Insulin, Insulin-Like Growth Factor-I, and Risk of Breast Cancer in Postmenopausal Women

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Background The positive association between obesity and postmenopausal breast cancer has been attributed, in part, to the fact that estrogen, a risk factor for breast cancer, is synthesized in adipose tissue. Obesity is also associated with high levels of insulin, a known mitogen. However, no prospective studies have directly assessed associations between circulating levels of insulin and/or insulin-like growth factor (IGF)-I, a related hormone, and the risk of breast cancer independent of estrogen level.

Methods We conducted a case–cohort study of incident breast cancer among nondiabetic women who were enrolled in the Women's Health Initiative Observational Study (WHI-OS), a prospective cohort of 93 676 postmenopausal women. Fasting serum samples obtained at study entry from 835 incident breast cancer case subjects and from a subcohort of 816 randomly chosen WHI-OS subjects were tested for levels of insulin, glucose, total IGF-I, free IGF-I, insulin-like growth factor binding protein-3, and estradiol. Multivariable Cox proportional hazards models were used to estimate associations between levels of the serologic factors and baseline characteristics (including body mass index [BMI]) and the risk of breast cancer. All statistical tests were two-sided.

Results Insulin levels were positively associated with the risk of breast cancer (hazard ratio [HR] for highest vs lowest quartile of insulin level = 1.46, 95% confidence interval [CI] = 1.00 to 2.13, P_{trend} = .02); however, the association with insulin level varied by hormone therapy (HT) use ($P_{interaction}$ = .01). In a model that controlled for multiple breast cancer risk factors including estradiol, insulin level was associated with breast cancer only among nonusers of HT (HR for highest vs lowest quartile of insulin level = 2.40, 95% CI = 1.30 to 4.41, P_{trend} < .001). Obesity (BMI ≥30 kg/m^2) was also associated with the risk of breast cancer among nonusers of HT (HR for BMI ≥30 kg/m^2 vs 18.5 to <25 kg/m^2 = 2.12, 95% CI = 1.26 to 3.58, P_{trend} = .003); however, this association was attenuated by adjustment for insulin (P_{trend} = .40).

Conclusion These data suggest that hyperinsulinemia is an independent risk factor for breast cancer and may have a substantial role in explaining the obesity–breast cancer relationship.

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Breast cancer is the most common malignancy among women in the United States. Approximately 182 000 new cases of breast cancer and more than 40 000 breast cancer–related deaths are expected in 2008 (1). One of the established risk factors for postmenopausal breast cancer, obesity (2–7), has reached epidemic proportions in the United States, and with more than one-third of women older than 40 years currently classified as obese (defined as a body mass index [BMI] ≥30 kg/m^2) (8), breast cancer incidence rates could soon rise. It is widely hypothesized that the association between obesity and postmenopausal breast cancer partly reflects the higher than average circulating estrogen levels present in obese women (9). However, obesity has additional endocrinologic effects that could play a role in breast cancer development.

Hyperinsulinemia—a consequence of insulin resistance or the impaired responsiveness of cells to insulin—is also more common in obese women than in normal-weight women (defined as a BMI of 18.5–25.0 kg/m^2) (10). Insulin has been shown to stimulate cell proliferation in normal breast tissue and in human breast cancer cell lines (11,12), and administration of exogenous insulin

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promotes breast tumor growth in animal models (13–15). Insulin may also increase the risk of breast cancer via alterations in circulating estrogen levels. For example, chronic hyperinsulinemia is associated with increased ovarian estrogen production, reduced hepatic secretion of sex hormone–binding globulin, and increased free estradiol levels (16,17).

Epidemiological data regarding the association between circulating insulin levels and the risk of breast cancer are limited. To our knowledge, only three small prospective studies (N < 200 cases each) have directly assessed the association between insulin levels and the risk of postmenopausal breast cancer (18–20). Two of these studies, both of which included women who were using hormone therapy (HT), reported no association (19,20), and the third study, which was conducted among nonusers of HT, found a positive association between hyperinsulinemia and postmenopausal breast cancer only among women who had a BMI that was greater than 25 kg/m^2 (18). In several other prospective investigations that lacked blood specimens from fasting patients, the circulating C-peptide level, a marker of insulin secretion, was positively associated with the risk of postmenopausal breast cancer (21–23). However, although in nonfasting patients, C-peptide levels are more stable than insulin levels, they do increase substantially postprandially (24), and the levels of C-peptide and insulin are not perfectly correlated (Spearman $r = .77$, $P < .001$), even when assessed in blood specimens from fasting patients (25). Given these considerations, further investigation of the insulin–breast cancer relationship is warranted.

Insulin-like growth factor (IGF)-I is a hormone related to insulin that has also attracted interest as an endocrine risk factor for breast cancer. Insulin-like growth factor-I and insulin share extensive sequence homology and downstream signaling pathways, but IGF-I exhibits stronger mitogenic and antiapoptotic effects (26). Insulin-like growth factor-I is a potent mitogen for both normal and transformed breast epithelial cells (27), and serum IGF-I level is associated with the development of mammary gland hyperplasia and cancer in a primate model (28). Most of the IGF-I in the circulation is produced by the liver and is bound to insulin-like growth factor binding proteins (IGFBPs); at least 75% of circulating IGF-I is bound to IGFBP-3 (29). Although only 1% of total serum IGF-I is unbound, this free fraction may be the most biologically active form of IGF-I (30).

Four systematic reviews and meta-analyses on IGF-I, IGFBP-3, and breast cancer (31–35) concluded that there was no overall association between total IGF-I or IGFBP-3 levels and the risk of postmenopausal breast cancer. Recently, results from two large prospective studies reported positive associations between total IGF-I levels and the risk of breast cancer only among women older than 50 years (36,37). The only prospective study to our knowledge to measure free IGF-I in serum reported a strong positive association between free IGF-I levels and postmenopausal breast cancer risk among overweight women not using HT, albeit on the basis of a small number of breast cancer cases (18). However, none of the investigations that prospectively investigated circulating IGF-I concentrations and breast cancer risk controlled for endogenous estrogen levels, which, given the strong association between estrogen level and breast cancer and the extensive overlap between the insulin–IGF and the sex hormone signaling pathways (38), may be necessary to assess the independent associations between insulin and IGF-I levels and the risk of breast cancer.

We investigated associations between incident breast cancer and fasting levels of insulin, total and free IGF-I, and estradiol in a large, prospective cohort of postmenopausal women. In addition, we assessed the degree to which these factors might explain the obesity–breast cancer relationship.

CONTEXT AND CAVEATS

Prior knowledge

Obesity has been linked to high circulating levels of insulin and estrogen and to an increased risk of postmenopausal breast cancer. However, it is unclear whether circulating levels of insulin and/or insulin-like growth factor-I (IGF-I), a related hormone, are associated with the risk of breast cancer independent of estrogen level.

Study design

A prospective case–cohort study among nondiabetic postmenopausal women enrolled in the Women's Health Initiative Observational Study (WHI-OS) to examine associations between levels of serologic factors (including insulin, total and free IGF-I, and estradiol) in fasting serum samples, baseline characteristics (including body mass index and hormone therapy use), and the risk of breast cancer.

Contribution

Hyperinsulinemia and high endogenous estradiol levels were independent risk factors for postmenopausal breast cancer and largely explained the association between obesity and the risk of breast cancer in postmenopausal women.

Implications

Interventions aimed at lowering fasting insulin levels or circulating estrogen levels may reduce the risk of breast cancer in postmenopausal women.

Limitations

Only baseline levels of the serologic factors were assessed. Some case subjects may have had subclinical breast cancer at WHI-OS recruitment. The observational study design does not establish a cause-and-effect association between the measured serum levels and breast cancer.

