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Cardiovascular Fat, Menopause and Sex Hormones in Women: The SWAN Cardiovascular Fat Ancillary Study

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Context: Cardiovascular risk increases in women after menopause. Mounting evidence demonstrates a role of cardiovascular fat (CF) in the pathogenesis of coronary heart disease (CHD), but no research has examined CF in relation to sex hormones or menopausal status in women.

Objective: To determine the relationship between CF depots, menopausal status, and endogenous sex hormones.

Design: Cross-sectional and longitudinal study designs.

Setting: The Study of Women's Health Across the Nation (SWAN) Heart.

Participants: 456 women (mean age: 50.75 years); 62% pre-/early peri-, and 38% late peri-/postmenopausal.

Intervention: Menopausal status, endogenous sex hormones measured simultaneously with CF volumes, and circulating estradiol available 4.80 years (median) prior to CF measures.

Main Outcome Measures: Volumes of CF (epicardial (EAT), paracardial (PAT), total heart (TAT=EAT+PAT) and aortic perivascular adipose tissues (PVAT))

Results: In final models, late peri-/postmenopausal women had 9.88% more EAT, 20.72% more PAT, and 11.69% more TAT volumes than pre-/early peri-menopausal women, $P < 0.05$. PVAT was not associated with menopausal status. In final models, lower estradiol concentrations were associated with greater volumes of PAT and TAT, $P < 0.05$. Women with the greatest reduction in estradiol since baseline had greater volumes of PAT compared to women with the least reduction, $P = 0.02$.

Conclusions: Late peri-/postmenopausal women have greater volumes of heart fat compared with pre-/early peri-menopausal women independent of age, obesity and other covariates. Endogenous sex hormones are associated with CF. Perhaps CF plays a role in the higher risk of CHD reported in women after menopause.

Increased weight gain in women over the menopausal transition has been a matter of long-term debate (1). Most of the cross-sectional and longitudinal studies evaluating weight gain and menopause have concluded that weight gain at midlife is due to aging rather than to menopause (1–3). However, this conclusion does not consider changes in body composition during the menopausal transition. Several cross-sectional and a few longitudinal stud-

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Abbreviations:

ies found that postmenopausal women have greater abdominal visceral fat and/or waist circumference when compared to premenopausal women (4–8). Moreover, older and postmenopausal women tend to have less subcutaneous fat in the abdomen and/or legs (7, 8). The changes in body fat composition and distribution may be due to hormonal fluctuations that occur during the menopausal transition (9, 10).

The accumulation of abdominal visceral fat during the menopausal transition (4–8) may play a key role in explaining the higher rates of coronary heart disease (CHD) among postmenopausal women (11, 12). Visceral fat is metabolically active and produces several inflammatory markers with significant atherogenic features (13). The redistribution of fat deposition in women at midlife is not limited to visceral and subcutaneous fat depots. Increasing visceral fat and decreasing fat storage capability in subcutaneous adipose tissue are often indications of increased fat deposition and infiltration into other visceral tissues such as the heart (14).

The excess of fat around the heart and aorta, known as cardiovascular fat (CF), may be more detrimental for cardiovascular risk than visceral fat given its close anatomical location (15). Increasing evidence supports a role of CF in the pathogenesis of CHD (16–18). Whether CF increases in women transitioning through menopause parallels the increase in visceral abdominal fat is unknown. Evaluating the potential association between CF and menopause may reveal CF as a novel risk factor in women at midlife.

To the best of our knowledge, no previous study has evaluated whether CF is associated with menopausal status or endogenous sex hormone Concentrations in women at midlife. Studies were mainly limited to either postmenopausal women or hysterectomized women with/out bilateral oophorectomy. Premenopausal women were not included for comparisons in any of these studies; therefore the above question has not been addressed (19, 20). The SWAN CF study, an ancillary study to the Study of Women's Health Across the Nation (SWAN), was specifically designed to 1) determine the relationship between CF depots and menopausal status; 2) investigate the associations between CF depots and concentrations of endogenous sex hormones in a sample of women at midlife. We hypothesize that postmenopausal women will have greater volumes of CF compared to premenopausal women, and that lower concentrations of estradiol (E2) and (sex hormone binding globulin) SHBG, and higher concentrations of free androgen index (FAI) will be associated with higher volumes of CF in midlife women.

