Research Article

Plasma Leptin Levels and Risk of Breast Cancer in Premenopausal Women

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Abstract

Body mass index (BMI) is inversely related to the risk of premenopausal breast cancer, but the underlying biological mechanisms of this association are poorly understood. Leptin, a peptide hormone produced primarily by adipocytes, is a potential mediator of the BMI association because BMI and total body fat are positively associated with circulating leptin levels and leptin and its receptor are overexpressed in breast tumors. We conducted a prospective case–control study nested within the Nurses' Health Study II cohort examining the association between plasma leptin levels in premenopausal women and breast cancer risk. Leptin was measured in blood samples collected between 1996 and 1999. The analysis included 330 incident breast cancer cases diagnosed after blood collection and 636 matched controls. Logistic regression models, controlling for breast cancer risk factors, were used to calculate ORs and 95% CIs. After adjustment for BMI at age 18, weight change since age 18 to blood draw, and other breast cancer risk factors, plasma leptin levels were inversely associated with breast cancer risk (OR for top vs. bottom quartile = 0.55; 95% CI = 0.31–0.99; P_{trend} = 0.04). Adjustment for BMI at blood draw attenuated the association (OR = 0.69; 95% CI = 0.38–1.23; P_{trend} = 0.26). Our results suggest that leptin may be inversely associated with breast cancer risk, but it is unclear whether any part of this association is independent of BMI. *Cancer Prev Res; 4(9); 1–8. ©2011 AACR.*

Introduction

An inverse association between body mass index (BMI) and risk of premenopausal breast cancer has consistently been observed in a number of epidemiologic studies (1). Key and Pike have suggested that the reduced breast cancer risk in overweight and obese women may be due to irregular or long menstrual cycles and anovulatory infertility (2) because decreased lifetime ovulations may lead to lower estradiol and progesterone exposure (3). However, recent studies have provided evidence that factors other than anovulation likely mediate the apparent protective effect of BMI on premenopausal breast cancer risk (4–6). Furthermore, it has been observed that premenopausal women with a high BMI have lower sex hormone levels

even when accounting for anovulation, suggesting ovarian insufficiency (7, 8).

Hormones on the insulin axis, such as leptin, are also potential mediators of the BMI–breast cancer association because BMI and total body fat are positively associated with circulating leptin levels (9). Leptin, a peptide hormone produced primarily by adipocytes, controls feeding behavior, metabolic rate, and some aspects of reproductive function and also acts as a growth factor and regulator of cell proliferation (10, 11). When plasma levels increase, leptin acts in a negative feedback loop to reduce food intake and increase the metabolic rate (11). Leptin also plays an important role in reproductive processes (11, 12). For example, in experimental animal models exposed to long-term fasting, treatment with leptin reversed the decrease in the secretion of sex steroid hormones, which suggests that leptin plays a role in the pathways that control their release (11).

Mechanistically, leptin could increase or decrease the risk of breast cancer. Leptin and its receptor (Ob-R) are present in human breast tissue and are overexpressed in breast tumors (13–15). In genetically obese mice, the incidence of mammary tumors is decreased in animals that are either leptin deficient or leptin receptor deficient (10). Leptin may also be involved in breast carcinogenesis through cell proliferation or tumor progression (10, 16, 17). Leptin and estrogen may interact because estrogen stimulates leptin production (18, 19) and leptin promotes estrogen production (20, 21). However, leptin also

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has the potential to reduce breast cancer risk in premenopausal women, as it may play a role in ovarian folliculogenesis and at elevated levels may reduce follicular estradiol secretion (22, 23).

Epidemiologic studies that have examined the relation between leptin and breast cancer in premenopausal women have produced conflicting results. Most studies measured leptin levels postdiagnosis and were limited in sample size (23–33). The only prospective study to utilize prediagnostic leptin levels did not observe an association of leptin with breast cancer risk; however, only postmenopausal breast cancer was evaluated (34). We conducted a prospective case–control study nested within the Nurses' Health Study II cohort to investigate whether prediagnostic plasma leptin levels were associated with predominantly premenopausal breast cancer risk. We also examined whether this relation differed by cancer subtype, estradiol level, or BMI.

