Greater Body Fatness Is Associated With Higher Protein Expression of LEPR in Breast Tumor Tissues: A Cross-Sectional Analysis in the Women’s Circle of Health Study

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Background: The mechanisms underlying the association of overall and central body fatness with poorer breast cancer outcomes remain unclear; altered gene and/or protein expression of the adipokines and their receptors in breast tumors might play a role.

Methods: In a sample of Black and White women with primary invasive breast cancer, we investigated associations of body mass index (BMI), waist circumference, hip circumference, waist-to-hip ratio (WHR), fat mass index (FMI), and percent body fat with protein expression (log-transformed, n = 722) and gene expression (log2-transformed, n = 148) of leptin (LEP), leptin receptor (LEPR), adiponectin (ADIPOQ), and adiponectin receptors 1 and 2 (ADIPOR1, ADIPOR2). Multivariable linear models, adjusting for race, menopausal status, and estrogen receptor status, were used to assess these associations, with Bonferroni correction for multiple comparisons.

Results: In multivariable models, we found that increasing BMI (β = 0.0529, 95% CI: 0.0151, 0.0906) and FMI (β = 0.0832, 95% CI: 0.0268, 0.1397) were associated with higher LEP gene expression, corresponding to 34.5% and 38.3% increases in LEP gene expression for a standard deviation (SD) increase in BMI and FMI, respectively. Increasing BMI (β = 0.0028, 95% CI: 0.0011, 0.0045), waist circumference (β = 0.0013, 95% CI: 0.0005, 0.0022), and hip circumference (β = 0.0015, 95% CI: 0.0007, 0.0024), and FMI (β =
INTRODUCTION

Epidemiologic evidence suggests that increasing obesity, measured using body mass index (BMI), is associated with elevated risk of postmenopausal breast cancer (1, 2) and poorer breast cancer outcomes among both pre- and postmenopausal women (2–4). However, differences have been observed by estrogen receptor (ER) status (3, 5). While increased premenopausal obesity is associated with increased risk of ER– but not ER+ disease, postmenopausal obesity is similarly associated with increased risk of both ER- and ER+ disease (5, 6). On the other hand, increasing waist-to-hip ratio (WHR) is associated with increased risk of ER+ disease among premenopausal women and with increased risk of both ER+ and ER- disease among postmenopausal women (7).

While the molecular mechanisms underlying the impact of overall and central obesity on poorer breast cancer outcomes are not well understood, it has been hypothesized that the biological effects of the adipokines, adiponectin (ADIPOQ) and leptin (LEP), which are secreted by adipocytes (8–13), and their respective receptors (adiponectin receptors 1 and 2 [ADIPOR1, ADIPOR2] and leptin receptor [LEPR], respectively) might play a role. Further, exploration of the relationship between central adiposity (rather than overall body size as measured by BMI) and adipokines and adipokine receptors might be the missing link. Circulating ADIPOQ levels decrease with increasing BMI (14–16) and are associated with increased breast cancer risk (17–20). Conversely, circulating LEP levels increase with increasing BMI (21, 22) and are associated with increased breast cancer risk in some studies (17, 23–25). Less is known about adipokine receptor protein and gene expression levels in breast tumor tissues or their associations with more accurate and specific measures of body fatness derived from anthropometry (e.g., waist circumference, hip circumference, waist-to-hip ratio [WHR]) or from bioelectrical impedance analysis (BIA) (e.g., fat mass index [FMI], percent body fat [BF%]). These data might provide novel insights about the impact of body fatness and adiposity-related adipokine and adipokine receptor expression in breast tumors on long-term breast cancer outcomes.

ADIPOQ is the most abundantly secreted adipokine by adipocytes (15, 26), and along with its receptors, is expressed in histologically normal and malignant breast tissues (27, 28). ADIPOQ has anti-inflammatory and anti-atherogenic properties (26, 29), inhibits cellular proliferation, and promotes apoptosis (10, 13, 30), implying a protective role in breast carcinogenesis. LEP, also secreted by adipocytes, is expressed in histologically normal and malignant breast cells, as is the LEPR (31, 32), LEP, once bound to LEPR, induces the activation of several signaling pathways, promotes cell growth and proliferation, and promotes angiogenesis (33–38).

Data from our prior research were the first to examine correlations between circulating ADIPOQ and LEP levels in plasma and levels within the breast, demonstrating that circulating adipokine levels are generally poor surrogates for levels within the local organ (39). More recently, we demonstrated that adipokine and adipokine receptor protein and gene expression in breast tumor tissues are associated with more aggressive tumor features associated with worse prognosis (40, 41). Specifically, lower LEP protein expression was associated with ER- status, triple-negative (TN) subtype (40), while lower gene expression of ADIPOQ, ADIPOR2, LEP, and LEPR were associated with more aggressive breast tumor features, including higher tumor grade, larger tumor size, ER-status, and human epidermal growth factor receptor 2 (HER2)-enriched and TN subtypes (41).