From the Editors

Subjects and Methods

Study Population

Women's Health Initiative. This study was conducted among women enrolled in the Women's Health Initiative Observational Study (WHI-OS), a prospective cohort of 93 676 postmenopausal women aged 50–79 years who were recruited through 40 clinical centers across the United States between October 1, 1993, and December 31, 1998 (39,40). At study entry (ie, baseline), WHI-OS participants provided written informed consent and completed questionnaires regarding demographic and behavioral factors, medical history, and use of medications (including HT). Each woman underwent a physical examination that included waist, hip, height, and weight measurements, and provided a blood sample following an overnight fast of at least 8 hours; the blood samples

were centrifuged at 1300 *g* and the separated sera stored at −70°C within 2 hours of collection (41). Most cancer outcomes (including breast cancer) among cohort members were initially ascertained through annual self-administered questionnaires; breast case status and the detailed diagnosis were subsequently formally determined through centralized review of all pathology reports, discharge and consultant summaries, operative and radiology reports, and tumor registry abstracts. Clinical and pathological characteristics of the breast tumors (eg, status of estrogen receptor [ER] and progesterone receptor [PR]) were obtained by Women's Health Initiative from the pathology reports. Breast cancer cases were coded according to the National Cancer Institute's Surveillance, Epidemiology, and End Results program guidelines (42,43). As of February 29, 2004, the date when the subjects included in this breast cancer case–cohort study were selected, the mean follow-up time for WHI-OS participants was 77 months; 1.6% of the women had been lost to follow-up, and 4.7% were deceased.

Study Subjects. A case of incident breast cancer was defined as the diagnosis of disease after more than 1 year of follow-up in a woman with no history of breast cancer at baseline. We randomly selected 900 of the approximately 1800 case subjects who met these criteria at the time this study was initiated. We excluded diabetic patients (determined through self-report or self-reported use of diabetes medications or who had a fasting glucose level >125 mg/dL) as in previous studies (18,19) because of the uncertain effects of an abnormal (ie, diabetic) hormonal milieu on the relationship between breast cancer and the serologic factors being measured. After we excluded the diabetic patients (n = 65), our study included 835 case subjects, including 10 with in situ cancer. The comparison group was a subcohort of 816 subjects who were randomly chosen from among all WHI-OS subjects who met the same inclusion and exclusion criteria as the case subjects and excluded the 835 case subjects.

Laboratory Methods

Serum insulin and glucose levels were measured by the Medical Research Laboratories (Highland Heights, KY) using assays with sensitivities of 0.26 μIU/mL and 0.5 mg/dL, respectively. We used the homeostasis model assessment–insulin resistance (HOMA-IR) index to estimate insulin resistance (HOMA-IR index = fasting insulin [μIU/mL] × fasting glucose [mg/dL]/22.5) (44). Hyperinsulinemia was defined as a value in the highest quartile of fasting insulin levels among nondiabetic individuals (45). Serum estradiol levels were measured at the Esoterix Center for Clinical Trials (Calabasas Hills, CA) with the use of a Vitros-Eci Immunodiagnostic assay (Ortho-Clinical Diagnostics, High Wycombe, UK) with a sensitivity of 5 pg/mL. Serum concentrations of total IGF-I, free IGF-I, and IGFBP-3 were measured using enzyme-linked immunosorbent assays (Diagnostic Systems Laboratories, Webster, TX) with sensitivities of 0.01, 0.015, and 0.04 ng/mL, respectively, as previously described (46,47). The estradiol assays were completed in a single batch. All of the other assays were completed in two separate batches with equal proportions of case subjects and subcohort members distributed across each batch. All assays were conducted once in duplicate, and the mean value for each duplicate pair was used as the unit of analysis. Tests with intra-assay coefficients of variation (CVs) greater than 10% were repeated. For the assay of free IGF-I, a CV of greater than 20% was used as the threshold for repeating the assay, because free IGF-I levels are typically low, and from a purely mathematical perspective, the CV becomes sensitive to small changes in SD when the mean value of a parameter is low. The Esoterix Center for Clinical Trials conducted its own quality control tests and repeated any tests that had a CV of greater than 20%. We retested approximately 5% of the WHI-OS samples chosen at random in a blinded fashion and found that the assay values determined in the replicates were highly correlated with the values from initial testing (Pearson *r* values: total IGF-I = .96, free IGF-I = .90, IGFBP-3 = .90, insulin = .98, glucose = .95, and estradiol = .99). Average interassay CVs, which were determined using the 5% blinded replicates, were as follows: total IGF-I = 8.2%, free IGF-I = 11.2%, IGFBP-3 = 3.6%, insulin = 3.4%, glucose = 4.2%, and estradiol = 5.9%.

Statistical Analysis

Differences in the distributions of baseline characteristics between the case subjects and the subcohort members (limited to those who did not later become case subjects) were compared using the Wilcoxon rank sum test (for continuous data) or the Pearson X^2 test (for categorical data). All serologic variables were expressed as quartiles or tertiles based on the distributions of the data in the subcohort, as was done in previous studies of insulin and IGF levels and breast cancer (20,21,23,36,37). For the assays that were conducted in two separate batches, the quartiles were determined separately for each batch to minimize the possibility that unrecognized variations in laboratory results across batches might affect our findings. Correlations between these categorical serologic data, age, and BMI (calculated as weight [kg] divided by [height in meters]^2) were assessed using the Spearman correlation coefficient. To assess the impact of current use of HT on each of the measured serologic factors, we categorized the mean values for the factor according to three HT strata—users of unopposed estrogen, users of combined estrogen and progestin, or nonusers of HT—and compared these values using analysis of variance (ANOVA).

To examine the associations between the serologic factors and the risk of breast cancer, we estimated hazard ratios using Cox proportional hazard regression models that used the Self–Prentice method (48) for computing robust SE estimates (to account for the case–cohort design), with time from WHI-OS enrollment (in days) as the underlying time metric. Proportionality of the data was verified by graphical inspection. All models were adjusted for the following established breast cancer risk factors (chosen before data analysis): age (50–54 [referent], 55–59, 60–64, 65–69, 70–74, or 75–79 years); smoking status (never [referent], former, or current smoker); race and/or ethnicity (white [referent], black, Hispanic, or Asian or other); usual physical activity, assessed as metabolic equivalent tasks per hour per week (defined as the caloric need per kilogram of body weight per hour of activity divided by the caloric need per kilogram of body weight per hour at rest) and categorized as quartiles (<3.75, 3.75–9.82, 9.83–18.74, ≥18.75); BMI (<18.5, 18.5 to <25.0 [referent], 25.0 to <30.0, or ≥30.0 kg/m^2); alcohol consumption, assessed as the number of servings per week during the preceding 3 months (none [referent], <3, or ≥3); current use of nonsteroidal anti-inflammatory drugs (NSAIDs; yes or no); family

history of breast cancer (defined as having a first-degree relative with breast cancer; yes or no); ever use of oral contraceptives (yes or no); parity (0 [referent], 1, or ≥2 live births); age at first child's birth (<20 [referent], 20–24, 25–29, or ≥30 years); age at menarche (≤10 [referent], 11–12, or ≥13 years); age at menopause (≤42 [referent], 43–48, 49–51, or ≥52 years); and the highest education level (high school or lower [referent], college, or postgraduate education). The cut points for the risk factors were chosen based on those used in previous studies in which these variables were investigated in relation to breast cancer (4,21) or by visually inspecting the data and distributing the data evenly across categories. Endogenous estradiol levels were assessed only in current nonusers of HT because standard estradiol assays cannot accurately measure the 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 (referent), moderate, or high estradiol levels (defined in tertiles); users of unopposed estrogen; and users of combined estrogen and progestin. These groups were then parameterized as separate dummy variables, with low estradiol as the common referent, which allowed us to assess potentially different associations with estrogen use, estrogen and progestin use, and high endogenous estradiol (among nonusers of HT) in a single model.

