

# Metabolic Obesity, Adipose Inflammation and Elevated Breast Aromatase in Women with Normal Body Mass Index



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## Abstract

Obesity is associated with breast white adipose tissue (WAT) inflammation, elevated levels of the estrogen biosynthetic enzyme, aromatase, and systemic changes that have been linked to the pathogenesis of breast cancer. Here, we determined whether metabolic obesity, including changes in breast biology and systemic effects, occurs in a subset of women with normal body mass index (BMI). Breast WAT and fasting blood were collected from 72 women with normal BMI (<25 kg/m<sup>2</sup>) undergoing mastectomy for breast cancer risk reduction or treatment. WAT inflammation was defined by the presence of crown-like structures of the breast (CLS-B) which are composed of dead or dying adipocytes surrounded by macrophages. Severity of inflammation was measured as CLS-B/cm<sup>2</sup>. The primary objective was to determine whether breast WAT inflammation is associated with aromatase expression and activity. Secondary objectives included assessment of circulat-

ing factors and breast adipocyte size. Breast WAT inflammation was present in 39% of women. Median BMI was 23.0 kg/m<sup>2</sup> (range, 18.4–24.9 kg/m<sup>2</sup>) in women with breast WAT inflammation versus 21.8 kg/m<sup>2</sup> (range, 17.3–24.6 kg/m<sup>2</sup>) in those without inflammation ( $P = 0.04$ ). Breast WAT inflammation was associated with elevated aromatase expression and activity, which increased with severity of inflammation ( $P < 0.05$ ). Breast WAT inflammation correlated with larger adipocytes ( $P = 0.01$ ) and higher circulating levels of C-reactive protein, leptin, insulin, and triglycerides ( $P \leq 0.05$ ). A subclinical inflammatory state associated with elevated aromatase in the breast, adipocyte hypertrophy, and systemic metabolic dysfunction occurs in some normal BMI women and may contribute to the pathogenesis of breast cancer. *Cancer Prev Res*; 10(4); 235–43. ©2017 AACR.

See related article by Berger, p. 223–25

## Introduction

Obesity, defined as a body mass index (BMI) of 30 kg/m<sup>2</sup> or greater, is a risk factor for the development of estrogen receptor (ER)-positive breast cancer in postmenopausal women (1, 2). After breast cancer diagnosis, obesity is associated with increased

risk of relapse and decreased overall survival across all breast tumor subtypes (3–6). We previously reported an inflammatory state in breast white adipose tissue (WAT) that occurs in the majority of women with elevated BMI and is associated with increased levels of aromatase, the rate-limiting enzyme for estrogen biosynthesis (7–10). Aromatase is a key pharmacologic target for both breast cancer prevention and treatment, and its local expression in breast WAT is thought to drive the growth of tumors in postmenopausal women. Importantly, we and others have reported that breast WAT inflammation is associated with worse prognosis for patients with breast cancer including shortened time to recurrence and worse overall survival (10, 11). The existence of breast WAT inflammation in cancer-free women suggests that its presence could also contribute to the development of cancer (12).

Inflammation has been implicated in the pathogenesis of obesity-associated disorders that promote the growth of breast cancer (13–21). These include insulin resistance and other conditions grouped together as the metabolic syndrome, for example, hypertension, dyslipidemia, and fasting hyperglycemia (22). Several clinical trials involving energy balance, that is, dietary modification and/or physical activity, or pharmacological targeting of metabolic pathways that are commonly deregulated in the setting of elevated BMI, have been undertaken to attenuate the effects of obesity on breast cancer risk and mortality (23–30).

Currently, BMI is the most commonly used measure of obesity in clinical trials that aim to mitigate its negative impact

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on cancer risk and progression. However, metabolic syndrome disorders that are classically associated with elevated BMI have been reported to occur in a subset of individuals with a normal BMI (31, 32). When found in individuals with a normal BMI, these disorders are collectively termed metabolic obesity in normal weight (33, 34) and as such, misclassification of cardiometabolic health and risk of cardiometabolic diseases occurs when using BMI categories (35). Considering that breast WAT inflammation is also found in a subset of women with normal BMI (9), the same is likely to occur when assessing cancer risk. Thus, the public health impact of obesity on cancer risk and mortality may be far greater than originally anticipated by conventional BMI categories.