Abbreviations: ADIPOQ, adiponectin; ADIPOR1, adiponectin receptor 1; ADIPOR2, adiponectin receptor 2; BIA, bioelectrical impedance analysis; BMI, body mass index; CI, confidence interval; CT, computed tomography; DCIS, ductal carcinoma in situ; ER, estrogen receptor; ESA, effective staining area; ESI, effective staining intensity; FFPE, formalin-fixed paraffin-embedded; H&E, hematoxylin and eosin; HER2, human epidermal growth factor receptor 2; LEP, leptin; LEPR, leptin receptor; SD, standard deviation; TMA, tissue microarray; TN, triple negative; WCHS, Women’s Circle of Health Study; WHR, waist-to-hip ratio.

0.0041, 95% CI: 0.0015, 0.0067) were associated with higher LEPR protein expression. These associations equate to 16.8%, 17.6%, 17.7%, 17.2% increases in LEPR protein expression for a 1-SD increase in BMI, waist circumference, hip circumference, and FMI, respectively. Further, these associations were stronger among White and postmenopausal women and ER+ cases; formal tests of interaction yielded evidence of effect modification by race. No associations of body fatness with LEP protein expression, LEPR gene expression, or protein or gene expression of ADIPOQ, ADIPOR1, and ADIPOR2 were found.

Conclusions: These findings support an association of increased body fatness – beyond overall body size measured using BMI – with higher LEP gene expression and higher LEPR protein expression in breast tumor tissues. Clarifying the impact of adipokine-related adipokine and adipokine receptor expression in breast tumors on long-term breast cancer outcomes is a critical next step.

Keywords: adiposity, breast cancer, leptin (LEP), leptin receptor (LEPR), adiponectin (ADIPOQ), adiponectin receptors 1 and 2 (ADIPOR1, ADIPOR2), protein expression, gene expression
In the current study, we hypothesize that measures of body fatness are associated with LEPR, ADIPOR1, and ADIPOR2 expression profiles in the breast tumor microenvironment, which might contribute mechanistically to the development of more aggressive breast tumor phenotypes and poorer prognosis. To test this, we investigated associations of general obesity (BMI), body fat distribution (waist circumference, hip circumference, WHR), and body composition (FMI, BF%) with protein and gene expression of the adipokines and adipokine receptors in breast tissue specimens from participants in the Women’s Circle of Health Study (WCHS).

MATERIALS AND METHODS

Study Sample and Data Collection

Study participants were women diagnosed with primary invasive breast cancer from 2001 through 2015 and enrolled in the WCHS (40, 41). Briefly, WCHS enrolled newly diagnosed breast cancer cases with histologically confirmed ductal carcinoma in situ (DCIS, stage 0) or invasive breast cancer (stages I–IV), who self-identified as either Black/African American or White, were 20–75 years of age, able to complete an interview in English, and had no history of cancer except non-melanomatous skin cancer. Data collection for the WCHS was conducted through in-person assessments (approximately 10 months after diagnosis) and included computer-assisted interviewer-administered questionnaires, as well as standardized protocols for taking anthropometric measurements during a home visit, including height, weight, waist circumference, and hip circumference, and body composition using a portable BIA scale (42). The baseline interview ascertained information on sociodemographic factors as well as established or probable breast cancer risk factors, including family and personal health history, reproductive history, hormone therapy use, and lifestyle exposures.

Nearly all WCHS participants (98%) consented to medical records release and for these participants, medical and pathology records were requested and retrieved from providers and institutions where participants reported receiving breast cancer care. Relevant clinical and breast tumor clinicopathologic data were abstracted and entered in an electronic database (43, 44).

Collection of Archived Breast Tumor Specimens and Tissue Microarray Construction

Tumor blocks and/or slides for WCHS participants were retrieved from hospitals upon written consent, with a retrieval rate of approximately 85%. Upon receipt at the Data Bank and Biorepository (DBBR) at Roswell Park Comprehensive Cancer Center, a board-certified pathologist (TK) reviewed hematoxylin and eosin (H&E) slides and circled areas where cores were selected for tissue microarray (TMA) construction. TMA cores ranged in size from 0.6 mm to 1.2 mm in diameter, and most WCHS participants’ tumors were represented by at least two TMA cores (range: 1 to 6 cores), which were placed into a recipient formalin-fixed paraffin-embedded (FFPE) block. The location of each core was recorded in a detailed TMA map file. The completed TMAs were stored at room temperature.

