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Cancer Research

Insulin, Estrogen, Inflammatory Markers, and Risk of Benign Proliferative Breast Disease

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Abstract

Women with benign proliferative breast disease (BPBD) are at increased risk for developing breast cancer. Evidence suggests that accumulation of adipose tissue can influence breast cancer development via hyperinsulinemia, increased estrogen, and/or inflammation. However, there are limited data investigating these pathways with respect to risk of BPBD. We evaluated serologic markers from these pathways in a case–control study of postmenopausal women nested within the Women's Health Initiative Clinical Trial. Cases were the 667 women who developed BPBD during follow-up, and they were matched to 1,321 controls. Levels of insulin, estradiol, C-reactive protein (CRP), and adiponectin were measured in fasting serum collected at baseline. Conditional logistic regression models were used to estimate ORs for the association of each factor with BPBD risk. Among nonusers of hormone therapy, fasting serum insulin was associated with a statistically significant increase in risk of BPBD (OR for highest vs. lowest quartile = 1.80; 95% confidence interval, CI, 1.16–2.79; $P_{trend} = 0.003$) as were levels of estradiol (OR for highest vs. lowest tertile = 1.89; 95% CI, 1.26–2.83; $P_{trend} = 0.02$) and CRP (OR for highest vs. lowest quartile = 2.46; 95% CI, 1.59–3.80; $P_{trend} < 0.001$). Baseline adiponectin level was inversely associated with BPBD risk (OR for highest vs. lowest quartile = 0.47; 95% CI, 0.31–0.71; $P_{trend} < 0.001$). These associations persisted after mutual adjustment, but were not observed among users of either estrogen alone or of estrogen plus progestin hormone therapy. Our results indicate that serum levels of estrogen, insulin, CRP, and adiponectin are independent risk factors for BPBD and suggest that the estrogen, insulin, and inflammation pathways are associated with the early stages of breast cancer development. *Cancer Res; 74(12); 1–11. ©2014 AACR.*

Introduction

Benign proliferative breast disease (BPBD), the hallmark of which is epithelial proliferation, is a putative breast cancer precursor (1, 2). A history of BPBD is associated with a 2-fold increased risk of developing breast cancer, and this relative risk rises to approximately five if atypical hyperplasia is present (1). Despite the strong relationship between BPBD and breast cancer development, the etiology of BPBD is poorly characterized. Among postmenopausal women, obesity is an established risk factor for breast cancer (3, 4), and given the relationship between BPBD and breast cancer, the molecular perturbations associated with obesity may also be relevant to BPBD.

Adiposity has been hypothesized to increase the risk of breast cancer in postmenopausal women via (i) increased estrogen production in adipose tissue during the postmenopausal period (5, 6); (ii) increased circulating insulin associated with insulin resistance (4, 7); and (iii) altered levels of inflammatory factors that originate directly from adipose tissue and may promote or prevent carcinogenesis (8, 9). Although there is evidence to suggest that hyperinsulinemia, high estrogen levels, and inflammation contribute to the development of breast cancer independently of each other, there is also evidence for considerable biologic interaction between these pathways (10, 11). Therefore, further elucidation of the role of each pathway in the early stages of breast cancer requires that they be evaluated concurrently. To this end, we investigated the associations of fasting insulin, estradiol, C-reactive protein (CRP), and adiponectin with the risk of BPBD in a large prospective cohort of postmenopausal women.

Patients and Methods

Study population

This investigation was conducted as a nested case–control study within the Women's Health Initiative Clinical Trial

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(WHI-CT; ref. 12). Briefly, the WHI-CT recruited 68,133 postmenopausal women, ages 50 to 79, from 40 U.S. clinical centers between 1993 and 1998. At baseline, subjects completed questionnaires about demographic and behavioral factors, medical history, and use of medications (including hormone therapy). Each woman also underwent a physical examination, including measurement of height and waist, and of waist and hip circumference, and provided a fasting blood sample (13).

Histopathology

Every 6 months, participants in the trial completed medical questionnaires on clinical events including breast procedures. In the Benign Breast Disease Ancillary Study, which was conducted in all clinical centers participating in the WHI-CT (14, 15), women who had undergone a breast procedure were asked to provide consent for retrieval of the resulting histologic sections. These histologic sections were reviewed by the study pathologist (David L. Page) who was blinded to randomization assignment in the clinical trials and to other exposure information. Benign lesions were classified using well-established criteria as nonproliferative lesions, proliferative lesions without atypia, or atypical (ductal and/or lobular) hyperplasia (16, 17). To assess intrarater agreement, a repeatability study was carried out on 144 histologic sections that, following initial review, were assigned new identification numbers (to blind the pathologist to the results of the first set of readings) and then reviewed a second time. Assessment of agreement on histologic classification as described above yielded a kappa of 0.6 (95% CI, 0.4–0.7), consistent with estimates found in other studies (18, 19).

Definition of cases and controls

Cases in our study were women diagnosed with incident BPBD (with or without atypia) during follow-up in the WHI-CT. Controls were women who did not develop BPBD during the same follow-up period as the corresponding cases, and who did not have an abnormal mammogram or abnormal clinical breast exam during the same period.

