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ORIGINAL ARTICLE

Endogenous hormones and bone turnover markers in pre- and perimenopausal women: SWAN

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Abstract We tested the hypothesis that higher serum osteocalcin and urinary N-telopeptide of type I collagen (NTx) concentrations would be found in women with increasing cycle irregularity or increased follicle stimulating hormone concentrations. We studied 2,375 pre- and early perimenopausal women from the Study of Women's Health Across the Nation (SWAN), aged 42–52 years, who self-identified their race/ethnic origin as African-American (28.3%), Caucasian (49.4%), Japanese (10.5%) or Chinese (11.8%). Outcome measures were serum osteocalcin, a measure of bone formation, and NTx, a measure of bone resorption. The explanatory variables were menopausal status, based on self-reported regularity of menstrual bleeding, and circulating endogenous hormone concentrations including estradiol (E_2), testosterone (T), sex hormone binding globulin (SHBG) and follicle stimulating hormone (FSH) concentrations. Additionally, we evaluated the association of the bone turnover markers with the Free Androgen Index (FAI) and the Free Estradiol Index (FEI), ratios of total testosterone and estradiol concentrations to SHBG, respectively. Higher FSH concentrations were associated with higher NTx concentrations ($\beta = 0.003$, partial $r^2 = 2.1\%$, $p < 0.0001$), both before and after adjusting for other covariates (total explained variability of 9%). Higher FSH concentrations were also associated with higher osteocalcin concentrations ($\beta = -0.216$, partial $r^2 = 4.1\%$, $p < 0.0001$, total explained variability of 15.4%). There were no significant associations of the bone turnover markers with other endogenous hormones, following adjustment for covariates. Mean osteocalcin and NTx values were not significantly different in premenopausal women compared to early perimenopausal women. In these pre- and early perimenopausal women, higher FSH concentrations, but not other serum reproductive hormone concentrations, are positively associated with greater bone turnover prior to the last menstrual period.

Keywords Estrogen · Follicle stimulating hormone · N-telopeptides · Osteocalcin · Perimenopause

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Introduction

Bone loss in perimenopausal women is widely regarded as a key element contributing to the subsequent risk of osteoporosis. Some cross-sectional studies have reported no evidence of age-related bone differences among women with normal menstrual cycles [1, 2, 3], whereas others have reported somewhat lower bone mineral density (BMD) at the femoral neck with increasing age [4, 5, 6, 7]. By contrast, among perimenopausal women, the rates of bone loss have been reported to be 1–2% per year [8, 9, 10]. The increased likelihood of anovulatory cycles and the greater variation in menstrual patterns in the perimenopause may reflect a hormonal environment that is permissive for bone loss in women prior to the last menstrual period [11, 12].

Few studies have examined bone turnover markers during the menopausal transition though osteocalcin

and urinary N-telopeptide of type I collagen (NTx) have been used to characterize the association of bone turnover and years since menopause as well as responses to bone-related interventions in the postmenopausal women [13]. However, there is a paucity of information on bone turnover in women classified as pre-, perimenopausal and postmenopausal by either bleeding criteria or hormone status [14]. The few studies of osteocalcin and the menopausal transition are inconsistent. For example, Ebeling et al. [14] found that osteocalcin concentrations were increased approximately 70% and 150% in peri- and postmenopausal Australian women, respectively, compared with premenopausal women while Yasumura et al. [15] reported no difference in osteocalcin levels between premenopausal and postmenopausal women. NTx may be a more responsive measure of estrogen deficiency and its replacement than osteocalcin. Levels in postmenopausal women are reported to be nearly 2.5 times greater than those observed in premenopausal women [16, 17], suggesting that NTx may be a good biochemical marker of bone resorption during the menopausal transition.

An expanded understanding of the relationship between bone turnover markers, hormone concentrations in the perimenopause and the rate of subsequent bone loss will be important to improve our understanding of the total effect of menopause on bone. Additional study is needed to understand the time course of changes in levels of bone turnover markers during the menopausal transition.

The Study of Women's Health Across the Nation (SWAN), a study of women at the mid-life, affords the opportunity to address the following questions in pre- and perimenopausal women from the Study's baseline examination. Do perimenopausal SWAN enrollees have higher measures of bone turnover than premenopausal enrollees? Is bone turnover positively correlated with greater follicle stimulating hormone (FSH) concentration and inversely correlated with serum estradiol and testosterone concentrations? A secondary goal of this report was to identify correlates of bone turnover measures to assure that, with statistical adjustment, any associations observed with bleeding patterns and hormone measures were less subject to confounding.