Protein Expression Analysis

For each WCHS participant included in the protein expression analysis (n = 722), immunohistochemistry (IHC) was used to stain TMAs of breast tumor specimens for LEP, LEPR, ADIPOQ, ADIPOR1, and ADIPOR2 as previously described (40). Briefly, IHC staining was performed using Ventana Discovery XT Automated Slide Stainer (Ventana Medical Systems, Inc., Tucson, AZ, USA). Decapsulation, antigen retrieval, blocking, DAB detection, counterstain, post-counterstain, and slide cleaning steps were automated on the Discovery XT. Primary antibodies and secondary antibodies were manually applied at programmed steps. The following primary antibodies were used: rabbit monoclonal OB (LEP) antibody (1:40 dilution; Santa Cruz, cat #sc-842), mouse monoclonal OB-R (LEPR) antibody (1:25 dilution; Santa Cruz, cat #sc-8391), mouse monoclonal adiponectin antibody (1:30 dilution; Abcam, cat #ab22554), rabbit monoclonal ADIPOR1 antibody (1:350 dilution; Abcam, cat #ab126611), and goat polyclonal ADIPOR2 antibody (1:25 dilution; Abcam, cat #ab77612). Optimal staining on control slides (human breast tissue TMAs) was obtained for each individual antibody. IHC was performed using the optimized conditions on the experimental WCHS TMA slides as well as on additional control slides. Primary antibodies were incubated at 37°C for 1–2 h; secondary antibodies were incubated at 37°C for 1 h, followed by either the DAB Map Detection Kit (Ventana, 760-124) or ChromoMap DAB kit (Ventana, 760-159). Slides were counterstained with hematoxylin (Ventana, 760-2021) and bluing reagent (Ventana, 760-2037) before cover slipping. A digital pathology analysis platform (VisioPharm, Hoersholm Denmark) was used to quantify protein expression of the adipokine receptors on each tissue core (45). Quantitative results were reported as a protein expression score defined as effective staining intensity (ESI) within the effective staining area (ESA) (45). Specimen artifacts, such as tissue folding were manually excluded from quantification. A board-certified pathologist (MAC) semi-quantitatively evaluated IHC expression for each tissue core stained (45). Semi-quantitative expression results were reported as: 0 (negative), 1 (weak expression), 2 (moderate expression), or 3 (strong expression). We observed high concordance between unsupervised, quantitative scores and pathologist-generated, semi-quantitative scores for LEP (r = 0.70, P<0.0001) and LEPR (r = 0.71, P<0.0001) (40). In the present analysis we included only quantitative protein expression data for LEP, LEPR, ADIPOQ, ADIPOR1, and ADIPOR2, which were averaged for participants with multiple TMA cores. Log-transformed protein expression data were used in the subsequent analysis.

Gene Expression Analysis

For each WCHS participant included in the gene expression analysis (n = 148), RNA was extracted from two 10μm curls (from representative breast tumor blocks without any pre-selection based on either the tumor or stromal contents so as
to maintain and capture the entire tumor lesion and surrounding microenvironment) using the High Pure FFPET RNA Isolation Kit (Roche Molecular Systems, Inc., Pleasanton, CA, USA) and quantified using Qubit and Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Gene expression of ADIPOQ, ADIPOR1, ADIPOR2, LEP, and LEPR were quantitated using NanoString nCounter® technology (NanoString Technologies, Seattle, WA, USA) (41). Raw count data were subjected to a series of normalization steps, including positive controls, housekeeping genes, and background subtraction, and the normalized data were log2-transformed and used in subsequent analyses (41).

Statistical Analyses
Descriptive statistics (mean and standard deviation [SD] and frequency and proportions) were used to describe the study sample and Pearson’s correlation analysis was used to assess pairwise correlations between adipokine receptor protein and gene expression. Multivariable linear regression models were utilized to evaluate the associations of BMI, waist circumference, hip circumference, WHR, FMI, and BF% with protein and gene expression of the adipokines and adipokine receptors. The difference in protein and gene expression per increase in SD of body fatness measures was also estimated, and a percentage increase was estimated as \( \exp(\beta) - 1 \) x 100%. Models were adjusted for age, race, menopausal status, and ER status. All reported \( P \)-values are two-sided and \( P<0.05 \) was considered statistically significant. To account for multiple comparisons, we used Bonferroni correction with a criterion of \( P<0.0083 \) (i.e., \( 0.05/6 \)) for statistical significance, given that there were six tests of association for protein and gene expression of each marker of interest. Analyses were performed using STATA (version 17, StataCorp, College Station, TX).

RESULTS
Sociodemographic and tumor characteristics of the study sample included in the protein expression and the gene expression analytic samples are shown in Table 1. Across both groups, most participants met the criteria for increased metabolic risk (46, 47) based on overall obesity (BMI >30 kg/m\(^2\) [50.6%]), central obesity (waist circumference >88 cm [74.4%] and/or WHR >0.85 [64.1%]), elevated/abnormal FMI (≥9.5 kg/m\(^2\) [73.4%]), and BF% (>35% [76.2%]).

There was no significant correlation between LEP protein and LEP gene expression \( (r = 0.11, P = 0.21) \), a weak positive correlation between LEPR protein and LEP gene expression \( (r = 0.29, P = 0.0006) \), a weak positive correlation between ADIPOQ protein and ADIPOQ gene expression \( (r = 0.18, P = 0.04) \), no significant correlation between ADIPOR1 protein and ADIPOQ gene expression \( (r = -0.03, P = 0.69) \), and a very weak positive correlation between ADIPOR2 protein and ADIPOR2 gene expression \( (r = 0.18, P = 0.04) \). In models adjusting for age, race, menopausal status, and ER status, we found that there were no significant associations between measures of body fatness and LEP protein expression (Table 2). In contrast, we found that women with increasing BMI \( (\beta = 0.0529, 95\% \text{ CI: } 0.0151, 0.0906) \), waist circumference \( (\beta = 0.0247, 95\% \text{ CI: } 0.0065, 0.0429) \), hip circumference \( (\beta = 0.0259, 95\% \text{ CI: } 0.0066, 0.0453) \), FMI \( (\beta = 0.0832, 95\% \text{ CI: } 0.0268, 0.1397) \), and BF% \( (\beta = 0.0396, 95\% \text{ CI: } 0.0025, 0.0767) \) had higher LEP gene expression. Only the findings for BMI and FMI were significant with correction for multiple comparisons and corresponds to 34.5% and 38.3% increases in LEP gene expression for a 1-SD increase in BMI and FMI, respectively.