A total of 705 cases of BPBD were included in the present study. These were comprised of all cases of atypical hyperplasia that had serum available ($N = 275$), in addition to 430 cases randomly selected from the 1,501 cases without atypia. Controls were selected from eligible participants using risk-set sampling (20) and were individually matched to their corresponding case on age at baseline (within 2 years), race (non-Hispanic white, black, Hispanic, Asian or Pacific Islander, other or missing), randomization group (and intervention/nonintervention arm), and date of baseline blood draw (within 1 year). Two controls were selected for 690 of the cases; for the remaining 15 cases only one control could be matched, giving a total of 1,395 controls.

Laboratory methods

All laboratory testing was performed blinded to case–control status by the Biomarker Analytic Core Laboratory at the Albert Einstein College of Medicine, Bronx, NY. Serum insulin levels were determined by enzyme-linked immunosorbent assay (ALPCO Diagnostics), which has an assay sensitivity of 0.40 μIU/mL. Serum adiponectin was measured by radio immune assay (Millipore Corporation) with a sensitivity of 0.2 ng/mL. Serum CRP was measured using a latex-enhanced turbidimetric immunoassay (Sakisui Diagnostics) with a sensitivity of 0.05 mg/L. Serum estradiol was measured using the DELFIA time-resolved fluoroimmunoassay method (Perkin Elmer Corporation), which can measure estradiol concentrations that approach nondetectable limits (21). Approximately 5% of all samples were randomly selected to be retested as blind duplicates, and all analytes showed strong correlations between duplicates (Pearson r: insulin = 0.99, estradiol = 0.98, CRP = 0.99, and adiponectin = 0.94). Average interassay coefficients of variation determined using the blind duplicates were as follows: insulin = 6.0%, estradiol = 11.7%, CRP = 1.9%, and adiponectin = 9.0%.

Statistical analysis

A total of 112 participants (38 cases and 74 controls) had a self-reported history of diabetes. Significant differences in assay levels of insulin ($P < 0.001$), CRP ($P < 0.001$), and adiponectin ($P < 0.001$) were observed when comparing these women with those without a self-reported history of diabetes (Supplementary Table S1). Given these differences, all analyses here considered only those without a history of diabetes (667 cases and 1,321 controls).

Differences in baseline demographics between cases and controls were evaluated using Pearson χ^2 test for categorical variables and the Wilcoxon rank sum test for continuous variables. Correlations between categorical serologic data, age, and body mass index (BMI) were assessed using Spearman correlation coefficients.

Conditional logistic regression models were used to estimate OR and 95% confidence intervals (CI) for the association of each serologic factor with risk of BPBD. For these analyses, insulin, CRP, and adiponectin levels were categorized by quartiles based on the distributions of the measurements in the controls. Estradiol levels were assessed in baseline nonusers of hormone therapy (HT) only, as standard estradiol assays cannot accurately measure equine hormones present in most HT preparations. We therefore stratified the analyses of serum estradiol by creating five nonoverlapping groups, namely, nonusers of HT with low, moderate, or high estradiol levels (tertiles, based on the distribution in the controls); users of unopposed estrogen; and users of combined estrogen and progestin (22). These groups were then parameterized as separate indicator variables, with low estradiol as the common referent. To account for the differences between those with and without a history of diabetes, we introduced an interaction term involving the diabetes variable (no = 0, yes = 1) and the main effect variable to all analyses. This allowed us to effectively evaluate the associations separately for those without a history of diabetes while retaining information from the matched pairs. In addition to those for the serum markers, we also examined the associations of BMI and waist circumference with risk given our interest in adiposity-related pathways and risk of BPBD. Age-adjusted analyses were conducted first, followed by multivariate analyses in which adjustment was made for relevant confounders, including age (years,

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continuous), BMI (<25, 25–30, 30–35, ≥35 kg/m^2; for analysis of serologic factors only), age at menopause (≤42, 43–48, 49–51, ≥52 years), use of HT (never, past, or current), history of breast biopsy at baseline (yes, no), and annual income (<$35,000, $35,000–$74,999, ≥$75,000, unsure). These covariates were chosen because their inclusion changed the OR estimates for the main exposures by at least 10%. Adjusting for BMI as a continuous variable did not change results substantially when compared with the four category adjustment (data not shown). Additional adjustment for other variables (e.g., age at menarche, parity, oral contraceptive use, energy intake) had no substantial effect on the associations, so these variables were not included in the main models. Analyses were also conducted after stratifying by hormone use at baseline (nonusers of HT, unopposed estrogen users, estrogen plus progestin users), BMI based on the median in controls (<27.9 kg/m^2 and ≥27.9 kg/m^2), waist circumference based on the median in controls (<86 cm and ≥86 cm), and age based on the median in controls (<62 and ≥62 years). These stratified analyses were conducted by introducing interaction terms into multivariate models that also included the main effect variables. In addition, we conducted separate analyses for cases with and without atypical hyperplasia. For all analyses, tests for trend were performed by assigning median values to each quartile/tertile and modeling these categories as a continuous variable. All hypothesis tests were two-sided, and all analyses were done using the statistical software Stata S/E 13.0 for Windows (STATA Corporation).