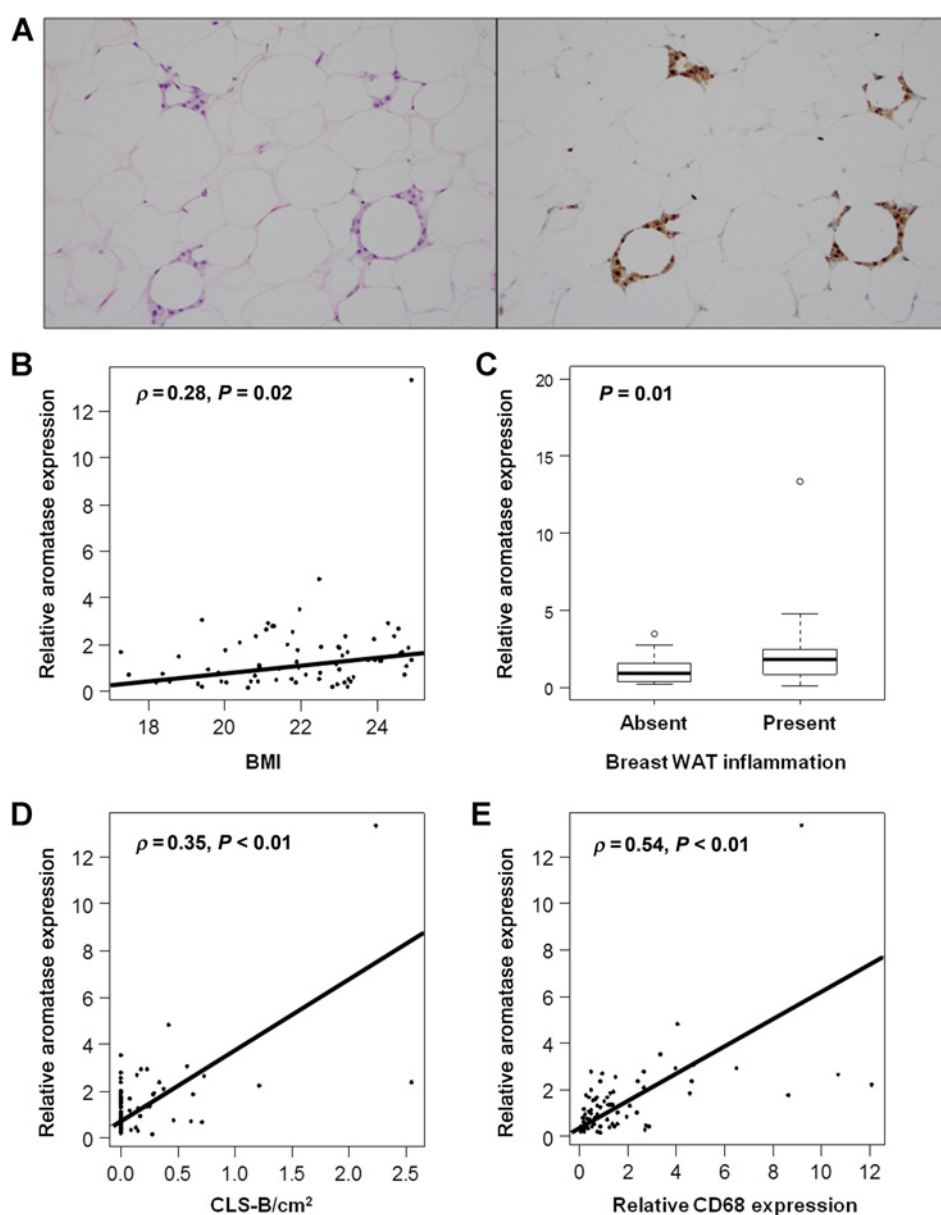
Aside from known inherited germline syndromes, the etiology of breast cancer in normal BMI individuals is not well-understood. We hypothesized that breast WAT inflammation could

reflect a metabolically obese state and a contributing cause of breast cancer despite normal BMI. Accordingly, we investigated whether breast WAT inflammation impacts aromatase levels in the breasts of women with normal BMI. We also investigated whether breast WAT inflammation in normal BMI women correlates with circulating metabolic and inflammatory factors implicated in the pathogenesis and progression of breast cancer.

## Materials and Methods

### Study design

Women considered to be normal weight by World Health Organization (WHO) criteria ( $BMI < 25 \text{ kg/m}^2$ ) and who were undergoing mastectomy for breast cancer risk reduction or treatment at Memorial Sloan Kettering Cancer Center (MSKCC; New York, NY) were eligible. Height and weight were recorded on the



**Figure 1.**

Elevated BMI and breast WAT inflammation are associated with increased aromatase expression in the breast. **A**, H&E (left) and anti-CD68 immunostaining (right) showing CLS-B (200 $\times$ ). **B**, Higher BMI correlates with elevated aromatase mRNA levels in breast tissue ( $\rho = 0.28$ ,  $P = 0.02$ ). **C**, Breast WAT inflammation is associated with higher breast aromatase expression ( $P = 0.01$ ). **D**, Increasing severity of breast WAT inflammation, measured as CLS-B/ $\text{cm}^2$ , correlates with higher breast aromatase mRNA levels ( $\rho = 0.35$ ,  $P < 0.01$ ). **E**, CD68 expression in breast tissue positively correlates with breast aromatase expression ( $\rho = 0.54$ ,  $P < 0.01$ ).

day of surgery and used to calculate BMI. Patients with known BRCA mutations were not eligible. At the time of surgery, non-tumor containing breast WAT and fasting blood samples were prospectively collected. This study was approved by the Institutional Review Boards of MSKCC, Weill Cornell Medical College (New York, NY), and Monash Health (Victoria, Australia).