DISCUSSION
Building on our prior research, here we examined the associations of body fatness measures with protein and gene expression of the adipokines, LEP and ADIPOQ, and the adipokine receptors, LEPR, ADIPOR1, and ADIPOR2 in breast tumor tissues. To our
knowledge, this is the first study to investigate these associations in women with breast cancer. Partially consistent with our hypothesis, we found that greater body fatness is associated with increased *LEP* gene expression and LEPR protein expression, although we observed no associations between body fatness and *LEPR* gene expression, nor with protein or gene expression of *ADIPOQ*, *ADIPOR1*, and *ADIPOR2*.

Past studies show that BMI is positively associated with circulating leptin concentrations and inversely associated with circulating adipokine concentrations, which are associated with increased risk of some obesity-related cancers including breast cancer (reviewed by Yoon et al. (48)). Our finding that increasing measures of body fatness are positively associated with *LEPR* protein expression in breast tumor tissues plays a role in breast carcinogenesis (49–54). Interestingly, our analysis showed significant effect modification by race (stronger among White women) and marginally significant effect modification by ER status (suggestion of stronger associations among ER+ cases, although our analysis was underpowered given the small sample of ER- cases). These findings further highlight the complex interplay among *LEPR* protein expression, adiposity, race, and breast tumor phenotype (55–58), which might require more precise adiposity measures, and identification and refinement of adiposity-associated biomarkers within breast tumor tissues that can predict breast cancer outcomes. We observed no significant associations between body fatness and *LEPR* gene expression, but we previously showed that gene expression of *LEPR* is significantly lower in ER- and TN breast tumors relative to ER+ and luminal A subtypes, respectively (41). While our sample with data on adipokine receptor gene expression was small and limited our statistical power, larger studies in the future will help clarify these findings. Nonetheless, our findings suggest that distribution of adiposity and adiposity-related expression profiles of the adipokines and adipokine receptors, especially *LEP* and *LEPR*, in

### TABLE 1 | Select characteristics of analytic samples included in the adipokine receptor protein expression and gene expression analysis.

| Sociodemographic and clinical characteristics       | Protein expression, N = 722a | Gene expression, N = 148b |
|-----------------------------------------------------|-------------------------------|----------------------------|
| Age at diagnosis (years), mean ± SD                 | 52.58 ± 10.83                | 53.08 ± 10.34              |
| Menopausal status                                   |                               |                            |
| Premenopausal                                       | 325 (46.43)                   | 68 (47.22)                 |
| Postmenopausal                                      | 375 (53.57)                   | 76 (52.78)                 |
| Race                                                |                               |                            |
| Black/African American                              | 541 (77.29)                   | 109 (75.69)                |
| White                                               | 159 (22.71)                   | 35 (24.31)                 |
| Body mass index (kg/m²), mean ± SD                 | 30.72 ± 6.99                  | 30.89 ± 7.50               |
| Waist circumference (cm), mean ± SD                | 98.63 ± 15.47                 | 99.15 ± 15.66              |
| Hip circumference (cm), mean ± SD                  | 112.13 ± 13.30                | 111.86 ± 13.88             |
| Waist-to-hip ratio, mean ± SD                       | 0.87 ± 0.08                   | 0.88 ± 0.07                |
| Fat mass index, mean ± SD                           | 12.44 ± 4.92                  | 12.61 ± 5.27               |
| Percent body fat (%), mean ± SD                     | 59.30 ± 7.77                  | 39.20 ± 8.07               |
| Breast tumor characteristics                        |                               |                            |
| Tumor grade                                         |                               |                            |
| Well differentiated                                 | 107 (16.85)                   | 13 (9.03)                  |
| Moderately differentiated                           | 219 (34.49)                   | 45 (31.25)                 |
| Poorly differentiated                               | 309 (48.66)                   | 86 (59.72)                 |
| Tumor size                                          |                               |                            |
| <1.0 cm                                            | 149 (20.64)                   | 22 (14.86)                 |
| 1.0-2.0 cm                                         | 281 (38.92)                   | 64 (43.24)                 |
| >2.0 cm                                            | 292 (40.44)                   | 62 (41.99)                 |
| AJCC stage                                          |                               |                            |
| Stage 0                                             | 62 (8.96)                     | 1 (0.72)                   |
| Stage I                                            | 257 (37.14)                   | 54 (39.13)                 |
| Stage II                                           | 271 (39.16)                   | 66 (47.83)                 |
| Stage III                                          | 96 (13.87)                    | 14 (10.14)                 |
| Stage IV                                           | 6 (0.87)                      | 3 (2.17)                   |
| ER status                                           |                               |                            |
| ER+                                                 | 505 (70.14)                   | 84 (56.76)                 |
| ER-                                                 | 215 (29.86)                   | 64 (43.24)                 |
| HER2 status                                         |                               |                            |
| HER2-                                               | 412 (61.58)                   | 112 (75.68)                |
| HER2+                                               | 93 (13.82)                    | 36 (24.32)                 |

aIn the protein expression sample, age was missing for 22 (3%); BMI was missing for 23 (3.2%); waist circumference, hip circumference, and waist-to-hip ratio were missing for 33 (4.6%); fat mass index was missing for 66 (9.1%); percent body fat was missing for 64 (8.9%); tumor grade was missing for 87 (12%); tumor stage was missing for 30 (4.2%); ER status was missing for 2 (0.3%), and HER2 status was missing for 18 (2.5%) participants.

