Menopause Is a Determinant of Breast Aromatase Expression and Its Associations With BMI, Inflammation, and Systemic Markers

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Context: Most estrogen-dependent breast cancers occur after menopause, despite low levels of circulating estrogens. Breast expression of the estrogen-biosynthetic enzyme, aromatase, is proposed to drive breast cancer development after menopause. However, the effects of menopause on breast aromatase expression are unknown.

Objective: To determine the effect of menopause on breast aromatase expression in relation to body mass index (BMI), white adipose tissue inflammation (WATi), and systemic markers of metabolic dysfunction.

Design, Setting, and Participants: Cross-sectional study of 102 premenopausal (age 27 to 56) and 59 postmenopausal (age 45 to 74) women who underwent mastectomy for breast cancer treatment/prevention.

Outcome: Breast tissue was assessed for the presence of crown-like structures and the expression and activity of aromatase. Systemic markers examined include interleukin (IL)-6, insulin, glucose, leptin, adiponectin, high-sensitivity C-reactive protein (hsCRP), cholesterol, and triglycerides. Multivariable analysis was performed for aromatase messenger RNA (mRNA) in relation to BMI, WATi, and blood markers.

Results: Postmenopausal women had higher BMI and more breast WATi than premenopausal women. Fasting levels of IL-6, glucose, leptin, hsCRP, and homeostatic model assessment 2 insulin resistance score were higher in the postmenopausal group. BMI was positively correlated with aromatase mRNA in both pre- and postmenopausal women. Aromatase levels were higher in breast tissue of postmenopausal women, with levels being higher in inflamed vs noninflamed, independent of BMI. Adipocyte diameter and levels of leptin, hsCRP, adiponectin, and high-density lipoprotein cholesterol were more strongly correlated with aromatase in postmenopausal than premenopausal women.

Conclusions: Elevated aromatase in the setting of adipose dysfunction provides a possible mechanism for the higher incidence of hormone-dependent breast cancer in obese women after menopause. (***J Clin Endocrinol Metab*** **102: 1692–1701, 2017)**

Abbreviations: BMI, body mass index; CLS, crown-like structures; CLS-B, crown-like structures of the breast; HOMA2-IR, homeostatic model assessment 2 insulin resistance; hsCRP, high-sensitivity C-reactive protein; IL, interleukin; mRNA, messenger RNA; MSKCC, Memorial Sloan Kettering Cancer Center; PCR, polymerase chain reaction; WAT, white adipose tissue; WATi, white adipose tissue inflammation.

Menopause is associated with significant changes in hormone levels, which contribute to physical and biological consequences that impact disease. For example, the menopausal transition has been associated with weight gain and central adiposity, elevated levels of systemic markers of inflammation, and metabolic dysfunction including C-reactive protein (CRP), interleukin (IL)-6, tumor necrosis factor-α, and leptin and increased incidence of metabolic syndrome and cardiovascular disease (1–4). Many cancers, including breast cancer, also occur more frequently after menopause. Despite low levels of circulating estrogens (5), the majority of breast cancers that occur after menopause are estrogen dependent (6), and the risk of breast cancer in these women is increased with obesity and metabolic syndrome (7).

Aromatase is the enzyme that catalyzes the final and key step in estrogen biosynthesis. Considering the low concentrations of circulating estrogens after menopause, it has been proposed that breast-derived estrogens are key drivers of breast cancer growth and that established tumors have the capacity to further increase the local production of estrogens (8). Consistent with this hypothesis, aromatase levels and activity have been reported to be highest in the breast quadrant containing the breast tumor (9–14). Aromatase is expressed in breast adipose stromal cells and is increased in response to a number of obesity-associated factors *in vitro*, including adipokines (*e.g.*, leptin) and inflammatory mediators (*e.g.*, prostaglandin E_2, IL-6, and tumor necrosis factor-α) (15–17). Studies in mice and women have demonstrated that elevated weight or body mass index (BMI), as well as associated white adipose tissue inflammation (WATi), are positively correlated with aromatase in mammary tissue (18, 19).

With obesity, adipocytes become dysfunctional and can undergo cell death. This leads to infiltration of immune cells, including macrophages, that form histologically visible crown-like structures (CLS) in visceral and subcutaneous fat (20). WATi also occurs in breast tissue, and CLS of the breast (CLS-B) have been reported to be associated with increased fasting glucose, insulin, triglycerides, leptin, high-sensitivity CRP (hsCRP), and IL-6, as well as breast aromatase expression (18, 19, 21). Menopause is also a significant determinant of breast WATi (22).

Despite the acknowledged importance of estrogens in driving breast cancer growth in postmenopausal women, the effect of menopause on breast aromatase has, to our knowledge, not been examined. The aim of this study was to determine whether menopause affects breast aromatase expression, and its association with BMI, breast WATi, and systemic markers of metabolic function.