#### Data and tissue collection

Clinicopathologic data were abstracted from the electronic medical record (EMR). Menopausal status was categorized as either premenopausal or postmenopausal on the basis of National Comprehensive Cancer Network criteria (36). Diagnoses of comorbidities including diabetes, hypertension, and dyslipidemia were recorded if documented in the EMR by the treating physician. Tumor subtype was classified as ER and/or progesterone receptor (PR) positive if >1% staining by immunohistochemistry (IHC) was reported. Human epidermal growth factor receptor-2 (HER2) was categorized as positive or negative if IHC 3+ or FISH amplification  $\geq 2.0$  (37).

For each subject, paraffin blocks and snap-frozen samples were prepared from breast WAT not involved by tumor on the day of mastectomy. Frozen samples were stored in the presence or absence of RNAlater (Ambion). A 30-mL fasting blood sample was obtained preoperatively on the day of surgery. Blood was separated into serum and plasma by centrifugation within 3 hours of collection and stored at  $-80^{\circ}\text{C}$ .

#### WAT inflammation

Consistent with established methods, the presence or absence of breast WAT inflammation was determined by histologic assessment (7, 9, 10). Breast WAT inflammation was defined by the presence of crown-like structures of the breast (CLS-B), which are composed of a dead or dying adipocyte surrounded by CD68-positive macrophages (7, 9, 10, 38). From each patient, 5 formalin-fixed, paraffin-embedded (FFPE) blocks were prepared, and 1 section per FFPE block (5- $\mu\text{m}$ -thick and  $\sim 2$  cm in diameter) was generated such that 5 sections were stained for CD68, a macrophage marker (mouse monoclonal KP1 antibody; Dako; dilution 1:4,000). Immunostained tissue sections were examined by the study pathologist (D.D. Giri) using light microscopy to detect the presence or absence of CLS-B and record the number of CLS-B per slide. Digital photographs of each slide were generated, and WAT area was measured with Image J Software (NIH, Bethesda, MD). The severity of WAT inflammation was quantified as number of CLS-B per square centimeter of WAT (CLS-B/ $\text{cm}^2$ ).

#### Adipocyte measurement

Two hematoxylin and eosin (H&E) sections were generated from FFPE breast tissue to measure adipocyte diameters as previously described (7, 9). The H&E sections were photographed at  $20\times$  using an Olympus BX50 microscope and MicroFire digital camera (Optronics). Mean diameters were calculated using measurements from 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 from frozen breast tissue using the RNeasy Mini Kit (Qiagen). One hundred nanograms of RNA was reverse-transcribed using the qScript cDNA Synthesis Kit (QuantaBio). The resulting cDNA was used for amplification. GAPDH

was used as an endogenous normalization control. Primers used were: CD68, forward 5'-GCTACATGGCGGTGGAGTACAA-3' and reverse: 5'-ATGATGAGAGGCAGCAAGATGG-3'; aromatase, forward: 5'-CACATCCTCAATACCAGGTCC-3' and reverse: 5'-CAG-AGATCCAGACTCGCATG-3'; GAPDH, forward: 5'-TTCTTTTG-CGTCGCCAGCCGA-3' and reverse: 5'-GTGACCAGGCCCCAA-TACGA-3'. Real-time PCR was conducted using Fast SYBR green PCR master mix on a 7500 HT real-time PCR system (Applied Biosystems), with expression determined using the  $\Delta\Delta\text{C}_T$  analysis protocol.

#### Aromatase activity and quantification

Microsomes were prepared from non-tumorous breast tissue lysates. Aromatase activity was quantified by measurement of the tritiated water released from  $1\beta$ -[ $^3\text{H}$ ] androstenedione (Perkin-Elmer Life Science; ref. 39). Aromatase activity was normalized to protein concentration and expressed as femtomoles per microgram of protein per hour.

To measure the levels of aromatase protein, tissue lysates were prepared from frozen non-tumorous breast tissue samples. Microsomal protein was isolated from tissue lysates using differential centrifugation. The microsomal suspension was subjected to immunoprecipitation with antisera to aromatase (anti-aromatase #677 mouse monoclonal antibody; Baylor College of Medicine, Houston, TX; ref. 40) followed by Western blotting (8). Protein levels of housekeeping gene  $\beta$ -actin were assessed from whole-tissue lysates by Western blotting.

Aromatase was also quantified in adipose stromal cells by immunofluorescence as previously described (41). Immunofluorescence was performed using anti-aromatase #677 mouse monoclonal antibody (dilution 1:500), anti-mouse Alexa Fluor 546 (Invitrogen, dilution 1:750), and Hoechst 33342 (Invitrogen, dilution 1:2,000). Imaging was performed using the Nikon inverted confocal microscope and perinuclear immunoreactivity quantified using Metamorph software (Molecular Devices).

