status and/or endogenous E2 levels on associations between heart fat depots and subclinical CVD. Previous studies were mainly limited to postmenopausal women.<sup>21,22</sup> Premenopausal women were not included for comparisons in any of these studies; therefore, determining whether PAT or EAT might be a menopause-specific CAD risk marker could not be evaluated in any of the previous studies. The SWAN Cardiovascular Fat Ancillary Study, an ancillary study to the Study of Women's Health Across the Nation (SWAN), was specifically designed (1) to assess the distinct associations between each heart fat depot and coronary artery calcification (CAC)—a robust subclinical measure of CAD<sup>23,24</sup>—in midlife women and (2) to investigate whether the associations between heart fat depots and CAC are modified by menopausal status and/or endogenous levels of E2. Given our recent findings showing greater volumes of EAT and PAT in postmenopausal than in premenopausal women and that levels of endogenous E2 could be an important contributor to PAT but not to EAT volumes,<sup>20</sup> we hypothesized that greater volumes of EAT and PAT would be significantly associated with CAC and that the association between PAT and CAC, but not between EAT and CAC, would be significantly modified by menopausal status and E2 levels in women at midlife.

## Participants and Methods

### Study Population

SWAN is an ongoing longitudinal community-based study of the menopausal transition.<sup>25</sup> Briefly, 3302 participants aged

42 to 52 years were recruited during the period 1996–1997 from 7 study sites (Boston, MA; Detroit, MI; Oakland, CA; Los Angeles, CA; Pittsburgh, PA; Chicago, IL; and Newark, NJ). The eligibility criteria for the SWAN study were (1) an intact uterus and  $\geq 1$  ovary, (2)  $\geq 1$  menstrual period within the past 3 months, and (3) no hormone therapy (HT) use within the past 3 months. At the Pittsburgh and Chicago sites, subclinical measures of atherosclerosis were collected as part of the SWAN Heart ancillary study. The SWAN Cardiovascular Fat Ancillary Study was designed to measure cardiovascular fat volumes among SWAN Heart study participants. To be part of the SWAN Cardiovascular Fat Ancillary Study, participants had to have electron beam computed tomography scans performed at the SWAN Heart baseline visit. Of 608 SWAN Heart participants, 564 had electron beam computed tomography scans to measure heart fat depots. For the current analyses, women were excluded if they were surgically menopausal, had undetermined menopausal status due to HT use, or were missing information on menopausal status ( $n=42$ ). Another 44 women were excluded either because of missing CAC data or inability to quantify heart fat depots because of technical issues, leaving 478 women in final analyses.

The institutional review board at each site approved the study protocol, and all participants signed informed consent prior to participation.

### Coronary Artery Calcification

Calcification in the coronary arteries was quantified using the C-150 Ultrafast CT Scanner (GE Imatron). An initial scout scan was performed to identify anatomic landmarks. To evaluate the coronary arteries, 30 to 40 contiguous 3-mm-thick transverse images were obtained from the level of the aortic root to the apex of the heart during maximal breath holding. All scan data were saved to an optical disk for central scoring, using a DICOM workstation and software by Accu-Image, Inc. This software program implements the Agatston scoring method.<sup>26</sup> 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. The scoring system had high reproducibility as measured in 40 consecutive participants from another study selected to have a wide range of calcium. The intraclass correlation for CAC scores was 0.99.<sup>27</sup>

### Heart Fat Depots

Electron beam computed tomography scans (GE Imatron C-150 Ultrafast CT Scanner) were used to quantify EAT, the adipose tissue within the pericardium, and PAT, the adipose tissue outside the pericardium. EAT and PAT were quantified

at the Biomedical Research Institute, Harbor- UCLA Medical Center, as described previously.<sup>20</sup> 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.<sup>21</sup> 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>21</sup>

## Menopausal Status

Menopausal status was determined based on frequency and regularity of menstrual bleeding as follows: (1) premenopause, no perceived change in bleeding; (2) early perimenopause, perceived change in cycle interval but at least 1 menstrual period within the past 3 months; (3) late perimenopause, 3 consecutive months of amenorrhea; (4) postmenopause, 12 consecutive months of amenorrhea. Because the sample size of premenopausal women was small ( $n=49$ ) and their characteristics and E2 and CAC levels were similar to those of early perimenopausal women (data not shown), pre- and early perimenopausal women were combined in 1 group in the current study. In contrast, only 50 women were classified as late perimenopausal; however, we did not combine those women with postmenopausal women, as we have done previously,<sup>20</sup> given that the 2 groups had different CAC levels. Late peri- and postmenopausal women were analyzed as separate groups in the current study.

