Research Article

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Circulating Adipokine Levels and Endometrial Cancer Risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial

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Abstract

Background: Circulating adipokine levels may be associated with endometrial cancer risk, yet few studies have evaluated these markers prospectively.

Methods: We conducted a nested case–control study of postmenopausal women in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial ($n = 78{,}216$), including 167 incident endometrial cancer cases and 327 controls that were matched on age, study center, race, study year of diagnosis, year of blood draw, time of day of blood draw, and menopausal hormone therapy (MHT) use. Adipokine and estradiol levels were categorized into tertiles (T). ORs and 95% confidence intervals (CIs) for the associations of adiponectin, leptin, and visfatin with endometrial cancer risk were estimated by conditional logistic regression, adjusting for known endometrial cancer risk factors, including body mass index (BMI) and circulating estradiol levels.

Results: Adiponectin levels were inversely associated with risk of endometrial cancer [$OR_{T3vsT1} = 0.48$; 95% CI, 0.29–0.80); $P_{trend} < 0.01$], whereas elevated leptin levels showed a positive association [2.77 (1.60–4.79); $P_{trend} < 0.01$]. These results remained significant after adjustment for estradiol, but not after further adjustment for BMI. When analyses were restricted to non-MHT users, associations of adiponectin and leptin were stronger and remained significant after adjustment for estradiol and BMI [0.25 (0.08–0.75); $P_{trend} = 0.01$ and 4.72 (1.15–19.38); $P_{trend} = 0.02$, respectively]. Nonsignificant positive associations were observed for visfatin.

Conclusion: Adipokines may influence endometrial cancer risk through pathways other than estrogen-mediated cell growth in postmenopausal women not currently on MHT.

Impact: Understanding how adipokines influence endometrial cancer risk may help to elucidate biological mechanisms important for the observed obesity–endometrial cancer association. *Cancer Epidemiol Biomarkers Prev; 22(7); 1304–12. ©2013 AACR.*

Introduction

Adipose tissue produces and secretes many metabolically active molecules, including adipokines such as adiponectin, leptin, and visfatin (1). Although obesity is a well-known risk factor for endometrial cancer, the relationship between these obesity-related factors and endometrial cancer risk remains largely unclear. To date, hypotheses about the mechanism by which obesity increases risk in postmenopausal women have largely centered around the aromatization of androgens in adipose tissue leading to increased circulating estradiol levels (2). Other mechanisms via inflammation, insulin resistance, and adipokines, however, are thought to be important.

Adiponectin, the most abundant adipokine, has been suggested to have anti-angiogenic, anti-inflammatory, and anti-apoptotic properties (3–5). In addition, increased adiponectin levels in serum have been shown to reduce blood glucose and insulin levels and thus are inversely correlated with obesity and type-2 diabetes (6, 7). Results from the limited number of epidemiologic studies evaluating circulating adiponectin levels and endometrial cancer are inconsistent. Case–control studies have reported an inverse association; however, these studies used postdiagnostic serum and thus could not address temporality (8–11). To date, only 3 prospective studies have been conducted; 1 study reported an inverse association with prediagnostic serum levels (12), whereas the other 2 reported no association (13, 14).

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Leptin has contrasting biological functions to adiponectin; it has been shown to promote cell proliferation, angiogenesis, and metastasis in certain cell lines (1, 5, 15). Serum leptin levels are positively correlated with obesity and function to regulate appetite, weight, metabolism, and fertility (16, 17). Results from case–control studies (8, 18, 19) that suggested a positive association between leptin levels and endometrial cancer have recently been corroborated in a prospective study (13). Few studies of adipokines and endometrial cancer risk have included measures of both adiponectin and leptin (8, 10, 13) and only 2 have reported associations for their ratio (8, 13). The leptin:adiponectin ratio has been suggested to be a surrogate marker of insulin resistance in both diabetic and nondiabetic women (20, 21) and has been shown to be positively associated with breast and colorectal cancer (22, 23).