bIn the gene expression sample, age, menopausal status, race, and BMI was missing for 4 (2.7%) participants; waist circumference, hip circumference, and waist-to-hip ratio were missing for 5 (3.4%) participants; fat mass index and percent body fat were missing for 12 (8.1%) participants; tumor grade was missing for 4 (2.7%) participants; and tumor stage was missing for 10 (6.8%) participants.
TABLE 2 | Multivariable-adjusted associations of body fatness measures with LEP protein and LEP gene expression in breast tumor tissues.

| Body Mass Index (kg/m²) | β (95% CI) | P      |
|------------------------|------------|--------|
| 635                    | 0.0013 (-0.0007, 0.0033) | 0.0650 | 0.189 |
| 144                    | 0.00529 (0.0151, 0.00906) | 0.2962 | 0.007** |

| Hip Circumference (cm) | β (95% CI) | P      |
|------------------------|------------|--------|
| 624                    | 0.0003 (-0.0006, 0.0013) | 0.0338 | 0.507 |
| 143                    | 0.0247 (0.0065, 0.0429) | 0.2880 | 0.009* |

| Waist-to-Hip Ratio | β (95% CI) | P      |
|--------------------|------------|--------|
| 624                | 0.00006 (-0.0004, 0.0016) | 0.0570 | 0.233 |
| 143                | 0.0259 (0.0096, 0.0453) | 0.2684 | 0.01* |

| Fat Mass Index (kg/m²) | β (95% CI) | P      |
|------------------------|------------|--------|
| 594                    | -0.0012 (-0.0019, 0.0044) | 0.0405 | 0.440 |
| 136                    | 0.0832 (0.0268, 0.1397) | 0.3245 | 0.005** |

| Percent Body Fat (%) | β (95% CI) | P      |
|---------------------|------------|--------|
| 596                 | -0.0002 (-0.0021, 0.0018) | -0.0103 | 0.843 |
| 136                 | 0.0396 (0.0025, 0.0767) | 0.2359 | 0.038* |

Protein expression scores reflect quantitative protein expression (using immunohistochemistry) of LEP as analyzed through an automated/unsupervised scoring (quantitative) methodology. The scores estimate the effective staining intensity (ESI) within the effective staining area (ESA) of the biomarker in question (mean±SD of log-transformed values are shown). Gene expression scores reflect normalized, log2-transformed gene expression of LEP as analyzed through the Nanostring nCounter Analysis System. Each model was generated using multiple linear regression adjusting for age, race, menopausal status, and estrogen receptor status.

*Statistically significant at P<0.05; **Statistically significant with correction for multiple comparisons (P<0.0083).

TABLE 3 | Multivariable-adjusted associations of body fatness measures with LEPR protein and LEPR gene expression in breast tumor tissues.

| Body Mass Index (kg/m²) | β (95% CI) | P      |
|------------------------|------------|--------|
| 571                    | 0.0028 (0.0011, 0.0045) | 0.155 | 0.002** |
| 144                    | 0.0062 (-0.0187, 0.0012) | 0.053 | 0.625 |

| Hip Circumference (cm) | β (95% CI) | P      |
|------------------------|------------|--------|
| 561                    | 0.0013 (0.0005, 0.0022) | 0.162 | 0.001** |
| 143                    | -0.0003 (-0.0122, 0.0116) | -0.005 | 0.962 |

| Waist-to-Hip Ratio | β (95% CI) | P      |
|--------------------|------------|--------|
| 561                | 0.0015 (0.0007, 0.0024) | 0.163 | 0.001** |
| 143                | -0.0006 (-0.0134, 0.0122) | -0.009 | 0.927 |

| Fat Mass Index (kg/m²) | β (95% CI) | P      |
|-----------------------|------------|--------|
| 534                   | 0.0041 (0.0015, 0.0067) | 0.159 | 0.002** |
| 136                   | 0.00091 (-0.0275, 0.0458) | 0.056 | 0.626 |

| Percent Body Fat (%) | β (95% CI) | P      |
|---------------------|------------|--------|
| 536                 | 0.0020 (0.0004, 0.0038) | 0.124 | 0.016* |
| 136                 | -0.0012 (-0.0248, 0.0228) | -0.008 | 0.941 |

Protein expression scores reflect quantitative protein expression (using immunohistochemistry) of LEPR as analyzed through an automated/unsupervised scoring (quantitative) methodology. The scores estimate the effective staining intensity (ESI) within the effective staining area (ESA) of the biomarker in question (mean±SD of log-transformed values are shown). Gene expression scores reflect normalized, log2-transformed gene expression of LEPR as analyzed through the Nanostring nCounter Analysis System. Each model was generated using multiple linear regression adjusting for age, race, menopausal status, and estrogen receptor status.