Subgroup analyses included stratification by current HT use (nonuser of HT, unopposed estrogen user, or estrogen and progestin user), endogenous estradiol level (stratified as higher vs lower than the median value in the subcohort because insufficient data were available to stratify by tertile), and BMI (categorized as normal [<25 kg/m^2] and overweight/obese [≥25 kg/m^2] according to World Health Organization criteria) (49). These stratified analyses were conducted by introducing interaction terms into multivariable models that also included the main effect variables. In addition, we conducted separate analyses for ER-positive and ER-negative breast cancer and for PR-positive and PR-negative breast cancer; however, the resulting hazard ratios could not be formally compared using standard tests of heterogeneity because the ER-positive and ER-negative breast cancers (and the PR-positive and PR-negative breast cancers) have a common comparison group (the representative subcohort), and therefore, the hazard ratios for the two breast cancer subtypes are not independent. Instead, the hazard ratios were compared qualitatively based on the point estimates and 95% confidence intervals.

All tests of statistical significance were two-sided, and *P* values less than .05 were considered statistically significant.

Results

Baseline Characteristics

Compared with women in the subcohort, the selected case subjects were older, had a later onset of menopause, had greater alcohol consumption, had a higher frequency of NSAID use, and more often reported being a former smoker (Table 1). They also were more likely than women in the subcohort to have a first-degree relative with breast cancer and to have been using HT at baseline (Table 1). The baseline characteristics of the randomly selected breast cancer case subjects were not statistically significantly different from those of the remaining breast cancer case subjects who were eligible for but not included in this study (data not shown).

Correlations between levels of each of the serologic factors, age, and BMI were estimated using Spearman correlation coefficients (Table 2). Among nonusers of HT, total IGF-I level was moderately positively correlated with the levels of free IGF-I (r = .17, P < .001) and IGFBP-3 (r = .40, P < .001) but not with BMI, whereas insulin level was strongly positively correlated with BMI (r = .57, P < .001). The level of endogenous estradiol had a moderately strong positive correlation with BMI (r = .32, P < .001) and insulin level (r = .17, P < .001) but was inversely correlated with free IGF-I level (r = −.34, P < .001). Among the users of unopposed estrogen, total IGF-I level was inversely correlated with age (r = −.30, P < .001) and positively correlated with free IGF-I (r = .24, P < .001) and IGFBP-3 (r = .45, P < .001) levels. Insulin level was strongly correlated with BMI (r = .58, P < .001) and moderately positively correlated with level of IGFBP-3 (r = .21, P = .006). Finally, among the women using estrogen and progestin, BMI was positively correlated with levels of free IGF-I (r = .23, P = .002), IGFBP-3 (r = .25, P < .001), and insulin (r = .58, P < .001), whereas insulin level was also correlated with free IGF-I (r = .16, P = .03) and IGFBP-3 (r = .25, P < .001) levels.

We also examined associations between current HT use and the serologic parameters. The levels of total IGF-I, free IGF-I, and IGFBP-3 were highest in women not using HT, intermediate in those using estrogen and progestin, and lowest in those using unopposed estrogen. For example, the mean level (SD) of free IGF-I was 0.47 ng/mL (0.39) in nonusers of HT, 0.40 ng/mL (0.39) in women who used estrogen and progestin, and 0.32 ng/mL (0.25) in those who used unopposed estrogen (P < .001, ANOVA). Mean insulin levels (SD) were also higher in nonusers of HT than in HT users (7.1 μIU/mL [5.0] for nonusers vs 6.0 μIU/mL [4.3] in estrogen-alone users and 5.7 μIU/mL [4.2] in estrogen and progestin users; P < .001, ANOVA).

Associations Between Incident Breast Cancer and Serologic Parameters and Obesity

Table 3 shows the associations between breast cancer and our major exposure variables in age-adjusted multivariable models that adjusted for established breast cancer risk factors. Fasting levels of insulin and endogenous estradiol were associated with a statistically significantly increased risk of incident breast cancer (hazard ratio [HR] for highest vs lowest quartile of insulin = 1.46, 95% confidence interval [CI] = 1.00 to 2.13, P_{trend} = .02; HR for highest vs lowest tertile of endogenous estradiol = 1.59, 95% CI = 1.00 to 2.55, P_{trend} = .04). Fasting levels of total IGF-I, free IGF-I, IGFBP-3, or glucose, and BMI were not associated with the risk of breast cancer in these models. The associations of levels of total and free IGF-I with breast cancer risk were not altered by adjustment for fasting levels of IGFBP-3 (data not shown). The magnitude of the association between the HOMA-IR index and the risk of breast cancer (HR for highest vs lowest quartile of HOMA-IR index = 1.35, 95% CI = 0.92 to 1.98, P_{trend} = .05) was similar to that for the association between fasting insulin level and breast cancer risk (data not shown).

We detected no statistically significant heterogeneity (all *P*s > .10) when we stratified the aforementioned results by BMI or by endogenous estradiol level (among nonusers of HT). The results

Table 1. Distributions of selected baseline characteristics among the selected case subjects and subcohort members*

Variable	Case subjects† (n = 841)	Subcohort members (n = 810)	*P*‡
Median age, y (IQR)	64.0 (54.0–74.0)	63.0 (51.0–75.0)	.01
Race/ethnicity, No. (%)			.28
White	737 (87.6)	695 (85.8)	
Black	56 (6.7)	51 (6.3)	
Hispanic	25 (3.0)	32 (4.0)	
Asian/other	19 (2.3)	29 (3.6)	
Missing	4 (0.4)	3 (0.3)	
Median weight, kg (IQR)	68.6 (49.6–87.6)	68.4 (51.5–85.3)	.36
Median body mass index, kg/m^2 (IQR)	25.8 (18.8–32.8)	26.0 (19.8–32.3)	.83
Median waist circumference, cm (IQR)	82.0 (65.0–99.0)	82.0 (65.1–98.9)	.23
Median waist-to-hip ratio (IQR)	0.8 (0.7–0.9)	0.8 (0.7–0.9)	.28
Age at menarche in y, No. (%)			.62
≤10	66 (7.8)	55 (6.8)	
11–12	347 (41.3)	328 (40.5)	
≥13	424 (50.4)	423 (52.2)	
Missing	4 (0.5)	4 (0.5)	
Age at menopause in y, No. (%)			.001
≤42	122 (14.5)	175 (21.6)	
43–48	182 (21.6)	192 (23.7)	
49–51	206 (24.5)	177 (21.9)	
≥52	253 (30.1)	200 (24.7)	
Missing	78 (9.3)	66 (8.1)	
Parity, No. (%)			.48
0	120 (14.3)	119 (14.7)	
1	62 (7.4)	72 (8.9)	
≥2	655 (77.9)	613 (75.7)	
Missing	4 (0.4)	6 (0.7)	
Age at first child's birth in y, No. (%)			.03
<20	78 (9.3)	78 (9.6)	
20–24	278 (33.1)	308 (38.0)	
25–29	228 (27.1)	171 (21.1)	
≥30	71 (8.4)	65 (8.0)	
Nulliparous/missing	186 (22.1)	188 (23.3)	
Ever use of oral contraceptives, No. (%)	345 (41.0)	328 (40.5)	.83
Current use of hormone therapy, No. (%)			.003
Currently using unopposed estrogen therapy	214 (25.9)	187 (23.1)	
Currently using combined estrogen + progestin therapy	228 (27.6)	181 (22.4)	
Currently not using hormone therapy	380 (45.5)	436 (53.8)	
Missing	13 (1.0)	6 (0.7)	
Current NSAID use, No. (%)	357 (42.5)	293 (36.2)	.01
First-degree relative with breast cancer, No. (%)	209 (25.0)	155 (19.0)	.02
Smoking status, No. (%)			.003
Never	405 (48.2)	435 (53.7)	
Former	387 (46.0)	311 (38.4)	
Current	37 (4.4)	53 (6.5)	
Missing	12 (1.4)	11 (1.4)	
Highest education level, No. (%)			.07
High school or less	209 (24.9)	253 (31.2)	
College	348 (41.4)	306 (37.8)	
Postgraduate education	268 (31.9)	244 (30.1)	
Missing	16 (1.8)	7 (0.9)	
Median alcohol consumption, servings per week (IQR)	0.7 (0–4.5)	0.4 (0–3.1)	.02
Median physical activity, METs h^{-1} wk^{-1} (IQR)	10.50 (0–27)	10.00 (0–26.3)	.99
Estrogen receptor status, No. (%)			NA
Positive	533 (63)	NA	
Negative	243 (29)	NA	
Missing	65 (8)	NA	

* IQR = interquartile range; NSAID = nonsteroidal anti-inflammatory drug; METs = metabolic equivalent tasks (defined as the caloric need per kilogram of body weight per hour of activity divided by the caloric need per kilogram of body weight per hour at rest); NA = not applicable.