Subjects and Methods

Study Population

SWAN is an ongoing community-based longitudinal study of the menopausal transition (21). Briefly, 3302 participants aged 42–52 years were recruited 1996–1997 from seven designated 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 ≥ 1 ovary, 2) ≥ 1 menstrual period within the past 3 months, 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 CF Ancillary Study was designed to measure CF among SWAN Heart study participants. To be part of the SWAN CF ancillary study, participants needed to have EBCT scans performed at the SWAN Heart baseline visit. Out of 608 SWAN Heart participants, 564 had CT scans to measure CF depots. For the current analyses, women were excluded if they were surgically menopausal, with undetermined menopausal status due to HT use, or missing menopausal status (n = 42). An additional 66 women were excluded due to missing covariates data, hormone data or due to use of HT, leaving 456 women in final analyses.

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

Cardiovascular Fat Depots

Computerized tomographic scans (GE-Imatron C150 EBCT, CA, USA) were used to quantify CF depots. Four CF depots were measured including: 1) Epicardial adipose tissue (EAT), the adipose tissue within the pericardial sac; 2) Paracardial adipose tissue (PAT), the adipose tissue outside the pericardial sac; 3) Total heart adipose tissue (TAT), the sum of EAT and PAT; 4) Perivascular adipose tissue (PVAT), the adipose tissue surrounding the descending thoracic aorta, **Supplemental Figure**. EAT, PAT, and TAT were quantified at the Biomedical Research Institute, Harbor- UCLA Medical Center, CA, USA, as described previously (18). In brief, EAT, PAT, and TAT volumes were 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 located around the proximal coronary arteries. The anterior border of the TAT volume was the chest wall and the posterior borders were the aorta and the bronchus. Using the volume analysis software (GE Healthcare, Waukesha, WI, USA), 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 2–3 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 from TAT volume. EAT and PAT measures have excellent reproducibility. Spearman correlation coefficients between-reader and within-reader were [mteq]0.97. Of the 456 women in the final analysis, 32 women did not have EAT, PAT or TAT due to technical issues, leaving 424 women for analyses of these measures. PVAT was quantified at the University of Pittsburgh Ultrasound Research Lab. Briefly, using an image analysis workstation equipped with Slice-O-Matic v4.3 (Tomovision, Magog, Quebec, Canada), PVAT was distinguished from other tissues by using the same Hounsfield thresholds as above. The pulmonary bifurcation

served as the proximal border, and the initial image of the first lumbar vertebrae marked the distal border. The borders surrounding the descending thoracic aorta were manually traced for every slice. The anterior borders included a horizontal line through the left bronchus, esophagus, and eventually the interior border of the crus of the diaphragm. The posterior border was a horizontal line tangent to the anterior border of the vertebral foramen. A similar protocol has been used before with excellent intra-reader and inter-reader intra-class coefficients of at least 0.99 (22). All women in the final analysis had PVAT measures (n = 456).

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 peri-menopause: perceived change in cycle interval, but at least one menstrual period within the past 3 months, 3) Late perimenopause: 3 consecutive months of amenorrhea, 4) Postmenopause: 12 consecutive months of amenorrhea. Due to small sample sizes of pre- (n = 48) and late peri-menopausal (n = 53) categories, and similar to previous publications from SWAN Heart study (23, 24) both preand early peri-menopausal women were combined in one group, while late peri- and postmenopausal women were combined in a second group.