Materials and Methods

Study population

The Nurses' Health Study II was established in 1989 when 116,430 registered nurses from 14 states completed a baseline questionnaire with questions about demographic and lifestyle factors, anthropometric variables, and prevalent disease. Follow-up questionnaires are sent to participants every 2 years to collect updated information on diseases, anthropometric factors, and other health-related topics. The racial/ethnic breakdown of the cohort is 96% white, 2% Asian, 2% African American, and 2% Hispanic.

From 1996 to 1999, blood samples were collected from 29,611 cohort members, aged 32 to 54 years (35). In brief, premenopausal women who had not used exogenous hormones and had not been pregnant or breast-fed within the past 6 months provided 2-timed blood samples ($n = 18,521$). The first timed sample was collected during the third to fifth days of the menstrual cycle (follicular sample); the second timed sample was collected 7 to 9 days before the anticipated start of their next cycle (luteal sample). Those who either declined or were unable to provide a timed sample (i.e., perimenopausal, postmenopausal, those with a simple hysterectomy, or currently using oral contraceptives or other hormones) provided an untimed sample ($n = 11,090$). Samples were shipped using an ice pack via overnight courier to our laboratory where they were processed and separated into plasma, red blood cell, and white blood cell components. Since collection, samples have been stored in the vapor phase of continuously monitored liquid nitrogen freezers.

The women completed a short questionnaire at blood collection. Those providing timed samples recorded the first day of the menstrual cycle in which the blood samples were drawn along with the dates, time, and number of hours since last food intake for both blood draws, whereas women providing untimed samples recorded the date, time, and number of hours since last food intake of the

sample. Information was also collected on current weight, recent medication use, and current smoking status from all women. The follow-up of the blood cohort was more than 96% in 2005.

We defined our cases as participants who provided a blood sample (timed or untimed) and reported a breast cancer diagnosis on a biennial questionnaire after blood collection and before June 1, 2005. All participants (or next of kin for those who had died) who reported breast cancer were asked for permission to review the relevant medical records and pathology reports to confirm the diagnosis. Estrogen receptor (ER) and progesterone receptor (PR) status information was obtained from pathology reports and was available for 97% of the invasive cases in this analysis. More detailed information on the identification of breast cancer cases has been described previously (36). The mean time from blood draw to breast cancer diagnosis was 3.8 years. Two controls from the blood cohort were selected for each case. Controls were matched to each case on age ($\pm$ 2 years), month and year of blood draw ($\pm$ 2 months), menopausal status at blood draw and diagnosis (premenopausal, postmenopausal, unknown status), race/ethnicity (white, Asian, African American, Hispanic, other), as well as time of day ($\pm$ 2 hours) and fasting status (<2, 2–4, 5–7, 8–11, >12 hours) for both blood draws as applicable. Cases providing timed samples were also matched on the luteal day of blood collection (date of next period minus date of luteal draw $\pm$ 1 day). Women ($n = 331$ cases and 643 controls) were defined as premenopausal at blood draw if they provided a timed sample, reported that their periods had not ceased, or reported having a hysterectomy but with at least 1 ovary remaining, and were 47 years or younger for nonsmokers or 45 years or younger for smokers. This study was approved by the Institutional Review Boards of the Harvard School of Public Health and Brigham and Women's Hospital (Boston, MA). Written informed consent was obtained from all participants.

Leptin measurements

Leptin was assayed in luteal and untimed samples at Children's Hospital (Boston, MA) by an ELISA that employs a quantitative sandwich enzyme immunoassay technique (R&D Systems; ref. 37) with a detection limit of 7.8 pg/mL. Samples from cases and matched controls were assayed together in a randomly determined order, with the laboratory blinded to case–control status. Each batch included blinded replicate samples to assess laboratory precision, and the average coefficient of variation from these samples was 14.1%. We screened for statistical outliers using the generalized extreme studentized deviate many-outlier approach and excluded these participants (1 case and 7 controls; ref. 38). The final analytic sample had 330 cases and 636 controls.