† Includes the six cases of breast cancer that arose in the subcohort.

‡ *P* values derived from the Wilcoxon rank sum test for continuous data or the Pearson chi-square test for categorical data.

Table 2. Spearman correlation matrix for age, BMI, and levels of the serologic factors among the representative subcohort members with stratification by current HT use*

Factor	Age	BMI	Total IGF-I	Free IGF-I	IGFBP-3	Insulin	Glucose	HOMA-IR index
Nonusers of HT								
Age	1.00							
BMI	−0.11	1.00						
Total IGF-I	−0.13	−0.08	1.00					
Free IGF-I	0.01	−0.01	0.17†	1.00				
IGFBP-3	−0.09	−0.01	0.40†	0.03	1.00			
Insulin	−0.04	0.57†	0.07	0.05	0.05	1.00		
Glucose	0.02	0.25	0.03	0.05	0.08	0.42†	1.00	
HOMA-IR	−0.02	0.57†	−0.06	0.05	0.06	0.99†	0.53†	1.00
Estradiol	−0.05	0.32†	0.04	−0.34†	−0.12	0.17†	0.06	0.16†
Unopposed estrogen users								
Age	1.00							
BMI	−0.07	1.00						
Total IGF-I	−0.30†	−0.08	1.00					
Free IGF-I	−0.06	0.03	0.24†	1.00				
IGFBP-3	−0.12	−0.01	0.45†	0.32†	1.00			
Insulin	−0.05	0.58†	0.12	0.09	0.21§	1.00		
Glucose	0.02	0.20‡	0.11	0.12	0.08	0.45†	1.00	
HOMA-IR	−0.05	0.56†	0.13	0.09	0.20‖	0.99†	0.55†	1.00
Estrogen + progestin users								
Age	1.00							
BMI	−0.03	1.00						
Total IGF-I	−0.13	0.01	1.00					
Free IGF-I	0.03	0.23¶	0.05	1.00				
IGFBP-3	−0.02	0.25†	0.39†	0.06	1.00			
Insulin	0.07	0.58†	0.10	0.16#	0.25†	1.00		
Glucose	0.17	0.13	0.16	0.14	0.15	0.31†	1.00	
HOMA-IR	0.10	0.58†	0.11	0.17	0.25†	0.99†	0.43†	1.00

* All *P* values are two-sided. BMI = body mass index; HT = hormone therapy; IGF-I = insulin-like growth factor-I; IGFBP-3 = insulin-like growth factor binding protein-3; HOMA-IR = homeostasis model assessment–insulin resistance.

† $P < .001$.

‡ $P = .005$.

§ $P = .006$.

‖ $P = .007$.

¶ $P = .002$.

$P = .03$.

were also essentially unchanged when we excluded women with in situ breast tumors (n = 10) or those who were diagnosed with breast cancer during an additional 2 years of follow-up (and hence, within 3 years of study entry, n = 140; data not shown). Endogenous estradiol level was the only major exposure variable that showed a differential association with breast cancer risk according to ER or PR status. Estradiol level was more strongly associated with ER-positive breast cancer (HR for highest vs lowest tertile of estradiol = 2.07, 95% CI = 1.16 to 3.67, $P_{trend} = .01$) than with ER-negative breast cancer (HR for highest vs lowest tertile of estradiol = 1.00, 95% CI = 0.50 to 2.05, $P_{trend} = .98$) and more strongly associated with PR-positive breast cancer (HR for highest vs lowest tertile of estradiol = 1.93, 95% CI = 1.09 to 3.82, $P_{trend} = .01$) than with PR-negative breast cancer (HR for highest vs lowest tertile of estradiol = 0.97, 95% CI = 0.46 to 2.09, $P_{trend} = .96$; data not shown).

Results Stratified by Current HT Use. Fasting insulin level was associated with a statistically significantly increased risk of incident breast cancer among nonusers of HT (HR for highest vs lowest quartile of insulin = 2.48, 95% CI = 1.38 to 4.47, $P_{trend} < .001$; Table 4), even after concurrent adjustment for BMI and levels of estradiol and free IGF-I (HR for highest vs lowest quartile of insulin = 2.40, 95% CI = 1.30 to 4.41, $P_{trend} < .001$; Table 5). The association between risk of breast cancer and insulin level differed statistically significantly among HT users ($P_{interaction} = .01$). We observed no association between fasting insulin level and incident breast cancer among women who used estrogen and progestin (HR for highest vs lowest quartile of insulin = 1.15, 95% CI = 0.34 to 3.84, $P_{trend} = .40$) and a possible inverse association among those who used estrogen alone (HR for highest vs lowest quartile of insulin = 0.33, 95% CI = 0.12 to 0.92, $P_{trend} = .31$; Table 4). Among nonusers of HT, insulin levels were more strongly associated with ER-positive breast cancer (HR for highest vs lowest quartile of insulin = 3.23, 95% CI = 1.62 to 6.49, $P_{trend} = .001$) than with ER-negative breast cancer (HR for highest vs lowest quartile of insulin = 1.37, 95% CI = 0.57 to 3.25, $P_{trend} = .99$); however, as discussed earlier, we could not formally test the results for heterogeneity (data not shown).

The association between BMI and the risk of incident breast cancer also varied by HT use, although this variation was not

Table 3. Age- and multivariable-adjusted HR (95% CI) for associations of baseline levels of insulin, total IGF-I, free IGF-I, IGFBP-3, glucose, endogenous estradiol, and BMI with the risk of incident breast cancer*

