Published in final edited form as:

Cancer Epidemiol. 2010 October ; 34(5): 580–586. doi:10.1016/j.canep.2010.05.014.

Do adipokines underlie the association between known risk factors and breast cancer among a cohort of U.S. women?

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Abstract

Obesity is a well-established risk factor for postmenopausal breast cancer, but mechanisms underlying the association are unclear. Adipocyte-derived, cytokine-like adipokines have been suggested as contributory factors. To evaluate their association with breast cancer risk factors and breast cancer risk, we conducted a nested case-control study of 234 postmenopausal breast cancer cases and 234 controls in a cohort of U.S. women with prospectively-collected serum samples obtained in the mid 1970's and followed for up to 25 years. Adiponectin, absolute plasminogen activator inhibitor-1 (aPAI-1), and resistin were measured by a multiplex immunoassay. Sex hormones were available for 67 cases and 67 controls. Among controls, we found that lower levels of adiponectin and higher levels of aPAI-1 were correlated with increasing levels of estradiol (Spearman $r = -0.26$, $p\text{-value} = 0.033$; $r = 0.42$, $p = 0.0003$), decreasing levels of sex hormone binding globulin ($r = 0.38$, $p = 0.0013$; $r = -0.32$, $p = 0.0076$), and increasing body mass index (BMI) ($r = -0.31$, $p < 0.0001$; $r = 0.39$, $p < 0.0001$). Hormones were not associated with resistin. Among the relatively small percentage of women using postmenopausal hormones at the time of blood collection (13.7%), aPAI-1 levels were higher than in nonusers ($p = 0.0054$). Breast cancer risk was not associated with circulating levels of adiponectin (age-adjusted p for linear trend = 0.43), aPAI-1 ($p = 0.78$), or resistin ($p = 0.91$). The association was not confounded by BMI, parity, age at first full-term birth, age at menopause, current postmenopausal hormone use, and circulating sex steroid hormones. Furthermore, adipokine associations were not modified by BMI ($p > 0.05$). The lack of association with risk may be due to measurement error of the laboratory assays. In conclusion, lower levels of adiponectin and higher levels of aPAI-1 measured in prospectively-collected serum from postmenopausal women were associated with increasing BMI but not breast cancer risk.

Introduction

Although obesity is a well-recognized risk factor for postmenopausal breast cancer [1], substantial uncertainties surround the underlying biologic mechanism of the association. Recent advancement in the understanding of adipokines have supported that these cytokine-

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like proteins are secreted as a consequence of inflammation secondary to the over-accumulation of fat in the adipocytes [2]. Adipokines have downstream effects on energy balance, lipid metabolism, and insulin sensitivity [2]. Circulating levels of adipokines have been associated with other chronic obesity-related diseases including diabetes mellitus, insulin resistance, and metabolic disorders [2]. They have also been linked to breast cancer cell proliferation, sex steroid hormone production, and angiogenesis induction [3]. However, there are limited epidemiologic data regarding the relationship of adipokines to breast cancer risk. Of the two published epidemiologic studies with prospectively-collected serum samples, one found an inverse association between levels of adiponectin and higher postmenopausal breast cancer risk, independent of overall body size [4], while the other found no association [5]. Other adipokines, such as absolute plasminogen activator inhibitor-1 (aPAI-1) and resistin, are also potential mediators of obesity in breast carcinogenesis but have not been previously studied in relation to breast cancer risk in prospective settings.

To further evaluate the association of these adipokines with established breast cancer risk factors and with risk of breast cancer, we conducted a nested case-control study of 234 postmenopausal breast cancer cases and 234 controls with prospectively-collected serum samples in the Columbia, MO Breast Cancer Serum Bank cohort.

Methods

Study Population

In 1977, the Breast Cancer Serum Bank was established as part of the National Cancer Institute's Biological Markers Project to identify serum markers of breast cancer. Blood collection sites were established in Columbia, MO for breast cancer free women, in Michigan for women with breast cancer, and in Delaware for women with benign breast disease. The current project utilizes the participants at the Columbia, MO site. These women were recruited from three sources, including the Breast Cancer Detection Demonstration Project, Women's Cancer Control Program at the Cancer Research Center (University of Missouri Hospital), and the Ellis Fischel Cancer Center. Consenting study volunteers were asked to provide blood samples (approximately 30ml) every year from 1977 to 1987. At the time of each blood collection clinical data, including age, height, weight, menstrual and reproductive histories, smoking, medication (including hormone) use, and family history of breast cancer, were obtained by self-report or medical record review. A total of 6,915 women who were initially free of cancer donated blood at least once.