Endogenous Sex Hormones

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 as either days 2–5 or outside that period. Blood was prepared and serum shipped to the Clinical Ligand Assay Satellite Services (CLASS) Central Laboratory at the University of Michigan. Endogenous sex hormones were measured using the Automated Chemilumisence System –180 automated analyzer (Bayer Diagnostics Corp., Norwood, MA). Estradiol (E2) was measured using a modified, off-line Automated Chemilumisence System: 180 (E2–6). The lower limit of detection (LLD) was between 1 and 7 pg/mL. The interand intra-assay coefficients of variation were 10.6% and 6.4%, respectively. Follicle stimulating hormone (FSH) was measured by a modified manual assay kit (Bayer Diagnostics) utilizing two monoclonal antibodies directed to different regions on the beta subunit. The LLD was between 0.4 and 1.0 mIU/mL. The interand intra-assay coefficients of variation were 11.4% and 3.8%, respectively. Serum testosterone (T) concentration was evaluated with the Automated Chemilumisence System: 180 total T assay, modified to increase precision in the low ranges. The LLD was between 2 and 2.2 ng/dL. The interand intra-assays coefficients of variation were 10.5% and 8.5%, respectively. Sex hormone binding globulin (SHBG) was measured with a two-site chemiluminescent immunoassay. The LLD was between 1.9 and 3.2 nM. The interand intra-assay coefficients of variation were 9.9% and 6.1%, respectively. The Free androgen index (FAI) was used to estimate the amount of testosterone unbound by SHBG and thus, immediately biologically active. FAI was calculated as 100×T/(28.84×SHBG). Only E2 assays were conducted in duplicate. The average for the duplicate measures was calculated and reported (coefficients of variation of 3% to 12%). Endogenous sex hormone values between zero and the LLD were replaced with a random value between zero and the LLD.

Study Covariates

Weight and height were measured to calculate body mass index (BMI) (kg/m^2), and obesity was defined as BMI ≥ 30 kg/m^2. Race/ethnicity and educational level were self-reported. Age, smoking status and alcohol consumption were derived from questionnaires. Physical activity was self-reported, and was assessed -via a modified Baecke score of habitual physical activity (25), with higher scores indicating more physical activity. Morbidity was defined as yes if participant reported a history of hypertension, diabetes, angina, stroke or myocardial infarction. Medication use was defined as yes if participant reported use of medications for hypertension, diabetes, or high cholesterol.

Statistical Analyses

CF volumes and hormone values were log transformed to achieve normality. Separate linear regression models were developed to evaluate the associations between each log-transformed CF depot as an outcome with menopausal status or each log-transformed hormone as the main independent variable. For ease of interpretation, % differences/changes and 95% CI in each CF volumes were calculated (26, 27). For multivariable analyses, all variables that were found to be significantly associated with study outcomes in the univariate analyses were considered as potential covariates. Since E2 was found to be associated with PAT in the current study, and to better understand the potential role of changes in E2 over the menopausal transition on PAT, E2 concentrations at the SWAN Parent baseline visit (median (Q1, Q4): 4.80(4.09, 5.10) years before the SWAN CF ancillary study) were available for 417 participants, and were utilized to calculate and evaluate quartiles of E2 relative change since baseline in relation to log-transformed PAT volume. Similar analyses were conducted with other CF depots, and results were not significant (data not shown). Statistical test were two sided with a significance level of 0.05, unless otherwise specified. SAS software (version 9.03; SAS Institute Inc, Cary, NC) was used for the analysis.

Results

Participants' characteristics in the total sample and by menopausal status are presented in Table 1. Participants were 50.75 ± 2.83 years old; 38% African American, and 62% pre-/early peri-menopausal. In unadjusted analyses, late peri-/postmenopausal women had greater volumes of all CF depots, **Figure 1**. Late peri-/postmenopausal women had 9.88% more EAT, 20.72% more PAT, and 11.69% more TAT compared to pre-/early peri-menopausal women ($P < .05$). These differences were independent of study covariates. Menopausal status was not associated with PVAT in final models, Table 2. Volumes of CF depots were very similar between preand early peri-menopausal women ($P \geq .05$ for all) as well as between late peri- and postmenopausal women ($P \geq .05$ for all), **Supplemental Table**.