**Table 1.** Baseline characteristics stratified by breast WAT inflammation

Characteristics	Breast WAT Inflammation		P
	Absent (n = 44)	Present (n = 28)	
BMI, $\text{kg}/\text{m}^2$			
Median (range)	21.8 (17.3–24.6)	23.0 (18.4–24.9)	0.04
Age, y			
Median (range)	47 (31–63)	49 (31–64)	0.26
Menopause, n (%)			
Pre	35 (80%)	19 (68%)	
Post	9 (20%)	9 (32%)	0.28
Race, n (%)			
Caucasian	34 (89%)	21 (88%)	
Black	1 (3%)	0 (0%)	
Asian	1 (3%)	3 (12%)	
Other	2 (5%)	0 (0%)	0.36
Not reported	7 (16%)	3 (11%)	0.73
Dyslipidemia, n (%)	0 (0%)	5 (19%)	<0.01
Statin use, n (%)	0 (0%)	3 (11%)	0.06
Diabetes mellitus, n (%)	0 (0%)	2 (7%)	0.15
Hypertension, n (%)	3 (7%)	3 (11%)	0.67
Tumor subtype, n (%)			
HR <sup>+</sup>	22 (63%)	12 (57%)	
HER2 <sup>+</sup>	9 (26%)	6 (29%)	
Triple-negative	4 (11%)	3 (14%)	0.86
Noninvasive or benign histology	9 (20%)	7 (25%)	0.77

Abbreviation: HR, hormone receptor.

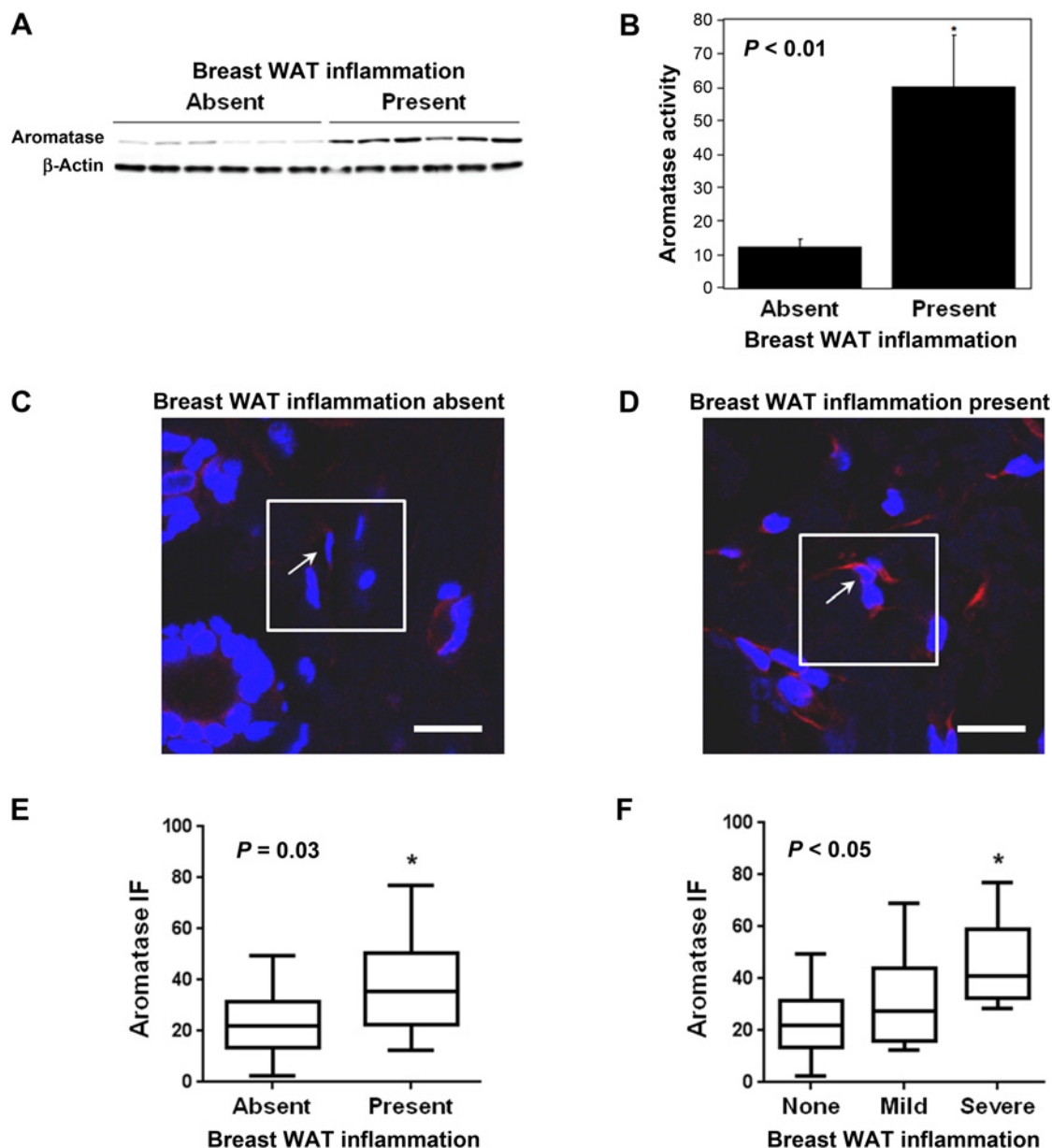
**Blood assays**

Plasma levels of leptin, adiponectin, high-sensitivity C-reactive protein (hsCRP), IL-6 (R&D Systems), and insulin (Merckodia) were measured by ELISA. Glucose levels were measured using the EnzyChrom Glucose Assay Kit (BioAssay Systems). Serum lipids including total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, and triglycerides were measured in the clinical chemistry laboratory at MSKCC. Intra-

assay coefficients of variation for quality control samples were less than 7%.