Materials and Methods

Study population and specimen collection

Breast tissue (uninvolved by tumor) and blood were obtained from women undergoing mastectomy for the treatment or prevention of breast cancer (n = 161). Breast tissue was either snap frozen or formalin fixed and paraffin embedded. Blood was separated into plasma and serum, and stored at –80°C. Clinicopathological data were obtained from electronic medical records and quality assured through independent data review. BMI was calculated from height and weight measurements obtained prior to surgery. Women were considered postmenopausal if they had reported cessation of menses or had undergone bilateral oophorectomy at least 1 year prior to biospecimen collection, in accordance with criteria established by the National Comprehensive Cancer Network (23). The study was approved by the Institutional Review Boards of Memorial Sloan Kettering Cancer Center (MSKCC) and Weill Cornell Medical College (New York, NY), and informed consent was obtained from women at MSKCC.

Measurement of systemic factors

Glucose (BioAssay Systems), insulin (Mercodia), leptin, adiponectin, hsCRP, and IL-6 (R&D Systems) were measured in plasma using an enzyme-linked immunosorbent assay. Fasting serum levels of triglycerides, as well as total, low-density, and high-density lipoprotein (HDL) cholesterol were measured by the clinical chemistry laboratory at MSKCC. Quality control was ensured, with intra-assay coefficients of variation being less than 7%.

Assessment of breast WATi and adipocyte diameters

Breast WATi was assessed by measuring the number of CLS-B as previously described (18). Briefly, immunohistochemistry for CD68 (mouse monoclonal KP1 antibody; Dako; 1:4,000) was performed on five sections of breast white adipose tissue (WAT) per case. The study pathologist (D.D.G.) assessed cases as CLS-B-positive (CLS-B+) or negative (CLS-B–) and recorded the number of CLS-B per case. To determine the total WAT area examined, exclusive of epithelial and fibrotic tissue, digital photographs of each slide were generated and measured with Image J Software (National Institutes of Health). In cases where CLS-B were detected, those with #CLS-B/cm^2 above the median value (0.40) were defined as having severe inflammation, whereas those with values below the median were defined as having mild inflammation.

Adipocyte diameter measurements were performed as previously described (18). Briefly, breast WAT hematoxylin and eosin–stained sections were photographed at 20× using an Olympus B ×50 microscope and MicroFire digital camera (Optronics). Mean diameters were then calculated from the digitized images by measuring 30 or more individual adipocytes for each patient using the linear dimensional tool in the Canvas 11 Software (ACD Systems International, Inc.).

Quantitative real-time PCR

Total RNA was isolated using the RNeasy Mini Kit (Qiagen). RNA (100 ng) was reverse transcribed using the qScript cDNA Synthesis Kit (QuantaBio) and the resulting cDNA used for real-time polymerase chain reaction (PCR) amplification with Fast SYBR green PCR master mix on a 7500HT real-time PCR system (Applied Biosystems). GAPDH was

used as an endogenous normalization control. Primers used were as follows: aromatase, forward: 5′-CACATCCTCAA-TACCAGGTCC-3′ and reverse: 5′-CAGAGATCCAGACTCG-CATG-3′; and GAPDH, forward: 5′-TTCTTTTGCGTCGCCA-GCCGA-3′ and reverse: 5′-GTGACCAGGCGCCCAATACGA-3′. Relative fold-induction was determined using the $\Delta\Delta C_T$ analysis protocol.

Aromatase activity

Aromatase activity was measured in microsomal preparations from breast tissue lysates using the tritiated water-release assay (24). Aromatase activity is expressed as femtomoles substrate converted per microgram of protein per hour.

Statistical analyses

The difference in each clinicopathologic feature between premenopausal and postmenopausal patients was examined using the nonparametric Wilcoxon rank-sum test for a continuous feature and Fisher's exact test or χ^2 test where appropriate for a categorical feature. The association between a continuous variable, such as the expression of aromatase or levels of a systemic marker of metabolic dysfunction, and menopausal status was examined using the Wilcoxon rank-sum test. The association between a continuous variable and menopausal status adjusting for other covariates, such as BMI and/or CLS-B status, was examined using the multiple linear regression model. The strength of the correlation between aromatase expression and each blood parameter in the study cohort and in premenopausal and postmenopausal women was examined using Spearman's method. The difference in the strength of correlation between premenopausal and postmenopausal women was further examined using a multiple linear regression model with an interaction term between the blood biomarker and the menopausal status. Levels of aromatase expression and several blood parameters, including IL-6, insulin, glucose, leptin, adiponectin, and hsCRP, were log transformed and the number of CLS-B/cm^2 was square-root transformed when linear regression analysis was used to ensure the underlying model assumptions are satisfied. A P value of ≤ 0.05 is considered statistically significant.