Visfatin, also known as nicotinamide phosphoribosyltransferase (Nampt) or pre–B-cell colony-enhancing factor, is an adipokine that was recently discovered in visceral fat. It has been implicated in a variety of metabolic and stress functions as well as cellular energy metabolism (24–26). Recent epidemiologic studies have shown direct associations of serum visfatin levels with gastric carcinoma, colorectal adenocarcinoma, and postmenopausal breast cancer (27–29). In addition, a few studies have suggested a link between visfatin and polycystic ovary syndrome, a risk factor for endometrial cancer (30–32); however, to date, the association of visfatin with endometrial cancer risk has not been evaluated.

To shed further light on relationships of adiponectin, leptin, and visfatin with endometrial cancer risk, we measured prediagnostic serum levels of these adipokines using a case–control study nested within the Prostate, Lung, Colorectal and Ovarian (PLCO) cancer screening trial. We adjusted for body mass index (BMI), estradiol, and other factors known to influence endometrial cancer risk to determine whether serum adipokine levels are independently associated with endometrial cancer.

Materials and Methods

Study population

The design of the PLCO cancer screening trial has been described previously (33). In brief, between 1993 and 2001, 78,216 women aged 55 to 74 years were recruited at 10 screening centers across the United States and randomized to either an intervention (screening) or control (usual care) arm. Incident cancers were ascertained primarily by self-report on an annual study update form and confirmed by medical record abstraction. The current study included women who were randomized to the screening arm of the trial and met the following criteria at baseline: intact uterus; no previous cancer diagnosis; available serum sample; completion of a detailed questionnaire, which captured basic demographics, lifestyle factors, and reproductive history, and informed consent for studies of cancer. Cancer cases were defined as women having a first primary diagnosis of an invasive epithelial tumor of the endometrium between their initial screening visit and April 1, 2006. Controls had to be alive at the time of the diagnosis of the case and were matched 2:1 on age (5-year intervals), study center, race (white/black/other), individual study year of diagnosis, individual year of blood draw, time of day of blood collection (AM/PM), and menopausal hormone therapy (MHT) use at baseline (never/former/current/unknown). Subjects with missing information on BMI, diabetes, and smoking information ($n = 2$ for cases and $n = 4$ controls), 1 case with insufficient serum volume for assays and 1 case whose serum sample was later determined to have been drawn after cancer diagnosis were excluded from analyses. The final study population consisted of 167 cases and 327 matched controls; there were 7 cases with 1 matched control after applying exclusion criteria.

Laboratory assays

Serum concentrations of adiponectin, leptin, visfatin, and estradiol were measured in the Reproductive Endocrine Research Laboratory of Dr. Frank Stanczyk (University of Southern California, Los Angeles, CA). Leptin and adiponectin were measured by radioimmunoassay (RIA) using reagents obtained from Millipore Linco Research (St. Charles, MO). The leptin assay sensitivity is 0.5 ng/mL, and the interassay coefficients of variation (CV) are 6.2%, 4.7%, and 3.6% at 4.9, 10.4, and 25.6 ng/mL, respectively. The adiponectin assay sensitivity is 1.0 ng/mL, and the interassay CVs are 9.2%, 6.9%, and 9.2% at 1.5, 3.0, and 7.5 ng/mL, respectively. Visfatin was measured by an ELISA (ALPCO). The assay sensitivity is 0.12 ng/mL, and the interassay CVs are 4.7%, 5.7%, and 7.2% at 0.72, 1.01, and 2.92 ng/mL, respectively. Estradiol was measured by RIA following organic solvent extraction and Celite column partition chromatography. The assay sensitivity is 2 pg/mL and the interassay CVs are 11%, 13%, and 12% at 15, 36, and 101 pg/mL, respectively.