*Statistically significant at P<0.05; **Statistically significant with correction for multiple comparisons (P<0.0083).

TABLE 4 | Multivariable-adjusted associations of body fatness measures with ADIPOQ protein and ADIPOQ gene expression in breast tumor tissues.

| Body Mass Index (kg/m²) | β (95% CI) | P      |
|------------------------|------------|--------|
| 618                    | 0.0009 (-0.0013, 0.0032) | 0.0406 | 0.418 |
| 144                    | 0.0036 (-0.0015, 0.0087) | 0.0140 | 0.897 |

| Hip Circumference (cm) | β (95% CI) | P      |
|------------------------|------------|--------|
| 608                    | 0.0008 (-0.0003, 0.0019) | 0.0765 | 0.137 |
| 143                    | -0.0065 (-0.0328, 0.0198) | -0.0518 | 0.629 |

| Waist-to-Hip Ratio | β (95% CI) | P      |
|--------------------|------------|--------|
| 608                | 0.0008 (-0.0004, 0.0019) | 0.0654 | 0.176 |
| 143                | 0.0041 (-0.0241, 0.0023) | 0.2921 | 0.775 |

| Fat Mass Index (kg/m²) | β (95% CI) | P      |
|-----------------------|------------|--------|
| 583                   | 0.0019 (-0.0016, 0.0053) | 0.0565 | 0.285 |
| 136                   | -0.0060 (-0.0890, 0.0770) | -0.0160 | 0.888 |

| Percent Body Fat (%) | β (95% CI) | P      |
|---------------------|------------|--------|
| 584                 | 0.0006 (-0.0015, 0.0027) | 0.0281 | 0.589 |
| 136                 | -0.0280 (-0.813, 0.0254) | -0.1144 | 0.306 |

Protein expression scores reflect quantitative protein expression (using immunohistochemistry) of ADIPOQ as analyzed through an automated/unsupervised scoring (quantitative) methodology. The scores estimate the effective staining intensity (ESI) within the effective staining area (ESA) of the biomarker in question (mean±SD of log-transformed values are shown). Gene expression scores reflect normalized, log2-transformed gene expression of ADIPOQ as analyzed through the Nanostring nCounter Analysis System. Each model was generated using multiple linear regression adjusting for age, race, menopausal status, and estrogen receptor status.

*Statistically significant at P<0.05; **Statistically significant with correction for multiple comparisons (P<0.0083).

the local organ might have differential impacts on breast cancer based on tumor subtype, and the crosstalk between ER and adipokine biomarkers and other inflammatory biomarkers might play a role (59).

Prior analysis from WCHS reported a lack of association between BMI and breast cancer risk, but higher hip circumference and waist circumference were associated with more than 2-fold increased risk of pre-menopausal breast cancer among women in the fourth quartiles for each measure compared to the first quartile.42 Further, findings from WCHS also showed that compared to BMI, WHR was more strongly associated with overall and breast cancer-specific mortality among Black women. Specifically, compared to the first quartile, women in the fourth quartile of WHR had 61% and 68% increased risk of overall and breast cancer specific death, respectively, while women with class I and class II obesity (compared to normal weight) had statistically non-significant increased risk of death ranging from 17—33% (44). From the combination of these findings, investigations of the associations between more accurate measures of adiposity and adipose tissue distribution (including overall adiposity, visceral adiposity, and subcutaneous adiposity assessed through computed tomography [CT]), in association with adipokine receptor protein
and gene expression are critical to elucidating the impact of adiposity on breast carcinogenesis and progression. Such data are critical to determining the clinical utility of adipokine receptor expression profiles as biomarkers of breast cancer risk and prognosis and might contribute to the development of novel interventions and therapeutics targeting high breast tumor expression of these markers, particularly of LEPR, as a means of improving outcomes.

An important strength of this study is that it adds new knowledge regarding the potential impact of overall and central body fatness on adiposity-related biomarkers in breast tumor tissues. Our findings suggest that measures of body fatness are associated with the expression of adipokine receptors – primarily LEP and LEPR – in breast tumors. From this, we generated new hypotheses about the mechanisms linking central adiposity with breast cancer outcomes.

Protein expression scores reflect quantitative protein expression (using immunohistochemistry) of ADIPOR1 as analyzed through an automated/unsupervised scoring (quantitative) methodology. The scores estimate the effective staining intensity (ESI) within the effective staining area (ESA) of the biomarker in question (mean±SD of log-transformed values are shown).

Protein expression scores reflect normalized, log2-transformed gene expression of ADIPOR1 as analyzed through the Nanostring nCounter Analysis System. Each model was generated using multiple linear regression adjusting for age, race, menopausal status, and estrogen receptor status.

Protein expression scores reflect quantitative protein expression (using immunohistochemistry) of ADIPOR2 as analyzed through an automated/unsupervised scoring (quantitative) methodology. The scores estimate the effective staining intensity (ESI) within the effective staining area (ESA) of the biomarker in question (mean±SD of log-transformed values are shown).

Protein expression scores reflect normalized, log2-transformed gene expression of ADIPOR2 as analyzed through the Nanostring nCounter Analysis System. Each model was generated using multiple linear regression adjusting for age, race, menopausal status, and estrogen receptor status.