Table 1. Participants Characteristics by Menopausal Status[a]

Characteristics	Total n = 456	Pre -/early peri-menopausal n = 282 (61.84%)	Late peri-/postmenopausal n = 174 (38.16%)	P-value
Age, year, mean±SD	50.75 ± 2.83	49.5 ± 2.21	52.63 ± 2.70	<0.001
African American, n(%)	173 (37.94)	99 (35.11)	74 (42.53)	0.11
Educational Level, n(%)				0.08
≤ High school	65 (14.77)	33 (12.13)	32 (19.05)	
Some college/vocational	229 (52.05)	151 (55.51)	78 (46.43)	
College degree, or higher	146 (33.18)	88 (32.35)	58 (34.52)	
Income, n(%)				0.16
Low:≤ 20k-34k	63 (13.88)	33 (11.74)	30 (17.34)	
Medium:35k-75k	179 (39.43)	109 (38.79)	70 (40.46)	
High: ≥76k	212 (46.70)	139 (49.47)	73 (42.20)	
Alcohol consumption, n(%)				0.9
0-< = 1/month	170 (37.28)	105 (37.23)	65 (37.36)	
>1/month-1/week	175 (38.38)	110 (39.01)	65 (37.36)	
>2/week	111 (24.34)	67 (23.76)	44 (25.29)	
Morbidity [b], n(%)	191 (41.89)	108 (38.30)	83 (47.70)	0.048
Medication [c], n(%)	91 (19.96)	52 (18.44)	39 (22.41)	0.3
BMI, kg/m^2, mean±SD	29.52 ± 6.45	29.23 ± 6.57	29.98 ± 6.24	0.22
Obesity (BMI≥30Kg/m^2), n(%)	184 (40.35)	106 (37.59)	78 (44.83)	0.12
Physical Activity scores, mean±SD	7.93 ± 1.76	8.04 ± 1.75	7.76 ± 1.75	0.09
Smoker, n(%)	76 (16.67)	47 (16.67)	29 (16.67)	0.99
E2, pg/ml, median(Q1, Q3)	29.88 (16.25, 77.85)	46.05 (25.00 109.10)	16.45 (11.90,27.25)	<0.001
FSH, mIU/ml, median(Q1, Q3)	32.55 (14.05,83.65)	17.60 (11.00,35.90)	85.55 (57.30 106.10)	<0.001
FAI, median(Q1, Q3)	3.14 (1.76, 5.24)	2.71 (1.63,4.68)	3.47 (2.25,6.04)	0.0004
SHBG, nM, median(Q1, Q3)	41.80 (28.10,61.55)	43.80 (28.90,63.30)	38.35 (26.30,56.30)	0.039

[a] E2: estradiol; FAI: free androgen index; FSH: Follicle stimulating hormone; SHBG: sex hormone binding globulin

[b] History of any of the following conditions: hypertension, diabetes, angina, stroke or heart attack.

[c] Use of any of the following medications: Lipid lowering, blood pressure, or diabetic medications.

Table 2. Unadjusted and Adjusted % Differences in Cardiovascular Fat Volumes by Menopausal Status [a]

