Serum follicle stimulating hormone and five-year change in adiposity in healthy postmenopausal women

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Abstract

Background: Evidence from animal studies suggests that the gradual rise in follicle stimulating hormone (FSH) during reproductive senescence may contribute to the change in adiposity distribution characteristic of menopause. The potential independent role the interrelationships of FSH and estradiol may play in postmenopausal adiposity changes are not well studied. Methods: In a sample of 667 postmenopausal women from the Women's Health Initiative Buffalo OsteoPerio Ancillary Study, we studied the associations of serum FSH and estradiol levels with dual x-ray absorptiometry (DXA)-derived adiposity measures via cross-sectional and longitudinal analyses (5-year follow-up). Results: In cross-sectional analyses, FSH levels were inversely associated with all measures of adiposity in models adjusted for age, years since menopause, smoking status, pack years, and hormone therapy (HT) use; these associations were not influenced by adjustment for serum estradiol. In longitudinal analyses, the subset of women who discontinued HT over follow-up (n=242) experienced the largest increase in FSH (+33.9 mIU/mL) and decrease in estradiol (-44.3 pg/mL) and gains in all adiposity measures in unadjusted analyses. In adjusted analyses, an increase in FSH was associated with a gain in percent total body fat, total body fat mass, and subcutaneous adipose tissue. Conclusions: While cross-sectional findings suggest that FSH is inversely associated with adiposity, our longitudinal findings suggest that greater increases in FSH were associated with greater increases in percent total body fat, total body fat mass and subcutaneous adipose tissue. Future studies are needed to provide additional insight into FSH-adiposity mechanisms in larger samples.

Introduction

Menopause is often accompanied by weight gain and/or changes in adiposity distribution, including increases in central fat mass and visceral adipose tissue, which are associated with adverse health conditions.[1-3] The menopausal transition includes decreasing levels of endogenous estrogen and rising follicle stimulating hormone (FSH); the FSH rise is attributable to loss of negative feedback by ovarian estrogen.[4, 5]

Evidence from animal studies suggests that FSH may contribute to adiposity, where interrupting the interaction of FSH with its receptor (via a polyclonal antibody) reduced body fat, visceral and subcutaneous adipose tissue, and enhanced thermogenesis in mice.[6, 7] The few clinical observational studies in humans to date on the association between FSH concentrations and adiposity in postmenopausal women are small and/or studied body mass index (BMI), a less robust measure of adiposity. In contrast to pre-clinical findings, cross-sectional studies in postmenopausal women (or stratified by menopausal status) suggest an inverse association of FSH with either BMI or visceral fat.[8-12] In longitudinal studies, an inverse association of FSH and BMI has also been reported [13, 14]; however, studies utilizing more robust measures of adiposity (visceral and subcutaneous fat or overall fat mass) found either no association [15], or a significant positive relationship of FSH and adiposity gain [16]. These studies had fewer than 200 postmenopausal women, did not evaluate estradiol, or did not account for changes in hormone therapy (HT) use.

Here, we performed both cross-sectional and longitudinal studies on the associations of FSH with dual x-ray absorptiometry (DXA)-assessed adiposity and adiposity change over a 5-year period in a sample of postmenopausal women, while also accounting for circulating estradiol and HT use.

Methods

Study sample. Our sample includes postmenopausal women enrolled in the Buffalo Osteoporosis and Oral Bone Loss (OsteoPerio) Study, an ancillary study to the Women's Health Initiative Observational Study. Study recruitment and data collection protocols have been described previously.[17] In brief, postmenopausal women between 50 and 79 years old at baseline were recruited for the OsteoPerio study from the group of women enrolled into the WHI Observational study in Buffalo, NY. Inclusion criteria included having six or more teeth, free of bone disease, no previous history of hip replacement, cancer in the last 10 years, or other serious illness. After all exclusions, a total of 1,341 postmenopausal women were eligible, enrolled, and completed the first study visit.

Of the 1,341 women who completed the OsteoPerio study baseline visit, 1,026 women (75%) completed the 5-year follow-up visit. Our sample was restricted to 667 women who had a stored blood sample at both visits to assess reproductive hormone levels [17]. Dual X-ray absorptiometry (DXA) measures were available for all participants. Written informed consent was obtained, and the study was approved by the University at Buffalo Institutional Review Board.