**Biostatistical analyses**

For continuous variables, the differences between patients with versus without breast WAT inflammation were examined using the nonparametric Wilcoxon rank-sum test. Severity of breast WAT inflammation was examined as a continuous variable and



**Figure 2.**

Women with breast WAT inflammation have higher aromatase protein levels and greater enzyme activity in the breast. **A**, Levels of aromatase protein assessed by Western blot analysis ( $n = 6/\text{group}$ ) are higher in women with breast WAT inflammation. **B**, Aromatase activity is higher in inflamed than uninflamed breast tissue ( $P < 0.01$ ). Columns, means; bars, SD,  $n = 6/\text{group}$ . **C** and **D**, Representative confocal images demonstrating aromatase (red) staining in adipose stromal cells from patients without breast WAT inflammation (**C**) and with inflammation (**D**). Arrows indicate adipose stromal cells. Scale bars, 10  $\mu\text{m}$ ; Hoechst 33342 nuclear stain (blue). **E**, Aromatase average staining intensity in adipose stromal cells is significantly higher in patients with breast WAT inflammation versus without inflammation [ $P = 0.03$ ;  $n = 19$  (breast WAT inflammation absent), 12 (breast WAT inflammation present)]. **F**, Aromatase average staining intensity is increased with severity of inflammation [ $P < 0.05$ ;  $n = 19$  (no inflammation), 6 (mild), 6 (severe)]. Asterisks denote effects that are significantly different. IF, immunofluorescence.

categorically using median number of CLS-B/cm<sup>2</sup> (0.29) among subjects with CLS-B as follows: no inflammation (CLS-B absent), mild inflammation ( $\leq 0.29$  CLS-B/cm<sup>2</sup>), and severe inflammation ( $> 0.29$  CLS-B/cm<sup>2</sup>). Associations between a categorical variable and the severity of inflammation categories were examined using the  $\chi^2$  or Fisher exact test where appropriate. To examine relationships between 2 continuous variables, the Spearman correlation was used. Differences in a continuous variable across multiple categories were examined using the nonparametric Kruskal-Wallis test. For circulating factors measured in 2 batches, the association between the level of a circulating factor and a covariate was examined using multiple linear regression analysis while adjusting for potential batch effects. Levels of a circulating factor were log-transformed where appropriate to ensure the underlying model assumption was satisfied. For all analyses, statistical significance was set at 2-tailed  $P < 0.05$ . All statistical analyses were conducted using R software (R Foundation for Statistical Computing).

## Results

### Study population

A total of 72 women with normal BMI were enrolled between January 2011 and August 2013. Breast WAT inflammation (Fig. 1A) was present in 39% of participants. Baseline char-

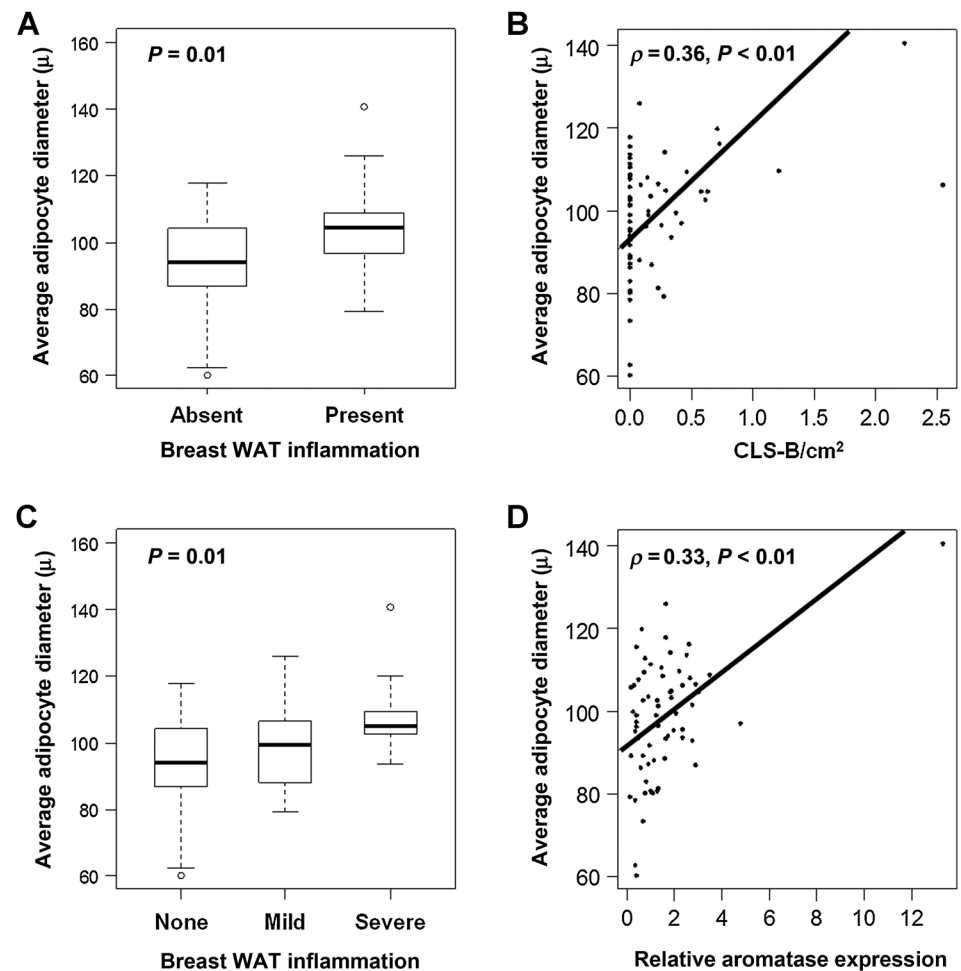
acteristics stratified by the presence or absence of breast WAT inflammation are shown in Table 1. Overall, median BMI was 21.9 kg/m<sup>2</sup> (range, 17.3–24.9 kg/m<sup>2</sup>) and was higher in women with breast WAT inflammation ( $P = 0.04$ ).