	EAT[b] n = 424		PAT[b] n = 424		TAT[b] n = 424		PVAT[b] n = 456	
Models	%Diff(95%CI)	P	%Diff(95%CI)	P	%Diff(95%CI)	P	%Diff(95%CI)	P
Unadjusted								
Late peri-/Postmenopausal	16.02 (5.91, 27.08)	0.002	25.86 (8.92, 45.44)	0.002	17.53 (7.11, 28.95)	0.001	9.52 (2.11, 7.47)	0.01
Pre-/Early peri-menopausal	—		—		—		—	
Model 1[c]								
Late peri-/Postmenopausal	9.25 (-0.07, 19.44)	0.05	20.00 (4.30, 38.06)	0.01	11.00 (1.72, 21.13)	0.02	1.78 (-4.80, 8.80)	0.60
Pre-/Early peri-menopausal	—		—		—		—	
Model 2[d]								
Late peri-/Postmenopausal	9.88 (0.411, 20.25)	0.04	20.72 (4.73, 39.15)	0.009	11.69 (2.25, 22.00)	0.01	1.67 (-4.95, 8.74)	0.63
Pre-/Early peri-menopausal	—		—		—		—	

[a] EAT: Epicardial adipose tissue; PAT: Paracardial adipose tissue; PVAT: Perivascular adipose tissue of the descending aorta; TAT: Total heart adipose tissue

[b] Log transformed. β coefficients and related 95% CI from linear regression were presented as % differences between menopausal status using the following formula: (e^{β}-1)*100, (26).

[c] Model1: Adjusted for study site, race, income, age, current smoking, physical activity, and obesity (BMI≥30Kg/m^2)

[d] Model2: Model 1 + alcohol consumption, medication use and comorbidities.

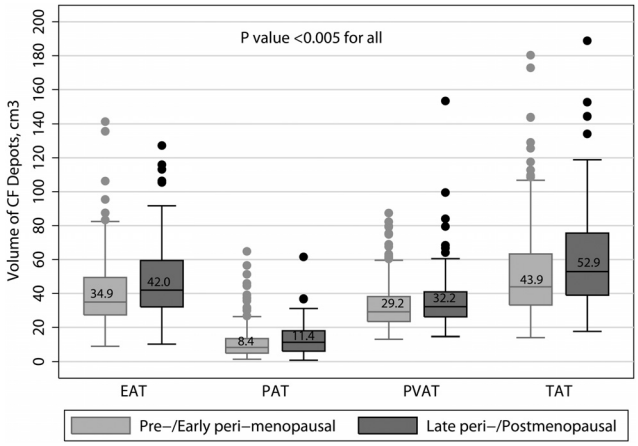

Figure 1. Cardiac Fat Volumes by Menopausal Status EAT: Epicardial adipose tissue; PAT: Paracardial adipose tissue; PVAT: Perivascular adipose tissue of the descending aorta; TAT: Total heart adipose tissue

When evaluating associations between CF depots and endogenous sex hormones, Table 3, higher concentrations of E2 were associated with lower volumes of EAT, PAT, and TAT, but not PVAT in unadjusted analyses. In final models, higher concentrations of E2 remained associated with lower volumes of PAT and TAT. Although higher concentrations of FAI were associated with greater CF volumes, in unadjusted models, only associations with PVAT remained significant in final models. Higher concentrations of SHBG were associated with lower volumes of all CF depots in all models. There were no associations between FSH and CF volumes.

Quartiles of E2 relative change to the baseline visit of the SWAN parent study were evaluated in relation to PAT to better understand the association between change in E2

Table 3. Unadjusted and Adjusted % Change in Cardiovascular Fat Volumes for Each 20% Increment in Endogenous Sex Hormones [a]