Hormone measurements. Fasting blood samples were collected by a trained phlebotomist and processed according to a standardized protocol [18]. Samples were processed within 30 minutes and stored in 0.5 ml straws in liquid nitrogen at -196ºC then moved to a -80 ºC immediately before being sent for analysis [18]. Twenty-seven pooled quality control (QC) serum samples were included and tested along with participants samples (one per each batch of 100 samples). Samples were sent to the Clinical & Epidemiologic Research Laboratory (Children's Hospital, Boston, MA) for measurement of serum estradiol and FSH.

Measured E_2 concentration was measured at baseline and follow-up by competitive electrochemiluminescence immunoassay (Roche E Modular system, Roche Diagnostics,

Indianapolis, IN) (Roche Catalog # 06656021190, RRID:AB_2905575). The limit of detection (LOD) of this assay is 5 pg/mL [18]. The intra-assay CV for the pooled QC samples was 4.1 percent. There were 154 participants at baseline and 411 at follow-up with serum E_2 below the LOD.

FSH levels were measured at both study visits by a sandwich electrochemiluminescence immunoassay on the Roche Cobas 6000 system (Roche Diagnostics, Indianapolis, IN) (Roche Catalog # 11775863122, RRID:AB_2800499). The lower LOD is 0.10 mIU/mL and the upper limit of detection is 200 mIU/mL. The intra-assay CV was 3.3 percent. One participant had baseline and follow-up serum FSH above the upper LOD.

Circulating free estradiol (fE_2) was calculated using measured serum E_2, serum SHBG, and albumin via the calculations described in Mazer et al. [19] and based on the method of Rinaldi et al. We calculated fE_2 at the baseline visit only because SHBG and albumin were only measured at baseline.[20] For participants with baseline serum E_2 below the lower LOD, the midpoint (2.5 pg/mL) was imputed and used for calculation of fE_2. Sex hormone binding globulin (SHBG) was measured by a competitive electrochemiluminescence immunoassay on the Roche E Modular system (Roche Diagnostics, Indianapolis, IN) (Roche Catalog# 03052001160, RRID: AB_2891165).

Anthropometry. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. Weight was measured to the nearest 0.1 kg on a balance bean scale. BMI was calculated as weight (kg)/height (m^2).

DXA body composition measurements. All participants completed a whole body dual x-ray absorptiometry (DXA) scan at the baseline visit (QDR-4500A; Hologic Inc., Bedford, MA) and at the 5-year follow-up visit (Discovery A; Hologic Inc., Bedford, MA) according to a standardized protocol by a trained and certified DXA technician. DXA instruments were calibrated by Hologic

due to scanner upgrades. Standardized procedure for participant positioning and scan analysis was used. Phantom scans and a random sampling of scans were reviewed to monitor machine and technician performance.

Whole-body DXA scans were used to estimate total body fat mass (TBF, kg), visceral adipose tissue (VAT, cm^2), and subcutaneous adipose tissue (SAT, cm^2). Percent total body fat was computed (TBF, kg/ total body mass, kg). To estimate abdominal VAT and SAT, DXA images at both time points were re-analyzed using new software (Hologic APEX 4.0 software toolbox). The proprietary procedures outlined in the Hologic Operator Manual (MAN-03644 Revision 005) were used to estimate VAT and SAT in an abdominal region of interest 5 cm wide at approximately the 4th lumbar vertebrae, avoiding the iliac crest and limiting bony interference with the soft tissue measures.

Covariate measurements. Demographic information and potential confounding variables were collected via self-administered questionnaires including age, self-identified race and ethnicity, years since menopause, education, diabetes, smoking status at baseline, pack years of smoking at baseline, and hormone therapy (HT) use at baseline and the 5-year follow-up visit.

For HT use, we categorized women at baseline and at the 5-year follow-up visit into the following categories: never/never, former/former, current/former, or current/current, where never/never users were women who had no history of HT use at baseline and were not using HT at year 5. Those in the ‘never/never’ and ‘former/former’ category were combined due to sample size. There were 15 women categorized as ‘former/current’ or ‘never/current’, or ‘never/former’, and two were missing information on HT use at baseline or follow-up; these women were excluded from longitudinal analyses.