## Blood Assays

Women provided fasting blood samples during the early follicular phase (days 2–5 of the menstrual cycle) at each visit. Fasting samples were obtained within 90 days of the recruitment anniversary date if a timed sample could not be

obtained. Accordingly, cycle day of blood draw was reported either as days 2 to 5 or as being outside that period. Blood was prepared and serum shipped to the Clinical Ligand Assay Satellite Services Central Laboratory at the University of Michigan. E2 was measured using a modified, off-line Automated Chemiluminescence System 180 (E2-6). E2 assays were conducted in duplicate. The average for the duplicate measures was calculated and reported (coefficients of variation of 3–12%). The lower limit of detection was between 1 and 7 pg/mL. The inter- and intra-assay coefficients of variation were 10.6% and 6.4%, respectively. E2 values between zero and the lower limit of detection were replaced with a random value between zero and the lower limit of detection.

Lipids, glucose, and insulin were assayed at the Medical Research Laboratories in Lexington, Kentucky, certified by the National Heart, Lung, and Blood Institute, Centers for Disease Control and Prevention Part III program. Total cholesterol and triglyceride levels were analyzed using enzymatic methods on a Hitachi 747 analyzer (Boehringer Mannheim Diagnostics),<sup>28</sup> and high-density lipoprotein was isolated using heparin-manganese.<sup>29</sup> Low-density lipoprotein was calculated using the Friedewald equation.<sup>30</sup> Serum insulin was measured by a radioimmunoassay (Coat-a-Count; Diagnostic Products Corp) procedure and monitored as part of the monthly quality assurance program by the Diabetes Diagnostic Laboratory at the University of Missouri. Glucose was measured with a hexokinase-coupled reaction (Boehringer Mannheim Diagnostics). The homeostasis model assessment index was calculated from fasting insulin and glucose as follows:  $(\text{insulin [in } \mu\text{U/ml]} \times \text{glucose [in mmol/L]}) / 22.5$ .<sup>31,32</sup>

## Study Covariates

Weight and height were measured to calculate body mass index (BMI;  $\text{kg/m}^2$ ). Blood pressure was averaged from 2 sequential measures in the right arm with the participant seated after at least 5 minutes of rest. Race and ethnicity, age, smoking status, use of HT, and educational level were self-reported. Physical activity was self-reported and was assessed via a modified Baecke score of habitual physical activity,<sup>33</sup> with higher scores indicating more physical activity. Morbidity was defined as *yes* if participant reported a history of hypertension, diabetes mellitus, angina, stroke, or heart attack. Medication use was defined as *yes* if the participant reported use of medications for hypertension, diabetes mellitus, or high cholesterol. Visceral adipose tissue (VAT) area was measured using 6-mm-thick transverse electron beam computed tomography scan (C-150 Ultrafast CT Scanner; GE Imatron) between L4 and L5 obtained during suspended respiration. Computed tomography scans were read by a single individual at the University of Pittsburgh. A

pixel range of  $-30$  to  $-190$  Hounsfield units was used to define fat in the scan circumference. VAT area was defined using image analysis (Accu-Image software). A region-of-interest line was drawn at the interior of abdominal musculature along the fascial plane. Fat within this area was considered VAT area. Intraobserver reliability was 0.94 for the VAT area.

## Statistical Analyses

Heart fat volumes, E2, triglycerides, and homeostasis model assessment index were log transformed to reduce skewed distributions. CAC was evaluated at 2 different levels: (1) by the presence of CAC (CAC Agatston score  $\geq 10$ ) and (2) by the extent of any CAC (log CAC Agatston score  $> 0$ ). Study variables were compared by the presence of CAC and by menopausal status (data not shown) using ANOVA, *t* tests, or chi-square tests, as appropriate. Separate logistic regression (for presence of CAC) and linear regression (for extent of CAC) models were developed to evaluate the associations between each CAC measure as an outcome, with each log-transformed heart fat depot volume as the main independent variable. Results from logistic and linear regression were presented per 1-SD increase in each log heart fat depot volume. For ease of interpretation, beta coefficients from linear regression models of log heart fat depots as related to log CAC  $> 0$  (CAC extent) were presented as percentage changes and 95% CI in CAC extent. For multivariable analyses, age, race, study site, and menopausal status were a priori selected covariates and were forced into the base model regardless of statistical significance. All other variables that were found to be significantly associated with study outcomes (Table 1) and independent variables in the univariate analyses (data not shown) were considered as potential covariates to be adjusted for in model 2. To build the best parsimonious model 2, step-up regression was used. Models with the best fit statistics (highest C or  $R^2$  statistics) were chosen. Because of the high correlations between adiposity measures (BMI, VAT) and heart fat depots (EAT, PAT), we could not adjust for both BMI and VAT in the same model. Models adjusted for BMI fit the data better than models adjusted for VAT; however, for some models, adjusting for BMI resulted in significant associations in the direction opposite what we expected, which is a sign of collinearity. Adjusting for obesity (BMI  $\geq 30$ ) instead did not severely affect model stability, and thus final models were adjusted for obesity instead of BMI. Model 3 was additionally adjusted for log E2, HT use, and cycle day of the blood draw (days 2–5 versus outside that period) to assess the impact of endogenous E2 and exogenous HT use on the tested associations. Effect modifications by menopausal status or by log E2 were tested, and stratified analyses were presented if interactions