Models Sex Hormones[b]	EAT[b] $n = 424$		PAT[b] $n = 424$		TAT[b] $n = 424$		PVAT[b] $n = 456$	
	%Change (95%CI)	*P*	%Change (95%CI)	*P*	%Change (95%CI)	*P*	%Change (95%CI)	*P*
Unadjusted								
E2	−0.95 (-1.67,-0.22)	0.01	−1.72 (-2.85, −0.57)	0.004	−1.04 (-1.78, −0.30)	0.006	−0.53 (-1.09, 0.03)	0.07
FSH	0.48 (-0.32, 1.28)	0.24	0.14 (-1.12, 1.42)	0.83	0.36 (-0.46, 1.18)	0.39	−0.03 (-0.64, 0.59)	0.92
FAI	2.30 (1.29, 3.31)	<0.001	3.80 (2.19, 5.43)	<0.001	2.53 (1.51, 3.56)	<0.001	2.66 (1.92, 3.41)	<0.001
SHBG	−3.25 (-4.43, −2.06)	<0.001	−4.38 (-6.23, −2.48)	<0.001	−3.34 (-4.54, −2.13)	<0.001	−2.97 (-3.86, −2.07)	<0.001
Model 1[c]								
E2	−0.59 (-1.21, 0.04)	0.07	−1.16 (-2.14, −0.18)	0.02	−0.64 (-1.26, −0.03)	0.04	−0.12 (-0.59, 0.36)	0.63
FSH	0.30 (-0.47, 1.07)	0.45	0.04 (-1.16, 1.25)	0.95	0.19 (-0.56, 0.95)	0.62	−0.38 (-0.95, 0.20)	0.20
FAI	0.52 (-0.35, 1.39)	0.24	0.82 (-0.54, 2.20)	0.24	−0.53 (-0.32, 1.38)	0.22	1.27 (0.63, 1.92)	0.0001
SHBG	−1.29 (-2.35, −0.22)	0.02	−1.91 (-3.56, −0.22)	0.03	−1.27 (-2.31, −0.22)	0.02	−1.56 (-2.35, −0.77)	0.0001
Model 2[d]								
E2	−0.57 (-1.20, 0.07)	0.08	−1.15 (-2.14, −0.16)	0.02	−0.63 (-1.25, −0.01)	0.04	−0.08 (-0.56, 0.40)	0.74
FSH	0.32 (-0.45, 1.11)	0.41	0.09 (-1.13, 1.32)	0.89	0.22 (-0.54, 0.99)	0.56	−0.39 (-0.97, 0.19)	0.18
FAI	0.45 (-0.40, 1.34)	0.29	0.81 (-0.56, 2.20)	0.25	0.49 (-0.37, 1.34)	0.26	1.25 (0.61, 1.90)	0.0001
SHBG	−1.21 (-2.28, −0.13)	0.03	−1.89 (-3.56, −0.19)	0.03	−1.20 (-2.25,-0.14)	0.03	−1.56 (-2.35, −0.76)	0.0002

[a] EAT: Epicardial adipose tissue; E2: estradiol, FAI: free androgen index; FSH: Follicle stimulating hormone; PAT: Paracardial adipose tissue; PVAT: Perivascular adipose tissue of the descending aorta; SHBG: sex hormone binding globulin; TAT: Total heart adipose tissue

[b] Log transformed. % Change and 95% CI in each CF volumes per 20% increase in each endogenous sex hormones were calculated using he following formula:$(e^{\beta^{*}\log(1.2)} - 1)^{*}100$, (27).

[c] Model1: Adjusted for study site, cycle day of the blood draw, race, income, age, current smoking, physical activity, and obesity (BMI≥30Kg/m^2)

[d] Model2: Model 1 + alcohol consumption, medication use and comorbidities .

and PAT. Women with the greatest reduction in E2 since baseline had significantly greater volumes of PAT compared to women with the least reduction in E2 since baseline (Q1 vs. Q4). These differences were independent of study covariates, including age, **Figure 2.**

Discussion

Using data from a large sample of midlife women, the current study showed for the first time that late peri-/postmenopausal women have significantly greater volumes of heart fat depots independent of age, race, obesity, physical activity, smoking, alcohol consumption, medications use, and comorbidity. These differences may be attributed to concentrations of endogenous sex hormones at midlife. We were able to show that lower concentrations of E2, measured concomitantly with CF volume, and a decline in E2 concentrations over 4.80 years were significantly associated with greater PAT volumes in midlife women. Additionally, concentrations of FAI and SHBG were associated with volumes of certain CF depots.