Statistical analysis. Descriptive statistics were computed to characterize the sample and examine distributions for normality. Bivariate associations were analyzed using parametric and

non-parametric one-way ANOVA for continuous variables and chi-square tests for categorical variables, as appropriate.

Cross-sectional analyses. Age-adjusted partial Spearman correlation coefficients between serum hormones and adiposity measures at baseline were calculated.

We used multivariate linear regression models to test the associations of baseline FSH and free estradiol with baseline DXA-derived adiposity measures (VAT, SAT, total body fat mass, percent total body fat, and BMI). FSH and fE_2 were entered simultaneously and separately into models, with adjustment for the covariates. Covariates were selected based on an association with FSH and adiposity in our sample and were parameterized as follows: age (years), years since menopause, HT use (dummy variables; current, former, never), smoking status (current, former, never), smoking (pack-years).

Longitudinal analyses. We used separate multivariate linear regression models to estimate associations of baseline FSH (on a continuous scale) and 5-year change in adiposity measures (VAT, SAT, total body fat mass, percent total body fat, and BMI; on a continuous scale), as well as 5-year change in FSH and 5-year change in adiposity measures. Both models adjusted for age (years), years since menopause, free estradiol at baseline (pg/ml), pack-years of smoking (years), smoking status (current, former, never), change in E_2 (pg/mL), change in weight (kg), and the respective baseline adiposity measure. In models of percent total body fat and BMI, change in weight was not included in the final model as the measures of change in percent total body fat and BMI fundamentally includes changes in mass.

Exploratory analyses. We explored the longitudinal associations of FSH and adiposity according to years since menopause ($\leq$10 or >10 years since menopause) using stratified linear regression models.

Hypotheses were tested using Wald tests of the regression coefficients at the alpha level of 0.05. All tests for multiplicative interaction were considered significant at an alpha level of 0.10. All analyses were performed using SAS (Release 9.4; SAS Institute, Inc., Cary, NC).

Results

Cross-sectional analyses

Demographic and adiposity statistics according to tertiles of FSH change over the 5-year follow-up are shown in **Table 1**. Women who experienced a gain in FSH during follow-up (tertile 3) tended to be slightly younger, have higher free estradiol at baseline and were more likely to have stopped using HT (current/former HT user). Women who had the largest FSH gain also experienced the largest gain in SAT, total body fat, and percent total body fat compared to women with decreasing or more stable FSH. Women with a relatively stable FSH (tertile 2) were slightly older and had approximately two more years since menopause compared to women in tertiles 1 or 3.

Hormone concentrations and adiposity measures at baseline according to HT use are shown in **Table 2**. At the baseline visit, current HT users had lower FSH, higher E_2 and higher free E_2 compared to never and former HT users (all p-values <0.001). Current HT users also had significantly lower VAT (p<0.017) and lower percent total body fat (p<0.003) than never and former HT users.

Women who stopped using HT (current/former) during follow-up had the largest decrease in circulating E_2 (44.3 compared to 3.8 pg/mL in non-current users) and the largest increase in FSH (33.9 versus 0.16 mIU/mL in non-current users); **Table 2**. We observed significant differences in all adiposity measures according to HT use category. Women who stopped using HT (current/former) experienced gains in all measures of adiposity; these adiposity gains were larger in magnitude compared to women who stayed on HT.

In cross-sectional analyses at the baseline visit, we observed significant inverse age-adjusted Spearman correlations between FSH and adiposity measures (correlation coefficients ranging from -0.12 to -0.22 across the DXA measures). We observed weak and non-significant positive age-adjusted Spearman correlations between free estradiol and adiposity (ranging from 0.02-0.07 across the DXA measures); data not shown.

Cross-sectional linear regression models are shown in **Table 3.** FSH is inversely associated with all measures of adiposity when entered in the model without free estradiol (all p-values<0.001). When free estradiol was entered into each adiposity model without FSH, free estradiol was positively associated with all adiposity measures (all p-values<0.05). When both FSH and free estradiol were entered into the models simultaneously, the significant inverse associations between FSH and adiposity measures persisted and were minimally influenced by the addition of free estradiol into the model.