Results

Postmenopausal women had significantly higher BMI, more severe WATi, and higher breast expression of aromatase

One hundred sixty-one women who underwent mastectomy for breast cancer treatment or prevention were included in the study: 102 were premenopausal and 59 were postmenopausal. Clinicopathological features are presented in Table 1. Compared with premenopausal

Table 1. Clinicopathologic Features of Study Population Based on Menopausal Status

Variables	All (n = 161)	Pre (n = 102)	Post (n = 59)	*P*
Age, median (range)	48 (27 to 74)	44.5 (27 to 56)	55 (45 to 74)	**<0.001**
BMI, median (range)	25.73 (17.27 to 62.58)	24.75 (17.27 to 49.98)	27.95 (18.36 to 62.58)	**0.001**
CLS-B/cm^2, n (%)				
No	68 (43.04%)	51 (51%)	17 (29.31%)	
Mild	45 (28.48%)	30 (30%)	15 (25.86%)	
Severe	45 (28.48%)	19 (19%)	26 (44.83%)	**0.002**
Missing	3 (1.86%)	2 (1.96%)	1 (1.69%)	1
Diabetes, n (%)				
No	55 (96.27%)	101 (99.02%)	54 (91.53%)	
Yes	6 (3.73%)	1 (0.98%)	5 (8.47%)	**0.025**
Dyslipidemia, n (%)				
No	142 (88.2%)	99 (97.06%)	43 (72.88%)	
Yes	19 (11.8%)	3 (2.94%)	16 (27.12%)	**<0.001**
Hypertension, n (%)				
No	135 (84.38%)	92 (91.09%)	43 (72.88%)	
Yes	25 (15.62%)	9 (8.91%)	16 (27.12%)	**0.003**
Missing	1 (0.62%)	1 (0.98%)	0 (0%)	1
Race, n (%)				
Asian	7 (4.79%)	7 (7.61%)	0 (0%)	
Black	13 (8.9%)	5 (5.43%)	8 (14.81%)	
Other	4 (2.74%)	3 (3.26%)	1 (1.85%)	
White	122 (83.56%)	77 (83.7%)	45 (83.33%)	**0.043**
Missing	15 (9.32%)	10 (9.8%)	5 (8.47%)	1
Invasive tumor present				
No	29 (18.47%)	18 (18.18%)	11 (18.97%)	
Yes	128 (81.53%)	81 (81.82%)	47 (81.03%)	1
Missing	4 (2.48%)	3 (2.94%)	1 (1.69%)	1
Tumor subtype, n (%)				
HR+	88 (69.84%)	52 (65.82%)	36 (76.6%)	
HER2+	20 (15.87%)	14 (17.72%)	6 (12.77%)	
TNBC	18 (14.29%)	13 (16.46%)	5 (10.64%)	0.491
N/A	35 (21.74%)	23 (22.55%)	12 (20.34%)	0.844

P values in bold reflect comparisons that are statistically different.

Abbreviations: HR+, hormone receptor positive; N/A, not applicable; TNBC, triple negative breast cancer.

women, postmenopausal women were significantly older [median (range): 55 (45 to 74) vs 44.5 (27 to 56); $P < 0.001$] and had a higher BMI [median (range): 27.95 (18.36 to 62.58) vs 24.75 (17.27 to 49.98); $P = 0.001$]. Women in the postmenopausal group were also more likely to have type II diabetes mellitus, hypertension, dyslipidemia, and more severe breast WATi, measured as the number of CLS-B/cm^2, compared with premenopausal women. There were no differences in breast cancer subtypes that developed in pre- vs postmenopausal women.

Aromatase messenger RNA (mRNA) levels were significantly higher in postmenopausal compared with premenopausal women [Fig. 1(a); median (range): 1.52 (0.28 to 18.91) vs 0.81 (0.1 to 4.79); $P < 0.001$]. Similarly, aromatase activity was also higher in the postmenopausal group [Fig. 1(b)]. Moreover, in the multivariable linear regression model including both age and menopausal status, only menopausal status was significantly associated with aromatase expression. There was a significant positive correlation between aromatase and BMI in both pre- and postmenopausal women [Fig. 1(c)]. In the postmenopausal group, being overweight or obese (BMI $\geq$ 25) was associated with higher levels of aromatase mRNA compared with having a healthy weight (BMI $<$ 25; $P < 0.001$), whereas the same comparison in premenopausal women did not achieve statistical significance. In women with a BMI $\geq$ 25, menopause was associated with higher aromatase [Fig. 1(d); $P < 0.001$].