Covariate assessment

Information on known and suspected risk factors for breast cancer was collected on baseline and biennial

questionnaires. Participants reported their age at menarche, height, and weight at age 18 at baseline. Age at first birth, parity, oral contraceptive use, family history of breast cancer (mother, sister, or grandmother), history of benign breast disease, physical activity, alcohol consumption, and current weight were reported at baseline and updated on biennial questionnaires. BMI was calculated as weight in kilograms divided by height in meters squared. Measurements of other hormones [estradiol, estrone, estrone sulfate, progesterone, prolactin, C-peptide, insulin, adiponectin, testosterone, insulin-like growth factor (IGF) I, IGF-binding protein 1 (IGFBP-1), IGFBP-3, growth hormone (GH), and sex hormone–binding globulin (SHBG)] were also available for a subset of women, and assays for these biomarkers have been described elsewhere (35, 39).

Statistical analysis

Quartiles of leptin were determined by the distribution among the controls. Conditional logistic regression was used to estimate ORs and 95% CIs for the association between leptin quartiles and breast cancer. In the covariate-adjusted models, we adjusted for the following *a priori* potential confounders: age at menarche (<12, 12, 13, $\geq$14), parity/age at first birth (nulliparous, age at first birth <25 years/1–4 children, age at first birth 25–29 years/1–4 children, age at first birth $\geq$30 years/1–4 children, age at first <25 years/$\geq$5 children, age at first birth $\geq$25 years/$\geq$5 children), family history of breast cancer (yes/no), history of benign breast disease (yes/no), and BMI at age 18 (continuous), and weight change from age 18 to blood draw (<5, 5–<20, $\geq$20 kg). In addition, physical activity, duration of oral contraceptive use, and height were also assessed on the basis of observed associations with plasma leptin or breast cancer. Of these, only physical activity was significantly associated with breast cancer risk and was included in the covariate-adjusted model along with the *a priori* selected covariates. We considered separate adjustment for BMI at blood draw for 2 reasons: (i) it may be on the pathway between leptin and breast cancer, and (ii) it was strongly correlated with leptin levels and, with such collinear terms, it may be difficult to disentangle the separate effects of each. In addition, we used the residual method to obtain a measure of leptin independent of BMI by computing the residuals from a linear regression model, with BMI at blood draw as the independent variable and leptin concentration as the dependent variable (40). The leptin residuals were then categorized into quartiles and included as independent variables in a conditional logistic regression model.

For secondary analyses, we used unconditional logistic regression, adjusting for matching factors. To assess the association of leptin and breast cancer independent of other hormones, we conducted analyses in the subset of women with these measures. Spearman correlations were calculated between leptin, BMI at blood draw, BMI at age 18, and weight change from age 18 to blood draw. Polytomous unconditional logistic regression adjusting for matching factors was used to test for heterogeneity in effect estimates between various case groups by ER and PR status, *in situ* versus invasive, as well as time since blood draw. As some risk factors for breast cancer differ by menopausal status at diagnosis, secondary analyses were conducted among women who were premenopausal at both blood draw and diagnosis. We also conducted analyses excluding cases diagnosed within 2 years of blood collection to ensure that the potential influence of leptin levels as a result of undiagnosed disease was minimized.

We examined whether the association between leptin and breast cancer varied by BMI (<25, $\geq$25) and estradiol levels (below/above median). Effect modification was assessed with a likelihood ratio test comparing a model with the cross-product term between the exposure variable and each potential effect modifier to the model with main effects only. When examining effect modification by BMI, leptin tertiles were used because of small numbers in some cells when leptin quartiles and BMI were cross-classified. Tests for trend were conducted using leptin quartile medians. χ^2 tests were used to obtain P values for the likelihood ratio statistics. All tests of statistical significance were 2 sided, and statistical analyses were carried out using SAS version 9.1 (SAS Institute Inc.).

Results

Among the 330 cases and 636 controls, the mean age at blood collection was 43 years (range, 32–52 years). Women in the highest quartile of leptin were slightly older, had a higher BMI at age 18 and at blood draw, were less likely to have a history of benign breast disease, had a younger age at menarche, and were less physically active than women in the lowest quartile (Table 1). Cases were more likely than controls to have a family history of breast cancer, a history of benign breast disease, to be nulliparous, and reported less physical activity (Table 2). Among the controls, the Spearman correlations between leptin and BMI at blood draw, BMI at age 18, and weight change from age 18 to blood draw were 0.79 (P < 0.0001), 0.33 (P < 0.0001), and 0.74 (P < 0.0001), respectively.