Matched sets were not retained within the same assay batch; however, the proportion of cases and controls was generally consistent across batches. We did not observe any systematic drifts in analyte levels based on batch, and adjustment for batch in subsequent analyses did not significantly change the results. In addition to the laboratory's quality control samples, 4 blinded quality control samples were included within each batch. Intrabatch CVs from these masked samples were 4.1, 4.2, 7.9, and 5.3; the interbatch CVs were 8.3, 4.5, 6.6, and 12.9; and the intraclass correlation (ICC) values were 0.677, 0.971, 0.938, and 0.915 for adiponectin, leptin, visfatin, and estradiol, respectively. Levels of visfatin were not available for 21 quality control samples due to insufficient serum volume; therefore, CV and ICC values for visfatin were based on 43 quality control samples. Samples that fell below the level of detection were set to the minimum detection level; 4 samples (3 controls and 1 case) were set to 1 pg/mL for estradiol and 2 samples (one case and one control) were set to 0.1 ng/mL for visfatin.

Statistical analyses

Differences in baseline characteristics and analyte levels were determined by χ^2 or Kruskall–Wallis tests, as appropriate. Spearman correlations were estimated for the associations between analytes and BMI among controls. ORs and 95% confidence intervals (CI) for the relationships between analyte levels and endometrial cancer were estimated using conditional logistic regression. Results from unconditional logistic regression models adjusted for matching factors were similar to those from conditional logistic regression models. Tertiles of analyte levels were determined based on the distribution in the control population. Multivariable models were adjusted for known endometrial cancer risk factors that were captured on the baseline questionnaire: oral contraceptive use (yes/no); family history of breast or endometrial cancer (yes/no); current smoking status (never/former/current); education level (less than high school/high school or equivalent/post–high school, no college/some college/college grad/postgraduate); parity (0, 1, 2, 3+ live births); and history of diabetes (yes/no). Additional adjustment was made for (i) BMI (continuous), (ii) estradiol (continuous), and (iii) BMI and estradiol. Linear trends were assessed by modeling categorical variables as ordinal variables. We conducted stratified analyses based on MHT status due to the potential effect of MHT use on adipokine levels. Because of the small number of former MHT users in the population, we combined never and former users into a single category for these analyses; however, similar results were observed when restricting to women who had never used MHT only. Statistical tests for interaction were conducted by likelihood ratio tests comparing unconditional logistic regression models that adjusted for all matching factors (excluding MHT status), BMI, and estradiol with and without an interaction term for current MHT use and each adipokine. All statistical analyses were conducted using STATA version 11.2 (STATA Corp.).

Results

The average age of endometrial cancer cases in this study was 66.4 ± 5.7 years and the average time from blood draw to diagnosis was 3.4 ± 2.8 years (Table 1). Cancer cases were more likely to be obese ($P < 0.001$) and less likely to have used oral contraceptives ($P = 0.03$) than matched controls. Other known endometrial cancer risk factors such as parity, diagnosis of diabetes, and smoking status did not differ significantly by case status. Furthermore, as expected, the distribution of matching factors did not differ substantially for cases compared with controls. Median levels of each adipokine differed by case status (Table 2). Levels of adiponectin were significantly lower among cases ($P = 0.002$), whereas median levels of leptin and visfatin were higher among cases compared to controls ($P < 0.001$ and $P = 0.05$, respectively).

Levels of each adipokine were significantly correlated with BMI; a negative correlation was seen for adiponectin, whereas the association was positive for leptin and visfatin (unadjusted Spearman correlation coefficients for adiponectin, leptin, and visfatin among controls: $r = -0.40$, 0.80, and 0.32, respectively; $P < 0.0001$ for all correlations). Similarly, among controls who were not currently using MHT, each adipokine was significantly correlated with estradiol ($r = -0.25$, 0.51, and 0.36, respectively; $P < 0.001$ for all correlations). In addition, significant correlations were observed between the adipokines, except for between visfatin and adiponectin. Leptin was negatively correlated with adiponectin but positively with visfatin ($r = -0.32$ and 0.31, respectively; $P < 0.0001$). Correlations between the adipokines and with estradiol and BMI were stronger among overweight/obese women compared with normal weight women (Supplementary Table S1).