Results

Cases were more likely than controls to have a lower BMI, to be using hormone replacement therapy, and to have a history of breast biopsy at baseline. Cases were also more likely to go on to develop invasive breast cancer (Table 1). Correlations between serologic factors were very similar in non-HT users, users of unopposed estrogen, and users of estrogen plus progestin (Supplementary Table S2). Considering nonusers of HT only, insulin levels were moderately positively correlated with CRP level ($r = 0.41$, $P < 0.001$) and strongly positively correlated with BMI ($r = 0.57$, $P < 0.001$) and waist circumference ($r = 0.60$, $P < 0.001$). Adiponectin levels were inversely correlated with insulin levels ($r = -0.49$, $P < 0.001$), CRP levels ($r = -0.30$, $P < 0.001$), BMI ($r = -0.33$, $P < 0.001$), and waist circumference ($r = -0.39$, $P < 0.001$), and positively correlated with age ($r = 0.15$, $P < 0.001$). CRP was strongly positively correlated with BMI ($r = 0.56$, $P < 0.001$) and waist circumference ($r = 0.54$, $P < 0.001$). Estradiol levels were assessed in nonusers of HT only and were moderately positively correlated with insulin levels ($r = 0.21$, $P < 0.001$), CRP levels ($r = 0.25$, $P < 0.001$), BMI ($r = 0.33$, $P < 0.001$), and waist circumference ($r = 0.27$, $P < 0.001$), and moderately inversely correlated with adiponectin levels ($r = -0.21$, $P < 0.001$; Supplementary Table S2).

We also examined associations between serum levels of the markers of interest and exogenous hormone use and found insulin levels to be higher in nonusers [mean, 5.3; interquartile range (IQR), 3.6–8.6] than in both estrogen users (mean, 4.7; IQR, 3.0–6.6) and estrogen plus progestin users (mean, 3.5; IQR, 2.8–6.5; $P < 0.001$, Kruskal–Wallis). CRP levels in nonusers of HT were lower (mean, 2.9; IQR, 1.1–5.0) than those in both estrogen users (mean, 4.9; IQR, 2.1–9.5) and estrogen plus progestin users (mean, 3.5; IQR, 1.7–7.1; $P < 0.001$, Kruskal–Wallis), whereas adiponectin levels were comparable in all three groups ($P = 0.93$, Kruskal–Wallis; Supplementary Table S3).

Associations between serologic factors and BPBD

After adjustment for relevant confounders (see Materials and Methods), fasting serum insulin was associated with a significant increase in risk of BPBD (OR for highest vs. lowest quartile = 1.40; 95% CI, 1.00–1.95; $P_{trend} = 0.01$; Table 2). Estradiol level was considered among non-HT users only and was associated with a significant increase in risk of BPBD (OR for highest vs. lowest tertile = 1.89; 95% CI, 1.26–2.83; $P_{trend} = 0.02$). Baseline CRP level was also associated with a significant increase in risk of BPBD (OR for highest vs. lowest quartile = 1.65; 95% CI, 1.18–2.29; $P_{trend} = 0.01$). In contrast, baseline level of adiponectin was associated with a decreased risk of BPBD (OR for highest vs. lowest quartile = 0.66; 95% CI, 0.49–0.90; $P_{trend} = 0.01$). After adjustment for confounding, there was no evidence of an association between BMI or waist circumference and risk of BPBD in the entire study population (Table 2). When subjects with a history of diabetes were reintroduced into these analyses, the results for estradiol, CRP, and adiponectin were attenuated slightly but remained statistically significant (data not shown). The insulin association was unchanged. On formal testing, there were no between-strata differences in the results when the analyses were stratified by median BMI, waist circumference, or age (Supplementary Tables S4–S6). Results were essentially the same when we repeated analyses separating cases into those with and without atypical hyperplasia (Supplementary Table S7). Given the strong association with history of breast biopsy at baseline and risk of BPBD (Table 1), we repeated the analyses after excluding those with a history of breast biopsy, but found no material change in estimates. We also repeated the analyses after excluding those who were randomized to the treatment arm of the HT trial, and again saw no substantial differences in estimates. Furthermore, we saw no substantial differences in the results when we evaluated the associations between serologic factors and risk of BPBD stratified by cases that went on to develop invasive breast cancer and cases that did not. However, numbers were very small for those who did develop subsequent breast cancer, and the follow-up time was relatively short.

Results stratified by use of hormone therapy

All of the associations observed between the serologic factors and risk of BPBD were stronger among nonusers of HT than among those using estrogen or estrogen plus progestin (Table 3). On formal testing, there was a significant interaction between HT use and CRP ($P = 0.04$), adiponectin ($P = 0.04$) and BMI ($P = 0.01$), but not insulin ($P = 0.24$), with respect to risk of BPBD. Considering nonusers of HT only, there was a strong positive association with fasting insulin (OR for highest vs. lowest quartile = 1.80; 95% CI, 1.16–2.79; $P_{trend} = 0.003$), a highly significant positive association with CRP level (OR for highest vs. lowest quartile = 2.46; 95% CI, 1.59–3.80; $P_{trend} < 0.001$), and a

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Table 1. Distribution of selected baseline characteristics in cases and controls