Protein expression scores reflect quantitative protein expression (using immunohistochemistry) of LEPR as analyzed through an automated/unsupervised scoring (quantitative) methodology. The scores estimate the effective staining intensity (ESI) within the effective staining area (ESA) of the biomarker in question (mean±SD of log-transformed values are shown). Each model was generated using multiple linear regression adjusting for age, race, menopausal status, and estrogen receptor status.

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**TABLE 5 | Multivariable-adjusted associations of body fatness measures with ADIPOR1 protein and ADIPOR1 gene expression in breast tumor tissues.**

| Measure                      | n     | \(\beta\) (95% CI) | \(P_{\text{standardized}}\) | \(P\)  |
|------------------------------|-------|--------------------|-----------------------------|-------|
| Body mass index (kg/m²)      | 654   | -0.0006 (-0.0028, 0.0013) | -0.028 | 0.473 |
| Waist circumference (cm)     | 575   | -0.0006 (-0.0015, 0.0009) | -0.032 | 0.497 |
| Hip circumference (cm)       | 575   | -0.0001 (-0.0014, 0.0012) | 0.0088 | 0.673 |
| Waist-to-hip ratio           | 575   | -0.0008 (-0.0273, 0.1995) | -0.017 | 0.749 |
| Fat mass index (kg/m²)       | 552   | 0.0002 (-0.0037, 0.0040)  | 0.044  | 0.939 |
| Percent body fat (%)         | 554   | 0.0001 (-0.0012, 0.0013)  | 0.031  | 0.557 |

**TABLE 6 | Multivariable-adjusted associations of body fatness measures with ADIPOR2 protein and ADIPOR2 gene expression in breast tumor tissues.**

| Measure                      | n     | \(\beta\) (95% CI) | \(P_{\text{standardized}}\) | \(P\)  |
|------------------------------|-------|--------------------|-----------------------------|-------|
| Body mass index (kg/m²)      | 594   | -0.0009 (-0.0028, 0.0023) | -0.010 | 0.844 |
| Waist circumference (cm)     | 445   | 0.0006 (-0.0001, 0.0014)  | 0.0791 | 0.101 |
| Hip circumference (cm)       | 445   | 0.0007 (-0.0012, 0.0015)  | 0.0169 | 0.735 |
| Waist-to-hip ratio           | 445   | 0.0267 (-0.1276, 0.1810)  | 0.0791 | 0.100 |
| Fat mass index (kg/m²)       | 428   | 0.0019 (-0.0042, 0.0042)  | 0.0791 | 0.302 |
| Percent body fat (%)         | 428   | 0.0008 (-0.0007, 0.0023)  | 0.0501 | 0.049* |

**TABLE 7 | Multivariable-adjusted associations of body fatness measures with LEPR protein expression in breast tumor tissues, stratified by race.**

| Measure                      | n     | \(\beta\) (95% CI) | \(P_{\text{standardized}}\) | \(P\)  |
|------------------------------|-------|--------------------|-----------------------------|-------|
| Body mass index (kg/m²)      | 455   | 0.0016 (0.0000, 0.0031) | 0.0906 | 0.049* |
| Waist circumference (cm)     | 445   | 0.0006 (-0.0001, 0.0014) | 0.0791 | 0.101 |
| Hip circumference (cm)       | 445   | 0.0007 (-0.0012, 0.0015) | 0.0169 | 0.735 |
| Waist-to-hip ratio           | 445   | 0.0267 (-0.1276, 0.1810) | 0.0791 | 0.100 |
| Fat mass index (kg/m²)       | 428   | 0.0019 (-0.0042, 0.0042) | 0.0791 | 0.302 |
| Percent body fat (%)         | 428   | 0.0008 (-0.0007, 0.0023) | 0.0501 | 0.049* |

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which will be pursued. Another strength was the opportunity to perform stratified analysis of the associations of interest by race, menopausal status, and ER status, yielding novel findings. Lastly, our population-based sample that included a large proportion of Black women with breast cancer was also a strength. This study also has some limitations worth noting, including a relatively small sample size (particularly in the gene expression analysis \(n = 148\)), which may have reduced the power to detect meaningful associations and limit our ability to fully evaluate the complex associations of body fatness with breast cancer. Relatedly, our analysis included multiple comparisons which may have increased the likelihood of observing statistically significant associations. However, we addressed this concern using Bonferroni correction.

Despite these limitations, the findings substantiated our hypothesis that measures of body fatness are associated with expression of adipokine biomarkers in breast tumors. These data are an important step towards understanding the biologic effects of and potential mechanisms linking adiposity with breast cancer risk and prognosis.