Higher BMI ($\geq$30 kg/m^2 vs. <25 kg/m^2) and estradiol levels were associated with an increased risk of endometrial cancer after mutual adjustment in multivariable models (OR = 2.69; 95% CI, 1.56–4.64; $P_{trend} < 0.001$ and $OR_{T3\ vs.\ T1} = 2.94$; 95% CI, 1.41–6.12; $P_{trend} = 0.02$, respectively). ORs for the association of adiponectin, leptin and visfatin with endometrial cancer, adjusted for (i) established endometrial cancer risk factors other than BMI and then additionally for (ii) BMI, (iii) estradiol, or (iv) BMI and estradiol, are presented in Table 3. In model (i), higher adiponectin levels were inversely associated with endometrial cancer risk ($OR_{T3\ vs.\ T1} = 0.48$; 95% CI, 0.29–0.80; $P_{trend} = 0.004$), whereas elevated levels of leptin were associated with increased risk ($OR_{T3vsT1} = 2.77$; 95% CI, 1.60–4.79; $P_{trend} < 0.001$). In addition, a significant reduction in risk was observed with an increased ratio of adiponectin:leptin ($OR_{T3\ vs.\ T1} = 0.35$; 95% CI, 0.20–0.61; $P_{trend} < 0.001$). Each of these associations remained significant after adjusting further for estradiol ($P_{trend} \leq 0.01$ for all associations). However, after adjustment for BMI, the estimates and linear trends were no longer significant. Models that adjusted for BMI and estradiol gave similar results to those adjusted for BMI alone.

Women currently using MHT were more likely to have a BMI <25 than those not on MHT (50% vs. 30%, respectively; $P < 0.0001$), suggesting MHT use may modify the association between adipokines and endometrial cancer risk. Therefore, associations for each adipokine, stratified by MHT use, are presented in Table 4. For these analyses, we combined never and former MHT users; however, similar results were observed when the population was restricted to never users only. Among women who were not using MHT at baseline, we observed stronger magnitudes of association than those for the overall population for adiponectin ($OR_{T3vsT1} = 0.25$; 95% CI, 0.08–0.75; $P_{trend} = 0.01$), leptin ($OR_{T3vsT1} = 4.72$; 95% CI, 1.15–19.38; $P_{trend} = 0.02$), and the ratio of adiponectin:leptin ($OR_{T3vsT1} = 0.15$; 95% CI, 0.03–0.61; $P_{trend} = 0.01$). Furthermore, the associations and linear trends remained statistically significant after adjustment for BMI and estradiol. No significant associations for adipokines were seen among women who were using MHT at baseline (Table 4).