However, the positive associations of free estradiol and adiposity were attenuated and became borderline or nonsignificant.

Longitudinal analyses

Figure 1 shows changes in FSH and percent fat between baseline and 5-year follow-up by change in HT use category, where never/never and former/former categories are collapsed into a 'non-current/non-current' category. The current/former HT user group experienced the largest mean increase in FSH from baseline to 5-year follow-up (34.0 mIU/mL). The current/current HT group experienced a relatively smaller FSH increase (15.7 mIU/mL). The non-current/non-current HT use group experienced no meaningful FSH change (0.16 mIU/mL). On average, the current/former HT group had a percent fat gain (mean percent fat gain= 1.0 %). The current/current HT group and the non-current/non-current HT group experienced percent fat fluctuations of 0.2% and -0.2%, respectively; **Figure 1, Panel B.**

We observed significant inverse associations between baseline FSH and 5-year change in percent body fat and total body fat that switched to positive associations when we modeled 5-year FSH change, where positive beta coefficients indicate that increasing FSH is associated with increased total body fat, percent total body fat and SAT; **Table 4.**

We evaluated associations of FSH change and adiposity change according to years since menopause (≤10 yrs, >10 yrs) and observed a similar association where FSH gain was associated with percent body fat gain in the >10 years since menopause strata (N=497) The association was of similar magnitude in the ≤10 years since menopause group (N=136) but did not reach significance. The p for interaction for years since menopause and FSH change among all models were non-significant ($p_{interaction}$>0.10); data not shown.

Discussion

To our knowledge, this is the largest longitudinal study of FSH and DXA-derived adiposity in postmenopausal women and the first to evaluate the influence of circulating estradiol and HT use on this association. This consideration of estrogen is compelled by the complexity of the FSH-E_2 feedback loop and dependence on either adipose-derived or HT-derived E_2 post ovarian failure. A unique aspect of this population is the decrease in HT use that occurred between our baseline (1997-2000) and five-year follow-up (2002-2005) visits, likely owing to the release of the WHI HT trial results in 2002 and 2004.[21, 22] Approximately 73% of current HT users at baseline stopped using HT during our study. As a result, an increase in FSH that is uncharacteristic of postmenopausal women was observed among these participants, providing a natural experiment for the study of the effects of FSH change. Our cross-sectional results suggest an inverse association between FSH and measures of adiposity, where women with higher FSH have lower values of all adiposity measures examined. However, evidence from our longitudinal study suggests that FSH gain is associated with percent total body fat,

total body fat mass, and SAT gain over a 5-year postmenopausal period, albeit the effect is small.

The previous two studies that utilized gold-standard estimates of body fat are in agreement. The first study was conducted in a sample of 77 women with adiposity measured via magnetic resonance imaging (MRI) from the Penn Ovarian Aging Study [12]. Women in the lowest VAT tertile had significantly higher mean FSH levels (90.5 mIU/mL) than women in the highest VAT tertile (40.0 mIU/mL). Similar results were reported for SAT, however differences were no longer significant after adjustment for age, race, and menopausal status [12].

In another cross-sectional study of 238 older postmenopausal women from the Reykjavik-AGES cohort who were not HT users, women in the highest quartile of FSH had significantly lower mean VAT (157.6 cm^2) measured via quantitative computed tomography (QCT) compared to the lowest FSH quartile (185.4 cm^2) following adjustment for age, subgroup, estradiol, and testosterone. This group reported similar non-significant inverse trends for SAT [11].

In our cross-sectional study, we corroborate these inverse association of FSH with all DXA-derived measures of adiposity and demonstrate that the associations are not influenced by E_2. Our findings further show a significant positive association of free estradiol and all DXA-derived adiposity measures. In both prior studies, FSH was more strongly associated with VAT than SAT, whereas we observed a stronger association for SAT. Differences in SAT findings may reflect age differences between study cohorts, as distribution of different compartments of abdominal fat (as well as VAT to SAT ratios) is dynamic early and late in the menopause years [23]. While the Penn Ovarian Aging cohort sample was closer to menopause (mean age of 52.2±3.18 yrs), the Reykjavik-AGES cohort was exclusively late postmenopausal women (mean age 80.8±4.2 yrs), whereas our sample has a broader age representation.