Menopause is associated with larger adipocytes and more severe breast WATi, a determinant of aromatase expression

Menopause was found to be associated with significantly larger breast adipocytes and a higher prevalence of breast WATi, measured by assessing the presence of CLS-B (Fig. 2). Specifically, the median adipocyte diameter in postmenopausal women was 111.05 μm compared with 101.14 μm in the premenopausal group [Fig. 2(a); $P < 0.001$]. Aromatase transcript levels were significantly associated with adipocyte diameter in postmenopausal [Fig. 2(b); $P < 0.001$], but not premenopausal women. Menopause was associated with a higher proportion of CLS-B+ cases (42 of the 59 cases, or 71%) compared with premenopausal women [51 of the 102 cases, or 50%; Fig. 2(c) and 2(d); $P = 0.01$]. Aromatase was correlated with CLS-B/cm^2 in both pre- and

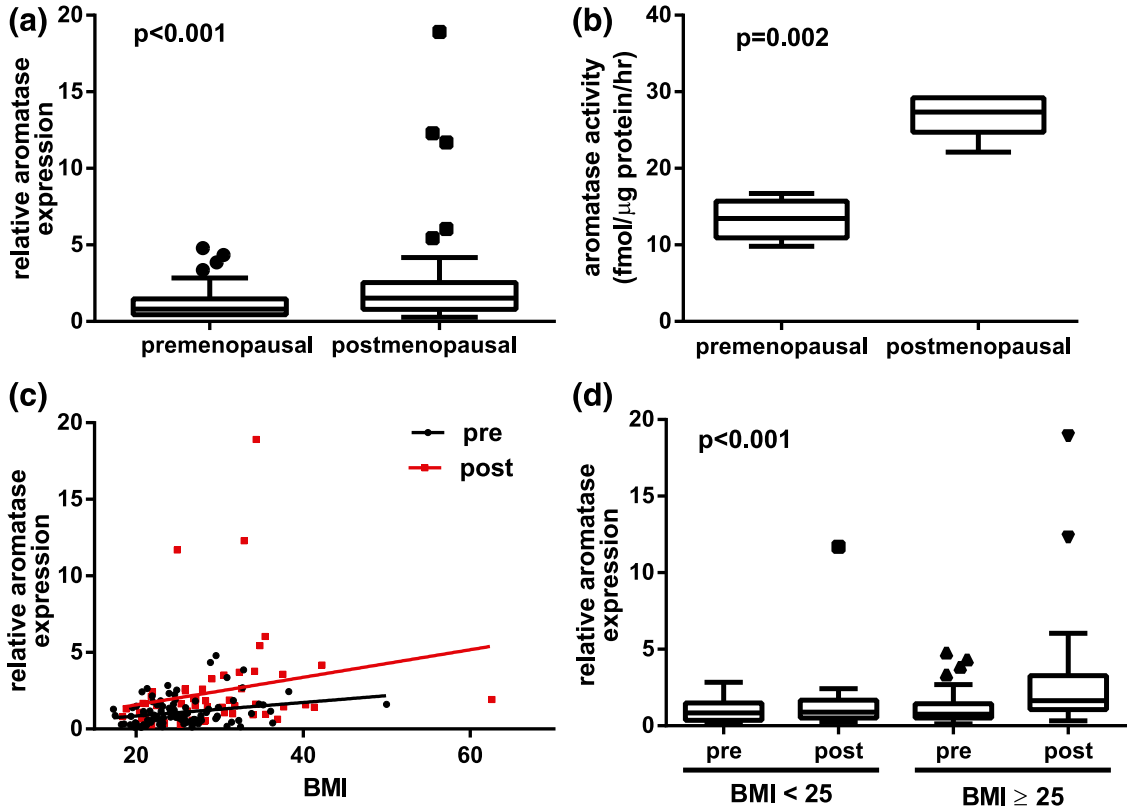

Figure 1. Effect of menopausal status on breast aromatase in relation to BMI. Breast levels of (a) aromatase mRNA and (b) activity are higher in postmenopausal compared with premenopausal women. (c) Aromatase transcript expression is positively correlated with BMI in pre- (black; ρ = 0.23; $P = 0.02$) and postmenopausal (red; ρ = 0.4; $P = 0.002$) women. (d) Menopause and overweight/obesity are associated with elevated levels of aromatase mRNA. Transcript expression was assessed in n = 161 (102 premenopausal, 59 postmenopausal), whereas aromatase activity was measured in n = 6/group.