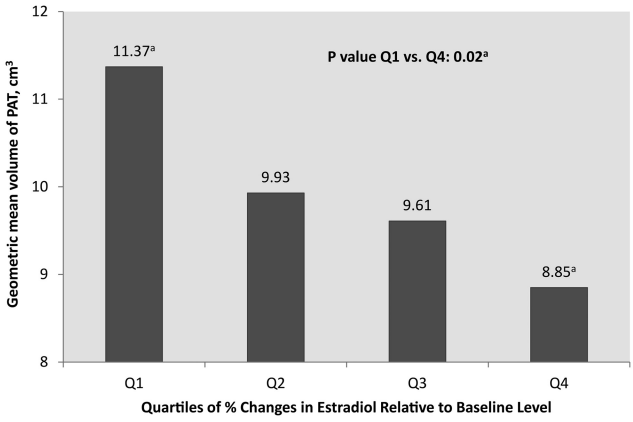

Figure 2. Geometric Means of Volumes of Paracardial Fat by Quartiles of % Changes in Estradiol relative to SWAN Parent Baseline Level [a] Model adjusted for age, site, ethnicity, income, smoking, physical activity, and obesity. P-value: adjusted for multiple comparisons. PAT: Paracardial adipose tissue

CF is a metabolically active organ that releases substances with known vascular actions and proinflammatory factors (15). Therefore; CF may locally modulate the function and morphology of the heart and vasculature, which could play a key role in adiposity-related atherosclerosis (15). Due to the absence of fascial boundaries, CF may have a local effect and modulate coronary and aortic arteries as well as myocardium via both paracrine and endocrine release of antiand proinflammatory adipokines (15). Higher CF has been associated with multiple CHD risk factors, presence of CAD, and coronary and aortic arteries calcification (16–18, 28). Our findings of greater volumes of all CF depots in late peri-/postmenopausal women suggest a potential role of menopause on body fat distribution to visceral organs such as the heart, and highlight CF as a possible risk factor for women at midlife. Interestingly, data from the Framingham study showed that the associations between both pericardal (TAT in our study) and peri-aortic fat, and CHD risk factors are significantly stronger in women than men (16, 17).

Although BMI and weight increase throughout the menopausal transition, abdominal visceral fat increases at least three times faster than BMI (10); in one study, it was the only fat depot that was significantly greater in postmenopausal than in premenopausal women, independent

of age (4). Researchers have evaluated associations between menopause and other visceral fat depots including liver fat. Interestingly, postmenopausal women showed greater liver fat compared with premenopausal women, which was found to be related to their lower concentrations of serum E2 (29, 30). These findings suggest that the menopausal transition is associated with changes in fat deposition location and not overall obesity. No previous study has evaluated associations between menopause and CF in humans. In animal models, after 12 weeks of ovariectomization, the weight of peri-aortic fat was significantly higher in the oophorectomized female rats compared to a sham group (31). Our findings are consistent with these results and extend them to the human population.

No previous study has directly evaluated associations between E2 concentrations and CF in midlife women. There is evidence from animal studies and studies of HT on other visceral fat depots that support our findings. Oophorectomized rats receiving 17-β E2 supplementation were found to have less weight and area of peri-aortic fat (31), and were protected from developing fatty liver (32). Further, aromatase gene knock-out mice, which cannot synthesize endogenous E2, exhibit a marked increase in gonadal and infrarenal fat pads that were reduced after receiving E2 therapy (33). In humans, women without fatty liver had significantly higher E2 concentrations (29). Additionally, HT was found to be associated with a reduction in central adiposity in randomized controlled trials (34). Our findings of significant and independent associations between lower concentrations and decline of E2, and higher volumes of PAT, suggest a potential role of E2 in explaining why late peri-/postmenopusal women showed greater volumes of CF depots compared to pre-/early peri-menopausal women in the current study. This hypothesis should be confirmed using a longitudinal study design.