The only longitudinal study that utilized QCT-derived measures of adiposity was in a sample of 162 postmenopausal women (mean age=82±4 yrs) from the Reykjavik-AGES cohort

who were not HT users [11]. The authors reported no significant association between baseline FSH and annualized absolute change in any measure of adiposity over 3 years of follow-up with adjustment for age, subgroup, estradiol, testosterone, diabetes status, eGFR, and current smoking status [15]. Our results for baseline FSH predicting adiposity change, albeit with absolute change over 5-year follow-up versus annualized change over 3 years, are largely congruent to the Reykjavik-AGES study, where most adiposity measures are not significant. The exception is the inverse finding of baseline FSH and percent body fat change that we identified. However, the older age of the sample, relatively small sample size, and shorter duration of follow-up contrast with our study and may explain the differences.

Results from two longitudinal studies that used BMI to represent adiposity are more in line with cross-sectional studies. In 196 women from the Penn Ovarian Aging Study who became postmenopausal by the end of 12-years of follow-up, obese women had significantly lower mean FSH levels (42.0 mIU/mL) on average compared to women with normal BMI (59.3 mIU/mL) at each respective timepoint, indicating an inverse relationship [13]. By contrast, in our study that included women who were postmenopausal at the start of the study, we found a gain in adiposity in women who had a rise in FSH levels. While we were focused on the influence of FSH on adiposity (as reflected in our modeling and hypotheses driven by pre-clinical data), other studies have analyzed the influence of adiposity on FSH, essentially modeling the linear associations in reverse. In the Study of Women Across the Nation (SWAN), that included women across the menopausal transition, BMI gain was associated with decreasing FSH concentrations over a 3-year period [14]. In menopause-stratified results, the largest effect size was observed among 114 women who were postmenopausal at the end of follow up [14]. However, neither study considered estradiol or HT use, and utilized BMI rather than directly measured adipose tissue.

By contrast, a longitudinal study of overall body fat (measured via bioelectrical impedance), indicated a positive association of FSH and adiposity that is similar to our own [16]. In a SWAN study of 543 women across the menopausal transition (including 81 women classified as postmenopausal by the end of 6-year follow-up), increasing FSH levels were associated with increasing total body fat with adjustment for age and baseline fat mass [16]. Our study also indicated a small but significant positive association between increasing FSH and increasing total body fat.

Tepper et al reported on E_2 and FSH trajectories in a sample of 1,316 postmenopausal women in SWAN, where measurements were included before and after the menopause transition [24]. When considering E_2 and FSH trajectories during the menopause transition, having a low FSH trajectory (out of three possible) and flat E_2 (out of four possible) trajectory was more often observed in obese women. This highlights the importance of characterizing obesity at different menopause stages in studying FSH-adiposity associations, because if women are already obese (at our baseline visit), they may have lower FSH levels due to obesity at the start of menopause that could influence the inverse associations we observed here in our postmenopausal sample.

In vivo studies support our findings of a positive association between FSH and adipose tissue. FSH receptor expression in visceral and subcutaneous fat provides evidence that adipose tissue is responsive to FSH signaling [25]. Recent studies of blocking binding of the ligand FSHβ to the FSH receptor (FSHR) reduced body fat, triggered adipocyte beiging, and increased thermogenesis in ovariectomized and intact mice [6, 7]. When considering these findings in the human context, these findings support our own results and previous longitudinal work by others, where increasing FSH is associated with body fat gain in postmenopausal women [16].

Our study has several strengths including the use of robust tools for measurement of adiposity (DXA). To date, this is also the largest study to evaluate the association between FSH

and adiposity in postmenopausal women. Other strengths include our ability to evaluate the influences of circulating estradiol (including free estradiol at baseline) and change in HT use over time. Change from current user to former HT user over 5-years of follow-up led to a spike in FSH over follow-up among aging postmenopausal women, which provided a natural experiment in this subset of participants.