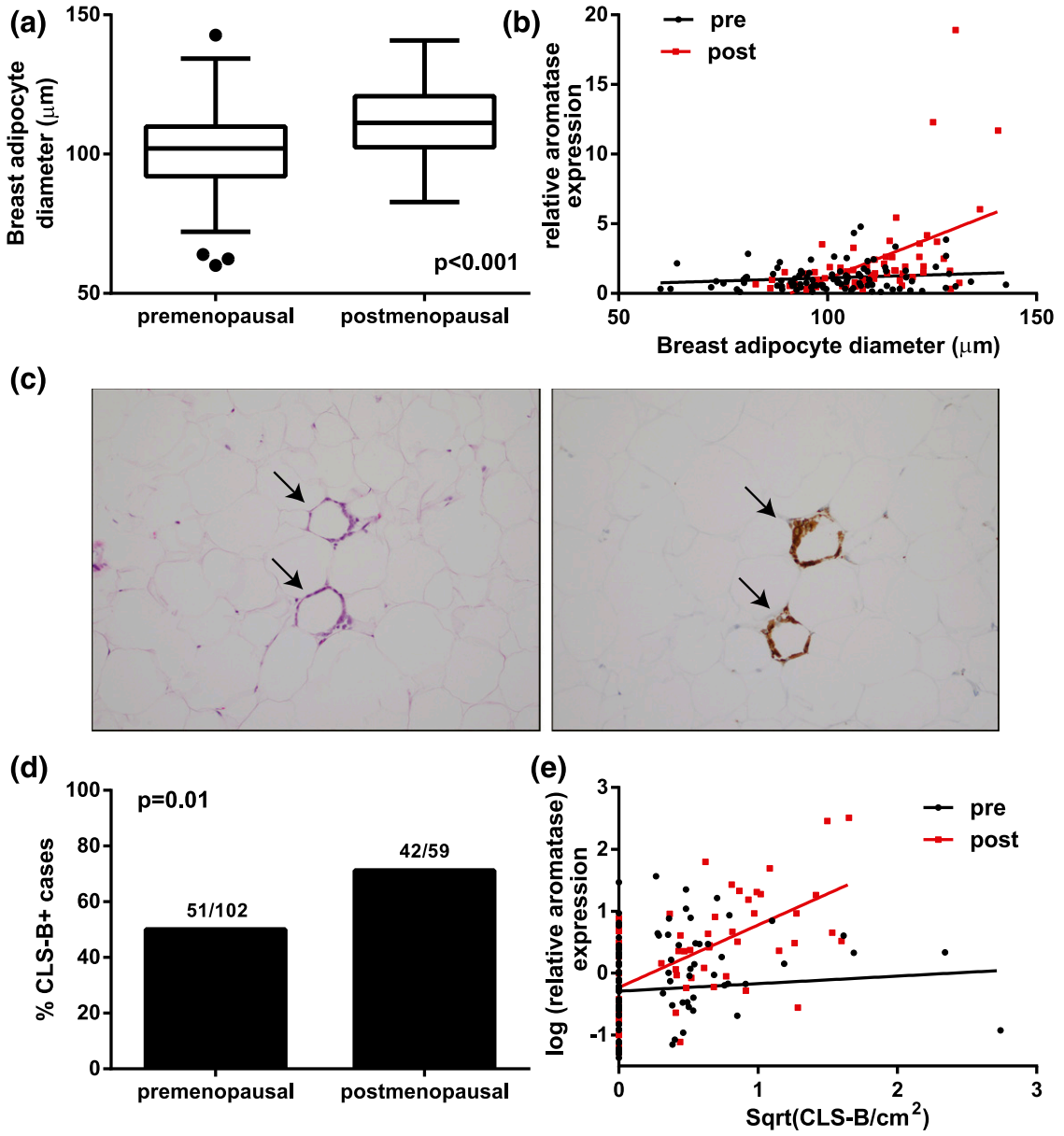

Figure 2. Impact of menopausal status on breast adipocyte diameter and WATi, and their association with aromatase. (a) Postmenopausal women have higher median breast adipocyte diameters than premenopausal women. (b) Aromatase mRNA levels are positively correlated with adipocyte diameters in postmenopausal (red; $\rho = 0.5$; $P < 0.001$), but not premenopausal women (black; $\rho = 0.11$; $P = 0.29$). (c) Representative images of WATi in breast. Hematoxylin and eosin (left) and CD68-stained (right) CLS-B (arrows). (d) The proportion of CLS-B+ cases is higher in postmenopausal compared with premenopausal women. (e) Aromatase is also positively correlated with CLS-B/cm^2 in both pre (black; $\rho = 0.21$; $P = 0.04$) and postmenopausal (red; $\rho = 0.57$; $P < 0.001$) women. The strength of the association is stronger in postmenopausal women compared with premenopausal women.

postmenopausal women, but the association was significantly stronger in postmenopausal women [Fig. 2(e); $P = 0.001$].

Menopause is associated with significant differences in levels of systemic markers

Circulating factors, stratified based on menopausal status, are presented in Table 2. Menopause was associated with a significant increase in the levels of IL-6 (median pre vs post: 0.99 pg/mL vs 1.68 pg/mL; $P < 0.001$), glucose (median pre vs post: 71.65 mg/dL vs 79.8 mg/dL; $P < 0.001$), leptin (median pre vs post: 11.85 ng/mL vs 21.08 ng/mL; $P < 0.001$), and hsCRP (media pre vs post: 0.75 μg/mL vs 2.06 μg/mL; $P < 0.001$). The homeostatic model assessment 2 insulin resistance (HOMA2-IR) score, which reflects insulin resistance, was also elevated in postmenopausal women (median pre vs post: 0.49 vs 0.59; $P = 0.044$).

