Obesity-related hormones and endometrial cancer among postmenopausal women: a nested case-control study within the B~FIT cohort

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Abstract

Endometrial cancer risk is strongly influenced by obesity, but the mechanisms of action remain unclear. Leptin and adiponectin, secreted from adipose tissue, reportedly play a role in such carcinogenic processes as cell proliferation, angiogenesis, and insulin regulation. In this case-control study, nested within the Breast and Bone Follow-up of the Fracture Intervention Trial (B~FIT) (n=15,595), we assessed pre-diagnostic serum leptin, total adiponectin, and high molecular weight (HMW) adiponectin in relation to endometrial cancer among postmenopausal women. During the 10-year follow-up, 62 incident endometrial cases were identified and matched to 124 controls on age, geographical site, time of fasting blood draw at baseline (1992-1993) and trial participation status. Adipokines and C-peptide were measured by enzyme-linked immunosorbent assays. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated via conditional logistic regression, with exposures categorized in tertiles (T). Multivariable models considered C-peptide, body mass index (BMI, kg/m^2) and estradiol (E2) as potential confounders. Endometrial cancer risk was significantly associated with higher leptin levels, adjusted for E2 and C-peptide (OR_{T3vsT1}=2.96, 95% CI: 1.21-7.25; *P*-trend<0.01). After further adjustment for BMI, the estimates were attenuated and the positive trend was no longer statistically significant (OR_{T3vsT1}=2.11, 95% CI: 0.69-6.44; *P*-trend=0.18). No significant associations were observed with adiponectin or HMW adiponectin and endometrial cancer. Our findings with leptin suggest that the leptin-BMI axis might increase endometrial cancer risk through mechanisms other than estrogen-driven proliferation. Continued exploration of these pathways in larger prospective studies may help elucidate mechanisms underlying observed obesity-endometrial cancer associations.

Introduction

Obesity is a well-established risk factor for endometrial cancer. Among postmenopausal women, this association may in part be explained by the increase in circulating estrogens that arise from the aromatization of androgens in adipose tissue (Calle and Kaaks 2004) or through mechanisms involving insulin (Lukanova, et al. 2004b) or adipose-derived hormones (van Kruijsdijk, et al. 2009). Adipose tissue, an active endocrine organ, also produces and secretes several bioactive peptides, including such adipokines as leptin and adiponectin (van Kruijsdijk et al. 2009).

Leptin, a product of the *ob* gene, is involved in the regulation of body weight, energy balance, and reproductive function (Paracchini, et al. 2005). Results from in vitro studies suggest that leptin exhibits both mitogenic and anti-apoptotic effects, depending on the cell line (Somasundar, et al. 2003). Leptin concentrations are elevated among obese individuals (Friedman and Halaas 1998) and are positively associated with endometrial cancer based on findings from case-control studies (Ashizawa, et al. 2010; Cymbaluk, et al. 2008; Petridou, et al. 2002). To date, no prospective studies have evaluated leptin in relation to endometrial cancer.

Contrary to leptin, adiponectin has been shown to decrease blood glucose and insulin concentrations (Lihn, et al. 2005); have anti-inflammatory, antiangiogenic and proapoptotic properties (Fantuzzi 2005; Roberts, et al. 2010); and be inversely correlated with obesity (Arita, et al. 1999). Epidemiologic studies that have assessed adiponectin in relation to endometrial cancer risk have generally found higher levels associated with reduced risks (Ashizawa et al. 2010; Cust, et al. 2007; Dal Maso, et al. 2004; Petridou, et al. 2003; Soliman, et al. 2006). However, several of these results derive from case-control studies (Ashizawa et al. 2010; Dal Maso et al. 2004; Petridou et al. 2003; Soliman et al. 2006), which utilized post-diagnostic blood samples and were unable to establish temporality. Only two of these case-control studies included measures of both leptin and adiponectin (post-diagnostic) (Ashizawa et al. 2010; Petridou et al. 2003), of which only one assessed the ratio of these two adipokines (Ashizawa et

al. 2010). The ratio of adiponectin to leptin may be a more sensitive parameter of insulin resistance than the individual adipokines (Cleary, et al. 2009; Inoue, et al. 2005); however, this ratio measure has yet to be evaluated in prospective studies of endometrial cancer. To date, only two prior prospective studies have evaluated pre-diagnostic adiponectin in relation to endometrial cancer risk, with one observing an inverse relation (Cust et al. 2007) and the other reporting no association (Soliman, et al. 2011). Recent evidence also suggests that high molecular weight (HMW) adiponectin may be more biologically active than total adiponectin (Pajvani, et al. 2004); however, prior studies have solely measured total adiponectin.