Materials and methods

Study sample

SWAN is a multi-site, longitudinal cohort study of mid-life being conducted in a community-based sample of 3,302 women [18]. Enrollees, aged 42–52 years, were still menstruating in the 3 months prior to screening and were not using oral contraceptives or hormone replacement therapy. This report is based on data from participants enrolled at five of the seven SWAN clinical sites located in Boston, the Detroit area, Los Angeles, Pittsburgh and Oakland that collected bone data. All SWAN sites enrolled Caucasians. Additionally, the Boston, Detroit area and Pittsburgh field sites enrolled African-American women, while Japanese and Chinese women were enrolled at the Los Angeles and Oakland sites, respectively. There were 2,356 women who had NTx data. Thirty-eight women were excluded because they were using medications known to affect bone or calcium metabolism.

Written informed consent was obtained from all participants and each site's protocol was conducted with approval from an Institutional Review Board.

Bone turnover and bone measurements

Serum osteocalcin and NTx excretion was measured in samples of venous blood and urine collected before 10:00 hours and in days 2 and 5 of the menstrual cycle. Serum osteocalcin was measured in duplicate using an immunoradiometric assay (ELSA-OSTEO, Cis Bio International, Bagnols/Ceze, France) that measures both the 1–49 amino acid intact human osteocalcin molecule and the 25–37 amino acid fragment. The lower limit of detection of the assay is 0.4 ng/ml and the intra- and interassay coefficients of variation are both $<6\%$. Urinary NTx was measured in duplicate using a competitive inhibition enzyme immunoassay (Osteomark, Ostex International, Seattle, WA). NTx is expressed as nanomoles of bone collagen equivalents per liter per millimole creatinine per liter (nM BCE/mM Cr). The lower limit of detection is 20 nM BCE and the intra- and interassay coefficients of variation are $<8\%$ and $<12\%$, respectively. Samples were reanalyzed when the coefficient of variation of the replicates exceeded 10%.

BMD was measured with Hologic 2000 (Pittsburgh and Oakland) and 4500A (Boston, Detroit area, and Los Angeles) instruments (Hologic, Waltham, MA). For this paper, hip bone area was used as a proxy for bone surface area, recognizing that bone turnover concentration is determined by total area available for turnover as well as the rapidity with which bone turns over.

Hormone measures

The hormones E_2, T, SHBG, thyroid stimulating hormone (TSH) and FSH, were assayed using the ACS-180 automated analyzer (Bayer Diagnostics, Norwood, MA). Serum estradiol concentrations were measured with a modified, off-line ACS:180 (E_2-6) immunoassay. Inter- and intra-assay coefficients of variation averaged 10.6% and 6.4%, respectively over the assay range. Serum FSH concentrations were measured with a two-site chemiluminometric immunoassay. Inter- and intra-assay coefficients of variation were 12.0% and 6.0%, respectively. Testosterone concentrations were determined with the ACS:180 total testosterone assay modified to increase precision in the lower ranges. Inter- and intra-assay coefficients of variation were 10.5% and 8.5%, respectively. DHEA-S concentrations were measured with a de novo assay using competitive binding of a DMAE-labeled DHEA-S derivative to a rabbit anti-DHEA-S antibody. The solid phase is goat anti-rabbit IgG conjugated to paramagnetic particles. Inter- and intra-assay coefficients of variation were 10.5% and 7.6%, respectively.

Insulin was measured in serum by solid phase RIA (Coat-A-Count, Diagnostics Product) and glucose was measured using a hexokinase-coupled reaction (Boehringer Mannheim Diagnostics, Indianapolis, IN). From the measurements of glucose and insulin, an insulin sensitivity index [(20 × insulin resistance)/(fasting glucose−3.5)] was calculated [19].

The de novo two-site chemiluminescent assay for SHBG involved competitive binding of DMAE-labeled SHBG to a commercially available rabbit anti-SHBG antibody and a solid phase of goat anti-rabbit IgG conjugated to paramagnetic particles. Inter- and intra-assay coefficients of variation were 9.9% and 6.1%, respectively. Total testosterone was indexed to SHBG to calculate the Free Androgen Index (FAI = 100×total testosterone/28.84×SHBG). Likewise, total estradiol was indexed to SHBG to calculate the Free Estradiol Index (FEI = 100×total estradiol/272×SHBG).