**DATA AVAILABILITY STATEMENT**

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

### TABLE 8 | Multivariable-adjusted associations of body fatness measures with LEPR protein expression in breast tumor tissues, stratified by menopausal status.

| Premenopausal women | n  | \(\beta\) (95% CI)    | \(\beta_{\text{standardized}}\) | \(P\)      |
|---------------------|----|----------------------|-------------------------------|------------|
| Body mass index (kg/m\(^2\)) | 264 | 0.0019 (-0.0002, 0.0040) | 0.1090 | 0.078 |
| Waist circumference (cm) | 261 | 0.0009 (-0.0001, 0.0019) | 0.1033 | 0.096 |
| Hip circumference (cm) | 261 | 0.0007 (-0.0004, 0.0018) | 0.0728 | 0.231 |
| Waist-to-hip ratio | 261 | 0.1446 (-0.0755, 0.3847) | 0.0818 | 0.199 |
| Fat mass index (kg/m\(^2\)) | 247 | 0.0026 (-0.0006, 0.0058) | 0.1023 | 0.111 |
| Percent body fat (%) | 247 | 0.0014 (-0.0007, 0.0034) | 0.0854 | 0.183 |

| Postmenopausal women | n  | \(\beta\) (95% CI)    | \(\beta_{\text{standardized}}\) | \(P\)      |
|---------------------|----|----------------------|-------------------------------|------------|
| Body mass index (kg/m\(^2\)) | 307 | 0.0027 (0.0007, 0.0048) | 0.1483 | 0.010* |
| Waist circumference (cm) | 300 | 0.0010 (0.0001, 0.0020) | 0.1244 | 0.033* |
| Hip circumference (cm) | 300 | 0.0015 (0.0004, 0.0025) | 0.1575 | 0.005** |
| Waist-to-hip ratio | 300 | -0.0420 (-0.2321, 0.1480) | -0.0259 | 0.665 |
| Fat mass index (kg/m\(^2\)) | 287 | 0.0039 (0.0008, 0.0069) | 0.1489 | 0.013* |
| Percent body fat (%) | 289 | 0.0021 (0.0001, 0.0040) | 0.1262 | 0.036* |

Protein expression scores reflect quantitative protein expression (using immunohistochemistry) of LEPR as analyzed through an automated/unsupervised scoring (quantitative) methodology. The scores estimate the effective staining intensity (ESI) within the effective staining area (ESA) of the biomarker in question (mean±SD of log-transformed values are shown). Each model was generated using multiple linear regression adjusting for age, race, and ER status.

*Statistically significant at \(P<0.05\); **Statistically significant with correction for multiple comparisons \((P <0.0083)\).

### TABLE 9 | Multivariable-adjusted associations of body fatness measures with LEPR protein expression in breast tumor tissues, stratified by ER status.

| ER+ cases | n  | \(\beta\) (95% CI)    | \(\beta_{\text{standardized}}\) | \(P\)      |
|-----------|----|----------------------|-------------------------------|------------|
| Body mass index (kg/m\(^2\)) | 405 | 0.0028 (0.0011, 0.0045) | 0.1620 | 0.011** |
| Waist circumference (cm) | 399 | 0.0014 (0.0006, 0.0022) | 0.1712 | 0.011** |
| Hip circumference (cm) | 399 | 0.0015 (0.0007, 0.0024) | 0.1717 | <0.0001** |
| Waist-to-hip ratio | 399 | 0.0540 (-0.1333, 0.2213) | 0.0342 | 0.527 |
| Fat mass index (kg/m\(^2\)) | 382 | 0.0041 (0.0015, 0.0067) | 0.1610 | 0.002** |
| Percent body fat (%) | 385 | 0.0020 (0.0004, 0.0037) | 0.1267 | 0.017* |

| ER- cases | n  | \(\beta\) (95% CI)    | \(\beta_{\text{standardized}}\) | \(P\)      |
|-----------|----|----------------------|-------------------------------|------------|
| Body mass index (kg/m\(^2\)) | 166 | 0.0011 (-0.0018, 0.0040) | 0.0613 | 0.440 |
| Waist circumference (cm) | 162 | 0.0001 (-0.0013, 0.0014) | 0.0047 | 0.954 |
| Hip circumference (cm) | 162 | -0.0001 (-0.0017, 0.0015) | -0.0105 | 0.894 |
| Waist-to-hip ratio | 162 | 0.0221 (-0.2635, 0.3076) | 0.0132 | 0.880 |
| Fat mass index (kg/m\(^2\)) | 152 | 0.0013 (-0.0020, 0.0053) | 0.0548 | 0.610 |
| Percent body fat (%) | 151 | 0.0011 (-0.0016, 0.0037) | 0.0654 | 0.432 |

Protein expression scores reflect quantitative protein expression (using immunohistochemistry) of LEPR as analyzed through an automated/unsupervised scoring (quantitative) methodology. The scores estimate the effective staining intensity (ESI) within the effective staining area (ESA) of the biomarker in question (mean±SD of log-transformed values are shown). Each model was generated using multiple linear regression adjusting for age, race, and ER status.

*Statistically significant at \(P<0.05\); **Statistically significant with correction for multiple comparisons \((P <0.0083)\).
ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Rutgers University Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AL: Grant funding, study conception and design, data collection, data analysis, data interpretation, and writing. JA: Literature search and data interpretation. T-YC: Data interpretation and manuscript editing. WC: Data collection and manuscript editing. MC: Pathology review, data collection, and manuscript editing. EC: Data interpretation and manuscript editing. BQ: Data collection, data interpretation, and manuscript editing. KL: Data analysis, data interpretation, and manuscript editing. BD: Grant funding, data collection, data interpretation, and manuscript editing. KD: Grant funding, data collection, data interpretation, and manuscript editing. All authors read and approved the final manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: Author EC is employed by the company Kaiser Permanente Northern California.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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