Within the Breast and Bone Follow-up of the Fracture Intervention Trial (B~FIT), we assessed pre-diagnostic leptin, total adiponectin and HMW adiponectin, and their ratios, in relation to incident endometrial cancer risk while also accounting for circulating estrogen and C-peptide levels.

Materials and Methods

We conducted a nested case-control study within B~FIT, a longitudinal cohort of participants screened for the Fracture Intervention Trial (FIT). FIT, which has previously been described (Black, et al. 1993), was a randomized, placebo-controlled trial designed to test whether alendronate, a bispohosphonate, would reduce the rate of fractures in women with low bone mineral density (Black et al. 1993). In 1992-1993, 22,695 postmenopausal women (ages 55-80) were screened for participation at 11 clinical centers in the United States. Potential participants underwent a bone mineral density scan, donated a baseline serum sample, provided clinical examination data (including measured anthropometric and blood pressure), and completed an extensive health history questionnaire that ascertained information on demographic, lifestyle, hormonal, and reproductive factors. Serum samples were originally stored at -20 °C for 3 years and then transferred to -70 °C for long-term storage. Primary results from FIT were reported in

1996 (Black, et al. 1996) and 1998 (Cummings, et al. 1998), and a subset of participants who had used alendronate for at least three years were invited to participate in the FIT Long Term Extension Trial (FLEX) (Black, et al. 2006).

B~FIT is a longitudinal cohort comprised of FIT screenees (N=15,595) from 10 of the original 11 FIT clinical centers; 1 clinic declined to participate in the follow-up study. Vital status and cause of death of screenees from the 10 participating clinics was determined using the National Death Index (NDI). From 2001 to 2004, surviving screenees were contacted by mail and/or telephone and invited to complete a follow-up questionnaire (64% of eligible women completed the BFIT questionnaire) that provided additional information on cancers, other health outcomes and reproductive surgeries that occurred since they were screened for FIT, family history of cancer, detailed hormone use, and preventive screening procedures. Women who reported an incident cancer or fracture were asked to give permission for medical record review of those events. In addition, women from the three clinical sites located in Surveillance Epidemiology and End Results (SEER) registry areas (Northern California, Washington, and Iowa) were linked to the cancer registry to identify and confirm cancer diagnoses. All women provided written informed consent. B~FIT was approved by the Institutional Review Board (IRB) of each participating site and the University of California, San Francisco Coordinating Center, as well as the National Cancer Institute.

Endometrial cancer ascertainment

Data on incident endometrial cancer were ascertained from the cancer registry linkages, medical reports, linkage with the National Death Index (NDI) for underlying cause of death, and self-report on the B~FIT follow-up questionnaire. Of the 81 cases of endometrial cancer identified among B~FIT participants since their screening visits, 93.8% were confirmed by medical record or linkage.

This analysis excluded cases based on the following criteria: baseline serum samples were unavailable or unusable (n=14); personal history of any cancer (other than non-melanoma skin cancer) before FIT baseline (n=2); non-Caucasian race (n=1); and missing BMI information (n=2). The final analysis included 62 endometrial cancer cases. No cases or controls reported using postmenopausal estrogens (oral, injection, or patch) within four months of their FIT interview/blood draw.

Selection of controls

Controls were chosen from among a subcohort of 515 B~FIT participants who were selected for analyses that utilized the archived serum specimens. This subcohort was randomly selected within 10-year age and clinic strata. The subcohort was further restricted to women with an intact uterus at FIT baseline and exclusions were applied based on the criteria described above (i.e., personal history of cancer before baseline, estrogen use within 4 months of baseline, unavailable/unusable samples, non-Caucasian race, or missing BMI data). For each case, we randomly selected two matched controls from among eligible non-cases who were alive and had not been diagnosed with endometrial, breast, ovarian, or colorectal cancer before the date of diagnosis of the case. Matching occurred in order of the following: (1) geographical site (10 FIT clinics), (2) age at baseline/blood draw ± 5 years, (3) time of blood draw ± 2 hours, and (4) trial participation status (screenee-only, FIT participant, FLEX participant) (this matching criteria was relaxed as necessary to select appropriate controls). Two matched controls were identified for 55 cases and one matched control for 6 cases. No eligible controls were available for one case; eight controls were selected for four cases that were later found to be ineligible. As adipokine measurements were available for these nine eligible women, we included them in the analysis. The final study population included 62 endometrial cancer cases and 124 controls.