TSH concentration was assessed using the commercial ACS:180 TSH assay (Bayer Diagnostics), which is a two-site sandwich chemiluminescent assay. Inter- and intra-assay coefficients of variation were 9.0% and 1.9%, respectively.

Menstrual status

Menstrual status was based on self-report at the time of screening for study eligibility. Menstrual status was classified as either premenopausal (menses in the 3 months prior to study entry without a change in regularity) or early perimenopause (menstrual bleeding in the 3 months prior to study entry but some change in the regularity of cycles).

Covariates

Weight and height data were used to calculate body mass index (BMI, kg/m^2). Self-administered questionnaires were used to assess current smoking status and physical activity using a modified Baecke [20] instrument that provides a summary score of active living, home, recreational physical activity, plus work activity. Dietary intake variables [alcohol (kcal/day), calcium (mg/day) and genistein (μg/day)] were assessed with a Block Food Frequency Questionnaire [21] modified to include common ethnic foods. Genistein and daidzein contents of food were based on Rienli and Block [22].

Standardized, self-report questionnaires, conducted in the language of the woman's preference, were used to determine age, education, marital status and difficulty in paying for basics. Women self-reported disease diagnoses (including diabetes, cardiovascular disease, arthritis, cancer and thyroid disease) and current medications. A participant was classified as having diabetes if she reported a diabetes diagnosis, using medications for diabetes, or having a fasting glucose concentration greater than 125 mg/dl. The physical functioning scale of the Medical Outcomes Scale (MOS-SF-36) [23, 24] was the basis of a three-level variable with values from 5 to -100 (best) and cutpoints at 35 and 85.

Data analysis

The major dependent variables were osteocalcin or NTx concentrations. The primary explanatory or independent variables were circulating endogenous hormone concentrations or menopausal status. After we had evaluated a number of variables as potential confounders with osteocalcin and NTx, the variables significantly associated with NTx concentrations included BMI, thyroid disease, insulin sensitivity, difficulty in paying for basics, race/ethnicity, seasonality and site. Those variables significantly associated with osteocalcin included smoking, physical activity, physical functioning, BMI, diabetes, insulin sensitivity, thyroid disease, race and site. These variables were eligible to enter into a multiple-variable model specific to either osteocalcin or NTx. A natural log or square root (for SHBG and TSH) transformation was applied to the continuous hormone measures to address skewness or to satisfy statistical modeling assumptions such as normally distributed residuals. Data management and data analyses were undertaken using SAS version 6.12 or 8.1 (SAS Institute, Cary, NC).

Regression analyses were used to evaluate the role of day of the menstrual cycle in the relationship between turnover markers and hormone concentrations. Though previous studies have been equivocal with respect to the association of bone turnover markers with menstrual cycle time [25, 26] we observed no association with the day of the menstrual cycle, and no variables were entered into the model to account for day of cycle.

Serum hormone concentrations or their associated indices and pre- versus early perimenopausal status were related to the bone turnover markers using multiple variable linear regression analyses. Model fit was assessed graphically and by using residual analyses as well as regression diagnostics. Analysis of covariance was used to calculate the least square mean concentrations of osteocalcin and NTx, according to pre- and early perimenopausal status, after adjusting for covariates. Transformed mean values were untransformed for presentation and appropriate adjustments made for the back-transformation of the variance estimates. FSH concentrations were categorized into quartiles to depict graphically the association with bone turnover markers.

Results

Table 1 describes the bone turnover, hormone, BMD and body size characteristics of the 2,375 women studied. The mean osteocalcin and NTx values were 16.1 ng/ml and 34.8 nM BCE, respectively, while the mean FSH and estradiol concentrations were 24.1 mIU/ml and 75.3 pg/ml, respectively, in this pre- and perimenopausal sample with a mean age of 46.4 years. The mean serum

Table 1 Bone turnover marker, body size, hormone and bone mineral density characteristics of the SWAN population ($n = 2375$) at baseline (*IQR* interquartile range)