Table 1. Baseline characteristics of study population

Characteristic	Incident endometrial cases ($n = 167$)	Matched controls ($n = 327$)	*P*-value[a]
	n (%)		
Age (at randomization)[b]			
<60	43 (25.8)	86 (26.3)	1.00
60–64	59 (35.3)	115 (35.2)	
65–69	48 (28.7)	92 (28.1)	
70+	17 (10.2)	34 (10.4)	
Race[b]			
White, non-Hispanic	157 (94.0)	309 (94.5)	0.94
Black, non-Hispanic	5 (3.0)	8 (2.5)	
Asian	5 (3.0)	10 (3.1)	
Time of blood draw[b]			
AM	103 (61.7)	203 (62.1)	0.93
PM	64 (38.3)	124 (37.9)	
Menopausal hormone use (MHT)[b]			
Never	62 (37.1)	125 (38.2)	0.68
Current	79 (47.3)	153 (46.8)	
Former	24 (14.4)	48 (14.7)	
Unknown	2 (1.2)	1 (0.3)	
BMI (kg/m^2)			
<25	46 (27.5)	142 (43.4)	<0.001
25–30	56 (33.5)	104 (31.8)	
30–35	29 (17.4)	59 (18.0)	
35+	36 (21.6)	22 (6.7)	
Education level			
Less than high school	7 (4.2)	14 (4.3)	0.38
High school or equivalent	34 (20.4)	89 (27.2)	
Post high school, not college	18 (10.8)	41 (12.5)	
Some college	50 (29.9)	72 (22.0)	
College graduate	33 (19.8)	60 (18.4)	
Postgraduate	25 (15.0)	51 (15.6)	
Number of live births			
0	23 (13.8)	34 (10.4)	0.38
1–2	48 (28.7)	86 (26.3)	
3+	96 (57.5)	207 (63.3)	
Smoking status			
Never	103 (61.7)	178 (54.4)	0.13
Current	8 (4.8)	30 (9.2)	
Former	56 (33.5)	119 (36.4)	
Oral contraceptive use	70 (41.9)	171 (52.3)	0.03
Family history of breast cancer	22 (13.3)	47 (14.4)	0.85
Family history of endometrial cancer	3 (1.8)	3 (0.9)	0.39
History of hypertension diagnosis	66 (39.5)	107 (32.7)	0.13
History of diabetes diagnosis	13 (7.8)	15 (4.6)	0.15
Age at diagnosis (years), mean ± SD (10th, 90th)	66.4 ± 5.7 (59, 74)		
Time from blood draw to diagnosis (years), mean ± SD (10th, 90th)	3.4 ± 2.8 (0.2, 7.3)		
Estradiol (pg/mL), median (10th, 90th)	20.3 (9.73, 47.2)	15.5 (6.6, 40.2)	<0.001[c]

[a]*P*-values based on χ^2 statistic.

[b]Matching factors. Other matching factors not included in the table were study center, study year of diagnosis, and year of blood draw; the distributions of these variables were not different between cases and controls.

[c]*P*-value based on Wilcoxon rank sum test for differences in medians.

Table 2. Median and interdecile range of serum adipokine levels by case status

Analyte	Incident endometrial cases ($n = 167$) Median (10^{th}, 90^{th})	Matched controls ($n = 327$) Median (10^{th}, 90^{th})	*P*-value[a]
Adiponectin (μg/mL)	12.16 (6.79, 22.92)	14.77 (7.49, 25.93)	0.002
Leptin (ng/mL)	19.82 (7.55, 57.44)	16.69 (5.98, 41.08)	<0.001
Visfatin (ng/mL)[b]	0.84 (0.41, 2.64)	0.76 (0.33, 1.77)	0.046

[a]*P*-value calculated by Wilcoxon rank sum test for differences in medians.
[b]Values based on 94 cases and 189 controls.

Discussion

In this case–control study, nested within the PLCO trial, we prospectively evaluated the relationship of prediagnostic serum levels of adiponectin, leptin, and visfatin with postmenopausal endometrial cancer risk, while also considering the role of circulating estradiol and BMI in these associations. In the overall population, elevated adiponectin and the ratio of adiponectin:leptin were associated with a significant decrease in endometrial cancer risk, whereas higher levels of leptin conferred an increased risk. These relationships remained significant after adjustment for estradiol, but not BMI. In addition, we observed evidence that MHT use modified the association of adiponectin and leptin with endometrial cancer. Stronger magnitudes of association were seen in women not currently using MHT, compared with the overall population. These estimates remained significant after adjustment for estradiol levels and BMI. However, no significant associations were observed in current MHT users.

Table 3. Multivariable adjusted associations of adipokine levels and endometrial cancer risk in the overall study population