were significant. For significant interactions between continuous measures of log E2 and PAT volumes as related to CAC, interactions were retested using tertiles of both E2 and PAT for easier interpretation. Statistical tests were 2-sided with a significance level of 0.05. SAS software (version 9.3; SAS Institute Inc) was used for the analysis.

## Results

Participants' characteristics in the total sample and by presence of CAC are presented in Table 1. Participants were aged  $50.9 \pm 2.9$  years and were 38% black; 58% were pre- or early perimenopausal, 10% were late perimenopause, and 32% were postmenopausal. Participants with CAC  $\geq 10$  were more likely to be older, postmenopausal, and obese and to have higher systolic blood pressure, BMI, VAT, low-density lipoprotein cholesterol, triglycerides, and homeostasis model assessment index and lower physical activity score and high-density lipoprotein cholesterol, and E2 levels. In addition, those participants were more likely to report comorbid conditions and use of medications (all  $P < 0.05$ ).

Associations between heart fat depots and presence of CAC and extent of CAC are presented in Table 2. In base models (model 1) adjusted for age, race, study site, and menopausal status, both EAT and PAT were significantly associated with greater odds of CAC presence and greater extent of log (CAC  $> 0$ ). Further adjustments for obesity, systolic blood pressure, smoking status, log triglycerides, and medication use explained the associations with PAT but not with EAT. Additional adjustment for log E2, HT use, and cycle day of the blood draw did not affect the associations between EAT and CAC measures. In contrast, the magnitude of the associations between PAT and CAC increased and the reported *P* values decreased when models were additionally adjusted for log E2 and HT use.

Significant interactions were found between PAT and menopausal status as related to presence (Table 3) and extent (Table 4) of CAC. Interestingly, the odds of CAC presence per 1-SD unit increase in log PAT were 102% higher in postmenopausal women compared with pre- or early perimenopausal women, independent of study covariates (model 2) but not independent of endogenous E2 levels and HT use (model 3). Additional adjustment for these 2 covariates explained the difference in risk of CAC presence between the 2 groups (Table 3). Similarly, postmenopausal women showed an 80% increase in CAC extent compared with pre- or early perimenopausal women per 1-SD unit increase in log PAT in model 2 (Table 4). These differences were attenuated after additional adjustment for endogenous E2 levels and HT use (model 3) (Table 4). Stratified analyses by