An important limitation is generalizability. The WHI OsteoPerio study is a cohort of predominantly white women, therefore our findings cannot necessarily be generalized to other race/ethnic groups. Randolph et al provided interesting data indicating differences in FSH and E_2 levels by race/ethnic group that will warrant further exploration in larger, more representative samples [14]. Because our WHI sample includes only postmenopausal women, we are unable to study FSH rise during the earlier part of menopause, nor do we have power to study associations stratified by HT where we observed the largest FSH rise (in former HT users). As this was not an intentional weight loss study, no wasting diseases were included, and there may have been insufficient follow-up to observe dramatic changes in body composition. Studying changes in abdominal adiposity earlier in menopause would more closely coincide with the FSH rise and may provide additional information on the FSH associations. Study size precluded robust stratified analyses; larger studies are needed.

The inverse cross-sectional association between FSH and adiposity may simply reflect weight status, while longitudinal results suggest a positive association between FSH and adiposity over time among post-menopausal women, in alignment with pre-clinical models. Replication of these findings in a larger diverse sample is critical in this population at high-risk for obesity-associated chronic diseases.

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This manuscript was prepared in collaboration with WHI investigators and has been reviewed and approved by the WHI. WHI investigators are listed at https://www-whi-org.s3.us-west-2.amazonaws.com/wp-content/uploads/WHI-Investigator-Short-List.pdf.

Data Availability Statement

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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Table 1. Demographic characteristics and key variables by tertiles of 5-year FSH change (n=666).

Mean (SD) or N (%)	FSH Change Tertile 1 [-77.2-0.30 mIU/mL] N=221	FSH Change Tertile 2 [0.33-19.7 mIU/mL] N=221	FSH Change Tertile 3 [19.7-121.3 mIU/mL] N=221	p-value*
Baseline variables:				
Age, yr	65.5 (6.8)	66.3 (6.7)	64.7 (6.5)	**0.039**
Years since menopause, yr	16.7 (8.4)	18.5 (8.6)	16.9 (8.1)	**0.047**
Education				
High school	43 (19.8%)	41 (18.8%)	44 (20.2%)	
College	91 (41.9%)	101 (46.3%)	93 (42.7%)	0.907
Post college	83 (38.3%)	76 (34.9%)	81 (37.2%)	
Missing	*4*	*3*	*3*	
Ethnicity				
Hispanic	2 (0.9%)	2 (0.9%)	2 (0.9%)	1.000
Non-Hispanic	219 (99.1%)	219 (99.1%)	219 (99.1%)	
Race				
White	215 (97.3%)	221 (100.0%)	219 (99.1%)	
American Indian/ Alaskan Native	3 (1.4%)	0 (0.0%)	1 (0.5%)	0.198
Asian American/ Pacific Islander	1 (0.5%)	0 (0.0%)	1 (0.5%)	
African American	2 (0.9%)	0 (0.0%)	0 (0.0%)	
Oophorectomy				
No	188 (85.8%)	185 (84.9%)	181 (83.4%)	
Yes	31 (14.2%)	33 (15.1%)	36 (16.6%)	0.777
Missing	*2*	*3*	*4*	
Diabetes				
No	210 (95.0%)	212 (95.9%)	217 (98.2%)	0.185
Yes	11 (5.0%)	9 (4.1%)	4 (1.8%)	
Pack-years of smoking, yr	9.2 (16.0)	7.9 (14.8)	8.9 (17.1)	0.424
Free E_2 (pg/mL)	0.23 (0.25)	0.41 (0.59)	0.78 (0.67)	**<0.001**
VAT, cm^2	100.6 (52.3)	109.5 (59.2)	98.3 (49.8)	0.236
SAT, cm^2	310.0 (115.5)	323.2 (117.4)	312.2 (118.9)	0.343
Total body fat, kg	28.2 (8.5)	29.7 (9.5)	28.0 (8.7)	0.147
% TBF	39.7 (5.4)	40.2 (5.5)	39.2 (5.4)	0.134
BMI, kg/m^2	26.2 (4.7)	27.2 (5.3)	26.3 (4.7)	0.073
Change during 5-year follow-up:				
Change in hormone therapy use				
Never/Never	88 (40.0%)	85 (38.6%)	15 (6.8%)	
Former/Former	83 (37.7%)	39 (17.7%)	5 (2.3%)	**<0.001**
Current/Current	20 (9.1%)	38 (17.3%)	32 (14.5%)	
Current/Former	18 (8.2%)	55 (25.0%)	168 (76.0%)	
Change in E_2, pg/mL	-3.1 (18.9)	-11.5 (21.0)	-48.6 (33.7)	**<0.001**
Change in VAT, cm^2	5.0 (21.6)	4.1 (24.8)	8.3 (24.2)	0.170
Change in SAT, cm^2	-1.7 (52.3)	-2.9 (52.6)	8.9 (56.4)	**0.033**
Change in total body fat, kg	-0.29 (3.2)	-0.51 (3.6)	0.58 (3.5)	**0.0003**
Change in %TBF	-0.02 (2.4)	0.03 (2.4)	0.89 (2.5)	**<0.001**
Change in BMI, kg/m^2	0.11 (1.8)	-0.07 (1.9)	0.20 (1.9)	0.290