	Case/control	Multivariable (MV) adjusted	MV and estradiol	MV and BMI	MV and BMI and estradiol
	N	OR (95% CI)			
Adiponectin (μg/mL)					
≤11.89	79/107	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
11.90–18.10	47/109	0.54 (0.34–0.87)	0.57 (0.35–0.92)	0.64 (0.39–1.05)	0.65 (0.40–1.08)
>18.10	41/111	0.48 (0.29–0.80)	0.53 (0.32–0.88)	0.71 (0.41–1.23)	0.73 (0.43–1.27)
P_{trend}		0.004	0.012	0.185	0.231
Leptin (ng/mL)					
≤11.03	34/109	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
11.04–22.55	52/109	1.61 (0.93–2.78)	1.59 (0.91–2.77)	1.20 (0.67–2.12)	1.21 (0.67–2.16)
>22.55	81/109	2.77 (1.60–4.79)	2.55 (1.46–4.46)	1.31 (0.65–2.64)	1.29 (0.64–2.61)
P_{trend}		<0.001	0.001	0.454	0.483
Visfatin (ng/mL)					
≤0.54	22/62	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
0.55–0.93	31/63	1.29 (0.55–3.01)	1.27 (0.54–2.97)	0.92 (0.37–2.30)	0.93 (0.37–2.30)
>0.93	41/64	1.67 (0.71–3.95)	1.68 (0.70–3.99)	1.25 (0.50–3.09)	1.24 (0.50–3.08)
P_{trend}		0.197	0.239	0.582	0.598
Adiponectin/leptin (μg)					
≤0.55	82/108	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
0.56–1.43	50/109	0.53 (0.32–0.89)	0.57 (0.34–0.96)	0.87 (0.48–1.56)	0.89 (0.50–1.61)
>1.43	35/110	0.35 (0.20–0.61)	0.39 (0.22–0.69)	0.74 (0.36–1.51)	0.78 (0.38–1.60)
P_{trend}		<0.001	0.001	0.409	0.504

Multivariable models: adjusted for family history of breast or endometrial cancer, education level, parity, history of diabetes diagnosis, oral contraceptive use, and current smoking status. OR and 95% CIs were estimated by conditional logistic regression (matched sets: 167 cases and 327 controls). Tertiles of adipokines based on the control distribution. Estradiol and BMI were included as continuous terms in the model.

Table 4. Multivariable adjusted associations of adipokine levels and endometrial cancer risk stratified by MHT use

	Noncurrent users[a]		Current users		
	Case/control *N*	MV and BMI and estradiol OR (95% CI)	Case/control *N*	MV and BMI and estradiol OR (95% CI)	*P*-value[b]
Adiponectin (μg/mL)					
≤11.89	53/57	Ref (1.00)	26/50	Ref (1.00)	0.005
11.90–18.10	23/57	0.56 (0.25–1.23)	22/51	0.67 (0.32–1.41)	
>18.10	10/59	0.25 (0.08–0.75)	31/52	1.19 (0.56–2.53)	
P_{trend}		0.011		0.645	
Leptin (ng/mL)					
≤11.03	6/46	Ref (1.00)	28/63	Ref (1.00)	0.003
11.04–22.55	21/61	2.00 (0.57–7.05)	30/47	1.18 (0.56–2.45)	
>22.55	59/66	4.72 (1.15–19.38)	21/43	0.70 (0.24–2.03)	
P_{trend}		0.019		0.640	
Visfatin (ng/mL)					
≤0.54	10/29	Ref (1.00)	12/33	Ref (1.00)	0.842
0.55–0.93	14/30	0.58 (0.08–4.06)	17/33	0.94 (0.16–5.37)	
>0.93	23/35	1.71 (0.15–20.12)	18/29	2.84 (0.53–15.11)	
P_{trend}		0.585		0.163	
Adiponectin/leptin (μg)					
≤0.55	62/65	Ref (1.00)	19/43	Ref (1.00)	<0.001
0.56–1.43	17/56	0.30 (0.10–0.84)	32/52	2.16 (0.86–5.43)	
>1.43	7/52	0.15 (0.03–0.61)	28/58	1.92 (0.62–5.91)	
P_{trend}		0.006		0.350	

MHT = menopausal hormone therapy; MV = multivariable: adjusted for family history of breast or endometrial cancer, education level, parity, history of diabetes diagnosis, oral contraceptive use, and current smoking status. OR and 95% CIs were estimated by conditional logistic regression (matched sets: 86 cases and 173 controls for never/former users and 79 cases and 153 controls for current users). Tertiles of adipokines based on the control distribution. Estradiol and BMI were included as continuous terms in the model.