An inverse association was observed between plasma leptin and breast cancer with an unadjusted OR of 0.66 (95% CI = 0.44–1.00; P_{trend} = 0.05) comparing the top to bottom quartile (Table 3). Adjustment for covariates slightly strengthened the inverse association (OR for top to bottom quartile = 0.62; 95% CI = 0.40–0.96; P_{trend} = 0.04), and further after adjustment for BMI at age 18 and weight change since age 18 to blood draw (OR for top to bottom quartile = 0.55; 95% CI = 0.31–0.99; P_{trend} = 0.04). Adjustment for BMI at blood draw (instead of BMI at age 18 and weight change from age 18 to blood draw) attenuated the association and widened the CI, potentially due to collinearity (OR = 0.69; 95% CI = 0.38–1.23; P_{trend} = 0.26). An inverse association was also observed between the BMI at blood draw–adjusted residuals of leptin and breast cancer, but this association was not statistically significant with a covariate-adjusted OR comparing the top to bottom quartile of 0.79 (95% CI = 0.50–1.23; P_{trend}

Table 1. Characteristics at blood collection by leptin quartiles among cases and controls in the Nurses' Health Study II, 1996–2005

	Leptin quartiles, ng/mL			
	≤9.38	9.39–16.19	16.2–26.69	≥26.7
N	245	246	251	224
Median leptin, ng/mL	6.4	12.2	20.4	35.9
Age, y	43.1 (4.1)	43.8 (3.8)	43.9 (4.1)	44.5 (3.6)
Height, inches	65.3 (2.5)	64.9 (2.6)	64.9 (2.6)	65.0 (2.5)
BMI at age 18 y, kg/m^2	20.1 (1.9)	20.4 (2.2)	20.6 (2.8)	22.7 (3.6)
BMI at blood draw, kg/m^2	21.1 (1.9)	23.2 (2.4)	25.4 (3.4)	31.6 (6.3)
Age at menarche, y	12.9 (1.5)	12.5 (1.4)	12.4 (1.4)	12.0 (1.3)
Nulliparous, %	21.3	18.1	17.0	21.0
Age at first birth, y	26.7 (4.4)	27.2 (4.5)	26.8 (4.7)	26.5 (4.9)
Number of children, among parous women	2.2 (0.8)	2.4 (1.0)	2.2 (0.9)	2.2 (1.0)
Physical activity, MET/wk	28.8 (39.4)	17.6 (17.5)	17.6 (18.3)	15.1 (20.0)
Family history of breast cancer, %	8.6	12.2	12.0	11.6
History of benign breast disease, %	20.0	17.5	17.1	16.5
Ever used oral contraceptives, %	83.3	87.8	85.7	87.5
Premenopausal at diagnosis, %	85.7	89.8	83.7	78.6

NOTE: Data represent mean (SD) unless otherwise indicated.
Abbreviation: MET, metabolic equivalent.

= 0.48), including adjustment for BMI at age 18 and weight change since age 18.

The results including only premenopausal women at diagnosis were similar to the full sample (covariate-adjusted OR including BMI at blood draw = 0.75; 95% CI = 0.40–1.42; P_{trend} = 0.37), as were the results excluding those diagnosed within 2 years of blood collection (comparable OR = 0.77; 95% CI = 0.40–1.51; P_{trend} = 0.53). The

Table 2. Characteristics at blood collection of cases and their matched controls in the Nurses' Health Study II, 1996–2005