Nested Case-Control Study

For the current analysis, the study population was selected after two periods of follow-up of the cohort. In the first period, cohort members were actively followed by mail until 1989. Study subjects were selected for a previous study to assess for associations between circulating sex steroid hormones and breast cancer risk [6,7]. Eligible women had at least 4 ml of available blood and at the time of blood collection: had no history of cancer other than non-melanoma skin cancer; were not previously diagnosed with benign breast disease; were postmenopausal; and, did not report taking postmenopausal hormones. At the time of the previously published analyses [6,7], 72 cases were diagnosed with histological-confirmed breast cancer. For each case, two controls were selected from among eligible women using incidence density sampling. Controls were alive and free of cancer (except non-melanoma skin cancer) at the age of the case's diagnosis and were matched to the case on exact age and on date (± 1 year) and time (± 2 hour) of blood draw. For our analysis, 66 cases and 132 controls met our additional inclusion criterion that eligible blood draws must have occurred at least 2 years since menopause. Of the two originally matched controls for each case, we

selected only one control that most closely matched the case's age at blood draw to age at menopause.

In a second follow-up period during 1999 to 2002, the vital status of 6,720 cancer-free women with available serum samples was determined. Participants were contacted by mail to complete a self-administered questionnaire to ascertain updated information on their personal cancer history including cancer site, dates of first diagnosis, and location of diagnosis. A total of 4,319 (64.3% of 6,720) women completed the questionnaire, 591 (8.8%) women were not located, 59 (0.9%) refused to participate, 49 (0.7%) were too ill to participate, 1,692 (25%) were deceased (1,257 were identified as deceased prior to follow-up and 435 were identified as deceased during follow-up), and 10 (0.1%) women were located, agreed to participate but did not respond by the end of the study. The response rate (excluding deceased women) was 79%. Self-reported vital status and cancer diagnoses were supplemented with linkage to the Missouri Cancer Registry (current through May 2003), the Breast Cancer Detection Demonstration Project Cohort files, and the National Death Index (current through December 2000). Loss to follow-up among women not known to be deceased was less than 10%.

Based on the second follow-up period, we selected our study population from all postmenopausal women, who began the study cancer-free (with the exception of non-melanoma skin cancer) and still had at least 2ml of serum available that was drawn at least 2 years after self-reported age of menopause. Women were not excluded based on exogenous hormone use or because of a previous diagnosis of benign breast disease. Women were censored on the date of their cancer diagnosis, death, or the end of follow-up (12/31/2002). An additional 168 cases were diagnosed with primary breast cancer. Serum samples drawn 2 or more years before diagnosis were used for analysis. Exclusions among breast cancer cases included 4 women with a missing date of breast cancer diagnosis and 10 women with no available eligible serum samples. We used a nested case-control design. Controls were selected using incidence density sampling. Women were considered eligible as a control if they were alive and had no prior diagnosis of cancer (except non-melanoma skin cancer) at the age of the case, and had a similar number of years between menopause and blood draw (categorized as 2–3, 4–5, 6–10, and 11 or more years). Controls were randomly selected without replacement. A total of 15 women who served as controls were later included as cases. Cases and controls were similar with respect to year, season (winter, spring, summer, fall), and time of day (morning, mid-day, and afternoon) of blood draw, although we did not match for these factors.

Serum Samples

Serum specimens were prepared using standard procedures. Approximately 30ml of blood was collected into red-top vacutainer tubes. Blood was allowed to stand at room temperature for at least 30 minutes or until it was thoroughly clotted and then refrigerated. Within two hours of blood collection, blood was centrifuged and serum (approximately 12 ml) was aliquoted into 1.1 ml amounts into sterile glass vials. Vials were labeled, sealed with rubber stoppers, and stored at -70°C . All women gave informed consent before donating serum to the serum bank. For these analyses, a 1.1 ml aliquot that had never been thawed was used.