Factor	Quantile 1	Quantile 2	Quantile 3	Quantile 4	P_{trend}†
Insulin					
Quartile cut points, μIU/mL	<3.6	3.6 to <5.7	5.7 to <9.5	≥9.5	
No. case subjects/No. subcohort members	185/197	184/190	236/205	222/202	
Age-adjusted HR (95% CI)	1.00 (referent)	1.04 (0.78 to 1.39)	1.24 (0.94 to 1.65)	1.21 (0.92 to 1.61)	.10
Multivariable-adjusted HR‡ (95% CI)	1.00 (referent)	1.14 (0.81 to 1.59)	1.55 (1.09 to 2.20)	1.46 (1.00 to 2.13)	.02
Total IGF-I					
Quartile cut points, ng/mL	<94.2	94.2 to <119.6	119.6 to <151.0	≥151.0	
No. case subjects/No. subcohort members	211/202	259/204	176/202	192/202	
Age-adjusted HR (95% CI)	1.00 (referent)	1.22 (0.93 to 1.60)	0.86 (0.65 to 1.15)	0.94 (0.71 to 1.25)	.25
Multivariable-adjusted HR‡ (95% CI)	1.00 (referent)	1.30 (0.95 to 1.77)	0.92 (0.65 to 1.28)	1.21 (0.85 to 1.72)	.92
Free IGF-I					
Quartile cut points, ng/mL	<0.21	0.21 to <0.36	0.36 to <0.55	≥0.55	
No. case subjects/No. subcohort members	192/195	238/195	210/195	166/195	
Age-adjusted HR (95% CI)	1.00 (referent)	1.24 (0.93 to 1.65)	1.11 (0.83 to 1.47)	0.86 (0.64 to 1.15)	.23
Multivariable-adjusted HR‡ (95% CI)	1.00 (referent)	1.33 (0.96 to 1.85)	1.30 (0.93 to 1.81)	1.09 (0.77 to 1.54)	.67
IGFBP-3					
Quartile cut points, ng/mL	<3604.0	3604.0 to <4053.6	4053.6 to <4583.5	≥4583.5	
No. case subjects/No. subcohort members	243/203	203/202	192/201	201/203	
Age-adjusted HR (95% CI)	1.00 (referent)	0.86 (0.65 to 1.13)	0.82 (0.62 to 1.09)	0.84 (0.64 to 1.11)	.20
Multivariable-adjusted HR‡ (95% CI)	1.00 (referent)	0.80 (0.59 to 1.10)	0.77 (0.56 to 1.07)	0.77 (0.55 to 1.08)	.26
Glucose					
Quartile cut points, mg/dL	<86.0	86.0 to <91.5	91.5 to <100	≥100	
No. case subjects/No. subcohort members	206/198	233/209	196/194	201/205	
Age-adjusted HR (95% CI)	1.00 (referent)	1.06 (0.80 to 1.40)	0.95 (0.72 to 1.26)	0.92 (0.69 to 1.21)	.40
Multivariable-adjusted HR‡ (95% CI)	1.00 (referent)	1.14 (0.82 to 1.59)	0.99 (0.70 to 1.38)	0.92 (0.65 to 1.29)	0.50
Estradiol§					
Tertile cut points, pg/mL	<8.0	8.0 to <14.0	≥14.0	NA	
No. case subjects/No. subcohort members	92/146	155/156	137/134	NA	
Age-adjusted HR (95% CI)	1.00 (referent)	1.59 (1.12 to 2.25)	1.76 (1.22 to 2.54)	NA	.003
Multivariable-adjusted HR‡ (95% CI)	1.00 (referent)	1.44 (0.95 to 2.18)	1.59 (1.00 to 2.55)	NA	.04
BMI					
Category cut points, kg/m^2	<18.5	18.5 to <25.0	25.0 to <30.0	≥30	
No. case subjects/No. subcohort members	10/14	341/308	269/290	211/180	
Age-adjusted HR (95% CI)	0.56 (0.23 to 1.32)	1.00 (referent)	0.84 (0.66 to 1.05)	1.10 (0.85 to 1.42)	.47
Multivariable-adjusted HR‡ (95% CI)	0.68 (0.27 to 1.69)	1.00 (referent)	0.81 (0.62 to 1.04)	1.13 (0.83 to 1.55)	.31

* The total numbers of case subjects and subcohort members vary for each serologic variable due to assay failure for some serum specimens. HR = hazard ratio; CI = confidence interval; IGF-I = insulin-like growth factor-I; IGFBP-3 = insulin-like growth factor binding protein-3; BMI = body mass index; NA = not applicable.

† Statistical tests for trend (two-sided) were calculated using ordinal quantile variables (1–4 for quartiles and 1–3 for tertiles) entered into the model as a single continuous variable.

‡ Multivariable model adjusted for age, race, alcohol consumption, smoking, family history of breast cancer, parity, age at menopause, age at menarche, age at first child's birth, use of oral contraceptives, use of nonsteroidal anti-inflammatory drugs, use of estrogen replacement therapy, use of hormone replacement therapy (estrogen and progestin), educational attainment, endogenous estradiol levels (in nonusers of HT) parameterized as described in the text, BMI, and physical activity.

§ Among nonusers of HT only.

statistically significant ($P_{interaction}$ = .07). Among nonusers of HT, high BMI was associated with an increased risk of breast cancer in multivariable models that adjusted for breast cancer risk factors (HR for BMI ≥30 kg/m^2 vs 18.5 to <25.0 kg/m^2 = 2.12, 95% CI = 1.26 to 3.58, P_{trend} = .003) (Table 5). Incorporating estradiol levels into these models resulted in only a modest (ie, 10%) reduction in the BMI–breast cancer association (HR for BMI ≥30 kg/m^2 vs 18.5 to <25.0 kg/m^2 = 1.91, 95% CI = 1.11 to 3.27, P_{trend} = .02), whereas adjustment for insulin levels attenuated this association by 30% (HR for BMI ≥30 kg/m^2 vs 18.5 to <25.0 kg/m^2 = 1.50, 95% CI = 0.80 to 2.83, P_{trend} = .40; Table 5). By contrast, among HT users, the association between BMI and the risk of breast cancer was highly variable according to the subgroup analyzed and not statistically significant for either estrogen and progestin users (HR for BMI ≥30 kg/m^2 vs 18.5 to <25.0 kg/m^2 = 0.41, 95% CI = 0.17 to 1.00, P_{trend} = .21) or users of estrogen alone (HR for BMI ≥30 kg/m^2 vs 18.5 to <25.0 kg/m^2 = 1.42, 95% CI = 0.64 to 3.14, P_{trend} = .97; Table 4). The association of obesity with breast cancer was of similar magnitude when BMI was categorized as quartiles or when waist circumference was used as the measure of obesity (data not shown).

We observed no statistically significant linear trends in the association between free IGF-I level and the risk of breast cancer, regardless of HT stratum (nonuser, unopposed estrogen user, estrogen and progestin user). However, among the nonusers of HT, compared with women in the lowest quartile of free IGF-I, those in the second and third highest quartiles were at increased

Table 4. Age- and multivariable-adjusted hazard ratios (95% confidence intervals) for associations between baseline levels of insulin, total IGF-I, free IGF-I, IGFBP-3, glucose, endogenous estradiol, and BMI, and the risk of incident breast cancer with stratification by hormone therapy (HT) use*