Menopause may modify the strength of the correlation between aromatase and systemic markers

To determine whether systemic factors that reflect metabolic health are associated with aromatase expression,

Table 2. Effect of Menopause on Systemic Markers of Metabolic Dysfunction

Variables, Median (Range)	All (n = 161)	Pre (n = 102)	Post (n = 59)	*P*
IL-6, pg/mL	1.19 (0.22 to 125.3)	0.99 (0.22 to 48.79)	1.68 (0.31 to 125.3)	**<0.001**
Insulin, mU/L	4.82 (1.26 to 20.59)	4.68 (1.26 to 17.66)	5.36 (1.38 to 20.59)	0.071
Glucose, mg/dL	75 (33.7 to 163.3)	71.65 (49.55 to 156.1)	79.8 (33.7 to 163.3)	**0.009**
Leptin, ng/mL	14.11 (0.72 to 96.78)	11.85 (0.72 to 78.24)	21.08 (2.79 to 96.78)	**<0.001**
Adiponectin, μg/mL	10.68 (1.8 to 36.36)	10.61 (1.8 to 29.18)	11.08 (2.51 to 36.36)	0.241
Leptin/adiponectin	1.37 (0.04 to 21.63)	1.21 (0.04 to 18.07)	2.2 (0.12 to 21.63)	**0.027**
hsCRP, μg/mL	0.86 (0.02 to 34.52)	0.75 (0.02 to 27.94)	2.06 (0.14 to 34.52)	**<0.001**
HOMA2-IR	0.51 (0.12 to 2.08)	0.49 (0.14 to 2.08)	0.59 (0.12 to 2.03)	**0.044**
Total cholesterol, mg/dL	197 (116 to 300)	199.5 (117 to 285)	191 (116 to 300)	0.813
LDL cholesterol, mg/dL	113 (38 to 195)	113.1 (38 to 184.8)	110 (48 to 195)	0.973
HDL cholesterol, mg/dL	64 (35 to 120)	64 (38 to 120)	63 (35 to 101)	0.431
Triglycerides, mg/dL	72 (26 to 225)	71 (29 to 225)	72 (26 to 192)	0.377

P values in bold reflect comparisons that are statistically different.

Abbreviation: LDL, low-density lipoprotein.

correlative analyses between blood markers and breast aromatase mRNA, in relation to menopausal status, were performed. Results are summarized in Table 3. Overall, there was a significant positive correlation between aromatase mRNA and levels of IL-6, insulin, glucose, leptin, hsCRP, HOMA2-IR, and triglycerides. A significant inverse correlation was observed between breast aromatase transcript expression and systemic levels of adiponectin and HDL cholesterol. When the study population was segregated based on menopausal status, associations between aromatase mRNA and glucose remained significant in both groups. However, correlations between aromatase transcript expression and IL-6, leptin, adiponectin, hsCRP, HOMA2-IR, and HDL cholesterol were only significant in the postmenopausal group. The strength of associations between aromatase and blood markers in pre- and postmenopausal groups was then assessed (Fig. 3). Findings demonstrate that aromatase mRNA levels are more strongly associated with leptin, hsCRP, adiponectin, and HDL cholesterol after menopause.

Discussion

Findings presented herein describe the impact of menopause on the expression of aromatase in normal breast tissue of women at risk for breast cancer and breast cancer patients, and its relationship to systemic and local measures of inflammation and metabolic function.

Consistent with other studies, postmenopausal women had significantly higher BMI than the premenopausal group [reviewed in (25)]. Weight gain during the menopausal transition has been attributed to hormonal changes, lower physical activity, and increased energy intake (26–28). The menopausal transition has also been shown to be associated with changes in body composition, including increased truncal fat mass, waist circumference, and visceral and abdominal subcutaneous

Table 3. Correlations Between Aromatase and Blood Parameters in Pre- vs Postmenopausal Women

Blood Biomarker	All (n = 161)	Premenopausal (n = 102)	Postmenopausal (n = 59)
IL-6	0.32 (<0.001)	0.18 (0.08)	**0.37 (0.005)**
Insulin	0.25 (0.002)	0.19 (0.06)	0.25 (0.06)
Glucose	0.28 (<0.001)	**0.22 (0.03)**	**0.28 (0.04)**
Leptin	0.31 (<0.001)	0.16 (0.12)	**0.38 (0.003)**
Adiponectin	−0.18 (0.02)	−0.12 (0.22)	**-0.40 (0.002)**
Leptin/adiponectin	0.32 (<0.001)	0.17 (0.09)	**0.47 (<0.001)**
hsCRP	0.35 (<0.001)	0.18 (0.08)	**0.46 (<0.001)**
HOMA2-IR	0.26 (<0.001)	0.19 (0.07)	**0.27 (<0.05)**
Total cholesterol	0.02 (0.83)	0.13 (0.19)	−0.17 (0.22)
LDL cholesterol	0.09 (0.26)	0.17 (0.09)	−0.03 (0.81)
HDL cholesterol	−0.27 (<0.001)	−0.14 (0.16)	**-0.49 (<0.001)**
Triglycerides	0.20 (0.01)	**0.20 (<0.05)**	0.17 (0.21)

Data presented as Spearman rank correlation coefficient (ρ). *P* values in bold reflect statistically significant correlations.