Pilot Study

We conducted a pilot study to determine whether adipokine values differed over multiple blood draws during a 2–3 year period. A total of 17 women, who had no prior history of breast cancer but had limited follow-up data available because they could not be located after 1999, were selected from the Columbia, MO cohort. These women were selected so that they had a range of BMI values ($20\text{--}37\text{ kg/m}^2$) and had at least two blood collections in

consecutive years (11 had 2 yearly blood draws and 6 had 3 yearly blood draws). Adiponectin, aPAI-1, and resistin were measured (see *below*). The year-to-year variability for each adipokine was estimated using the coefficient of variation (CV), which was the square root of the variation between yearly adipokine levels nested by individual.

Laboratory Assays

The pilot study samples and those from 234 cases and 234 matched controls were assayed for adiponectin, aPAI-1, and resistin using the Linco 3-plex Human Adipokine Panel (Millipore Diagnostic Services CAT#HADK1-61K-A). To estimate within-batch and between-batch variation, each of the 15 plates contained 4 blinded replicates from 3 ineligible women with known high, medium, and low adipokine values, as measured in the pilot effort. Samples from 12 matched case-control pairs were assayed on the same plate. Assays were performed in duplicate with the mean value used for statistical analyses. All plates were ordered from the same kit production batch. For the 132 study subjects in the first follow-up period, dehydroepiandrosterone (DHEA), DHEA sulfate (DHEAS), testosterone (T), sex hormone binding globulin (SHBG), estrone (E1), and estradiol (E2) were measured using commercially-available RIA kits, as previously described [6,7].

Statistical Analysis

Intraclass correlation coefficients (ICC) and between batch CVs were estimated as follows. First, to estimate the between-subject variability we estimated the variance among all of the 234 control subjects (σ_a^2). The variability between-batches (σ_b^2) and the variability associated within serum samples measured in the same batch (σ_c^2), among on the blinded replicate samples, were estimated using a nested, within-person ANOVA model with log-transformed data to achieve approximately normal distributions. Then, the variance estimates were used to compute the ICCs as $(\sigma_a^2 / [\sigma_a^2 + \sigma_b^2 + \sigma_c^2])$ and the between batch CVs as $\sqrt{\sigma_b^2}$. Similarly, year-to-year reproducibility was estimated as the CV ($\sqrt{\sigma_b^2}$) of model that also included a variable for year of blood draw.

Women with adipokine values above or below 3 standard deviations from the mean were considered outliers and set to missing (4 cases and 5 controls for adiponectin, 1 control for aPAI-1, and 2 controls for resistin). Adipokine values were assessed as categories using quartiles of the control distribution. BMI was calculated using weight (kg) divided by squared height (in meters (m)). These values were from self reports at baseline. The following breast cancer risk factors were assessed at the time of blood draw and treated as continuous variables, if applicable: years of education, parity, age at first birth (per 5 year increase), age at menarche, age at natural menopause (per 5 year increase), BMI (per 5 kg/m² unit increase), family history of breast cancer in a first degree relative, and current use of menopausal hormone therapy including unopposed estrogen or estrogen plus progestin use (ever, never).

Among controls, the distributions of adipokines were compared with characteristics of key breast cancer risk factors and blood specimen collection characteristics using the Wilcoxon rank sum test for factors with two categories, and the Kruskal-Wallis test for factors with three or more categories. Spearman correlation coefficients were estimated between continuous levels of adipokines and hormones, and adjusted for age at reference (defined as the date of diagnosis for cases and date of interview for controls). Conditional logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between individual adipokines and breast cancer risk conditional on the case-control matched pairs. We assessed linear trend by the inclusion of quartiles of adipokine values as an ordinal variable in statistical models. Crude models were adjusted only for age at reference, because inclusion of breast cancer risk factors did not change ORs

by >15%. BMI at blood draw was assessed as a possible effect modifier on the multiplicative scale using the log-likelihood ratio test to compare logistic models with and without the interaction term. In sensitivity analyses, we estimated ORs after excluding women who were currently using menopausal hormone therapy at the time of blood draw.

All statistical analyses were performed with SAS (Version 9).