HT use, factor	Quantile 1	Quantile 2	Quantile 3	Quantile 4	P_{trend}†
Nonusers of HT					
Insulin					
Quartile cut points, μIU/mL	<3.9	3.9 to <5.6	5.6 to <8.8	≥8.8	
No. case subjects/No. subcohort members	77/108	70/106	97/108	134/106	
Age-adjusted HR (95% CI)	1.00 (referent)	0.95 (0.62 to 1.45)	1.29 (0.85 to 1.95)	1.94 (1.30 to 2.90)	<.001
Multivariable-adjusted HR‡ (95% CI)	1.00 (referent)	1.04 (0.59 to 1.84)	1.45 (0.81 to 2.58)	2.48 (1.38 to 4.47)	<.001
Total IGF-I					
Quartile cut points, ng/mL	<106.0	106.0 to <133.0	133.0 to 162.0	≥162.0	
No. case subjects/No. subcohort members	97/109	99/110	94/108	94/109	
Age-adjusted HR (95% CI)	1.00 (referent)	1.04 (0.70 to 1.55)	1.02 (0.68 to 1.53)	1.03 (0.69 to 1.55)	.90
Multivariable-adjusted HR‡ (95% CI)	1.00 (referent)	1.09 (0.67 to 1.76)	1.08 (0.67 to 1.75)	0.99 (0.59 to 1.64)	.72
Free IGF-I					
Quartile cut points, ng/mL	<0.26	0.26 to <0.42	0.42 to <0.63	≥0.63	
No. case subjects/No. subcohort members	71/107	114/104	102/107	85/104	
Age-adjusted HR (95% CI)	1.00 (referent)	1.56 (1.03 to 2.36)	1.39 (0.91 to 2.12)	1.17 (0.76 to 1.80)	.72
Multivariable-adjusted HR‡ (95% CI)	1.00 (referent)	1.83 (1.11 to 3.02)	1.82 (1.09 to 3.04)	1.24 (0.73 to 2.10)	.41
IGFBP-3					
Quartile cut points, ng/mL	<3749.3	3749.3 to <4231.3	4231.3 to <4695.6	≥4695.6	
No. case subjects/No. subcohort members	110/110	88/107	81/110	104/108	
Age-adjusted HR (95% CI)	1.00 (referent)	0.81 (0.54 to 1.21)	0.78 (0.52 to 1.16)	1.02 (0.68 to 1.51)	.99
Multivariable-adjusted HR‡ (95% CI)	1.00 (referent)	0.73 (0.45 to 1.18)	0.75 (0.46 to 1.23)	0.95 (0.57 to 1.59)	.94
Glucose					
Quartile cut points, mg/dL	<87	87 to <93	93 to <100	≥100	
No. case subjects/No. subcohort members	96/110	95/98	91/114	100/111	
Age-adjusted HR (95% CI)	1.00 (referent)	1.07 (0.72 to 1.60)	0.87 (0.58 to 1.29)	1.00 (0.67 to 1.50)	.76
Multivariable-adjusted HR‡ (95% CI)	1.00 (referent)	1.02 (0.62 to 1.67)	0.74 (0.45 to 1.20)	0.88 (0.52 to 1.47)	.47
Estradiol					
Tertile cut points, pg/mL	<8.0	8.0 to <14.0	≥14.0	NA	
No. case subjects/No. subcohort members	92/146	155/156	137/134	NA	
Age-adjusted HR (95% CI)	1.00 (referent)	1.59 (1.12 to 2.25)	1.76 (1.22 to 2.54)	NA	.003
Multivariable-adjusted HR‡ (95% CI)	1.00 (referent)	1.44 (0.95 to 2.18)	1.59 (1.00 to 2.55)	NA	.04
BMI					
Category cut points, kg/m^2	<18.5	18.5 to <25.0	25.0 to <30.0	≥30	
No. case subjects/No. subcohort members	2/7	125/155	126/151	75/65	
Age-adjusted HR (95% CI)	0.29 (0.06 to 1.41)	1.00 (referent)	1.04 (0.74 to 1.46)	1.51 (1.05 to 2.16)	.01
Multivariable-adjusted HR‡ (95% CI)	0.46 (0.08 to 2.79)	1.00 (referent)	1.15 (0.74 to 1.77)	1.91 (1.11 to 3.27)	.02
Unopposed estrogen users					
Insulin					
Quartile cut points, μIU/mL	<3.0	3.0 to <4.8	4.8 to <8.2	≥8.2	
No. case subjects/No. subcohort members	59/46	48/44	65/45	38/45	
Age-adjusted HR (95% CI)	1.00 (referent)	0.72 (0.38 to 1.36)	1.22 (0.69 to 2.17)	0.66 (0.36 to 1.22)	.52
Multivariable-adjusted HR‡ (95% CI)	1.00 (referent)	0.43 (0.17 to 1.09)	1.03 (0.42 to 2.52)	0.33 (0.12 to 0.92)	.31
Total IGF-I					
Quartile cut points, ng/mL	<75.2	75.2 to <95.3	95.3 to <118.2	≥118.2	
No. case subjects/No. subcohort members	52/47	56/47	47/46	58/47	
Age-adjusted HR (95% CI)	1.00 (referent)	1.14 (0.63 to 2.06)	1.03 (0.56 to 1.88)	1.37 (0.73 to 2.56)	.41
Multivariable-adjusted HR‡ (95% CI)	1.00 (referent)	1.34 (0.52 to 3.43)	1.02 (0.42 to 2.48)	2.41 (0.81 to 7.18)	.72
Free IGF-I					
Quartile cut points, ng/mL	<0.16	0.16 to <0.27	0.27 to <0.57	≥0.57	
No. case subjects/No. subcohort members	54/45	52/46	57/45	42/45	
Age-adjusted HR (95% CI)	1.00 (referent)	1.05 (0.58 to 1.91)	1.06 (0.58 to 1.91)	0.87 (0.47 to 1.62)	.72
Multivariable-adjusted HR‡ (95% CI)	1.00 (referent)	1.99 (0.73 to 5.44)	1.42 (0.57 to 3.53)	1.44 (0.53 to 3.87)	.83
IGFBP-3					
Quartile cut points, ng/mL	<3440.0	3440.0 to <3863.3	3863.3 to <4367.2	≥4367.2	
No. case subjects/No. subcohort members	63/47	40/47	54/46	57/47	
Age-adjusted HR (95% CI)	1.00 (referent)	0.67 (0.37 to 1.22)	1.03 (0.57 to 1.89)	1.05 (0.59 to 1.88)	.60
Multivariable-adjusted HR‡ (95% CI)	1.00 (referent)	0.68 (0.30 to 1.53)	0.93 (0.41 to 2.09)	0.91 (0.39 to 2.08)	.94

(Table continues)

Table 4 (continued).

HT use, factor	Quantile 1	Quantile 2	Quantile 3	Quantile 4	P_{trend}†
Glucose					
Quartile cut points, mg/dL	<85	85 to <89	89 to <96	≥96	
No. case subjects/No. subcohort members	49/49	55/46	62/45	47/46	
Age-adjusted HR (95% CI)	1.00 (referent)	1.42 (0.77 to 2.59)	1.48 (0.83 to 2.63)	1.21 (0.66 to 2.21)	.48
Multivariable-adjusted HR‡ (95% CI)	1.00 (referent)	1.93 (0.76 to 4.89)	2.11 (0.86 to 5.19)	1.29 (0.48 to 3.44)	.83
BMI					
Category cut points, kg/m^2	<18.5	18.5 to <25.0	25.0 to <30.0	≥30	
No. case subjects/No. subcohort members	2/1	88/70	74/80	47/35	
Age-adjusted HR (95% CI)	0.99 (0.08 to 11.59)	1.00 (referent)	0.67 (0.41 to 1.08)	1.16 (0.65 to 2.07)	.99
Multivariable-adjusted HR‡ (95% CI)	8.99 (0.30 to 267.7)	1.00 (referent)	0.77 (0.40 to 1.48)	1.42 (0.64 to 3.14)	.97
Estrogen + progestin users					
Insulin					
Quartile cut points, μIU/mL	<3.1	3.1 to <4.5	4.5 to <7.8	≥7.8	
No. case subjects/No. subcohort members	54/44	50/45	79/46	41/45	
Age-adjusted HR (95% CI)	1.00 (referent)	0.93 (0.51 to 1.68)	1.55 (0.87 to 2.75)	0.78 (0.42 to 1.44)	.96
Multivariable-adjusted HR‡ (95% CI)	1.00 (referent)	1.22 (0.45 to 3.29)	2.43 (0.90 to 6.52)	1.15 (0.34 to 3.84)	.40
Total IGF-I					
Quartile cut points, ng/mL	<91.1	91.1 to <114.4	114.4 to <138.7	≥138.7	
No. case subjects/No. subcohort members	50/46	78/45	43/45	55/45	
Age-adjusted HR (95% CI)	1.00 (referent)	1.58 (0.88 to 2.83)	0.91 (0.48 to 1.72)	1.08 (0.58 to 1.99)	.70
Multivariable-adjusted HR‡ (95% CI)	1.00 (referent)	2.39 (0.97 to 5.90)	0.88 (0.37 to 2.14)	1.18 (0.49 to 2.85)	.97
Free IGF-I					
Quartile cut points, ng/mL	<0.20	0.20 to <0.36	0.36 to <0.52	≥0.52	
No. case subjects/No. subcohort members	58/43	62/42	58/43	36/43	
Age-adjusted HR (95% CI)	1.00 (referent)	1.12 (0.62 to 2.04)	1.12 (0.61 to 2.06)	0.62 (0.32 to 1.19)	.21
Multivariable-adjusted HR‡ (95% CI)	1.00 (referent)	1.33 (0.52 to 3.37)	1.60 (0.62 to 4.10)	1.38 (0.43 to 4.42)	.99
IGFBP-3					
Quartile cut points, ng/mL	<3615.4	3615.4 to <4075.7	4075.7 to <4559.2	≥4559.2	
No. case subjects/No. subcohort members	66/46	71/45	52/46	38/44	
Age-adjusted HR (95% CI)	1.00 (referent)	1.12 (0.63 to 1.96)	0.84 (0.47 to 1.51)	0.56 (0.31 to 1.02)	.04
Multivariable-adjusted HR‡ (95% CI)	1.00 (referent)	1.29 (0.52 to 3.20)	0.47 (0.18 to 1.24)	0.25 (0.08 to 0.80)	.10
Glucose					
Quartile cut points, mg/dL	<85	85 to <90	90 to <96	≥96	
No. case subjects/No. subcohort members	55/45	67/47	39/43	65/46	
Age-adjusted HR (95% CI)	1.00 (referent)	1.16 (0.65 to 2.06)	0.76 (0.41 to 1.40)	1.03 (0.57 to 1.84)	.75
Multivariable-adjusted HR‡ (95% CI)	1.00 (referent)	1.12 (0.48 to 2.62)	0.62 (0.22 to 1.75)	1.16 (0.51 to 2.65)	.99
BMI					
Category cut points, kg/m^2	<18.5	18.5 to <25.0	25.0 to <30.0	≥30	
No. case subjects/No. subcohort members	6/6	122/81	64/57	32/33	
Age-adjusted HR (95% CI)	0.50 (0.12 to 1.98)	1.00 (referent)	0.76 (0.46 to 1.24)	0.65 (0.35 to 1.18)	.26
Multivariable-adjusted HR‡ (95% CI)	0.43 (0.05 to 3.67)	1.00 (referent)	0.48 (0.23 to 0.99)	0.41 (0.17 to 1.00)	.21