Laboratory assays

Leptin, adiponectin (total and HMW), and C-peptide assays were conducted in the laboratory of Dr. Michael Pollak (Jewish General Hospital, Montreal, Quebec, Canada). Fasting serum concentrations of leptin, adiponectin (total and HMW), and C-peptide were measured in duplicate using standard commercially available enzyme-linked immunosorbent assays (ELISA); reagents of these assays were supplied from Millipore Corporation, Billerica, MA.

All samples were analyzed using single kit lots. Cases and their matched controls were run on the same plates. Laboratory personnel were blinded to case status of samples. In addition to the laboratory's quality control (QC) samples, three blinded QC samples were included within each batch. Coefficients of variation (within- and between-batch) from masked QC samples ranged from 3.0 -11.3 for all analytes. The ranges of detection were: leptin, 0.5 – 100 ng/ml; adiponectin, 787.80 – 50,500 ng/ml; HMW adiponectin, 312 – 50,500 ng/ml; and C-peptide, 0.2 – 20 ng/ml. For one subject, the C-peptide value was set to the lowest detection limit (0.2 ng/ml). Circulating total estradiol, estrone and estriol were measured using liquid chromatography mass spectrometry; the published lower limit of quantitation for these serum estrogens is 8 pg/ml(Xu, et al. 2007). Coefficients of variation (within- and between-batch) for serum estrogens were <1%.

Statistical Analysis

Differences in baseline characteristics and circulating analyte levels by case status were assessed using t-tests, Wilcoxon Mann-Whitney, Kruskal Wallis or Chi-square tests as appropriate. Spearman partial correlations, adjusting for age at blood draw, were estimated for the associations between analytes, BMI, waist circumference, and circulating estradiol, estrone, and estriol. Conditional logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the relationship between each analyte and endometrial cancer.

Tertile categories for each analyte were determined based on the distribution among the controls. In addition to levels of leptin, adiponectin, and HMW adiponectin, we also assessed the ratio of adiponectin to leptin and HMW adiponectin to leptin. Multivariable models were fitted to assess whether each adipokine was associated with endometrial cancer risk, after adjusting for estradiol, C-peptide and BMI. The multivariable models included adjustment for (1) estradiol only, (2) BMI only, (3) BMI and estradiol, (4) estradiol and C-peptide, and (5) BMI, estradiol and C-peptide. Models were adjusted for estradiol rather than estrone or estriol due to the strong main effect of estradiol on endometrial cancer in this study population. C-peptide was included as an adjustment factor to account for insulin secretion. Similar models were performed using waist to hip ratio (WHR) as the adjustment measure in lieu of BMI. WHR was categorized based on the tertile distribution among controls and missing values were retained in the model as a separate category. Additional adjustment for other baseline covariates (diabetes, hypertension, smoking, gravidity, number of live births, and postmenopausal hormone use) did not substantially alter the results and were not included in the final models. Tests for linear trend were assessed by modeling each exposure as an ordinal variable (coded as 0,1, 2). All statistical analyses were performed using the SAS software package, version 9.2 (SAS Institute, Cary, NC). All *P*-values were two-sided.

Results

On average, cases and controls were 67 years of age at blood draw (Table 1). Among cases, the mean age at endometrial cancer diagnosis was 73.8 ± 5.7 years while the interval between blood draw and diagnosis was 6.4 ± 3.2 years. Cases were more likely to be obese (40% versus 22%, p=0.01), to have reported a history of diabetes and hypertension at baseline, to have never smoked and to have never been pregnant as compared to controls (Table 1). Distributions of each adipokine and C-peptide were similar for cases and controls with the exception of leptin

(Table 2); median leptin levels (ng/ml) were significantly higher for cases than controls [median (10^{th}, 90^{th}): 42.4 (12.5, 92.7) and 25.1 (8.3, 65.7), respectively; p=0.005].

Spearman partial correlation coefficients, adjusted for age, showed that serum leptin levels were positively correlated with BMI (r=0.73, p <.0001) whereas total adiponectin and HMW adiponectin were negatively correlated with BMI (r=-0.24 and -0.25, respectively, p<.01). Circulating total adiponectin was highly correlated with HMW adiponectin (r=0.95, p<.0001) and both analytes shared similar correlations with the remaining analytes and anthropometric measures (Table 3).