### Breast WAT inflammation and aromatase

Levels of aromatase mRNA in breast tissue positively correlated with BMI within the normal range ( $\rho = 0.28$ ,  $P = 0.02$ ; Fig. 1B). Women with breast WAT inflammation had higher breast aromatase mRNA levels than those without inflammation ( $P = 0.01$ ; Fig. 1C). Higher breast aromatase expression correlated with increasing severity of breast WAT inflammation ( $\rho = 0.35$ ,  $P < 0.01$ ; Fig. 1D). A positive correlation between aromatase and CD68 mRNA levels in the breast was also observed ( $\rho = 0.54$ ,  $P < 0.001$ ; Fig. 1E). The correlations between breast aromatase mRNA levels and breast WAT inflammation were stronger than with BMI. Furthermore, women with breast WAT inflammation had higher aromatase protein levels and enzyme activity in the breast than women without inflammation (Fig. 2A and B). In addition, aromatase immunoreactivity was detected in breast adipose stromal cells (Fig. 2C and D). Women with breast WAT inflammation had higher aromatase average staining intensity in breast adipose stromal cells than those without inflammation ( $P = 0.03$ ; Fig. 2E), which increased with severity of breast WAT inflammation ( $P < 0.05$ ; Fig. 2F).

**Figure 3.**

Adipocyte hypertrophy is present in women with breast WAT inflammation and adipocyte size correlates with increasing severity of inflammation and aromatase expression. **A**, Average adipocyte diameters are larger in women with breast WAT inflammation versus without inflammation ( $P = 0.01$ ). **B**, Average adipocyte diameter positively correlates with severity of breast WAT inflammation ( $\rho = 0.36$ ,  $P < 0.01$ ). **C**, Women with severe breast WAT inflammation had the largest breast adipocytes ( $P = 0.01$ ). **D**, Average adipocyte diameter positively correlates with breast aromatase mRNA level ( $\rho = 0.33$ ;  $P < 0.01$ ).



### Adipocyte hypertrophy, breast WAT inflammation, and aromatase

Women with breast WAT inflammation had larger average adipocyte diameters ( $103.6 \pm 13.1 \mu$ ) than women without inflammation ( $94.3 \pm 13.8 \mu$ ,  $P = 0.01$ ; Fig. 3A). In addition, breast adipocyte diameter correlated with the severity of breast WAT inflammation ( $\rho = 0.36$ ,  $P = 0.003$ ; Fig. 3B), and the largest adipocytes were found in those with severe inflammation (Fig. 3C). Larger adipocyte size correlated with higher aromatase mRNA levels in the breast ( $\rho = 0.33$ ,  $P = 0.006$ ; Fig. 3D).

### Circulating factors, breast WAT inflammation, and aromatase

Circulating factors measured in fasted blood are presented in Table 2 and are stratified by the presence or absence of breast WAT inflammation. Breast WAT inflammation was associated with elevated circulating levels of hsCRP ( $P = 0.05$ ), leptin ( $P = 0.01$ ), insulin ( $P = 0.02$ ), and triglycerides ( $P < 0.01$ ). Of note, breast WAT inflammation was associated with a clinical diagnosis of dyslipidemia ( $P < 0.01$ ; Table 1). Breast WAT inflammation was also associated with insulin resistance ( $P < 0.01$ ) as evidenced by the homeostasis model assessment of insulin resistance (HOMA2-IR, Table 2). In addition, circulating levels of leptin were positively correlated with adipocyte size ( $P < 0.01$ ; Fig. 4A), severity of breast WAT inflammation ( $P = 0.01$ ; Fig. 4B), and aromatase expression ( $P = 0.02$ ; Fig. 4C).