[a]Includes never and former MHT users.

[b]*P*-value calculated from a likelihood ratio test comparing unconditional multivariable logistic regression models with and without an interaction term for the adipokine and current MHT use adjusting for matching factors and the variables included in the MV models.

Previous studies evaluating adiponectin and leptin levels and endometrial cancer risk have had various inclusion criteria for menopausal status and MHT use; one study included only postmenopausal women (13), whereas the others stratified by menopausal status (12, 14) and women were either matched on MHT use (14) or current users were excluded (12, 13). Overall, these studies suggest an inverse association of adiponectin levels with endometrial cancer risk, which is consistent with the results from this study, although some differences are noted. Specifically, in our stratified analysis, we observed significant associations of adiponectin and leptin with endometrial cancer after adjusting for estradiol and BMI. The 2 previous studies that excluded MHT users, the European Prospective Investigation into Cancer and Nutrition (EPIC) and the Breast and Bone Follow-up of the Fracture Intervention Trial (B∼FIT) reported a nonsignificant inverse association or no association among postmenopausal women who were not MHT users, respectively, after adjusting for BMI and/or estradiol (12, 13). Potential reasons for the observed discrepancies include differences in study population demographics, sample size, and assay limitations. Although the sample size for this study was similar to that of EPIC, the average level of adiponectin was much higher in our population (12 vs. 8 μg/mL, for cases in PLCO and EPIC, respectively). Similar results are seen when comparing tertiles with similar ranges of adiponectin levels between the 2 studies (second tertile of PLCO to third of EPIC). Reasons for differences in adiponectin levels between the study populations could be related to the assays used to measure adipokines (RIA vs. ELISA) or differences in population demographics such as BMI levels or other characteristics. Similarly, differences in observed associations reported from B∼FIT could be related to sample size, only 62 cases were included in B∼FIT (compared with 86 in the

stratified models for the current study) or population characteristics such as the older age of diagnosis for B∼FIT cases compared with this study (74 vs. 66 years). The only other prospective study of adiponectin, the Nurses' Health Study (NHS) did not report associations for postmenopausal women who were not MHT users; however, the results from the overall population in this study are similar to those reported for postmenopausal women in NHS.

Our observed association of leptin with endometrial cancer risk is generally consistent with the only other prospective study to evaluate this adipokine (13), which reported a nonsignificant positive association, after adjusting for BMI among postmenopausal women not on MHT. However, we observed a stronger elevated risk among women not on MHT, with over a 4-fold increased risk among women with leptin levels in the third tertile compared with the first. The median levels of leptin reported in B∼FIT were higher than those in the current population (25 ng/mL vs. 17 ng/mL, respectively), which could be related to the different assays used or population characteristics. When estimates from comparable leptin levels are compared between the 2 studies, similar results are observed. In addition, associations for the ratio of adiponectin:leptin with endometrial cancer reported in this study are similar with those reported in B∼FIT (13), however continued evaluation is needed to determine if this ratio is indeed a more sensitive measure of insulin resistance, as has been suggested (20, 21), and its relationship with endometrial cancer risk.

From our stratified analyses, we observed evidence that MHT use significantly modified the association of adiponectin and leptin with endometrial cancer risk, with significant associations observed only in non-MHT users. These results are similar to what has been reported for the relationship of BMI and MHT with endometrial cancer. The effect of BMI on endometrial cancer risk is restricted to women who have not used MHT (34) and conversely, MHT use has been shown to increase the risk of endometrial cancer among women with a normal body weight, whereas no additional risk was observed among obese women (35, 36). This suggests that MHT and BMI can influence endometrial cancer risk through similar pathways, namely estrogen-driven proliferation. Furthermore, these data indicate that including MHT users when evaluating hormonally related factors may mask potential associations.