Crude and adjusted odds ratios from conditional logistic regression models for each adipokine and BMI in relation to endometrial cancer are described in Table 4. Higher BMI was associated with increased endometrial cancer risk (*P*-trend=0.02); women with a BMI of 30 kg/m^2 or greater were 2 times as likely to develop endometrial cancer as compared to women with a BMI < 25 kg/m^2 (OR_{T3vsT1}=2.44, 95% CI: 1.14, 5.21), even after adjustment for estradiol and C-peptide (*P*-trend=0.05). In crude models, higher serum leptin levels were associated with a significant increase in endometrial cancer risk (OR_{T3vsT1}=3.29 (95% CI: 1.41, 7.69; *P*-trend=0.004); this positive dose-response persisted even after adjustment for estradiol and C-peptide (OR_{T3vsT1}=2.96 (95% CI: 1.21, 7.25; *P*-trend=0.02). However, in models adjusted only for BMI, this association was attenuated and the linear trend was no longer statistically significant (OR_{T3vsT1}=2.11, 95% CI: 0.69-6.44; *P*-trend=0.18). Models adjusted for (1) BMI and estradiol or (2) BMI, estradiol and C-peptide resulted in similar findings as those observed in the models adjusted only for BMI (data not shown). Similar results were observed when models were adjusted for WHR in lieu of BMI; WHR was not significantly associated with endometrial cancer risk in this study (data not shown). Leptin remained positively associated with increased risk after adjustment for WHR (OR_{T3vsT1}=3.70, 95% CI: 1.4-9.2; *P*-trend=0.004) and in models adjusted for WHR, estradiol and c-peptide (OR_{T3vsT1}=2.71, 95% CI: 1.00-7.33; *P*-

trend=0.009).No statistically significant associations were observed between total adiponectin or HMW adiponectin and endometrial cancer risk (Table 4). However, in analyses examining the ratio of total adiponectin to leptin or HMW adiponectin to leptin, significant inverse trends were observed (*P*-trend=0.02 and 0.005, respectively). A higher adiponectin to leptin ratio was associated with decreased endometrial cancer risk in crude and estradiol adjusted models (estradiol adjusted OR_{T3vsT1}=0.43, 95% CI=0.18, 1.01; *P*-trend=0.03) while an elevated HMW adiponectin to leptin ratio was inversely related to endometrial cancer risk even after adjustment for both estradiol and C-peptide (OR_{T3vsT1}=0.38, 95% CI=0.15, 0.94; *P*-trend=0.02). However, the linear trends were no longer statistically significant in models adjusted for BMI. Findings from models adjusted for WHR instead of BMI are similar to the results of crude models presented in Table 4 (data not shown).

Discussion

In this nested-case control study, we evaluated leptin, total adiponectin, and HMW adiponectin in relation to postmenopausal endometrial cancer risk while adjusting for markers of other possible biological pathways underlying the obesity-endometrial cancer association. We observed significantly increased endometrial cancer risk with increasing levels of leptin and the ratio of either total adiponectin or HMW adiponectin to leptin. Women in the highest tertile of circulating leptin had approximately three times the risk of women in the lowest tertile. This significant positive trend persisted after adjustment for estradiol and C-peptide but was attenuated and no longer statistically significant once BMI was included in the model. Furthermore, women in the highest tertile of either ratio had an approximate 60% reduction in endometrial cancer risk after adjustment for estradiol. While we adjusted models for BMI for comparability of results with prior studies, adipokines and BMI are correlated; further, BMI is on the causal pathway for endometrial cancer and inclusion as a covariate may result in over

adjustment. This is particularly relevant for the leptin models given that leptin and BMI were highly correlated.

Despite the correlation between leptin and BMI in our analysis, endometrial cancer was more strongly associated with leptin than BMI, even after adjustment for estradiol and C-peptide. Data from prior studies are limited and based on case-control analyses, where samples were collected after diagnosis (Ashizawa et al. 2010; Cymbaluk et al. 2008; Petridou et al. 2002), but generally support a positive association with leptin, such as the three-fold increase in endometrial cancer risk for women with leptin levels in the highest tertile reported by Ashizawa et al. (Ashizawa et al. 2010) However, these prior case-control studies measured post-diagnostic leptin levels, which raises questions about the extent to which levels may have been affected by the presence of endometrial cancer. Our prospective cohort study measured leptin in pre-diagnostic samples collected well before diagnosis. Thus, the positive association with leptin in our study adds methodologically rigorous support for a potential role of leptin in endometrial carcinogenesis.