	Cases	Controls
N	330	636
Age, y	44.1 (4.0)	43.6 (3.9)
Height, inches	65.2 (2.6)	64.9 (2.6)
BMI at age 18 y, kg/m^2	20.8 (3.0)	21.0 (2.8)
BMI at blood draw, kg/m^2	24.9 (4.8)	25.4 (5.7)
Age at first birth, y	27.1 (4.6)	26.7 (4.6)
Number of children, among parous women	2.2 (0.8)	2.3 (0.9)
Physical activity, MET/wk	17.6 (21.2)	21.0 (28.1)
Family history of breast cancer, %	15.5	8.8
History of benign breast disease, %	21.8	15.7
Age at menarche, y	12.5 (1.4)	12.5 (1.5)
Nulliparous, %	22.7	17.6
Ever used oral contraceptives, %	84.2	87.0
Premenopausal at diagnosis, %	83.9	84.9
Median leptin (10th–90th percentile), ng/mL	15.5 (6.5–33.8)	16.2 (5.4–41.4)
Luteal phase sample	14.1 (6.5–32.9)	15.9 (5.6–42.0)
Untimed sample	17.1 (6.6–46.4)	17.3 (4.5–40.3)

NOTE: Data represent mean (SD) unless otherwise indicated.
Abbreviation: MET, metabolic equivalent.

Table 3. ORs and 95% CIs of breast cancer by quartile of leptin concentration among premenopausal women at blood collection in the Nurses' Health Study II

| | Leptin concentrations, ng/mL | | | | |
	$\leq$9.38	9.39–16.19	16.2–26.69	$\geq$26.7	P_{trend}[a]
Cases/controls	86/159	87/159	95/156	62/162	
Simple model[b]	1.00 (reference)	0.98 (0.68–1.41)	1.07 (0.74–1.54)	0.66 (0.44–1.00)	0.05
Covariate-adjusted model[c]	1.00 (reference)	0.95 (0.64–1.40)	1.09 (0.74–1.60)	0.62 (0.40–0.96)	0.04
Covariate-adjusted model + BMI at blood draw[d]	1.00 (reference)	0.96 (0.64–1.43)	1.14 (0.75–1.73)	0.69 (0.38–1.23)	0.26
Covariate-adjusted model + BMI at age 18 and weight change from age 18 to blood draw[e]	1.00 (reference)	0.90 (0.59–1.36)	0.98 (0.63–1.54)	0.55 (0.31–0.99)	0.04

Abbreviation: MET, metabolic equivalent.
[a]Determined using quartile medians.
[b]Conditional logistic regression. Cases and controls were matched on age, menopausal status at blood collection and diagnosis, race/ethnicity, date, time of day, and fasting status at blood collection, and luteal day of blood draw (timed cases only).
[c]Conditional logistic regression adjusted for age at menarche (<12, 12, 13, $\geq$14), parity/age at first birth (nulliparous, age at first firth <25 years/1–4 children, age at first birth 25–29 years/1–4 children, age at first birth $\geq$30 years/1–4 children, age at first birth <25/$\geq$5 children, age at first birth $\geq$25/$\geq$5 children), family history of breast cancer (yes/no), history of benign breast disease (yes/no), and physical activity (<3, 3–<9, 9–<18, 18–<27, 27–<42, and $\geq$42 MET/wk).
[d]Conditional logistic regression adjusted for the variables in the covariate-adjusted model plus BMI at blood draw (continuous).
[e]Conditional logistic regression adjusted for the variables in the covariate-adjusted model plus BMI at age 18 (continuous) and weight change from age 18 to blood draw (<5, 5–<20, $\geq$20 kg).

results were also similar when examined by time between blood draw and diagnosis (data not shown). Among the subset of women who had other hormone measurements, individual adjustment for these hormones (estradiol, estrone, estrone sulfate, progesterone, prolactin, C-peptide, insulin, adiponectin, testosterone, IGF-I, IGFBP-1, IGFBP-3, GH, and SHBG) did not materially alter the association with the exception of insulin and IGFBP-1, which slightly attenuated the association (Supplementary Table S1).