Although the leading hypothesis for the obesity-related increase in endometrial cancer risk in postmenopausal women centers around elevated circulating estradiol levels through the aromatization of androgens in adipose tissue, other non-hormonal pathways may also be important (2). After accounting for estradiol levels and obesity (BMI), we observed a significant association of adiponectin and leptin with endometrial cancer risk among non-MHT users, suggesting adipokines may influence risk through mechanisms other than estrogen-mediated proliferation. Results from these stratified analyses should be interpreted with caution due to the limited range of BMI among the 2 strata of MHT use and the small number of non-MHT users in the lowest (leptin) and highest (adiponectin) tertiles, which is not surprising given the interrelatedness of BMI, MHT, and adipokines. These small numbers could lead to unstable estimates, which may not reflect the true association. However, the magnitudes of association for adiponectin and leptin were greater than that of BMI alone among non-MHT users (OR = 4.00, 4.72, and 3.07, respectively), further supporting potential roles for adipokines in non-hormonal pathways associated with cancer risk. Cell culture studies have implicated adiponectin in anti-inflammatory and insulin resistance pathways, whereas leptin has been shown to act in carcinogenic pathways such as cell proliferation and metastasis (1, 2). Future research into these functions could elucidate additional mechanisms of action for adipokines in endometrial carcinogenesis. It is also possible that adiponectin and leptin levels may serve as better markers of the biological mechanisms that underlie the obesity-related associations for endometrial cancer risk, especially among non-MHT users. Future studies are needed to distinguish between these possibilities.

Few epidemiological studies have evaluated the relationship of visfatin with cancer risk and none have examined endometrial cancer. Our analyses with visfatin were limited by inadequate serum volume to measure levels in all samples, which could have affected our power to detect associations. Nevertheless, median levels of visfatin were significantly higher among cases compared with controls. In addition, point estimates from regression analyses suggest increased cancer risk among the highest tertile compared to the lowest, which is in agreement with previous epidemiologic studies of visfatin and other obesity-related cancers (27–29). Continued evaluation is needed to fully understand the potential role of visfatin in endometrial carcinogenesis and its relationship to risk.

The main strengths of this study include the prospective nature of the PLCO trial, the measurement of multiple adipokines, and adjustment for key covariates for endometrial cancer such as BMI and estradiol. In addition, cases and controls were matched on time of day of blood draw, age, race, and several other important variables, limiting the possibility that these factors influenced the observed associations. Finally, this is the first study to report the association of visfatin with endometrial cancer risk.

Despite these strengths, we lacked information on other anthropometric measures of obesity, such as waist-to-hip ratio, which may be a more accurate measure of central adiposity than BMI. Other studies have not seen further attenuation of associations for adipokines and endometrial cancer when including both BMI and waist circumference in the model (12). We also did not have information on fasting status at the time of blood draw, which could influence adipokine levels. However, previous studies have shown that adiponectin levels are not significantly affected by fasting status (12, 37) and we matched on time of day of blood draw to minimize these effects. In addition, approximately 25% of the serum samples in this study were collected within one year

of cancer diagnosis, making it difficult to establish temporality. However, sensitivity analyses excluding these samples resulted in similar patterns. Finally, this study included a large portion of women who were current MHT users at baseline (46%), leading to small numbers of women in the stratified models and potentially unstable estimates of association.

In summary, this study supports the notion that adiponectin, leptin, and the ratio of the 2 may be important predictors of endometrial cancer risk. In contrast, in this first-time evaluation, visfatin did not seem related. Our study also expanded on previous knowledge by specifically assessing the potential interactions between MHT use and these analytes, showing that adiponectin and leptin were only related to risk among non-users of MHT. Together, these studies provide evidence for potential biological mechanisms that underlie the obesity-related endometrial cancer risk and suggest that in addition to estradiol, adiponectin, and leptin play an important role in endometrial carcinogenesis. Given the interrelatedness of adipokines with BMI and MHT, larger sample sizes from pooling efforts or consortia may be necessary to fully evaluate the roles of these adipokines in endometrial cancer development.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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