Variable	Cases ($n = 667$)	Controls ($n = 1,321$)	$P_{difference}$
Median age, y (IQR)	61.6 (56.7–67.3)	61.8 (56.8–67.5)	0.66
Race/ethnicity, *n* (%)			
White	589 (88.3)	1,175 (89.0)	
Black	40 (6.0)	74 (5.6)	
Hispanic	17 (2.6)	32 (2.4)	
Asian/other	14 (2.1)	28 (2.1)	
Unknown	7 (1.1)	12 (0.9)	0.99
Median BMI, kg/m^2 (IQR)	27.1 (24.1–31.1)	27.7 (24.5–31.9)	0.03
Median waist circumference, cm (IQR)	85.3 (77.0–94.5)	86.0 (78.0–96.0)	0.11
Age at menarche, y, *n* (%)			
≤10	40 (6.0)	79 (6.0)	
11–12	273 (40.9)	549 (41.6)	
≥13	352 (52.8)	687 (52.0)	
Missing	2 (0.3)	6 (0.5)	0.95
Age at menopause, y, *n* (%)			
≤42	117 (17.5)	229 (17.3)	
43–48	167 (25.0)	334 (25.3)	
49–51	136 (20.4)	251 (19.0)	
≥52	144 (21.6)	272 (20.6)	
Missing	103 (15.4)	235 (17.8)	0.72
Parity, *n* (%)			
0	67 (10.0)	136 (10.3)	
1	42 (6.3)	99 (7.5)	
≥2	556 (83.4)	1,080 (81.8)	
Missing	2 (0.3)	6 (0.5)	0.72
Age at first child's birth, y, *n* (%)			
<20	80 (12.0)	206 (15.6)	
20–29	426 (63.9)	795 (60.2)	
≥30	47 (7.1)	78 (5.9)	
Nulliparous/missing	114 (17.1)	242 (18.3)	0.10
Ever use of oral contraceptives, *n* (%)	340 (51.0)	673 (51.0)	0.99
Current use of hormone therapy, *n* (%)	340 (51.0)	545 (41.3)	
Currently using unopposed estrogen therapy	176 (26.4)	279 (21.1)	
Currently using combined estrogen + progestin therapy	164 (24.6)	266 (20.1)	
Currently not using hormone therapy	326 (48.9)	774 (58.6)	
Missing	1 (0.2)	2 (0.2)	0.001
Smoking status, *n* (%)			
Never	336 (50.4)	711 (53.8)	
Former	282 (42.3)	506 (38.3)	
Current	40 (6.0)	93 (7.0)	0.20
Ever breast biopsy, *n* (%)	219 (32.8)	185 (14.0)	<0.001
First-degree relative with breast cancer, *n* (%)	136 (20.4)	237 (17.9)	0.19
Highest education level, *n* (%)			
High school or less	185 (27.7)	430 (32.6)	
College	274 (41.1)	519 (39.3)	
Postgraduate education	203 (30.4)	368 (27.9)	
Missing	5 (0.8)	4 (0.3)	0.08
Income, *n* (%)			
<$35,000	207 (31.0)	467 (35.4)	
$35,000 to $74,999	267 (40.0)	536 (40.6)	
≥$75,000	152 (22.8)	251 (19.0)	
Do not know/missing	41 (6.2)	67 (5.1)	0.09

(Continued on the following page)

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Table 1. Distribution of selected baseline characteristics in cases and controls (Cont'd)

Variable	Cases (n = 667)	Controls (n = 1,321)	$P_{difference}$
Median energy intake, calories (IQR)	1,633 (1277–2040)	1,639 (1254–2100)	0.84
Median alcohol consumption, servings per week (IQR)	0.4 (0–2.9)	0.4 (0–2.8)	0.96
Median physical activity, METs hours per week (IQR)	5.9 (0–15.0)	6.0 (0–15.0)	0.84
Developed invasive breast cancer, *n* (%)	46 (6.9)	42 (3.2)	<0.001

highly significant inverse association with adiponectin (OR for highest vs. lowest quartile = 0.47; 95% CI, 0.31–0.71; P_{trend} < 0.001) and risk of BPBD. There was also a borderline significant positive association between waist circumference and BPBD risk (Table 3). None of these associations was observed among women using either type of HT.

The associations between the serologic factors and BPBD risk persisted, but were attenuated when all four markers were included in the same model (Table 4). Adjustment for other factors (e.g., age at menarche, parity, OC use, and energy intake) had no substantial effect on the associations observed in models containing all four serologic factors.

Table 2. Age- and multivariable-adjusted OR (95% CI) for associations between baseline levels of serologic factors and BPBD risk