Results

Description of Study Population

In this study population defined in the late 1970s, the prevalence of obesity (23.8%) and postmenopausal hormone use (13.7%) was low (Table 1). Use of exogenous hormones at the time of the blood draw was associated with a higher risk of breast cancer (OR=1.68, 95% CI 1.02–2.77). Overweight (BMI 25.1–30.0 kg/m²: 39.7% vs. 32.2%), but not obesity (BMI >30.0 kg/m²: 17.9% vs. 23.8%), was more common among cases than controls. The corresponding ORs (95% CIs) of breast cancer risk were 1.34 (0.88–2.04) for overweight and 0.77 (0.47–1.28) for obesity, compared to a BMI<25kg/m². Reproductive factors such as nulliparous women and women with an older age at first birth were at higher risk of breast cancer. Twenty-two percent of the control population had a family history of breast cancer, which was associated with a 50% increase in breast cancer risk (OR=1.50, 95% CI 0.97–2.30). The distribution of blood collection characteristics, including age, year, hour, and season of collection were similar for cases and controls from the first and second follow-up periods (data not shown); therefore, data were pooled for all analyses.

Among controls, the geometric means (95% CI) of adiponectin, aPAI-1, and resistin were 23,388 (95% CI 21,647–25,246) ng/ml, 11,908 (95% CI 11,111–12,982) pg/ml, and 6,891 (95% CI 6,360–7,481) pg/ml, respectively. The quality control replicates across batches provided the reproducibility data for each adipokine. For adiponectin, the ICC value was 63.8% and the CV was 26.7%; for aPAI-1, the ICC was 80.5%, the CV 17.4; and, for resistin, the ICC was 62.8% and the CV was 26.1%.

Pilot Results for Reproducibility of Adipokines by Calendar Time

The year-to-year reproducibility of the adipokines varied. Adiponectin levels remained relatively stable with a year-to-year variability estimated at 11.3%, which was lower than the between batch variation. The CV (27.1%) for aPAI-1 indicated more variance in levels over time. Resistin levels were not reproducible for individuals at the multiple time points (CV=66.6%).

Interrelationships between Adipokines, Breast Cancer Risk Factors and Blood Collection Characteristics

Among controls, increasing levels of aPAI-1 were correlated with lower levels of adiponectin ($r=-0.25$, $p\text{-value}<0.0001$) and higher levels of resistin ($r=0.25$, $p\text{-value}=0.0001$). Adiponectin and resistin were not related ($r=0.00$, $p\text{-value}=0.93$) (data not shown in tables).

Controls with higher BMI had lower levels of adiponectin ($p\text{-value}<0.0001$) and higher levels of aPAI-1 ($p\text{-value}<0.0001$) (Table 2). Resistin levels were higher for increasing BMI values, but the relationship was not statistically significant ($p=0.14$). All adipokines increased with age but differences in distributions were not statistically significant. With the exception of higher median levels of aPAI-1 among women who were using postmenopausal hormones at the time of blood draw ($p=0.0054$), there was no relationship between adipokines and other known breast cancer risk factors, including height, family

history of breast cancer, number of children, and age at first birth among parous women (Table 2).

Levels of adiponectin, aPAI-1, and resistin did not vary by the number of years between menopause and the blood draw. Specimens collected during spring had significantly higher levels of resistin than blood collected during other seasons ($p=0.0018$), but season of blood draw did not affect levels of adiponectin or aPAI-1. Levels of resistin tended to be lower in older blood samples, with median levels of 6,207 pg/ml for serum collected 1977–1978, 8,092 pg/ml for bloods from 1979, and 10,058 pg/ml for bloods collected between 1980–1987 ($p\text{-value}<0.0001$). Levels of aPAI-1 showed a similar but not significant trend of lower values with longer sample storage. aPAI-1 levels were higher in blood specimens drawn in the morning compared to those drawn in the afternoon ($p=0.0019$).

Restriction of analyses to women who were not taking postmenopausal hormones at the time of blood collection did not alter relative risks (data not shown). The relative risk associated with adipokines and breast cancer risk were not modified by BMI (data not shown).

Association between Adipokines and Breast Cancer Risk

The age-adjusted models did not reveal any associations between adipokines and postmenopausal breast cancer risk (Table 3). Further adjustment for age at reference, BMI, number of births, age at first full term birth, years between blood collection and menopause, and current postmenopausal hormone use did not alter risk estimates.