Measures	Unit	*n*	Mean	SD	Median	IQR
Bone turnover markers						
Osteocalcin	ng/ml	2,356	16.1	6.2	15.2	12.1–19.1
NTx/Creatinine	nM BCE/mM Cr	2,353	34.8	20.1	30.5	21.9–41.9
Body size measures						
Weight	kg	2,357	73.9	20.6	69.2	58.0–84.9
Body mass index (BMI)	kg/m^2	2,336	27.9	7.3	26	22.5–31.8
Height	cm	2,346	162.5	6.6	162.5	158.0–167.0
Hormones and indices						
FSH	mIU/ml	2,367	24.1	25.4	15.8	11.0–26.4
SHBG	nM	2,365	45.5	24.6	41.1	28.1–58.1
Estradiol	pg/ml	2,368	75.3	76.3	55	33.2–87.2
Free Estradiol Index		2,365	0.77	1.04	0.51	0.32 –0.86
Testosterone	ng/ml	2,366	46.7	28.6	41.5	29.6–55.5
Free Androgen Index		2,364	5.05	5.70	3.53	2.13–6.01
TSH	mIU/ml	2,344	2.3	1.9	1.9	1.3–2.8
Insulin sensitivity		2,259	2.3	2.0	1.8	1.4–2.6
Bone mineral density						
Lumbar spine	g/cm^2	2240	1.078	0.14	1.075	0.980–1.164
Femoral neck	g/cm^2	2291	0.846	0.14	0.836	0.747–0.930
Total hip	g/cm^2	2292	0.963	0.15	0.952	0.859–1.053
Hip bone area	cm^2	2292	33.0	3.4	32.7	30.7–35.1
Age	years	2375	46.4	2.7	46.2	44.1–48.4
Physical activity score		2371	7.68	1.83	7.65	6.40–8.95

FSH concentration (unadjusted for covariates) was 25% greater in perimenopausal women compared with the mean value for premenopausal women. Fifty-four percent of women were classified as premenopausal while 46% were classified as early perimenopausal, based on variability in menstrual bleeding (Table 2).

As shown in Table 3, serum FSH and SHBG concentrations were significantly and positively associated with both bone turnover measures. NTx was inversely and weakly associated with estradiol concentrations ($r = -0.06$, $p < 0.05$). The FAI was inversely and weakly associated with both osteocalcin ($r = -0.05$, $p < 0.05$) and NTx ($r = -0.07$, $p < 0.001$). TSH concentrations and insulin sensitivity were negatively associated with NTx concentrations while insulin sensitivity was negatively associated with osteocalcin concentrations.

Though osteocalcin and NTx concentrations are somewhat higher in the early perimenopausal women these differences were very small (2.3% and 2.8%, respectively) and not significantly different from each other.

Table 2 The number and frequencies of SWAN participants with bone turnover data according categorical variables for site, demographic information and health status

Variable	Level	*n* (%)
Race/ethnicity	African-American	671 (28.3)
	Caucasian	1174 (49.4)
	Chinese	249 (10.5)
	Japanese	281 (11.8)
Site of study	Michigan	528 (22.2)
	Boston	443 (18.7)
	Davis	456 (19.2)
	Los Angeles	494 (20.8)
	Pittsburgh	463 (19.1)
Menopausal status	Early perimenopausal	1077 (46.0)
	Premenopausal	1265 (54.0)
Smoking	Never	1374 (58.8)
	Past only	587 (25.1)
	Present	375 (16.1)
Diabetes (treated or glucose >125 mg/dl)	No	2207 (93.0)
	Yes	167 (7.0)
Currently using thiazide medication	No	2273 (95.7)
	Yes	102 (4.3)

Table 3 The correlation coefficients between bone markers and serum reproductive hormones or their indices, unadjusted for covariates

		Osteocalcin	NTx/Creatinine ratio
FSH	mIU/ml	0.11***	0.11***
SHBG	nM	0.09***	0.09***
Total estradiol	pg/ml	0.04	0.06*
Free Estradiol Index		0.02	0.10***
Total testosterone	ng/dl	0.01	0.02
Free Androgen Index		0.05*	0.07**
TSH	mIU/ml	0.01	−0.10***
Insulin sensitivity		0.10***	−0.06*

p value for test that observed correlation is not equal to zero: *$p < 0.05$;**$p < 0.001$; ***$p < 0.0001$

Multiple-variable models

In a multiple-variable model that explained approximately 9% of the variation in NTx values (Table 4), FSH concentrations were positively associated with bone resorption, explaining 2.1% of the total explained variation (8.9%). As seen in Fig. 1, there was a higher mean adjusted NTx in those women in the highest quartile of the FSH distribution (values greater than 26 mIU/ml). Variables for season of the year (lower NTx in the summer) and thiazide antihypertensive use (lower NTx with use) each contributed approximately 1% of the total 9% explained variability.

As seen in Fig. 2, there was a higher mean adjusted osteocalcin in the highest quartile of FSH. While FSH concentrations remained positively associated with osteocalcin concentrations, contributing 1% of the total 15% explained variation ($p < 0.0001$), diabetes explained approximately one-fourth of the total explained variation ($p < 0.0001$).