Abbreviation: LDL, low-density lipoprotein.

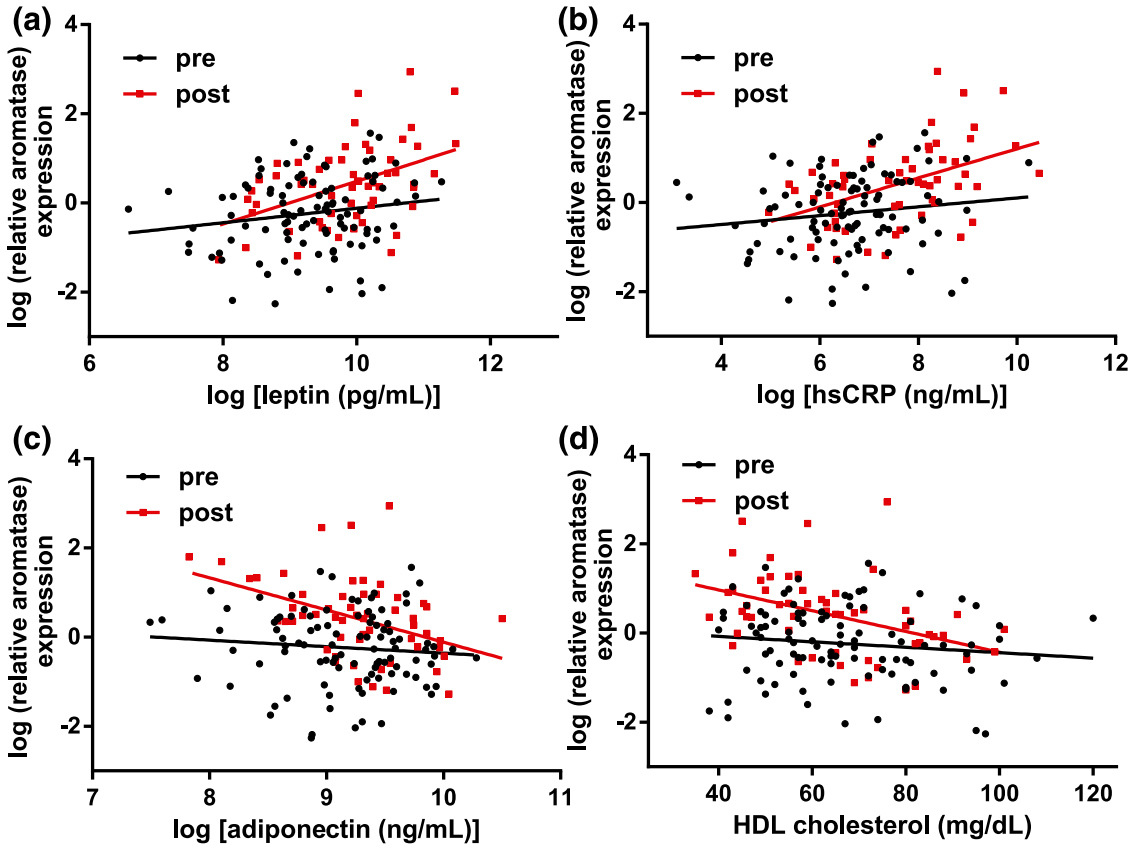

Figure 3. Effect of menopause on correlations between aromatase and markers of metabolic function. Aromatase mRNA is positively correlated with (a) leptin ($\rho = 0.38$; $P = 0.003$) and (b) hsCRP ($\rho = 0.46$; $P < 0.001$) in postmenopausal women (red), but not premenopausal women (black). An inverse correlation is observed between aromatase transcript expression and (c) adiponectin ($\rho = 0.4$; $P = 0.002$) and (d) HDL cholesterol ($\rho = 0.49$; $P < 0.001$) levels in the postmenopausal (red), but not the premenopausal (black) group.

fat (29–31). This is associated with metabolic dysfunction that increases the risk of metabolic syndrome and a number of comorbidities. In the current study population, menopause was associated with higher systemic levels of IL-6, glucose, leptin, and hsCRP. These findings are consistent with previous reports demonstrating that metabolic profiles in plasma are menopause dependent (1, 32). Moreover, postmenopausal women were more likely to have metabolic syndrome disorders including higher HOMA2-IR scores, type II diabetes mellitus, hypertension, and dyslipidemia, and a proportion of these reported use of medication, that is, antihypertensive, antidiabetic, or statins. Because of the limited number of cases, it is not possible to determine whether medication use affects WATi and aromatase in these women, and whether this is dependent on menopausal status. Future studies are required to address these questions.