Factor	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}
Insulin					
Quartile cut points, μIU/mL	<3.3	3.3–<4.9	4.9–<8.0	≥8.0	
Number of cases/controls	174/333	142/326	174/318	167/314	
Age-adjusted OR (95% CI)	1.0[Ref.]	0.83 (0.63–1.08)	1.04 (0.80–1.34)	0.99 (0.76–1.29)	0.61
Multivariable-adjusted OR[a] (95% CI)	1.0[Ref.]	0.94 (0.70–1.26)	1.41 (1.05–1.89)	1.40 (1.00–1.95)	0.01
Estradiol (non-HT users only)					
Tertile cut points, pg/mL	<22.1	22.1–<28.9	≥28.9		
Number of cases/controls	74/235	121/237	117/240		
Age-adjusted OR (95% CI)	1.0[Ref.]	1.77 (1.23–2.54)	1.78 (1.22–2.60)		0.02
Multivariable-adjusted OR[a] (95% CI)	1.0[Ref.]	1.78 (1.22–2.60)	1.89 (1.26–2.83)		0.02
CRP					
Quartile cut points, mg/L	<1.4	1.4–<2.9	2.9–<6.3	≥6.3	
Number of cases/controls	139/325	157/322	167/321	192/322	
Age-adjusted OR (95% CI)	1.0[Ref.]	1.14 (0.87–1.51)	1.23 (0.93–1.62)	1.42 (1.08–1.86)	0.02
Multivariable-adjusted OR[a] (95% CI)	1.0[Ref.]	1.26 (0.93–1.70)	1.35 (0.99–1.84)	1.65 (1.18–2.29)	0.01
Adiponectin					
Quartile cut points, μg/mL	<10.3	10.3–<14.3	14.3–<19.3	≥19.3	
Number of cases/controls	178/336	178/329	172/329	139/325	
Age-adjusted OR (95% CI)	1.0[Ref.]	1.02 (0.79–1.33)	0.99 (0.76–1.29)	0.81 (0.61–1.06)	0.11
Multivariable-adjusted OR[a] (95% CI)	1.0[Ref.]	0.93 (0.70–1.22)	0.86 (0.64–1.14)	0.66 (0.49–0.90)	0.01
BMI					
Category cut points, kg/m^2	<25	25–<30	30–<35	≥35	
Number of cases/controls	221/393	239/448	126/283	77/181	
Age-adjusted OR (95% CI)	1.0[Ref.]	0.94 (0.75–1.18)	0.78 (0.60–1.03)	0.75 (0.55–1.03)	0.03
Multivariable-adjusted OR[a] (95% CI)	1.0[Ref.]	0.99 (0.78–1.26)	0.82 (0.62–1.09)	0.85 (0.61–1.09)	0.15
Waist circumference					
Quartile cut points, cm	<78	78–<86	86–<96	≥96	
Number of cases/controls	196/351	153/311	161/328	156/320	
Age-adjusted OR (95% CI)	1.0[Ref.]	0.87 (0.67–1.13)	0.87 (0.67–1.13)	0.87 (0.67–1.12)	0.31
Multivariable-adjusted OR[a] (95% CI)	1.0[Ref.]	0.91 (0.69–1.20)	0.94 (0.71–1.23)	0.97 (0.74–1.27)	0.90

[a]Multivariable models adjusted for age, BMI (serologic factors only; <25, 25–<30, 30–<35, ≥35 kg/m^2), age at menopause (≤42, 43–48, 49–51, ≥52), use of HT (never, past, current), ever had breast biopsy at baseline (yes/no), and annual income (<$35,000, $35,000–$74,999, ≥$75,000, unsure).

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Table 3. Age- and multivariable-adjusted OR (95% CI) for associations between baseline levels of serologic factors and BPBD risk after stratification by HT use

HT use, factor	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}
Nonusers of HT					
Insulin					
Quartile cut points, μIU/mL	<3.3	3.3–<4.9	4.9–<8.0	≥8.0	
Number of cases/controls	55/164	67/189	88/190	112/216	
Age-adjusted OR (95% CI)	1.0[Ref.]	1.06 (0.70–1.60)	1.38 (0.93–2.05)	1.49 (1.02–2.19)	0.02
Multivariable-adjusted OR[a] (95% CI)	1.0[Ref.]	1.06 (0.68–1.64)	1.64 (1.07–2.51)	1.80 (1.16–2.79)	0.003
Estradiol					
Tertile cut points, pg/mL	<22.1	22.1–<28.9	≥28.9		
Number of cases/controls	74/235	121/237	117/240		
Age-adjusted OR (95% CI)	1.0[Ref.]	1.77 (1.23–2.54)	1.78 (1.22–2.60)		0.02
Multivariable-adjusted OR[a] (95% CI)	1.0[Ref.]	1.78 (1.22–2.60)	1.89 (1.26–2.83)		0.02
CRP					
Quartile cut points, mg/L	<1.4	1.4–<2.9	2.9–<6.3	≥6.3	
Number of cases/controls	82/241	74/199	81/178	81/136	
Age-adjusted OR (95% CI)	1.0[Ref.]	1.05 (0.72–1.52)	1.28 (0.89–1.84)	1.77 (1.21–2.59)	0.002
Multivariable-adjusted OR[a] (95% CI)	1.0[Ref.]	1.34 (0.90–1.98)	1.65 (1.11–2.45)	2.46 (1.59–3.80)	<0.001
Adiponectin					
Quartile cut points, μg/mL	<10.3	10.3–<14.3	14.3–<19.3	≥19.3	
Number of cases/controls	106/200	83/186	77/190	60/197	
Age-adjusted OR (95% CI)	1.0[Ref.]	0.85 (0.60–1.21)	0.76 (0.53–1.08)	0.56 (0.38–0.82)	0.002
Multivariable-adjusted OR[a] (95% CI)	1.0[Ref.]	0.78 (0.54–1.12)	0.66 (0.45–0.96)	0.47 (0.31–0.71)	<0.001
BMI					
Category cut points, kg/m^2	<25	25–<30	30–<35	≥35	
Number of cases/controls	80/224	117/242	75/171	52/128	
Age-adjusted OR (95% CI)	1.0[Ref.]	1.40 (0.99–1.97)	1.23 (0.84–1.81)	1.17 (0.77–1.76)	0.68
Multivariable-adjusted OR[a] (95% CI)	1.0[Ref.]	1.39 (0.97–1.98)	1.25 (0.84–1.87)	1.18 (0.77–1.82)	0.63
Waist circumference					
Quartile cut points, cm	<78	78–<86	86–<96	≥96	
Number of cases/controls	68/197	75/164	80/196	102/207	
Age-adjusted OR (95% CI)	1.0[Ref.]	1.36 (0.92–2.00)	1.16 (0.79–1.70)	1.42 (0.99–2.05)	0.11
Multivariable-adjusted OR[a] (95% CI)	1.0[Ref.]	1.36 (0.91–2.03)	1.21 (0.81–1.80)	1.51 (1.03–2.21)	0.06
Unopposed estrogen users					
Insulin					
Quartile cut points, μIU/mL	<3.3	3.3–<4.9	4.9–<8.0	≥8.0	
Number of cases/controls	61/81	32/65	43/75	35/48	
Age-adjusted OR (95% CI)	1.0[Ref.]	0.63 (0.36–1.08)	0.83 (0.50–1.37)	0.95 (0.56–1.62)	0.75
Multivariable-adjusted OR[a] (95% CI)	1.0[Ref.]	0.63 (0.35–1.11)	0.95 (0.55–1.62)	1.18 (0.66–2.10)	0.29
CRP					
Quartile cut points, mg/L	<1.4	1.4–<2.9	2.9–<6.3	≥6.3	
Number of cases/controls	19/36	42/60	41/68	71/108	
Age-adjusted OR (95% CI)	1.0[Ref.]	1.41 (0.71–2.80)	1.17 (0.60–2.29)	1.28 (0.67–2.43)	0.80
Multivariable-adjusted OR[a] (95% CI)	1.0[Ref.]	1.39 (0.68–2.87)	1.30 (0.64–2.64)	1.51 (0.76–2.99)	0.39
Adiponectin					
Quartile cut points, μg/mL	<10.3	10.3–<14.3	14.3–<19.3	≥19.3	
Number of cases/controls	40/67	48/73	51/76	37/61	
Age-adjusted OR (95% CI)	1.0[Ref.]	1.14 (0.66–1.97)	1.13 (0.67–1.90)	1.04 (0.60–1.80)	0.92
Multivariable-adjusted OR[a] (95% CI)	1.0[Ref.]	1.10 (0.62–1.96)	1.05 (0.60–1.82)	0.97 (0.54–1.74)	0.85
BMI					
Category cut points, kg/m^2	<25	25–<30	30–<35	≥35	
Number of cases/controls	62/75	71/110	34/60	9/27	