Discussion

Studies of skeletal biology and the menopause have been largely confined to studies of estradiol concentrations, the duration of postmenopause or the replacement of estrogen around the menopause. This study extends previous work by identifying that in pre- and early perimenopausal women, higher FSH concentrations are associated with greater bone turnover. While FSH was associated with both bone turnover markers, there was no association of bone turnover markers with estradiol or testosterone concentrations, after accounting for important covariates in pre- and perimenopausal women. This study indicates that higher FSH concentrations, prior to the last menstrual period, were associated with greater bone formation and resorption activity. However, this association was discernible only when the transition was characterized by FSH and not identified in a classification system defined by bleeding regularity. Though the positive association of both osteocalcin and NTx with FSH would suggest greater bone turnover, the data are not indicative of an uncoupling of formation and resorption processes.

To best understand the pattern of bone turnover change in the transition period will require longitudinal study, something that SWAN has undertaken. Currently, there is a single report of a longitudinal study following 15 women (with 6 remaining premenopausal); urinary pyridinoline and deoxypyridinoline concentrations were approximately 25–33% greater in the women who became postmenopausal, while no difference was reported in the pre- and perimenopausal women. These concentrations were not correlated with reproductive hormones [27].

Cross-sectional studies have been inconsistent in describing the magnitude of osteocalcin changes with menopause. Some investigators have found that osteo-

Table 4 Multiple variable regression models to characterize the sources of variation in urinary N-telopeptide (NTx) and serum osteocalcin concentrations in the women of SWAN

Variable	Parameter estimate	Coefficient of partial determination	*p* value	Coefficient of determination
NTx (nM BCE/mM Cr) model[a]				
FSH (mIU/ml)	0.003	0.021	< 0.0001	8.9%
Thiazide medication use	−0.285	0.013	< 0.0001	
Measured in summer	−0.087	0.010	< 0.0001	
TSH (mIU/ml)	−0.065	0.005	< 0.0001	
TSH	0.004	0.006	< 0.0001	
SHBG (nM)	0.002	0.007	0.0001	
African-American	−0.078	0.004	< 0.004	
Diabetes	−0.077	0.002	< 0.05	
Osteocalcin (ng/ml) model[a]				
Diabetes	−0.216	0.041	< 0.0001	15.4%
Total hip area (cm^2)	0.009	0.022	< 0.0001	
$_{log}$BMI	−0.249	0.015	< 0.0001	
FSH (mIU/ml)	0.002	0.012	< 0.0001	
Japanese	−0.196	0.025	< 0.0001	
Chinese	−0.174	0.010	< 0.0001	
Current smoker	−0.098	0.009	< 0.0001	
African-American	−0.089	0.006	< 0.0001	
SHBG (nM)	0.001	0.003	< 0.006	
Free Androgen Index	0.001	0.002	< 0.02	

[a]Models include adjustment for individual study site with Michigan as the reference site; Caucasians were the reference race/ethnic group

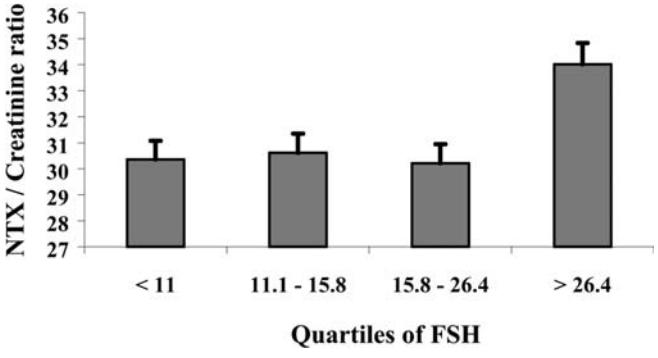

Fig. 1 Mean NTx (nM BCE/mM Cr), adjusted for covariates, according to quartiles of the FSH distribution in the pre- and perimenopausal women of SWAN