**Table 1.** Characteristics of the Study Population by Presence of CAC

| Characteristics   | All participants<br>n=478 | CAC <10<br>n=382 (79.92%) | CAC ≥10<br>n=96 (20.08%) | P Value |
|---|---------------------------|---------------------------|--------------------------|---------|
| Age (y), mean±SD  | 50.93±2.92                | 50.63±2.92                | 52.14±2.57               | <0.0001 |
| Black, n (%)  | 183 (38.28)               | 139 (36.39)               | 44 (45.83)               | 0.09    |
| Menopausal status, n (%)                                  |                           |                           |                          | 0.01    |
| Pre-/early perimenopausal                                 | 275 (57.53)               | 232 (60.73)               | 43 (44.79)               |         |
| Late perimenopausal                                       | 50 (10.46)                | 39 (10.21)                | 11 (11.46)               |         |
| Postmenopausal  | 153 (32.01)               | 111 (29.06)               | 42 (43.75)               |         |
| Educational level, n (%)                                  |                           |                           |                          | 0.56    |
| High school or less                                       | 72 (15.69)                | 59 (16.08)                | 13 (14.13)               |         |
| Some college/vocational                                   | 234 (50.98)               | 190 (51.77)               | 44 (47.83)               |         |
| College degree or higher                                  | 153 (33.33)               | 118 (32.15)               | 35 (38.04)               |         |
| SBP (mm Hg), mean±SD                                      | 119.80±16.24              | 117.93±14.95              | 127.23±18.92             | <0.0001 |
| BMI (kg/m <sup>2</sup> ), mean±SD                         | 29.41±6.32                | 27.94±5.07                | 35.25±7.36               | <0.0001 |
| BMI ≥30, n (%)  | 191 (39.96)               | 119 (31.15)               | 72 (75.00)               | <0.0001 |
| Physical activity scores, mean±SD                         | 7.93±1.78                 | 8.05±1.76                 | 7.44±1.78                | 0.004   |
| HDL-C (mg/dL), mean±SD                                    | 57.28±14.59               | 58.45±14.82               | 52.62±12.69              | 0.0004  |
| LDL-C (mg/dL), mean±SD                                    | 118.46±31.95              | 116.53±31.33              | 126.11±33.39             | 0.01    |
| Triglycerides (mg/dL), median (Q1–Q3)                     | 99.00 (76.00–138.00)      | 79.00 (73.00–129.00)      | 117.50 (86.00–193.00)    | <0.0001 |
| HOMA index, median (Q1–Q3)                                | 2.04 (1.45–3.35)          | 1.87 (1.39–2.80)          | 3.09 (2.08–5.30)         | <0.0001 |
| E2 (pg/mL), median (Q1–Q3)                                | 28.80 (16.00–72.45)       | 32.45 (16.15–79.60)       | 23.23 (14.68–41.35)      | 0.01    |
| Morbidity*, n (%)   | 203 (42.475)              | 145 (37.96)               | 58 (60.42)               | <0.0001 |
| Use of medication†, n (%)                                 | 98 (20.50)                | 68 (17.80)                | 30 (31.25)               | 0.004   |
| Use of HT, n (%)  | 26 (5.44)                 | 22 (5.76)                 | 4 (4.17)                 | 0.54    |
| Smoker, n (%)   | 87 (18.20)                | 72 (18.85)                | 15 (15.63)               | 0.46    |
| Visceral fat area (cm <sup>2</sup> ), median (Q1–Q3)      | 111.75 (73.75–163.95)     | 100.53 (69.37–145.24)     | 177.33 (126.04–221.31)   | <0.0001 |
| Epicardial fat volume (cm <sup>3</sup> ), median (Q1–Q3)  | 36.85 (27.93–51.46)       | 34.80 (26.14–46.82)       | 51.30 (38.18–67.83)      | <0.0001 |
| Paracardial fat volume (cm <sup>3</sup> ), median (Q1–Q3) | 9.03 (5.44–14.94)         | 8.32 (5.00–13.38)         | 14.43 (8.63–22.01)       | <0.0001 |
| CAC score, median (Q1–Q3)                                 | 0.00 (0.00–6.520)         | 0.00 (0.00–2.06)          | 24.38 (15.79–46.35)      | ...     |
| CAC score >0, n (%)                                       | 226 (47.28)               | ...                       | ...                      | ...     |

BMI indicates body mass index; CAC, coronary artery calcification; E2, estradiol; HDL-C, high-density lipoprotein cholesterol; HOMA index, homeostatic model assessment insulin resistance index; HT, hormone therapy; LDL-C, low-density lipoprotein cholesterol; Q1, first quartile; Q3, third quartile; SBP, systolic blood pressure.

\*Morbidity: history of hypertension, diabetes mellitus, angina, stroke, or heart attack.

†Use of medication: antihypertensive, lipid lowering, or antidiabetic.

menopausal status showed stronger associations and larger effect sizes in the relationships of PAT with presence of CAC (Table 5) and the extent of CAC (Table 6) in postmenopausal women than in pre- or early perimenopausal women. Adjusting for VAT instead of obesity in model 2 in Tables 3 through 6 provided similar findings (data not shown).

Association between PAT and CAC extent was dependent on E2 levels, adjusting for study site, age, race and ethnicity, menopausal status, obesity, systolic blood pressure, smoking, log triglycerides, medication use, cycle day of the blood draw, and HT use (interaction  $P=0.004$ ). Similar results were seen if

the model was adjusted for VAT instead of obesity (data not shown). Higher volumes of PAT were significantly associated with greater CAC extent only among women with E2 levels in the lowest tertile ( $E2 \leq 18.65$  pg/mL; trend  $P=0.038$ ) (Figure 1).

Associations between EAT and CAC measures were not significantly modified by menopausal status. In addition, E2 levels did not significantly modify these associations, except for a weak interaction between E2 and EAT related to extent of calcification ( $P=0.04$ ) that became nonsignificant once models were adjusted for VAT instead of obesity.