## Discussion

Findings from this study establish the presence of an inflammatory state that can occur in the breasts of women with normal BMI. This subclinical condition includes (i) WAT inflammation defined by the presence of CLS-B, (ii) increased aromatase expression and activity in the breast, and (iii) systemic metabolic

dysfunction. In addition, the presence of adipocyte hypertrophy in normal BMI women with breast WAT inflammation suggests a dysmorphic body composition, that is, hyperadiposity despite normal BMI. Collectively, these findings identify a population of women with normal BMI but underlying pathophysiology associated with the development and progression of breast cancer.

Our findings support the role of breast WAT inflammation and associated metabolic obesity in breast cancer development and growth for some normal BMI women. We previously reported that breast WAT inflammation is present in approximately 90% of patients with BMI  $> 30 \text{ kg/m}^2$  and is associated with activation of NF- $\kappa$ B, a transcription factor that induces expression of proinflammatory mediators, and increased levels of aromatase (7–9). Furthermore, women with breast WAT inflammation have increased circulating levels of proinflammatory mediators as well as insulin resistance and dyslipidemia—which confer increased risk and poorer survival for breast cancer (10, 13, 17, 18, 21). In the current study, we found that the presence of breast WAT inflammation in normal BMI individuals is indicative of increased aromatase locally and systemic alterations that mirror the pathophysiology typically associated with elevated BMI. Consistent with these biologic alterations, breast WAT inflammation is associated with shortened survival, independent of BMI, in patients with breast cancer (10, 11).

Although we found a correlation between breast aromatase expression and BMI within the normal range, the strength of correlation was stronger between aromatase and severity of breast WAT inflammation. Given that CD68-expressing macrophages makeup CLS-B, we compared CD68 and aromatase expression in the breast and again found that aromatase expression correlated more strongly with CD68 than BMI. Notably, the majority of women in this study were premenopausal, a group in which elevated BMI has not been shown to increase breast cancer risk. Our findings in these normal BMI women may help explain the inadequacy of BMI for cancer risk assessment, particularly in premenopausal women.

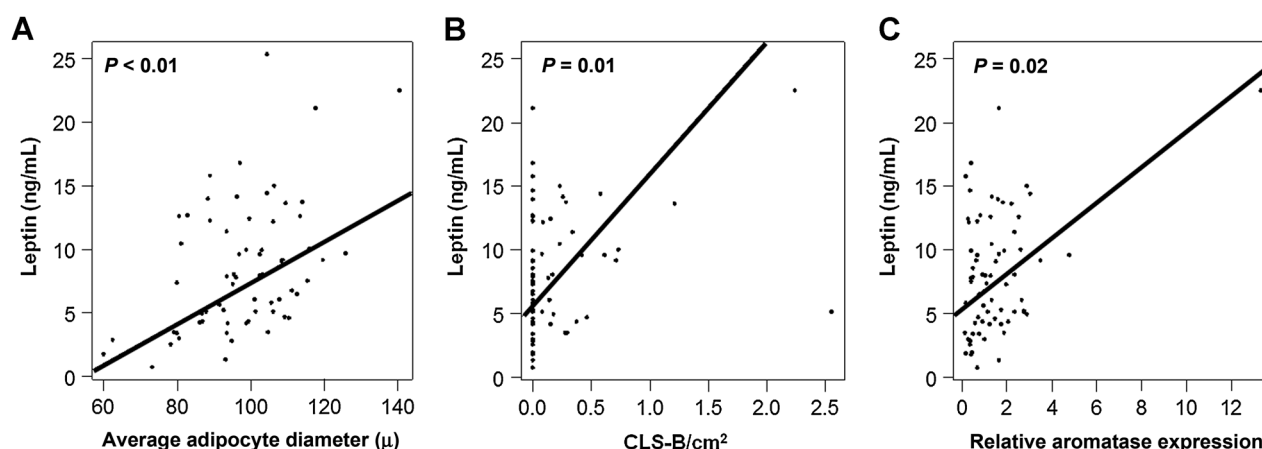
Findings in blood link metabolic obesity in normal weight to a breast microenvironment supportive of tumor growth. Macrophage infiltration and inflammation of abdominal subcutaneous WAT have been reported to occur in metabolically obese normal weight individuals (42). Consistently, women with breast WAT inflammation had biochemical changes characteristic of the metabolic syndrome including higher levels of insulin and triglycerides despite having a normal BMI. Furthermore, these normal-weight women with breast WAT inflammation had higher circulating levels of hsCRP, a cardiovascular risk factor. Because a variety of proinflammatory mediators are known inducers of aromatase (43, 44), it is possible that these mediators are contributing to the observed increase in aromatase. Moreover, elevated levels of hsCRP and insulin are each associated with increased breast cancer risk in normal BMI individuals (19, 20). In addition, circulating levels of the adipocyte-secreted hormone, leptin, were increased in normal BMI women with breast WAT inflammation. In addition to stimulating cancer cell proliferation and survival (45), leptin has been shown to regulate aromatase in adipose stromal cells (44). Here, we found increased aromatase in breast adipose stromal cells of women with WAT inflammation. Supporting these findings, higher circulating levels of leptin correlated with increased aromatase expression in the breast. Leptin levels are elevated in the setting of