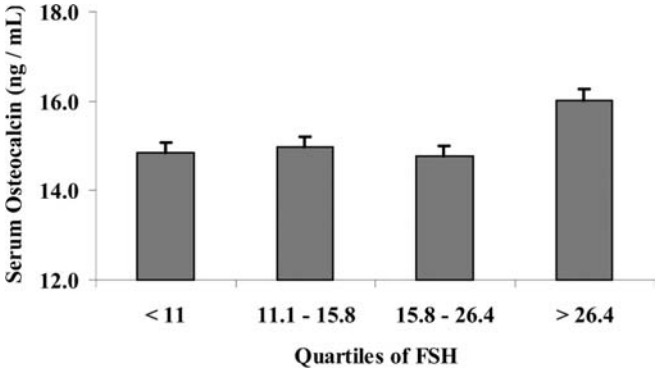

Fig. 2 Mean osteocalcin (ng/ml), adjusted for covariates, according to quartiles of the FSH distribution in the pre- and perimenopausal women of SWAN

calcin concentrations were approximately 30% higher in postmenopausal women than in premenopausal women [28, 29]. Ebeling et al. [14] reported that osteocalcin concentrations were approximately 70% and 150% higher in peri- and postmenopausal women, respectively, compared with premenopausal women. These differences in magnitude could potentially reflect greater bone turnover at different stages of the menopause transition or a different magnitude of bone turnover relative to the time since the last menstrual period. It is unknown whether these differences in magnitude also reflect the use of single point-in-time measures of osteocalcin or different osteocalcin assay methodologies.

With respect to bone resorption markers, Ebeling et al. [14] reported that NTx was approximately 30% greater in peri- and postmenopausal women, respectively, compared with premenopausal women. When Garton et al. [30] classified 68 women into three groups according to FSH levels (< 10, 10–35 and > 35 U/I), they reported that pyridinoline crosslink concentrations were approximately 30% higher in those women whose FSH levels were greater than 35 U/I. This supports our findings that indicated higher FSH values were associated with higher NTx concentrations.

There is surprisingly little information about health or medical conditions and lifestyle behaviors related to bone turnover, particularly conditions and behaviors that may have their origins or primary expression at the mid-life and the menopause. Diabetes was associated with both osteocalcin and NTX concentrations, indicating the importance of the metabolic processes associated with diabetes in this transitional period. Women with diabetes have been variously reported to have higher or lower risk of osteoporotic fracture, and their BMD may be higher not lower than other women with osteoporotic fracture [31, 32, 33, 34, 35, 36]. To explain a higher fracture risk in diabetics in the presence of higher BMD, investigators have cited the increased risk of falls, limitations in functional ability, impact of neuropathy, use of medications and poor vision, but have not

considered a role for bone metabolism prior to the menopause. Our study suggests that impaired carbohydrate metabolism may influence bone metabolism during the time of the menopausal transition.

Greater SHBG concentrations were related to higher levels of both osteocalcin and NTx, though not to the same degree as FSH. In other studies, SHBG concentrations have been positively associated with age and with bone change in the menopausal transition [37]. Higher serum SHBG concentrations were associated with lower levels of spine BMD, but not with total hip or femoral neck BMD [12]. It has been speculated that higher serum SHBG levels are associated with lower peak bone mass by diminishing the availability of free or biologically active testosterone and estradiol [37].

We included a measure of seasonality in our regression models, recognizing that our study sites were geographically dispersed and that enrollees from the California sites in Oakland and Los Angeles had more opportunity for sunlight exposure than women in Michigan, Pennsylvania and Massachusetts. There were lower bone turnover concentrations in samples collected during the North American summer. Though 25-hydroxyvitamin D was not measured, we speculated that the effect on the bone turnover markers could be attributed to subclinical vitamin D deficiency during the winter period, as has been proposed by other investigators [38, 39]. We evaluated dietary calcium intake in the population and identified no association with either osteocalcin or NTx concentrations.

In summary, this cross-sectional study of women at the mid-life identifies that higher FSH concentrations are positively associated with measures of osteocalcin and NTx, measures thought to represent bone turnover. Serum estradiol and testosterone concentrations, as well as the indices calculated with SHBG, were not associated with these bone turnover markers.

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Appendix. SWAN

Clinical Centers University of Michigan, Ann Arbor, MI (U01 NR04061, MaryFran Sowers, PI); Massachusetts General Hospital, Boston, MA (U01 AG12531, Joel S. Finkelstein, PI); University of California/Kaiser, Davis, CA (U01 AG12554, Ellen Gold, PI); University of California, Los Angeles, CA (U01 AG12539, Gail Greendale PI); and the University of Pittsburgh, Pittsburgh, PA (U01 AG12546, Karen Matthews, PI).

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Coordinating Center University of Pittsburgh (Kim Sutton-Tyrrell, PI).

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Steering Committee Chair Jennifer L. Kelsey.

The manuscript was reviewed by the Publications and Presentations Committee of SWAN and has its endorsement.

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