**Table 2.** Associations Between Heart Fat Depots and Presence of CAC and Extent of CAC in Women at Midlife

| Heart Fat Depots<br>Separate Models | Presence of CAC (CAC $\geq 10$ )* |         |                        |         |                        |         |
|-------------------------------------|-----------------------------------|---------|------------------------|---------|------------------------|---------|
|                                     | Model 1                           |         | Model 2                |         | Model 3                |         |
|                                     | OR (95% CI)                       | P Value | OR (95% CI)            | P Value | OR (95% CI)            | P Value |
| Log EAT                             | 2.46 (1.86–3.27)                  | <0.0001 | 1.56 (1.12–2.17)       | 0.01    | 1.58 (1.12–2.23)       | 0.01    |
| Log PAT                             | 2.17 (1.66–2.84)                  | <0.0001 | 1.37 (0.99–1.90)       | 0.06    | 1.40 (0.99–1.96)       | 0.05    |
|                                     | Extent of CAC (Log CAC >0)*       |         |                        |         |                        |         |
|                                     | % Change (95% CI)                 |         | % Change (95% CI)      |         | % Change (95% CI)      |         |
|                                     | % Change (95% CI)                 | P Value | % Change (95% CI)      | P Value | % Change (95% CI)      | P Value |
| Log EAT                             | 33.34 (9.22–62.79)                | 0.01    | 27.29 (0.47–61.29)     | 0.04    | 27.29 (0.19–62.04)     | 0.04    |
| Log PAT                             | 25.89 (3.02–53.85)                | 0.02    | 19.52 (–5.07 to 50.46) | 0.12    | 24.04 (–2.20 to 57.32) | 0.08    |

Model 1: adjusted for age, race, study site, and menopausal status. Model 2: model 1 plus obesity, systolic blood pressure, smoking, log triglycerides, and medication use. Model 3: model 2 plus log estradiol, cycle day of the blood draw, and hormone therapy use. CAC indicates coronary artery calcification; EAT, epicardial adipose tissue; OR, odds ratio; PAT, paracardial adipose tissue.

\*Results were presented per 1-SD unit greater in each log heart fat depot.

## Discussion

To the best of our knowledge, this study is the first to assess associations between volumes of heart fat depots and CAC in women at different stages of the menopausal transition and to evaluate the impact of menopausal status and endogenous E2 levels on these associations. We demonstrated that greater volumes of EAT are significantly associated with presence and extent of CAC, independent of age, race, menopausal status, and traditional CVD risk factors. In addition, we reported that the associations between PAT and CAC measures are significantly modified by women's menopausal status and E2 levels independent of age, race, obesity (or VAT; data not shown), and other CVD risk factors; similar effect modifications were not found for EAT as related to CAC measures.

Our findings of significant associations of CAC measures with EAT volumes in women at midlife are in agreement with previous studies in other populations, which suggests a significant role of EAT in the pathogenesis of CAD. Higher heart fat volumes are associated with CVD risk factors, CVD events, and CAC.<sup>13–18</sup> It is well recognized that heart fat is a metabolically active organ that releases various substances with known vascular actions that, in turn, could locally modulate the morphology and functions of the heart and thus contribute to increase CVD risk.<sup>12</sup>

We demonstrated that the associations between CAC measures and EAT do not depend on women's menopausal status or E2 levels, whereas the associations between CAC measures and PAT were menopause-specific and may be

**Table 3.** ORs of the Effect Modifications of Menopausal Status on the Association Between Heart Fat Depots and Presence of CAC

| Presence of CAC (CAC $\geq 10$ )        |                  |         |                  |         |                  |         |
|---|------------------|---------|------------------|---------|------------------|---------|
| Separate Models for Log EAT and Log PAT | Model 1          |         | Model 2          |         | Model 3          |         |
|   | OR (95% CI)      | P Value | OR (95% CI)      | P Value | OR (95% CI)      | P Value |
| Per 1-SD unit greater in log EAT        |                  |         |                  |         |                  |         |
|   |                  | 0.30    |                  | 0.20    |                  | 0.47    |
| Pre-/early perimenopausal               | ...              |         | ...              |         | ...              |         |
| Late perimenopausal                     | 0.58 (0.26–1.31) | 0.19    | 0.52 (0.22–1.21) | 0.13    | 0.62 (0.25–1.57) | 0.32    |
| Postmenopausal                          | 1.12 (0.61–2.08) | 0.71    | 1.15 (0.62–2.15) | 0.66    | 1.14 (0.60–2.17) | 0.68    |
| Per 1-SD unit greater in log PAT        |                  |         |                  |         |                  |         |
|   |                  | 0.004   |                  | 0.001   |                  | 0.01    |
| Pre-/early perimenopausal               | ...              |         | ...              |         | ...              |         |
| Late perimenopausal                     | 0.49 (0.23–1.05) | 0.07    | 0.35 (0.16–0.78) | 0.01    | 0.39 (0.17–0.92) | 0.03    |
| Postmenopausal                          | 2.09 (1.10–3.98) | 0.03    | 2.02 (1.02–3.99) | 0.04    | 1.76 (0.87–3.55) | 0.12    |

Model 1: adjusted for age, race, study site, and menopausal status. Model 2: model 1 plus obesity, systolic blood pressure, smoking, log triglycerides, and medication use. Model 3: model 2 plus log estradiol, cycle day of the blood draw, and hormone therapy use. CAC indicates coronary artery calcification; EAT, epicardial adipose tissue; OR, odds ratio; PAT, paracardial adipose tissue.