*Chi square tests used for categorical variables; non-parametric one-way ANOVA used for continuous variables.
FSH: follicle stimulating hormone; E_2: estradiol; VAT: visceral adipose tissue; SAT: subcutaneous adipose tissue; %TBF: percent total body fat; BMI: body mass index

Table 2. Hormone concentrations and adiposity characteristics at baseline and follow-up according to hormone therapy use.

Baseline characteristics (N=666).

Mean (SD)	Never HT use (N=202)	Former HT use (N=132)	Current HT use (N=332)	p-value*
FSH, mIU/mL	70.3 (25.6)	72.2 (28.3)	43.2 (24.2)	**<0.001**
Estradiol, pg/mL	10.6 (13.1)	11.5 (14.6)	51.1 (33.3)	**<0.001**
Free estradiol, pg/mL	0.22 (0.3)	0.23 (0.3)	0.72 (0.6)	**<0.001**
VAT, cm^2	111.2 (57.9)	107.5 (58.1)	95.9 (48.9)	**0.017**
SAT, cm^2	322.6 (117.6)	322.1 (132.8)	307.0 (109.8)	0.370
Total body fat mass, kg	29.8 (8.9)	29.3 (10.4)	27.7 (8.2)	0.059
% TBF	40.7 (5.1)	39.9 (6.2)	38.9 (5.3)	**0.003**
BMI, kg/m^2	27.1 (5.1)	26.9 (5.9)	26.1 (4.4)	0.085

Changes in hormones and adiposity during 5-year follow-up (N=650).

Mean (SD)	Non-HT use/ Non-HT use** (N=318)	Current/ Former HT use** (N=242)	Current/ Current HT use** (N=90)	p-value*
5-year change in FSH, mIU/mL	0.16 (12.3)	33.9 (26.6)	15.8 (26.2)	**<0.001**
5-year change in estradiol, pg/mL	-3.8 (16.0)	-44.3 (30.3)	-23.5 (40.6)	**<0.001**
5-year change in VAT, cm^2	3.2 (23.8)	9.7 (24.4)	6.1 (19.0)	**0.008**
5-year change in SAT, cm^2	-4.1 (53.1)	10.4 (55.7)	-1.16 (49.5)	**0.001**
5-year change in total body fat mass, kg	-0.61 (3.4)	0.68 (3.6)	-0.08 (2.8)	**<0.001**
5-year change in %TBF	-0.11 (2.4)	0.91 (2.5)	0.20 (2.4)	**<0.001**
5-year change in BMI, kg/m^2	-0.07 (1.8)	0.32 (2.0)	0.01 (1.7)	**0.021**

*Chi-square tests or Kruskal-Wallis one-way analysis of variance, as appropriate.
**Non-HT use/Non=HT users were not using HT at baseline or at 5-yr follow-up. Current/Former HT users stopped using HT during follow-up. Current/Current HT users were using HT at baseline and follow-up.
HT: hormone therapy; FSH: follicle stimulating hormone; VAT: visceral adipose tissue; SAT: subcutaneous adipose tissue; %TBF: percent total body fat; BMI: body mass index

Table 3. Cross-sectional study, linear regression models for FSH and fE_2 with adiposity measures. FSH and fE_2 modeled separately and together (n=654).