* The total numbers of case subjects and subcohort members vary for each serologic variable due to assay failure for some serum specimens. IGF-I = insulin-like growth factor-I; IGFBP-3 = insulin-like growth factor binding protein-3; BMI = body mass index; HT = hormone therapy; HR = hazard ratio; CI = confidence interval; NA = not applicable.

† Statistical tests for trend (two-sided) were calculated using ordinal quantile variables (1–4 for quartiles and 1–3 for tertiles) entered into the model as a single continuous variable.

‡ Multivariable model adjusted for age, race, alcohol consumption, smoking, family history of breast cancer, parity, age at menopause, age at menarche, age at first child's birth, use of oral contraceptives, use of nonsteroidal anti-inflammatory drugs, use of estrogen replacement therapy, use of hormone replacement therapy (estrogen and progestin), educational attainment, endogenous estradiol levels (in nonusers of HT) parameterized as described in the text, BMI, and physical activity.

risk of breast cancer (HR for quartile 2 vs quartile 1 = 1.83, 95% CI = 1.11 to 3.02; HR for quartile 3 vs quartile 1 = 1.82, 95% CI = 1.09 to 3.04), whereas those in the highest quartile of free IGF-I were not (HR for quartile 4 vs quartile 1 = 1.24, 95% CI = 0.73 to 2.10), suggesting a possible curvilinear association between free IGF-I level and the risk of breast cancer (Table 4). However, inclusion of insulin levels in the multivariable model attenuated this association (HR for quartile 2 vs quartile 1 = 1.55, 95% CI = 0.94 to 2.57; HR for quartile 3 vs quartile 1 = 1.50, 95% CI = 0.91 to 2.49) (Table 5). Nevertheless, in a multivariable model that included BMI and estradiol and insulin levels as well as established breast cancer risk factors, the curvilinear association between free IGF-I level and the risk of breast cancer (assessed by including a quadratic term for free IGF-I in the multivariable model) was nearly statistically significant ($P_{curvilinearity}$ = .05).

Discussion

We observed a strong positive association between the risk of breast cancer and fasting insulin levels in postmenopausal women

Table 5. Multivariable hazard ratios (95% confidence intervals) of incident breast cancer among women not using hormone therapy for baseline levels of insulin, free IGF-I, endogenous estradiol, and BMI*

Factor, model	Quantile 1	Quantile 2	Quantile 3	Quantile 4	P_{trend}†
Insulin					
Quartile cut points, μIU/mL	<3.9	3.9 to <5.6	5.6 to <8.8	≥8.8	
No. case subjects/No. subcohort members	77/108	70/106	97/108	134/106	
Multivariable‡	1.00 (referent)	1.00 (0.60 to 1.67)	1.59 (0.96 to 2.62)	2.65 (1.61 to 4.36)	<.001
Multivariable + BMI	1.00 (referent)	1.00 (0.57 to 1.75)	1.39 (0.79 to 2.46)	2.42 (1.35 to 4.31)	<.001
Multivariable + estradiol	1.00 (referent)	1.02 (0.60 to 1.73)	1.57 (0.94 to 2.62)	2.56 (1.54 to 4.25)	<.001
Multivariable + free IGF-I	1.00 (referent)	1.04 (0.61 to 1.79)	1.62 (0.96 to 2.72)	2.64 (1.58 to 4.43)	<.001
Full model§	1.00 (referent)	1.03 (0.56 to 1.89)	1.40 (0.76 to 2.57)	2.40 (1.30 to 4.41)	<.001
Free IGF-I					
Quartile cut points, ng/mL	<0.26	0.26 to <0.42	0.42 to <0.63	≥0.63	
No. case subjects/No. subcohort members	71/107	114/104	102/107	85/104	
Multivariable‡	1.00 (referent)	1.74 (1.08 to 2.80)	1.65 (1.01 to 2.68)	1.19 (0.72 to 1.96)	.52
Multivariable + BMI	1.00 (referent)	1.72 (1.06 to 2.79)	1.68 (1.02 to 2.76)	1.17 (0.70 to 1.97)	.52
Multivariable + estradiol	1.00 (referent)	1.85 (1.13 to 3.01)	1.77 (1.08 to 2.92)	1.24 (0.75 to 2.06)	.44
Multivariable + insulin	1.00 (referent)	1.55 (0.94 to 2.57)	1.50 (0.91 to 2.49)	1.10 (0.65 to 1.85)	.77
Full model§	1.00 (referent)	1.67 (0.98 to 2.85)	1.72 (1.00 to 2.93)	1.24 (0.72 to 2.14)	.47
Estradiol					
Tertile cut points, pg/mL	<8.0	8.0 to <14.0	≥14.0		
No. case subjects/No. subcohort members	92/146	155/156	137/134	NA	
Multivariable‡	1.00 (referent)	1.53 (1.02 to 2.27)	1.85 (1.19 to 2.89)	NA	.004
Multivariable + BMI	1.00 (referent)	1.44 (0.95 to 2.18)	1.59 (1.00 to 2.55)	NA	.04
Multivariable + insulin	1.00 (referent)	1.55 (1.02 to 2.37)	1.77 (1.11 to 2.84)	NA	.02
Multivariable + free IGF-I	1.00 (referent)	1.55 (1.03 to 2.33)	2.03 (1.27 to 3.22)	NA	.003
Full model§	1.00 (referent)	1.59 (1.02 to 2.49)	1.87 (1.11 to 3.15)	NA	.03
BMI					
Category cut points, kg/m^2	<18.5	18.5 to <25.0	25.0 to <30.0	≥30.0	
No. case subjects/No. subcohort members	2/7	125/155	126/151	75/65	
Multivariable‡	0.40 (0.07 to 2.40)	1.00 (referent)	1.20 (0.78 to 1.83)	2.12 (1.26 to 3.58)	.003
Multivariable + estradiol	0.46 (0.08 to 2.79)	1.00 (referent)	1.15 (0.74 to 1.77)	1.91 (1.11 to 3.27)	.02
Multivariable + insulin	0.80 (0.12 to 5.43)	1.00 (referent)	0.97 (0.60 to 1.57)	1.50 (0.80 to 2.83)	.40
Multivariable + free IGF-I	0.52 (0.08 to 3.30)	1.00 (referent)	1.24 (0.80 to 1.94)	2.19 (1.27 to 3.77)	.004
Full model§	1.19 (0.14 to 10.29)	1.00 (referent)	0.94 (0.56 to 1.58)	1.36 (0.70 to 2.67)	.80

* The total numbers of case subjects and subcohort members vary for each serologic variable due to assay failure in some serum specimens. IGF-I = insulin-like growth factor-I; BMI = body mass index; NA = not applicable.