**Table 4.** Effect Modifications of Menopausal Status on the Association Between Heart Fat Depots and Extent of CAC

| Extent of CAC (Log CAC >0)              |                          |         |                          |         |                          |         |
|---|--------------------------|---------|--------------------------|---------|--------------------------|---------|
| Separate Models for Log EAT and Log PAT | Model 1                  |         | Model 2                  |         | Model 3                  |         |
|   | % Change (95% CI)        | P Value | % Change (95% CI)        | P Value | % Change (95% CI)        | P Value |
| Per 1-SD unit greater in log EAT        |                          |         |                          |         |                          |         |
|   |                          | 0.40    |                          | 0.41    |                          | 0.62    |
| Pre-/early perimenopausal               | ...                      |         | ...                      |         | ...                      |         |
| Late perimenopausal                     | -10.12 (-51.74 to 67.39) | 0.74    | -17.33 (-55.61 to 53.97) | 0.55    | -0.92 (-48.98 to 91.51)  | 0.97    |
| Postmenopausal                          | 26.71 (-16.56 to 92.40)  | 0.27    | 21.52 (-20.34 to 85.38)  | 0.36    | 22.09 (-21.08 to 89.74)  | 0.37    |
| Per 1-SD unit greater in log PAT        |                          |         |                          |         |                          |         |
|   |                          | 0.003   |                          | 0.002   |                          | 0.03    |
| Pre-/early perimenopausal               | ...                      |         | ...                      |         | ...                      |         |
| Late perimenopausal                     | -26.25 (-58.38 to 31.63) | 0.31    | -31.53 (-61.64 to 23.12) | 0.20    | -21.74 (-58.99 to 48.24) | 0.45    |
| Postmenopausal                          | 83.88 (19.52-180.81)     | 0.01    | 79.83 (16.88-176.67)     | 0.01    | 68.20 (6.91-164.61)      | 0.03    |

Model 1: adjusted for age, race, study site, and menopausal status. Model 2: model 1 plus obesity, systolic blood pressure, smoking, log triglycerides, and medication use. Model 3: model 2 plus log estradiol, cycle day of the blood draw, and hormone therapy use. CAC indicates coronary artery calcification; EAT, epicardial adipose tissue; PAT, paracardial adipose tissue.

modulated by women's endogenous levels of E2. In our previous work, although late peri- and postmenopausal women had greater volumes of EAT and PAT than pre- and early perimenopausal women,<sup>20</sup> late peri- and postmenopausal women had greater volumes of PAT (20% higher) than EAT (10% higher) compared with pre- and early perimenopausal women. In addition, we reported that women with the greatest E2 decline over almost 5 years of follow-up had 20% higher PAT volumes compared with women with the least decline in E2 over time.<sup>20</sup> We hypothesized, based on these previous findings, that heart fat could play a role in the higher risk of CVD reported in women after menopause. In the current study, we showed that postmenopausal women had ≈80% greater CAC extent than pre- and early perimenopausal women per 1 SD greater log PAT. The current results, together with our noted previous findings,<sup>20</sup> support our hypothesis that PAT is a potential menopause-specific CAD

risk marker in women at midlife. Further research is needed to confirm our hypothesis using a longitudinal study design.

Our findings of significant impact of E2 levels on associations of CAC with PAT but not with EAT add to the lines of evidence that EAT and PAT are distinct heart fat depots with different endocrine properties and thus should be evaluated separately.<sup>12</sup> EAT and PAT have different embryological origins; EAT originates from the splanchnopleuric mesoderm, whereas PAT originates from the primitive thoracic mesenchyme. Moreover, the coronary circulation supplies blood to EAT but not to PAT. Finally, EAT is the energy source for the heart, with high free fatty acid release and uptake and low glucose requirements; in contrast, to date, there are no known cardioprotective functions of PAT.<sup>34</sup>

It is not clear why E2 levels modulated the associations of CAC with PAT but not EAT. We previously reported significant associations between lower levels and greater declines of E2