	FSH model* (no fE_2 in model)		fE_2 model* (no FSH in model)		FSH and fE_2 entered together in model*			
	FSH β (SE) (mIU/mL)	**p-value**	**fE_2 β (SE) (pg/mL)**	**p-value**	**FSH β (SE) (mIU/mL)**	**p-value**	**fE_2 β (SE) (pg/mL)**	**p-value**
VAT, cm^2	**-0.60 (0.1)**	**<0.001**	**13.4 (4.2)**	**0.001**	**-0.57 (0.1)**	**<0.001**	**8.1 (4.1)**	**0.048**
SAT, cm^2	**-1.30 (0.2)**	**<0.001**	**28.5 (9.1)**	**0.002**	**-1.24 (0.2)**	**<0.001**	16.9 (8.9)	0.059
Total fat, kg	**-0.10 (0.01)**	**<0.001**	**1.9 (0.7)**	**0.007**	**-0.10 (0.01)**	**<0.001**	0.97 (0.7)	0.153
% TBF	**-0.04 (0.01)**	**<0.001**	**0.95 (0.4)**	**0.026**	**-0.04 (0.01)**	**<0.001**	0.56 (0.4)	0.190
BMI, kg/m^2	**-0.06 (0.01)**	**<0.001**	**1.3 (0.4)**	**0.001**	**-0.06 (0.01)**	**<0.001**	**0.77 (0.4)**	**0.039**

*Adjusted for age, years since menopause, smoking status, pack years, HT use.
FSH (follicle stimulating hormone); fE_2 (free estradiol); VAT (visceral adipose tissue); SAT (subcutaneous adipose tissue); total fat (total body fat mass); %TBF (percent total body fat); BMI (body mass index); HT (hormone therapy)

Table 4. Longitudinal analysis, linear regression models of baseline and 5-year FSH change predicting 5-year change in adiposity measures (N=633).

	Baseline FSH (mIU/mL) predicting change in adiposity				5-year change in FSH (mIU/mL) predicting change in adiposity			
	Unadjusted model		Adjusted model*		Unadjusted model		Adjusted model*	
	FSH β (SE)	**p-value**	**FSH β (SE)**	**p-value**	**FSH β (SE)**	**p-value**	**FSH β (SE)**	**p-value***
Δ VAT, cm^2	-0.05 (0.03)	0.131	-0.04 (0.03)	0.137	0.04 (0.04)	0.260	0.03 (0.03)	0.383
Δ SAT, cm^2	-0.08 (0.07)	0.286	-0.10 (0.06)	0.076	0.14 (0.08)	0.083	**0.17 (0.07)**	**0.016**
Δ Total fat, kg	-0.01 (0.01)	0.151	**-0.005 (0.003)**	**0.042**	**0.01 (0.01)**	**0.006**	**0.01 (0.003)**	**<0.001**
Δ TBF, %	**-0.01 (0.03)**	**0.004**	**-0.01 (0.004)**	**0.005⁺**	**0.02 (0.004)**	**<0.001**	**0.01 (0.005)**	**0.011⁺**
Δ BMI, kg/m^2	-0.001 (0.003)	0.622	-0.004 (0.003)	0.169⁺	-0.001 (0.003)	0.804	-0.004 (0.003)	0.198⁺

*Adjusted for age, years since menopause, baseline free E_2, smoking status, pack years, change in E_2, change in weight, and respective adiposity measure at baseline.
⁺Adjusted for age, years since menopause, baseline free E_2, smoking status, pack years, change in E_2, and respective adiposity measure at baseline.
FSH (follicle stimulating hormone); VAT (visceral adipose tissue); SAT (subcutaneous adipose tissue); total fat (total body fat mass); % TBF (percent total body fat); BMI (body mass index); free E_2 (free estradiol); E_2 (measured estradiol)