The inverse association between leptin and breast cancer seemed stronger for PR-negative tumors (OR comparing top quartile to combined first, second, and third quartiles = 0.21; 95% CI = 0.06–0.66) than PR-positive tumors (OR = 1.08; 95% CI = 0.62–1.87; $P_{heterogeneity}$ = 0.009); however, these results are based on small numbers and should be interpreted with caution (Table 4). No significant difference was observed across ER status ($P_{heterogeneity}$ = 0.12), although a stronger association was also observed for ER-negative tumors (comparable OR = 0.38; 95% CI = 0.12–1.17) than for ER-positive tumors (OR = 0.99; 95% CI = 0.58–1.70). Although not statistically significant ($P_{heterogeneity}$ = 0.14), the association between leptin and breast cancer seemed stronger among *in situ* than invasive cases (comparable OR = 0.42; 95% CI = 0.19–0.91; P_{trend} = 0.66 for *in situ* cases and OR = 0.80; 95% CI = 0.49–1.16; P_{trend} = 0.98 for invasive cases). When the BMI-adjusted residuals of leptin were examined, there was also no significant difference observed between *in situ* and invasive cases or by ER or PR status ($P_{heterogeneity}$ > 0.41).

The leptin association did not significantly differ by BMI or estradiol levels (luteal or follicular), although the association between leptin and breast cancer was suggestively stronger among women with a lower BMI ($P_{heterogeneity}$ = 0.08). Women in the highest leptin tertile with a BMI less than 25 had a covariate-adjusted OR of 0.50 (95% CI = 0.24–1.03) compared with women with a BMI less than 25 in the lowest tertile, whereas women in the highest leptin tertile with a BMI 25 or more had a covariate-adjusted OR of 0.93 (95% CI = 0.50–1.74) compared with the same reference group; however, these results were based on small numbers.

Discussion

In this study including 330 cases of primarily premenopausal breast cancer, we observed a borderline significant inverse association between plasma leptin levels in the highest quartile and breast cancer risk. However, after adjustment for concurrent BMI, the association was no longer statistically significant.

Consistent with our results, in 2 retrospective case–control studies, inverse associations between leptin levels and premenopausal breast cancer risk were also observed. Petrido and colleagues reported an OR of 0.1 (95% CI = 0.0–0.9) for 1 SD change in serum leptin level comparing 14 cases to 15 controls, and Falk and colleagues reported an OR for the highest versus lowest quartile leptin of 0.71 (95% CI = 0.5–1.3) with 233 cases and 251 controls

Table 4. Covariate-adjusted[a] ORs and 95% CIs of breast cancer by leptin level by tumor characteristics among premenopausal women at blood collection in the Nurses' Health Study II

	Leptin concentrations, ng/mL		
	≤26.69	≥26.7	$P_{heterogeneity}$[b]
ER/PR status			
ER+	1.00 (reference)	0.99 (0.58–1.71)	0.12
Cases	130	40	
ER−	1.00 (reference)	0.38 (0.12–1.18)	
Cases	37	7	
PR+	1.00 (reference)	1.08 (0.62–1.87)	0.009
Cases	113	40	
PR−	1.00 (reference)	0.21 (0.06–0.66)	
Cases	55	5	
In situ vs. invasive			
In situ	1.00 (reference)	0.42 (0.19–0.91)	0.14
Cases	83	13	
Invasive	1.00 (reference)	0.80 (0.49–1.31)	
Cases	175	48	

Abbreviation: MET, metabolic equivalent.

[a]Polytomous logistic regression adjusted for matching factors, age at menarche (<12, 12, 13, >14), parity/age at first birth (nulliparous, age at first birth <25 years/1–4 children, age at first birth 25–29 years/1–4 children, age at first birth ≥30 years/1–4 children, age at first birth <25/≥5 children, age at first birth ≥25/≥5 children), family history of breast cancer (yes/no), history of benign breast disease (yes/no), physical activity (<3, 3–<9, 9–<18, 18–<27, 27–<42, and ≥42 MET/wk), and BMI at blood draw (continuous).

[b]Determined using polytomous logistic regression.

(23, 29). Conversely, another case–control study reported a significant positive association between plasma leptin levels and premenopausal breast cancer but did not adjust for any covariates (31). Five other retrospective case–control studies have reported nonsignificant positive associations between leptin levels and premenopausal breast cancer risk (26–28, 32, 33), and 1 case–control study reported no association with premenopausal breast cancer (30). These conflicting results may partly be explained by the measurement of leptin postdiagnosis, small sample sizes, varying level of control for confounders, differences in sample collection and measurement techniques, and heterogeneity of associations with pre- and postmenopausal breast cancer.