**Table 5.** ORs of the Associations Between Paracardial Fat Volumes and Presence of CAC Stratified by Menopausal Status

| Presence of CAC (CAC ≥10) |                                   |         |                            |         |                        |         |
|---------------------------|-----------------------------------|---------|----------------------------|---------|------------------------|---------|
| Log PAT                   | Pre-/Early Perimenopausal (n=275) |         | Late Perimenopausal (n=50) |         | Postmenopausal (n=153) |         |
|                           | OR (95% CI)*                      | P Value | OR (95% CI)*               | P Value | OR (95% CI)*           | P Value |
| Model 1                   | 1.92 (1.34-2.74)                  | 0.0004  | 0.97 (0.44-2.16)           | 0.93    | 3.87 (2.21-6.78)       | <0.0001 |
| Model 2                   | 1.16 (0.75-1.81)                  | 0.51    | 0.76 (0.23-2.54)           | 0.66    | 2.59 (1.34-5.01)       | 0.01    |
| Model 3                   | 1.22 (0.78-1.91)                  | 0.39    | 1.03 (0.29-3.68)           | 0.97    | 2.76 (1.38-5.53)       | 0.004   |

Model 1: adjusted for age, race, and study site. Model 2: model 1 plus obesity, systolic blood pressure, smoking, log triglycerides, and medication use. Model 3: model 2 plus log estradiol, cycle day of the blood draw, and HT use. HT use and cycle day of blood draw were not included in models for late perimenopausal and postmenopausal because the model would not converge if they were included. CAC indicates coronary artery calcification; HT, hormone therapy; OR, odds ratio; PAT, paracardial adipose tissue.

\*ORs (95% CIs) were presented per 1-SD unit greater in log PAT.

**Table 6.** Percentage Change and 95% CI in CAC Extent as Related to Paracardial Fat Volumes Stratified by Menopausal Status

| Extent of CAC (Log CAC >0) |                                  |         |                            |         |                       |         |
|----------------------------|----------------------------------|---------|----------------------------|---------|-----------------------|---------|
| Log PAT                    | Pre/Early Perimenopausal (n=123) |         | Late Perimenopausal (n=27) |         | Postmenopausal (n=76) |         |
|                            | % Change (95% CI)*               | P Value | % Change (95% CI)*         | P Value | % Change (95% CI)*    | P Value |
| Model 1                    | 7.71 (−18.54 to 42.84)           | 0.58    | −18.17 (−53.82 to 44.98)   | 0.47    | 86.63 (28.16–170.57)  | 0.002   |
| Model 2                    | 2.25 (−24.59 to 38.66)           | 0.89    | −23.46 (−71.71 to 105.55)  | 0.57    | 99.53 (27.78–209.28)  | 0.003   |
| Model 3                    | 3.78 (−25.15 to 43.90)           | 0.83    | −4.36 (−66.44 to 174.62)   | 0.93    | 90.84 (17.75–206.99)  | 0.01    |

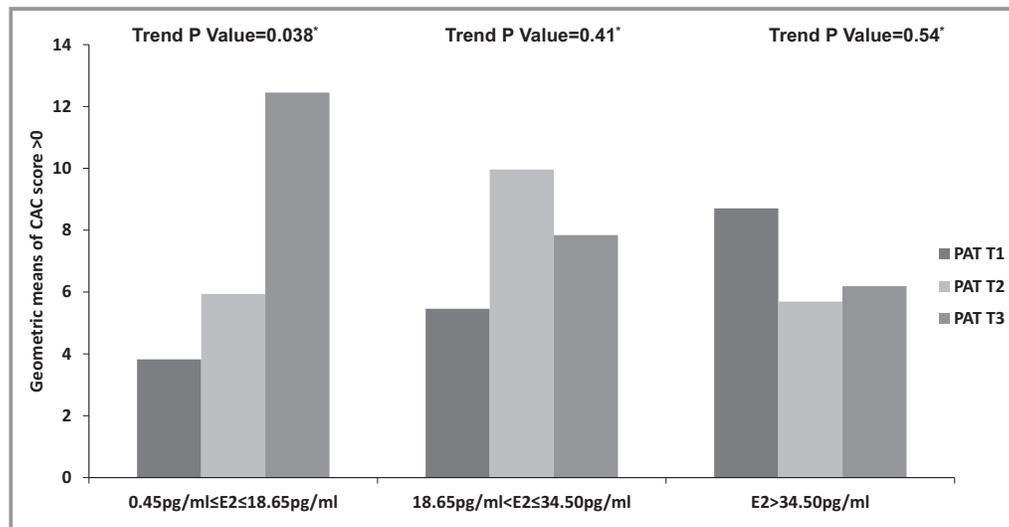
Model 1: adjusted for age, race, study site, and menopausal status. Model 2: model 1 plus obesity, systolic blood pressure, smoking, log triglycerides, and medication use. Model 3: model 2 plus log estradiol, cycle day of the blood draw, and hormone therapy use. CAC indicates coronary artery calcification; PAT, paracardial adipose tissue.