Adipokines and Sex Steroid Hormones

Data from 67 cases and 67 controls were available to explore the interrelationships between sex hormones and adipokines in this population. Among controls, women with higher adiponectin had lower levels of E2 (age-adjusted $r=-0.25$, $p\text{-value}=0.040$) and free E2 ($r=-0.27$, $p\text{-value}=0.026$), and higher FSH ($r=0.43$, $p\text{-value}=0.0003$) and SHBG ($r=0.38$, $p\text{-value}=0.0017$). aPAI-1 was positively correlated with E1 ($r=0.30$, $p\text{-value}=0.015$), E2 ($r=0.42$, $p\text{-value}=0.0004$), E1S ($r=0.39$, $p\text{-value}=0.0013$), and T ($r=0.37$, $p\text{-value}=0.0024$), but negatively correlated with SHBG ($r=-0.32$, $p\text{-value}=0.0090$). Resistin was positively correlated with SHBG ($r=0.25$, $p\text{-value}=0.044$).

Among these 67 cases and 67 controls, the ORs (Table 4) of adipokines and risk of breast cancer were imprecise. However, adjustment for E2 and SHBG, hormones with the strongest relation with adipokines, did not alter the ORs for the association between breast cancer risk and levels of adiponectin, aPAI-1, and resistin.

Discussion

In our study of 234 postmenopausal breast cancer cases and 234 controls with prospectively-collected serum samples, we found no association between breast cancer risk and circulating levels of adiponectin, aPAI-1, or resistin. These findings were not confounded by known breast cancer risk factors including BMI. Among the participants with measured levels of serum sex hormones (approximately 25% of the study population), lower levels of adiponectin and higher levels of aPAI-1 correlated with increasing levels of estradiol, and decreasing levels of SHBG. aPAI-1 was higher among women who were taking postmenopausal hormones at the time of blood collection than non-users.

The lack of association between adiponectin and breast cancer risk in our study is in contrast to our hypothesis and a previous prospective study of 858 postmenopausal cases and 1,309 controls [4]. They reported that the body size-adjusted relative risk for the highest versus lowest serum levels of adiponectin was 0.73 (95% CI 0.55–0.98), which was stronger for

women with low circulating estrogen levels and non-users of postmenopausal hormones. An inverse association for adiponectin [8–10] is also supported by three smaller epidemiologic studies as well as breast cancer cell line studies that demonstrate adiponectin activates cell apoptosis and inhibits cell cycle progression [11–13]. However, consistent with our study, a recent prospective study of 561 pre- and post-menopausal breast cancer cases and 561 nested controls reported that higher levels of adiponectin were not associated with postmenopausal breast cancer risk (p-value for linear trend=0.95) [5]; although, as in our study, obesity was not associated with postmenopausal breast cancer risk.

Additional studies in prospective cohorts are needed to clarify the role of adipokines on breast cancer risk. Although aPAI-1 has been examined as potential mediator of the adverse effects of obesity and has been detected in breast cancer tissue [14,15], it has not been evaluated in relation to breast cancer risk prior to this study. A pooled analysis of 8,377 breast cancer patients [16] convincingly showed high levels of PAI-1 in breast tissue are a poor prognostic factor, predicting tumor invasion, metastasis and shorter survival. PAI-1 in normal breast tissue is thought to arise both from local production in breast adipocytes [17,18] and from the circulation. Circulating levels of aPAI-1 have been observed to be higher in obese individuals (27), particularly among those with greater stores of visceral fat [15,19]. We did not find an association between aPAI-1 levels and breast cancer risk.

Resistin is produced in breast and adipose tissue with 250% greater production in visceral, compared to subcutaneous fat [20,21]. It has been linked to inflammation, energy homeostasis, and insulin resistance [20]. Plasma resistin levels have been correlated with overall adiposity, percentage of body fat, and other components of metabolic syndrome [22,23]. Two previously published studies on less than 80 Asian cases and matched controls with retrospectively collected serum samples found higher levels of resistin among breast cancer cases than controls [24,25]. Our findings in a larger sample with prospectively-collected serum samples are in contrast to these earlier studies.