Leptin can promote estrogen production (20), or conversely, may limit follicular estradiol secretion (23), and thus, as is hypothesized for BMI, may influence breast cancer risk through hormonal pathways. However, it is very difficult to separate the independent effects of BMI and leptin due to their close biological relation. Leptin levels may change with adiposity because, as part of a negative feedback loop, leptin reduces appetite and increases the metabolic rate. Because of this circular relation between BMI and leptin, BMI may be a confounder or may be on the biological pathway of the leptin–premenopausal breast cancer relation and possibly approximately collinear with plasma leptin levels. We therefore presented our covariate-adjusted analyses in various ways: without adjustment for body size, with adjustment for BMI at age 18 and weight change from age 18 to blood draw (which is synonymous with BMI at blood draw but avoids collinearity with leptin concentrations because BMI at age 18 is less strongly correlated with plasma leptin levels than BMI at blood draw), and with adjustment for BMI at blood draw. In addition, to examine a measure of leptin uncorrelated with BMI, we used the residual method (40), which allows for more complete control of the strong confounding by BMI and avoids collinearity. We observed a nonsignificant inverse association between the highest quartile of BMI-adjusted residuals of leptin and breast cancer risk. This suggests that the association of leptin with breast risk is at least partially mediated through BMI.

Leptin has been reported to enhance aromatase activity in MCF-7 cell lines (41), which may enhance estrogen production and induce tumor cell growth (42). Similarly, leptin receptors are expressed in T47-D breast cancer cell lines and leptin induces proliferation of T47-D cells (15, 16). However, leptin may also reduce breast cancer risk through other mechanisms. Leptin is involved in the regulation of ovarian folliculogenesis (22) and at high levels may reduce follicular estradiol secretion (23). In addition, obese leptin receptor–deficient mice have a decreased incidence of mammary tumors (10), thus an individual with elevated leptin levels could be at a reduced risk for breast cancer due to leptin resistance (43, 44) and its association with reduced leptin receptor activity (45).

Moreover, the soluble leptin receptor (sOb-R), which may play an important role in the availability of circulating leptin (46), has been more strongly associated with risk of diseases such as diabetes (47) and polycystic ovary syndrome (PCOS; ref. 48) than plasma leptin.

A limitation of our study was that we only had a single plasma leptin measurement that may not accurately capture an individual's true long-term average level of leptin. However, the intraclass correlations reported for leptin over a 1- to 4-year period are high, ranging from 0.74 to 0.82 (34, 49). Another limitation is the potential for misclassification due to laboratory error (coefficient of variation = 14%), but differential misclassification was unlikely due to the blinding of laboratory personnel to case status and to assaying cases and controls in the same batch. Potential misclassification of leptin measurements are therefore most likely nondifferential with respect to breast cancer and would thus have attenuated our results.

Circulating hormones associated with both leptin and premenopausal breast cancer may confound the association (18–21). We were able to adjust for several hormones, including insulin, adiponectin, IGF-I, IGFBP-3, and estradiol, in this analysis and minimally changed the leptin association. Residual confounding by these biomarkers may remain due to random measurement error. BMI at age 18 and BMI at blood draw may have been affected by measurement error; however, the validity of these measures has been reported to range from 0.84 to 0.99 (50, 51). Residual confounding is likely to have attenuated any observed associations.

To our knowledge, this is the first study to examine the relation between leptin and primarily premenopausal breast cancer risk by using blood samples collected prior to cancer diagnosis. We also have high follow-up rates, cancer cases that have been confirmed by medical records, and data on many important covariates, including BMI at blood draw and at age 18, other hormones, and other risk factors for breast cancer.

In conclusion, our data do not support an increase in the risk of breast cancer with high leptin levels. Our results suggest that leptin may be inversely associated with breast cancer risk, but it is unclear whether any part of this association is independent of BMI. Future studies that examine the association between premenopausal breast cancer risk and leptin receptor levels, which are more strongly related with risk of diabetes and PCOS than plasma leptin concentrations, may further our understanding of this relation.

Disclosure of Potential Conflicts of interest

No potential conflicts of interest were disclosed.

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