(Continued on the following page)

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Table 3. Age- and multivariable-adjusted OR (95% CI) for associations between baseline levels of serologic factors and BPBD risk after stratification by HT use (Cont'd)

HT use, factor	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}
Age-adjusted OR (95% CI)	1.0[Ref.]	0.81 (0.51–1.28)	0.72 (0.42–1.22)	0.45 (0.21–0.97)	0.04
Multivariable-adjusted OR[a] (95% CI)	1.0[Ref.]	0.75 (0.47–1.21)	0.71 (0.40–1.24)	0.51 (0.24–1.11)	0.08
Waist circumference					
Quartile cut points, cm	<78	78–<86	86–<96	≥96	
Number of cases/controls	54/68	46/76	47/73	29/59	
Age-adjusted OR (95% CI)	1.0[Ref.]	0.70 (0.41–1.18)	0.84 (0.50–1.41)	0.64 (0.37–1.11)	0.17
Multivariable-adjusted OR[a] (95% CI)	1.0[Ref.]	0.62 (0.35–1.07)	0.82 (0.47–1.41)	0.67 (0.38–1.18)	0.30
Estrogen + progestin users					
Insulin					
Quartile cut points, μIU/mL	<3.3	3.3–<4.9	4.9–<8.0	≥8.0	
Number of cases/controls	57/88	43/71	43/51	20/50	
Age-adjusted OR (95% CI)	1.0[Ref.]	0.97 (0.58–1.62)	1.31 (0.77–2.20)	0.63 (0.34–1.17)	0.23
Multivariable-adjusted OR[a] (95% CI)	1.0[Ref.]	1.23 (0.72–2.13)	1.89 (1.08–3.33)	0.83 (0.42–1.62)	0.77
CRP					
Quartile cut points, mg/L	<1.4	1.4–<2.9	2.9–<6.3	≥6.3	
Number of cases/controls	38/48	40/63	45/74	40/76	
Age-adjusted OR (95% CI)	1.0[Ref.]	0.83 (0.45–1.55)	0.75 (0.42–1.35)	0.66 (0.37–1.19)	0.21
Multivariable-adjusted OR[a] (95% CI)	1.0[Ref.]	0.85 (0.44–1.63)	0.76 (0.41–1.39)	0.77 (0.41–1.43)	0.51
Adiponectin					
Quartile cut points, μg/mL	<10.3	10.3–<14.3	14.3–<19.3	≥19.3	
Number of cases/controls	32/67	47/68	44/63	41/67	
Age-adjusted OR (95% CI)	1.0[Ref.]	1.46 (0.84–2.53)	1.43 (0.83–2.49)	1.19 (0.67–2.09)	0.73
Multivariable-adjusted OR[a] (95% CI)	1.0[Ref.]	1.27 (0.71–2.27)	1.26 (0.70–2.27)	0.94 (0.51–1.73)	0.68
BMI					
Category cut points, kg/m^2	<25	25–<30	30–<35	≥35	
Number of cases/controls	78/94	51/93	17/51	16/26	
Age-adjusted OR (95% CI)	1.0[Ref.]	0.68 (0.43–1.06)	0.42 (0.23–0.77)	0.70 (0.35–1.40)	0.04
Multivariable-adjusted OR[a] (95% CI)	1.0[Ref.]	0.76 (0.48–1.23)	0.39 (0.21–0.73)	0.73 (0.35–1.50)	0.04
Waist circumference					
Quartile cut points, cm	<78	78–<86	86–<96	≥96	
Number of cases/controls	73/85	32/70	34/58	25/53	
Age-adjusted OR (95% CI)	1.0[Ref.]	0.53 (0.31–0.89)	0.68 (0.40–1.16)	0.55 (0.31–0.97)	0.04
Multivariable-adjusted OR[a] (95% CI)	1.0[Ref.]	0.65 (0.38–1.12)	0.73 (0.42–1.27)	0.55 (0.31–0.99)	0.05