Although our study benefited from prospectively collected serum samples, the study did have several limitations, particularly with regard to the laboratory reproducibility and biologic stability of the adipokines. Assay measurements for adiponectin and resistin had high batch-to-batch variability. Comparison of adipokine levels from one year to the next for any given women showed that of the analytes measured, only adiponectin, appears to be reliably measured with a single blood draw. Additionally, not all study subjects were fasting at the time of blood collection (<4 hours between blood collection and time since last meal), which is problematic for analytes such as aPAI-1 that have a distinct circadian pattern [26]. However, inclusion of time of blood draw did not appear to appreciably alter our results. Thus, measurement error arising from these sources may well have attenuated true, but weak, breast cancer risks. In addition, power may have been limited to detect smaller differences in adipokine levels between cases and controls.

In conclusion, while we found a strong correlation between BMI and circulating levels of adiponectin and aPAI-1, we did not detect associations between adipokines and postmenopausal breast cancer risk. Adipokines were not associated with reproductive risk factors but were related to levels of estradiol and SHBG. Further studies conducted in adipokines and breast cancer risk should control blood collection and assay parameters.

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Characteristics of established breast cancer risk factors and blood collection parameters among 234 cases and 234 controls nested in the Columbia, MO cohort

Table 1

Relationship between covariates and breast cancer risk						
Possible Covariates	Case		Control		OR ¹	95% CI
	N	%	N	%		
Breast Cancer Risk Factors						
Age at Reference						
≤50	10	4.4	22	9.3	1.00	
51–70	211	90.4	185	78.9	2.88	(1.12–7.36)
>70	13	5.2	27	11.9	1.25	(0.29–5.34)
BMI (kg/m ²) at Reference						
<25	97	42.4	100	44.1	1.00	
25.1–30.0	91	39.7	73	32.2	1.34	(0.88, 2.04)
>30	41	17.9	54	23.8	0.77	(0.47, 1.28)
Family History of Breast Cancer						
No	160	69.9	175	77.1	1.00	
Yes	69	30.1	52	22.9	1.50	(0.97, 2.30)
No. of children						
0	39	17.0	37	16.3	1.00	
1	24	10.5	32	14.1	0.66	(0.33, 1.32)
2+	166	72.6	158	69.7	0.89	(0.54, 1.46)
Age at First Birth (among parous women)						
≤20	24	10.5	29	12.8	1.00	
21–24	87	38.0	87	38.3	1.19	(0.64, 2.21)
25–29	59	25.8	54	23.8	1.37	(0.71, 2.67)
>29	20	8.7	20	8.8	1.22	(0.54, 2.77)
Age at Menopause						
≤47	87	38.0	84	37.0	1.00	
48–49	28	12.2	32	14.1	0.84	(0.46, 1.53)
50–51	53	23.1	49	21.6	1.05	(0.63, 1.75)
>51	61	26.6	61	26.9	0.98	(0.61, 1.57)

Relationship between covariates and breast cancer risk						
Possible Covariates	Case		Control		OR ¹	95% CI
	N	%	N	%		
Unknown	0	0.0	1	0.5		
Current Use of Exogenous Hormones						
No	178	79.0	196	86.3	1.00	
Yes	44	21.0	31	13.7	1.68	(1.02, 2.77)
Blood Collection Parameters						
Season of Blood Draw						
Fall	52	22.7	53	23.4		
Spring	66	28.8	77	33.9		
Summer	62	27.1	55	24.2		
Winter	49	21.4	42	18.5		
Year of Blood Draw						
1977–1978	126	55.0	101	44.5		
1979	62	27.1	82	36.1		
1980+	41	17.9	44	19.4		
Hour of Blood Draw						
7–10am	77	33.6	87	38.3		
11am–1pm	87	38.0	89	39.2		
2–5pm	65	28.4	51	22.5		
Assay Batch						
1	15		6.6	16	7.0	
2	17		7.4	16	7.0	
3	16		7.0	16	7.0	
4	16		7.0	14	6.2	
5	17		7.4	14	6.2	
6	17		7.4	17	7.5	
7	15		6.6	16	7.0	
8	15		6.6	16	7.0	
9	16		7.0	16	7.0	
10	16		7.0	16	7.0	