Estrogen plays a significant role in regulating adipocyte metabolism and sexual dimorphism of particular adipose depots via estrogen receptors Er-α (35) expressed in human subcutaneous and visceral adipose tissues (36). E2 can directly increase the number of antilipolytic α2A-adrenergic receptors in subcutaneous adipocytes (37), and lipolytic β-adrenergic expression in visceral adipocytes (38), which maintain the typical female type of fat distribution resulting in greater accumulation of fat in subcutaneous fat depot with minimal deposition in abdominal visceral fat depot (39). Therefore, it is possible that lower concentrations of E2 and decline in E2 over time in our study were associated with higher volumes of fat deposition around the heart through lowering antilipolitic and lipolitic receptors in subcutaneous and visceral fat, respectively. This in turn, would reduce the ability of subcutaneous fat to deposit fat and potentially increase the deposition and infiltration of fat in visceral tissues, including the heart and aorta.

The current study showed that higher concentrations of FAI and lower concentrations of SHBG were associated with greater volumes of certain CF depots. FAI concentrations were significantly associated with fat around the aorta, while concentrations of SHBG were significantly associated with fat in all evaluated CF depots in women at midlife. Other studies in populations known to have excess androgen, such as women with polycystic ovary syndrome and women with idiopathic hirsutism, showed that these women have significantly greater EAT compared to controls (40). These findings are in line with our findings and suggest that androgens might also influence adipose tissue deposition.

The incidence of CHD, the leading cause of death in women, increases after the age of 50 (11, 12). Identifying potential risk factors for CHD development in women at midlife will enhance our understanding of why women after menopause are subjected to a higher risk of CHD. Doubling of or 100% increase of EAT has been associated with a 54% increase in coronary events, adjusting for cardiovascular risk factors (hazard ratio (HR) [HR] [95% (CI)]: 1.54 [1.09 to 2.19]), (41). The current study found an average 20% higher PAT in late peri-/postmenopausal than in pre-/early peri-menopausal women, which could correspond to 11% increase risk of coronary events. Identifying possible prevention strategies to reduce CF in women at midlife may reduce CHD risk associated with excess CF. Weight management could be a potential prevention strategy. Weight-loss interventions of equal energy deficit with/without aerobic exercise significantly reduced pericardial fat (TAT in the current study) by 17% in postmenopausal women (42).

The current study has some limitations, including the cross-sectional design, which did not allow us to assess the temporality of the evaluated associations: 1) whether CF volumes progress overtime in midlife women; and 2) whether the dynamic changes in sex hormones are associated with greater progression in CF over the menopausal transition. Due to small sample sizes of preand late perimenopausal categories, we were not able to assess study aims in each of these categories separately. Late perimenopausal stage would be of great interest, since women are subjected to significant vascular remodeling during this period (43). Although levels of E2 and FSH may not be similar in late peri- and postmenopausal women, they were close. Same apply to levels of E2 and FSH in preand early peri-menopausal women. Additionally, volumes of CF were very similar between preand early peri-meno-

pausal women as well as between late peri- and postmenopausal women. Another limitation of the current analyses was the inability to adjust for total body fat as we did not have this measure in SWAN Heart participants. Despite these limitations, this study has several strengths, which include being the first to evaluate whether volumes of CF are associated with menopausal status and endogenous sex hormones in women at midlife. It utilized a well characterized cohort, the SWAN Study. Subclinical CHD measures and related risk factors are available through the SWAN parent study and will be utilized to assess the potential role of CF on CHD risk factors and subclinical measures in women at midlife.

In conclusion, late peri-/postmenopausal women have greater volumes of epicardial, paracardial and total heart adipose tissue depots compared with pre-/early perimenopausal women independent of age, obesity and other covariates. Endogenous sex hormones are associated with the CF volumes, with E2 being associated with total heart adipose tissue driven by the significant association with paracardial heart fat. Perhaps CF plays a role in the higher risk of CHD reported in women after menopause.

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