[a]Multivariable models adjusted for age, BMI (serologic factors only; <25, 25–<30, 30–<35, ≥35 kg/m^2), age at menopause (≤42, 43–48, 49–51, ≥52), ever had breast biopsy at baseline (yes/no), and annual income (<$35,000, $35,000–$74,999, ≥$75,000, unsure).

Discussion

To our knowledge, this is the first prospective study to examine the association between serum levels of insulin, estradiol, CRP, and adiponectin and risk of BPBD. We found independent positive associations of fasting serum levels of insulin, estradiol, and CRP with the risk of BPBD among nondiabetic postmenopausal women who were not using HT. We also observed an inverse association with serum adiponectin levels and risk of BPBD in this same group. These adiposity-related markers were highly correlated with BMI in these data. Although BMI was not associated with increased risk of BPBD in this study, there was a borderline statistically significant positive association between waist circumference, a measure of central adiposity, and risk of BPBD.

One of the most pronounced metabolic changes associated with increased deposits of adipose tissue is the development of an impaired responsiveness of cells to insulin, a condition known as insulin resistance (7, 23). This resistance results in elevated serum insulin or hyperinsulinemia. Insulin is a growth factor for a wide range of tissues, and has been shown to be mitogenic in normal breast tissue and breast cancer cell lines (24, 25) and to promote breast tumor growth in animal models (26, 27). Indirectly, insulin may also increase the risk of breast cancer by stimulating estrogen synthesis (28, 29). Several epidemiologic studies have prospectively investigated the association of fasting insulin levels with breast cancer incidence, and most have shown positive associations (22, 30–32). Data on the association of hyperinsulinemia with BPBD are limited, although C-peptide,

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Table 4. Multivariable-adjusted OR (95% CI) for associations between baseline levels of serologic factors and BPBD risk among non-HT users, adjusted for all serologic factors

Serologic factor	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}
Insulin					
Quartile cut points, μIU/mL	<3.3	3.3–<4.9	4.9–<8.0	≥8.0	
Number of cases/controls	51/147	64/173	82/172	105/192	
Multivariable-adjusted OR[a] (95% CI)	1.0[Ref.]	1.04 (0.65–1.66)	1.54 (0.98–2.44)	1.61 (1.00–2.59)	0.03
Estradiol					
Tertile cut points, pg/mL	<22.1	22.1–<28.9	≥28.9		
Number of cases/controls	72/227	116/227	114/230		
Multivariable-adjusted OR[a] (95% CI)	1.0[Ref.]	1.85 (1.26–2.74)	1.83 (1.20–2.78)		0.06
CRP					
Quartile cut points, mg/L	<1.4	1.4–<2.9	2.9–<6.3	≥6.3	
Number of cases/controls	77/220	69/172	79/165	77/127	
Multivariable-adjusted OR[a] (95% CI)	1.0[Ref.]	1.44 (0.94–2.19)	1.62 (1.06–2.47)	2.18 (1.37–3.45)	0.002
Adiponectin					
Quartile cut points, μg/mL	<10.3	10.3–<14.3	14.3–<19.3	≥19.3	
Number of cases/controls	98/177	80/167	70/166	54/174	
Multivariable-adjusted OR[a] (95% CI)	1.0[Ref.]	0.87 (0.59–1.29)	0.76 (0.50–1.15)	0.58 (0.37–0.93)	0.02
BMI					
Category cut points, kg/m^2	<25	25–<30	30–<35	≥35	
Number of cases/controls	74/195	109/216	70/152	47/113	
Multivariable-adjusted OR[a] (95% CI)	1.0[Ref.]	1.07 (0.72–1.60)	0.81 (0.51–1.28)	0.64 (0.38–1.09)	0.07

[a]Multivariable models adjusted for age, BMI (serologic factors only; <25, 25–<30, 30–<35, ≥35 kg/m^2), age at menopause (≤42, 43–48, 49–51, ≥52), use of HT (never, past, current), ever had breast biopsy at baseline (yes/no), annual income (<$35,000, $35,000–$74,999, ≥$75,000, unsure), insulin level (quartile), estradiol level (tertile), CRP level (quartile), and adiponectin level (quartile).

a marker of insulin secretion, has been associated with the prevalence of breast hyperplasia (33). Our finding that increased serum insulin is associated with an increase in risk for BPBD is in line with evidence linking insulin with breast cancer development. This association remained after adjustment for endogenous estradiol, suggesting that insulin acts to promote breast carcinogenesis independently of estradiol levels.