We have previously reported that menopause is an independent determinant of breast WATi (22). Consistent with these findings, postmenopausal women in the present cohort also had higher incidence of severe breast WATi than the premenopausal group. Previous studies have also demonstrated that aromatase expression and activity are elevated in abdominal and gluteal subcutaneous tissues in relation to age and menopause (33, 34). This study, however, highlights the potential importance of locally produced estrogens in the development of breast cancer after menopause. Notably, and similar to findings relating to breast WATi (22), the variation in breast aromatase expression levels appeared to be better explained by menopausal status than by age. The cohort examined, inclusive of pre- and postmenopausal women, mostly developed estrogen receptor–positive breast cancer, which is consistent with numerous other studies (6). The observation that postmenopausal women continue to develop estrogen-dependent breast cancer despite a drop in systemic levels of estrogens supports a causal role for the local production of estrogen. This is further supported by studies demonstrating that tissue-to-plasma ratios of estrogens, including estrone, estradiol, and sulfoconjugated estrogens, are higher in benign breast tissue from postmenopausal women compared with premenopausal women (35–37). A recent study also supports the increased aromatization of androgens in the breast in relation to WATi, demonstrating that women in the lowest tertile of breast WAT estrone-to-androstenedione ratios were less likely to have breast

WATi compared with those in the highest tertile (38). The current study examined normal breast tissue from women undergoing mastectomy for breast cancer treatment or prevention, raising the question of whether findings are unique to women with breast cancer or at high risk of the disease. Previous studies have demonstrated that the breast quadrant containing the tumor has the highest levels of aromatase expression and activity (9–14), presumably due to the effects of tumor-derived factors. Nevertheless, findings presented herein demonstrate that aromatase is higher in uninvolved quadrants of the breast of postmenopausal women compared with premenopausal women, suggesting that a hormonal milieu conducive to tumor growth was present in these women prior to tumor formation. To ensure that results were not confounded by the presence of a tumor, additional analyses were performed whereby aromatase and CD68 (surrogate for CLS-B) mRNA levels were compared between uninvolved quadrants of the ipsilateral breast and the contralateral breast. There was a strong concordance in the expression of aromatase or CD68 at these sites (data not shown), suggesting that results obtained reflect levels of breast WATi and aromatase that are unaffected by tumor and that findings can potentially be extrapolated to cancer-free women.

Across the range of BMI, postmenopausal women had higher breast aromatase expression than their premenopausal counterparts. Furthermore, the current study also demonstrates that certain obesity-related parameters are more strongly associated with aromatase after menopause. For example, having a BMI ≥ 25 was associated with higher levels of aromatase compared with women with a BMI < 25 in postmenopausal women, but not premenopausal women. It is plausible that this highlights the inaccuracy of BMI as a reflection of adiposity across life stages in women, and it has been proposed that the cut-off for "obesity" should be reduced in postmenopausal women due to changes in body composition after menopause (39, 40). Interestingly, markers of adipocyte and metabolic function, as well as lipid metabolism, were found to be more strongly correlated with aromatase in postmenopausal compared with premenopausal women. In particular, the adipokines leptin and adiponectin, previously shown to regulate aromatase in breast adipose stromal cells (15), were found to be associated with aromatase in postmenopausal women, but not premenopausal women. Whether these effects are due to differences in cellular composition of the breast after menopause (*e.g.*, more adipose stromal cells, or increased sensitivity to these factors) remains to be determined. Moreover, aromatase expression was more strongly correlated with WATi in postmenopausal women than women in the premenopausal group. Considering that inflammatory mediators have been shown to affect stromal cell proliferation (41, 42), differentiation into adipocytes, including dedifferentiation of adipocytes (43, 44), and aromatase expression (15–17), these data suggest that WATi may contribute to the increased local production of estrogens after menopause. These results also suggest that the biology of the breast is different after menopause and that these factors, including breast WATi, may be key predictive markers for breast aromatase and cancer risk after menopause. These studies may also explain why obesity has a greater effect on breast cancer risk in postmenopausal women compared with premenopausal women.

Overall, these findings provide a mechanism that includes interactions among inflammation, metabolic dysfunction, and aromatase in the breast of women at risk for breast cancer or those who have developed breast cancer that helps explain the observed increased risk of hormone-dependent breast cancer after menopause and why obesity increases breast cancer risk in post- but not premenopausal women. Additional studies are required to determine whether these findings apply to cancer-free women and whether reducing weight may be associated with lower levels of breast aromatase and reduced breast cancer risk in older women.

Acknowledgments

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