Relationship between covariates and breast cancer risk						
Possible Covariates	Case		Control		OR ¹	95% CI
	N	%	N	%		
	11	15	6.6	14	6.2	
	12	13	5.7	15	6.6	
	13	16	7.0	15	6.6	
	14	16	7.0	17	7.5	
	15	9	3.9	9	4.0	
Assay Day						
	1	98	42.8	93	41.0	
	2	131	57.2	134	59.0	
Years between Menopause and Blood Collection						
	2-3	30	13.1	32	14.1	
	4-5	28	12.2	27	11.9	
	6-10	58	25.3	54	23.8	
	11+	113	49.3	114	50.2	

Relationship between adipokines and known breast cancer risk factors and blood collection parameters among 234 controls, Columbia, MO cohort

Table 2

Characteristic	N	%	Adiponectin (ng/ml)		aPAI-1 (pg/ml)		Resistin (pg/ml)	
			median	p-value [†]	median	p-value [†]	median	p-value [†]
Breast Cancer Risk Factors								
Age at blood draw								
≤50	22	9.3	20,435	0.07	10,734	0.34	7,095	0.31
51–70	185	78.9	25,789		12,119		7,536	
>70	27	11.9	29,850		13,686		8,882	
Body mass index (kg/m ²) at blood draw								
<25	100	44.1	29,746	<0.0001	10,194	<0.0001	7,483	0.14
25.1–30.0	73	32.2	24,234		12,854		7,713	
>30	54	23.8	20,435		16,710		8,015	
Height (cm) at blood draw								
<157.0	38	16.7	25,522	0.74	12,171	0.28	7,896	0.81
157.1–161.5	73	32.2	24,234		13,839		7,516	
161.6–165.0	69	30.4	27,155		11,872		7,505	
>165.0	47	20.7	25,270		11,267		8,061	
Family history of breast cancer								
No	175	77.1	25,790	0.89	12,362	0.23	7,695	0.49
Yes	52	22.9	25,943		11,708		7,559	
Number of children								
0	37	16.3	26,547	0.20	12,252	0.81	7,734	0.98
1	32	14.1	25,659		13,252		7,447	
2	54	23.8	26,992		11,720		7,568	
3	51	22.5	26,152		11,686		7,554	
4+	53	23.4	23,450		12,937		8,085	
Age at first birth among parous women								
≤20	29	12.8	23,374	0.77	12,188	0.77	8,314	0.43
21–24	87	38.3	25,206		11,519		7,481	
25–29	54	23.8	26,911		12,513		7,530	

Characteristic	N	%	Adiponectin (ng/ml)		aPAI-1 (pg/ml)		Resistin (pg/ml)	
			median	p-value ^J	median	p-value ^J	median	p-value ^J
>29	20	8.8	28,539		13,249		7,865	
Using postmenopausal hormones at the time of blood draw								
No	196	86.3	24,432	0.73	10,476	0.0054	8,038	0.60
Yes	31	13.7	26,032		12,497		7,559	
Blood Collection Parameters								
Years between menopause and blood draw								
2-3	32	14.1	24,197	0.15	10,593	0.69	6,829	0.46
4-5	27	11.9	26,494		10,068		7,865	
6-10	54	23.8	24,871		13,130		7,221	
11+	114	50.2	26,243		12,252		7,676	
Season of blood draw								
Fall	53	23.4	25,728	0.88	11,240	0.53	6,960	0.0018
Spring	77	33.9	25,881		12,479		8,396	
Summer	55	24.2	26,494		11,703		7,150	
Winter	42	18.5	24,476		12,552		7,480	
Year of blood draw								
1977-1978	101	44.5	26,601	0.33	11,548	0.18	6,207	<0.0001
1979	82	36.1	24,197		12,577		8,092	
1980+	44	19.4	25,826		13,559		10,058	
Time of blood draw								
7- 10am	87	38.3	25,267	0.91	14,164	0.0019	7,655	0.39
11am-1pm	89	39.2	25,246		11,956		8,018	
2-5pm	51	22.5	26,477		10,419		7,470	

^J p-value calculated using Wilcoxon rank sum test if the risk factor had two categories and using the Kruskal-Wallis test if the risk factor had three or more categories

Odds ratios (OR) and 95% confidence intervals (CI) for the association between adipokines and breast cancer risk, Columbia, MO cohort (234 cases, 234 controls)