The association between obesity and postmenopausal breast cancer is thought to partly reflect the elevated circulating estrogen levels present in obese women (5, 6). The metabolism of estrogen creates mutagenic metabolites, and estrogen can also directly stimulate breast tissue growth (34). Epidemiologic studies have consistently shown a positive association between circulating estradiol levels and postmenopausal breast cancer (5, 34). There is also evidence to support a role for estrogens in the development of BPBD. Use of conjugated equine estrogens has been associated with an increased risk of BPBD (14, 35, 36). Data on the role of endogenous estrogens and BPBD are more limited, although plasma levels of free estradiol have been shown to be significantly higher in women with benign breast disease compared with normal patients (37). Our finding of an association with estradiol levels and risk of BPBD lends support to the idea that increasing levels of estradiol may increase breast cancer risk, at least in part, via their influence on the early stages of breast cancer development.

In addition to its endocrine/metabolic effects, obesity is considered to be a chronic proinflammatory state. Adipose tissue contributes approximately 30% of circulating IL6, an inflammatory cytokine, which induces hepatic synthesis of the acute-phase protein, CRP (38, 39). Levels of serum CRP are higher among obese women than normal weight women (40). Adipose tissue itself is essentially an endocrine organ secreting a large range of proteins, collectively called adipokines. Of interest, adiponectin has anti-inflammatory activity (41), and can function as an insulin-sensitizer (42). Adiponectin also strongly inhibits proliferation of endothelial cells (43, 44), and has been shown to exert antiproliferative effects on breast tissue (9). Although the factors that control adiponectin levels are not clearly defined, levels are lower in obese women (45). Epidemiologic evidence suggests a positive association between serum CRP and breast cancer risk (46), and there is evidence that high levels of adiponectin may be associated with decreased risk of postmenopausal breast cancer (47). Our findings of highly statistically significant associations with risk of BPBD and both increased CRP levels and decreased adiponectin levels indicate that inflammation may play an important role in the development of BPBD, and thus be important in early breast carcinogenesis.

Epidemiologic studies consistently show that the association between obesity and risk of postmenopausal breast cancer is limited to women who did not use postmenopausal HT

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(3, 48). Our data show that the associations between adiposity-related markers examined here and risk of BPBD are also limited to nonusers of HT. Despite these associations, we found no evidence linking BMI with increased risk of BPBD. This lack of association may reflect growing evidence that BMI is not an accurate measure of adiposity and that an estimated 10% to 25% of obese individuals are metabolically healthy, with respect to insulin resistance and inflammation (49). Nevertheless, we did observe a borderline statistically significant positive association between waist circumference—a measure of central adiposity—and risk of BPBD among non-HT users.

A major strength of this prospective study was the collection of prediagnostic blood samples at baseline, and ascertainment of subsequent (postbaseline) development of BPBD, thereby limiting bias. Furthermore, given the large sample size, we were also able to investigate all four serologic markers concurrently, enabling us to better elucidate the independence of each investigated factor. It should be noted that although we found statistical independence for each factor, we cannot be certain that this signifies biologic independence. One further study strength was the centralized histologic review of the benign breast lesions, which allowed the separation of cases into those with or without atypical hyperplasia. Atypical hyperplasia is considered to be more proximal to invasive breast cancer than proliferative disease without atypia, and is associated with a greater increase in risk of subsequent breast cancer development (2). Despite this, we did not find that the associations with serologic factors were stronger among atypical hyperplasia cases, although the numbers in each stratum were much reduced and therefore the statistical power to detect significant differences between the histologic subtypes was limited. An important limitation of this study was that measurements of each serologic factor were only taken once, at baseline. Repeated measurements over time might have resulted in more accurate estimates of marker levels. However, prior evidence indicates that the factors of interest exhibit sufficient stability to be reliable indicators of long-term exposure (50–52). Also, there is typically substantial heterogeneity in diagnosis of BPBD, which, if present here, may have biased our estimates. However, in this instance, any misclassification of outcome would have been nondifferential, and thus would have biased results toward the null (53). Finally, the WHI-CT participants were a nonrandom sample of the population, which may limit the generalizability of our findings. However, because the results reported here demonstrate associations between measured biologic markers and risk of BPBD, it seems plausible that these same biologic markers may have similar effects in other populations.

The multistep model of breast carcinogenesis posits that invasive carcinoma arises via a series of steps, in which nonatypical proliferative changes and proliferative disease with atypia represent successive steps preceding the development of *in situ* carcinoma and ultimately invasive cancer (1, 54). The data presented here indicate that relatively high levels of insulin, estrogen, and CRP, and low levels of adiponectin are independent risk factors for BPBD among nondiabetic, postmenopausal women. These findings suggest that disruptions of the estrogen, insulin, and inflammation pathways, indicative of accumulation of excess adipose tissue, are associated with the early stages of breast cancer development.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C. Catsburg, M.J. Gunter, T.E. Rohan

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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): T.E. Rohan

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