**Table 2.** Measured blood factors stratified by breast WAT inflammation

Factors	Breast WAT Inflammation		$P^a$
	Absent ( $n = 44$ )	Present ( $n = 28$ )	
hsCRP, ng/mL			
Median (range)	0.50 (0.02–7.06)	0.67 (0.07–7.5)	0.05 <sup>b</sup>
IL-6, pg/mL			
Median (range)	0.83 (0.22–48.79)	1.03 (0.24–15.00)	0.26 <sup>b</sup>
Leptin, pg/mL			
Median (range)	6.29 (0.72–21.08)	9.56 (3.43–25.32)	0.01 <sup>b</sup>
Adiponectin, mg/mL			
Median (range)	13.37 (1.99–23.05)	10.45 (3.02–23.85)	0.33 <sup>b</sup>
Leptin:Adiponectin ratio			
Median (range)	0.64 (0.04–4.02)	0.79 (0.23–4.2)	0.04
Glucose, mg/dL			
Median (range)	69 (54–95)	81 (34–106)	0.19 <sup>b</sup>
Insulin, mU/L			
Median (range)	3.74 (1.26–10.19)	4.99 (1.38–9.18)	0.02 <sup>b</sup>
HOMA2-IR			
Median (range)	0.40 (0.14–1.10)	0.55 (0.12–1.00)	$<0.01$
Total cholesterol, mg/dL			
Median (range)	192 (129–284)	195 (152–285)	0.54
LDL cholesterol, mg/dL			
Median (range)	103 (38–183)	108 (66–185)	0.41
HDL cholesterol, mg/dL			
Median (range)	74 (48–120)	68 (41–101)	0.15
Triglycerides, mg/dL			
Median (range)	62 (29–136)	69 (39–225)	$<0.01$

<sup>a</sup> $P$  values were obtained using linear regression adjusted for potential cohort differences.

<sup>b</sup>Log-transformed data were used to ensure the underlying model assumptions were met.



**Figure 4.**

Circulating levels of leptin positively correlate with adipocyte size, breast WAT inflammation, and aromatase expression. **A**, Blood leptin levels correlate with average adipocyte size ( $P < 0.01$ ). **B**, Circulating leptin levels correlate with severity of breast WAT inflammation ( $P = 0.01$ ). **C**, Blood leptin levels correlate with aromatase expression in the breast ( $P = 0.02$ ).

hyperadiposity (46). We found breast adipocyte hypertrophy in women with elevated leptin levels, suggesting hyperadipose body composition despite a normal BMI. These women with larger breast adipocytes also had greater aromatase expression in the breast. Thus, breast WAT inflammation in normal BMI individuals is indicative of a hyperadipose state, including higher circulating leptin levels, and increased aromatase in the breast. Measurement of body composition, for example, via radiographic techniques, may provide more accurate cancer risk assessment and prognostication for normal BMI individuals and additional studies are needed. Furthermore, quantification of fat mass could identify normal weight patients who may benefit from interventions aimed at attenuating the impact of obesity on cancer risk and progression.

Local production of estrogens in the breasts of women with WAT inflammation may contribute to the development and progression of estrogen-dependent tumors. Consistently, the majority of women in this study had ER-positive tumors; however, the incidence was not higher in women with breast WAT inflammation. It is plausible that the impact of local inflammation and estrogen production on hormone receptor-positive tumor development is more substantial in postmenopausal women, when ovarian estrogen biosynthesis has ceased. Consistently, 50% of postmenopausal women with normal BMI had breast WAT inflammation compared with 35% of premenopausal women. These findings are consistent with our prior report in which we demonstrate that the postmenopausal state is independently associated with breast WAT inflammation (9). In light of these observations, additional studies are needed to examine local aromatase expression and circulating markers in postmenopausal women. It is also likely that breast WAT inflammation and its associated systemic alterations promote tumors via hormone-dependent and -independent mechanisms. For example, WAT inflammation in the tongue confers shortened disease-specific survival for patients with early-stage squamous cell carcinoma of the oral tongue, a disease that is not thought to be hormonally regulated (47). Insulin can stimulate the synthesis of insulin-like growth factor 1 and both can activate the PI3K/Akt/mTOR

and Ras/Raf/MAPK pathways which are linked to breast tumor progression (48–50). Thus the presence of WAT inflammation is likely to stimulate multiple pathways that contribute to tumor development and growth.

In conclusion, this study identifies a population of metabolically obese normal weight women with inflammation and increased aromatase in the breast. Additional studies are needed to develop clinically feasible methods to identify this vulnerable population. Such methods could include body composition assessment and the development of blood biomarker signatures that accurately identify patients with WAT inflammation. This potentially high-risk population that is often thought to be healthy by visual inspection warrants further study to determine whether interventions typically offered to anthropometrically obese individuals will reduce breast cancer risk and mortality.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

#### Authors' Contributions

**Conception and design:** N.M. Iyengar, K.A. Brown, X.K. Zhou, A. Gucalp, C.A. Hudis, A.J. Dannenberg

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