† Statistical tests for trend (two-sided) were calculated using ordinal quantile variables (1–4 for quartiles and 1–3 for tertiles) entered into the model as a single continuous variable.

‡ Multivariable model adjusted for age, race, alcohol consumption, smoking, family history of breast cancer, parity, age at menopause, age at menarche, age at first child's birth, use of oral contraceptives, educational attainment, use of nonsteroidal anti-inflammatory drugs, and physical activity.

§ Multivariable model with adjustment for free IGF-I, insulin, estradiol, and BMI.

who were neither diabetic nor using HT. In these women, breast cancer incidence rates were 2.4-fold greater among those in the highest quartile compared with the lowest quartile of fasting insulin level, even after controlling for estradiol levels, BMI, free IGF-I level, and established breast cancer risk factors. Estradiol level was associated with ER-positive and PR-positive breast cancers only, and an initially strong association between BMI and postmenopausal breast cancer was greatly attenuated by adjustment for insulin level and, to a lesser degree, for estradiol level. Together, insulin and estradiol levels appeared to largely explain the association between obesity and postmenopausal breast cancer among the women in this study.

Obesity is a well-established risk factor for postmenopausal breast cancer (2–7). Results of previous studies have indicated that this association may be partly explained by the high levels of circulating estrogen in obese women. For example, two large prospective studies (50,51) demonstrated that the association between BMI and the risk of breast cancer was substantially reduced by controlling for estrogen levels. In this study, the association between BMI and the risk of breast cancer was attenuated more by controlling for insulin level than by controlling for estradiol level. Laboratory data support a direct role for insulin in the etiology of breast cancer. For example, the binding of insulin to the insulin receptor activates the mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-kinase pathways, which leads to an increase in cell proliferation (52,53). In addition, insulin stimulates cell growth in both normal and neoplastic breast tissue (11,12,27,54,55) and promotes breast tumor growth in animal models (13–15,28). Insulin may also play a role in breast carcinogenesis via the extensive cross talk that occurs between the insulin–IGF and the

estrogen signaling pathways in breast tissue. Insulin and estradiol can act in concert to promote cell cycle progression in breast cancer cells (56), and insulin activates estrogen receptor alpha–mediated transcription in breast cancer cell lines (57,58), even in the absence of estradiol (59), whereas estrogen stimulates the insulin signaling pathway by enhancing MAPK activation (60).

A strength of this study was our ability to control for endogenous estradiol levels, which allowed us to evaluate the association between insulin level and postmenopausal breast cancer independent of estradiol level. A previous investigation conducted among European women that measured C-peptide levels and adjusted for levels of estradiol and other sex hormones found that the association between C-peptide levels and the risk of postmenopausal breast cancer was attenuated after controlling for free estradiol level (21). By contrast, we observed a positive association between fasting insulin levels and the risk of postmenopausal breast cancer that was unaffected by adjustment for endogenous estradiol levels, supporting a mechanism that is independent of circulating estradiol. Similarly, the positive association of estradiol levels with breast cancer risk was unaltered by controlling for insulin levels, suggesting that hyperinsulinemia and high estrogen may represent two distinct mechanisms that underlie the obesity–breast cancer relationship. Although we directly measured fasting insulin levels, unlike the European study, we did not control for free estradiol level, which may be the more bioactive estradiol component in circulation. However, in previous studies (50,51), adjustment for free estradiol level resulted in only a slightly greater attenuation of the obesity–breast cancer association than adjustment for total estradiol level. Moreover, the association between fasting insulin level and the risk of breast cancer observed in this study was much more robust than that between C-peptide level and breast cancer risk reported by the European study.

Our finding that there was no association between total IGF-I level and breast cancer risk was largely consistent with that in earlier reports (31–35). However, we found a modest positive association between the level of free IGF-I and the risk of breast cancer among nonusers of HT in our study population. Free IGF-I is purported to be the main bioactive component of circulating IGF-I (30), and in two previous studies (18,46), the level of free IGF-I was more strongly associated with the risk of postmenopausal breast cancer than the level of total IGF-I. We did not find evidence for a linear association between free IGF-I levels and breast cancer risk; however, we observed a possible curvilinear association between free IGF-I levels and the risk of breast cancer in nonusers of HT. However, we note that this association was attenuated following adjustment for insulin levels, suggesting that the strong relation between insulin levels and breast cancer observed in this study may underlie the association between free IGF-I levels and breast cancer risk. Furthermore, given that we had not hypothesized a curvilinear association and the paucity of prospective data regarding the relationship between free IGF-I level and breast cancer risk, these data should be interpreted with caution.

We found no association between the risk of incident breast cancer and BMI, the fasting levels of insulin, or free IGF-I among women who used HT. This finding is consistent with data from several large prospective cohort studies that found that HT use interacts with the association between obesity and postmenopausal breast cancer (4–7). However, this interaction was not confirmed in subsequent clinical trials (61,62). It is possible that an association between insulin level and the risk of breast cancer may have been obscured by the high estrogen levels in the HT users. However, other physiologic changes that arise as a consequence of HT use may also explain why an association between insulin and breast cancer was not observed among HT users. In particular, orally administered HT exposes the liver to a large bolus of estrogen, which alters hepatic protein synthesis (63). Indeed, in this study, the levels of IGF-I and IGFBP-3, both of which are produced in the liver (63,64), were statistically significantly lower among the women who used HT (which in the WHI-OS is almost exclusively orally administered HT) than in those who did not. Insulin levels were also statistically significantly lower among the HT users than among the nonusers of HT in our dataset. The use of HT therefore complicates the interpretation of our data on the associations between insulin and IGF-I levels and breast cancer risk, and greater knowledge of the effects of orally administered HT on hepatic protein synthesis may be needed to better understand the associations between these factors and the risk of breast cancer among HT users.

Our study has some important limitations. First, we assessed only baseline serum levels of insulin, glucose, total IGF-I, free IGF-I, IGFBP-3, and estradiol. Measuring these factors over time may have allowed us to classify the study participants more precisely with respect to their circulating levels of these factors. However, plasma levels of insulin, total IGF-I, free IGF-I, IGFBP-3, and estradiol in postmenopausal women have been shown to be stable over at least 3 years (65), suggesting that any misclassification of study subjects according to levels of these factors is likely to have been limited. Second, we cannot exclude the possibility that some case subjects had subclinical breast cancer at WHI-OS recruitment and that the associations we observed were due in part to reverse causality. However, we limited this study to women who developed breast cancer after at least 12 months of follow-up, thereby minimizing the potential bias due to breast cancers that were undiagnosed at baseline. Although the latency period for the breast cancers in this study cannot be known with certainty, we subsequently conducted an exploratory analysis that included only women who developed breast cancer 3 or more years after the baseline blood draw and found that the results were essentially unaltered.

In summary, our data indicate that hyperinsulinemia and high endogenous estradiol levels are independent risk factors for postmenopausal breast cancer and largely explain the association between obesity and the risk of breast cancer in postmenopausal women. Interventions aimed at lowering fasting insulin levels or circulating estrogen levels—either through weight loss or increased physical activity or via pharmacologic approaches—may reduce the risk of breast cancer in postmenopausal women.

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