Table 3

Adipokines	Cases		Controls		Age-adjusted OR (95% CI)	p for linear trend ²	Multivariate-adjusted ¹ OR (95% CI)	p for linear trend ²
	N	%	N	%				
Adiponectin, ng/ml								
626–19,535	54	23.5	62	26.8	1.00		1.00	
19,536–25,758	57	24.8	57	24.7	1.15 (0.69–1.93)		1.02 (0.59–1.72)	
25,759–32,921	59	25.7	53	22.9	1.29 (0.76–2.20)		1.18 (0.68–2.08)	
32,922–73,588	60	26.1	59	25.5	1.19 (0.70–2.03)	0.43	1.04 (0.59–1.83)	0.70
Missing	4		3					
aPAI-1, pg/ml								
1,280–8,048	59	25.2	58	24.9	1.00		1.00	
8,049–12,236	60	25.6	56	24.0	1.07 (0.64–1.79)		1.17 (0.68–1.99)	
12,237–17,108	56	23.9	59	25.3	0.94 (0.57–1.56)		0.95 (0.56–1.60)	
17,109–57,786	59	25.2	60	25.8	0.98 (0.59–1.64)	0.78	1.30 (0.74–2.30)	0.62
Missing	0		1					
Resistin, pg/ml								
1,280–4,683	60	25.6	56	24.2	1.00		1.00	
4,684–7,551	55	23.5	61	26.4	0.88 (0.53–1.45)		0.90 (0.53–1.51)	
7,552–10,653	59	25.2	58	25.1	1.02 (0.60–1.71)		1.03 (0.60–1.76)	
10,654–31,407	60	25.6	56	24.2	1.05 (0.62–1.76)	0.91	1.07 (0.62–1.83)	0.85
Missing	0		1					

¹ Adjusted for age at reference, body mass index, number of births, age at first full term birth, age at menopause, and current postmenopausal hormone use

² p-value for linear trend was estimated using the categorical values as an ordinal variable in statistical models

Table 4

Age- and hormone-adjusted odds ratios (OR) and 95% confidence intervals (CI) for the association between adipokines and breast cancer risk, Columbia, MO cohort (67 cases, 67 controls)

Adipokines	Cases		Controls		Age-adjusted OR (95% CI)	p for linear trend ²	Estradiol-adjusted OR (95% CI)	p for linear trend ²	SHBG-adjusted OR (95% CI)	p for linear trend ²
	N	%	N	%						
Adiponectin, ng/ml										
626–19,535	14	21.5	13	19.4	1.00		1.00		1.00	
19,536–25,758	13	20.0	16	23.9	0.55 (0.17, 1.82)		0.65 (0.19–2.25)		0.55 (0.16–1.87)	
25,759–32,921	15	23.1	13	19.4	1.50 (0.43, 5.22)		2.11 (0.54–8.20)		1.49 (0.42–5.62)	
32,922–73,588	23	35.4	25	37.3	1.16 (0.41, 3.27)	0.90	1.32 (0.45–3.83)	0.34	1.15 (0.39–3.45)	0.39
aPAI-1, pg/ml										
1,280–8,048	20	30.3	18	26.9	1.00		1.00		1.00	
8,049–12,236	20	30.3	17	25.4	1.01 (0.38, 2.72)		1.10 (0.35–3.49)		1.27 (0.44–3.71)	
12,237–17,108	13	19.7	13	19.4	0.82 (0.27, 2.45)		0.67 (0.22–2.11)		0.99 (0.35–2.81)	
17,109–57,786	13	19.7	19	28.4	0.49 (0.16, 1.52)	0.25	0.33 (0.10–1.09)	0.06	0.53 (0.19–1.50)	0.23
Resistin, pg/ml										
1,280–4,683	20	30.3	18	26.9	1.00		1.00		1.00	
4,684–7,551	15	22.7	20	29.9	0.61 (0.23, 1.63)		0.36 (0.12–1.12)		0.43 (0.15–1.25)	
7,552–10,653	16	24.2	14	20.9	0.81 (0.27, 2.38)		0.87 (0.24–3.18)		0.83 (0.24–2.87)	
10,654–31,407	15	22.7	15	22.4	0.77 (0.28, 2.16)	0.93	0.81 (0.25–2.62)	0.97	0.80 (0.25–2.51)	0.93

² p-value for linear trend was estimated using the categorical values as an ordinal variable in statistical models