\*% Changes (95% CIs) were presented per 1-SD unit greater in log PAT.

with PAT but not EAT.<sup>20</sup> These findings support that volumes of PAT but not EAT may be modulated by E2. Estrogen receptor  $Er-\alpha$ ,<sup>35</sup> expressed in human subcutaneous and visceral adipose tissues,<sup>36</sup> plays a significant role in regulating adipocyte metabolism and sexual dimorphism of adipose tissue depots. E2 can directly increase the antilipolytic  $\alpha$ 2A-adrenergic receptors<sup>37</sup> and lipolytic  $\beta$ -adrenergic expression<sup>38</sup> to control the accumulation of fat in a certain adipose tissue depot. Consequently, it is possible that  $Er-\alpha$  estrogen receptors are more expressed in PAT than in EAT and could, in turn, make any changes in E2 levels affect PAT more than EAT. It would be of great interest to assess the potential role of HT use on heart fat volumes and their associations with

subclinical atherosclerotic measures such as CAC in future studies.

In the current study, greater PAT volume was associated with higher odds of CAC presence and greater CAC extent in postmenopausal women compared with pre- and early perimenopausal women. These findings were independent of study covariates but not of endogenous E2 levels and HT use. Given the current uncertainty about the cardioprotective impacts of HT use<sup>39</sup> and the lack of any publication on the impact of HT use on heart fat volumes, identifying other possible prevention strategies to decrease heart fat in general and PAT in particular in women at midlife may reduce CVD risk associated with excess heart fat. Interestingly, a recent



**Figure.** Predicted geometric means of CAC extent by tertiles of PAT and E2. \*Adjusted for study site, race/ethnicity, age, menopausal status, obesity, systolic blood pressure, smoking, log triglycerides, medication use, hormone therapy use, cycle day of the blood draw.  $P=0.04$  for interaction between PAT tertiles and E2 tertiles.  $P=0.004$  for interaction between PAT and E2 as continuous measures. E2 tertile 1,  $n=79$ ; E2 tertile 2,  $n=83$ ; E2 tertile 3,  $n=51$ . PAT T1:  $\geq 2.48$  and  $\leq 9.43$   $\text{mm}^3$ ; PAT T2:  $>9.43$  and  $\leq 16.02$   $\text{mm}^3$ ; PAT T3:  $>16.02$   $\text{mm}^3$ . CAC indicates coronary artery calcification; EAT, epicardial adipose tissue; E2, estradiol; PAT, paracardial adipose tissue; PAT T1, first tertile of paracardial adipose tissue volume; PAT T2, second tertile of paracardial adipose tissue volume; PAT T3, third tertile of paracardial adipose tissue volume.

systematic review and meta-analysis of studies assessing whether heart fat volumes could be modified and whether different strategies could be utilized concluded that it is possible to reduce heart fat and that significant reductions occurred with dieting and bariatric surgery but not with exercise.<sup>40</sup> Heart fat can be decreased by 17% in abdominally obese postmenopausal women on weight-loss interventions of equal energy deficit with or without aerobic exercise.<sup>41</sup>

Our findings should be viewed in the context of some limitations, including the cross-sectional design, which prevented us from testing whether the accumulation of heart fat is associated with CAC progression and whether the dynamic changes in E2 as women transition through menopause could modify this association. Our findings may not be generalizable to women of other ages or other racial and ethnic groups. Because of small sample sizes for the pre- and late perimenopausal categories, we may not have enough power to assess study aims in each category separately. In addition, our findings of the extent of CAC and PAT should be interpreted with caution, given the wide reported confidence intervals, which could be due to small sample size. This study has several strengths, which include using a well-characterized cohort (SWAN) and being the first to evaluate whether volumes of heart fat are associated with CAC in women at midlife and whether women's menopausal status and their E2 levels modify this association. Future studies should evaluate the role of HT use on heart fat volume accumulation and the impact of HT use on the associations between heart fat volumes and subclinical measures of atherosclerosis.

In conclusion, although greater volumes of EAT were significantly associated with CAC, only the association of CAC with volumes of PAT was dependent on menopausal status and E2 levels. Our findings suggest PAT as a potential menopause-specific CAD risk marker and maintain that EAT and PAT are distinct fat depots that should be evaluated separately. The current study supports the need to monitor and target heart fat depots for intervention in women at midlife.

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**Postmenopausal Women With Greater Paracardial Fat Have More Coronary Artery Calcification Than Premenopausal Women: The Study of Women's Health Across the Nation (SWAN) Cardiovascular Fat Ancillary Study**

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