With regards to total adiponectin and HMW adiponectin, no statistically significant associations were observed in this study. Only two prior prospective studies of adiponectin and endometrial cancer have been conducted (Cust et al. 2007; Soliman et al. 2011), with inconsistent results reported, and further, no prior studies of endometrial cancer have measured the HMW isoform. Within the Nurses' Health Study (NHS), Soliman et al. observed no association between adiponectin and endometrial cancer (n=146 cases), either before or after adjustment for BMI (Soliman et al. 2011). Within the European Prospective Investigation into Cancer and Nutrition (EPIC) (Cust et al. 2007) (n=284 cases), an approximate 50% reduction in endometrial cancer risk was seen among women in the highest quartile of plasma adiponectin compared to those in the lowest, after adjustment for BMI, waist circumference and various obesity-related circulating hormones including c-peptide, IGF binding protein-1 and 2, estrone

and testosterone. Findings from retrospective studies (Ashizawa et al. 2010; Dal Maso et al. 2004; Petridou et al. 2003; Soliman et al. 2006) also support inverse associations (~50% reductions) with higher levels of post-diagnostic total adiponectin. Possible explanations for the lack of an association with total or HMW adiponectin in our prospective study may include the time interval between blood draw and endometrial cancer development, as was also suggested by Soliman et al. (2011). In the present analysis, the time between blood draw and diagnosis was on average 6 years (interval: 1.8, 10.3) which is comparable with the NHS (average= 7.4; interval (2-13) but not with EPIC (average=3.0; interval: 47, 71.0); findings from EPIC support significant reductions in risk. Although the use of pre-diagnostic serum and the time interval between blood draw and endometrial cancer development reduces the possibility of reverse causation in our study, a shorter time interval may reflect changes in insulin levels associated with pre-clinical disease. The longer interval in our study may in part explain our null findings. However, more research on the representativeness of adiponectin levels over time and in relation to insulin markers is needed.

In our prospective study, higher ratios of total adiponectin to leptin and HMW adiponectin to leptin were associated with a lower risk of endometrial cancer. Both ratios resulted in similar reductions in risk, suggesting that either marker of adiponectin, when measured in relation to leptin, captures relevant biologic exposures and may provide more information than that of the individual adipokines alone. Only one prior study (n=146 cases) has evaluated the ratio of these adipokines using post-diagnostic serum (Ashizawa et al. 2010) with results suggesting a 6-fold increase in endometrial risk with a higher leptin to adiponectin ratio. Recent evidence suggests that the ratio of these adipokines may better predict insulin sensitivity (Ashizawa et al. 2010; Inoue et al. 2005) and further, that the balance of these markers, rather than their individual levels, may be important for carcinogenesis (Cleary et al. 2009).

Multiple inter-related pathways are likely to explain the association between obesity and increased endometrial cancer risk, including sex steroids, insulin, inflammation, growth factors, and adipokines (Calle and Kaaks 2004). Circulating estradiol is strongly associated with increased endometrial cancer risk among postmenopausal women, as observed in our study as well as others (Kaaks, et al. 2002; Lukanova, et al. 2004a). Although this strong estrogen link supports aromatization as a possible explanation for the increased risk of endometrial cancer with obesity, studies have shown significant independent effects of obesity on endometrial cancer even after adjustment for sex hormones (Calle and Kaaks 2004; Potischman, et al. 1996). Our observed positive association between leptin and endometrial cancer persisted after adjustment for estradiol but was notably attenuated after adjustment for BMI. This suggests that the leptin-BMI axis might also increase endometrial cancer risk through mechanisms other than estrogen-driven proliferation.

Findings from cell studies support a direct role of leptin on carcinogenic processes such as mitogenesis, angiogenesis and inflammation (Renehan, et al. 2008; Roberts et al. 2010). Leptin synthesis in adipocytes is regulated by various hormones implicated in these processes, including insulin, glucocorticoids, tumor necrosis factor alpha (TNF-α), and reproductive hormones (Garofalo and Surmacz 2006). Although elevated circulating insulin and glucose levels have been associated with increased endometrial cancer risk, these markers do not fully account for the association between obesity, as measured by BMI, and endometrial cancer risk (Troisi, et al. 1997). Other obesity-related hormones, such as leptin and adiponectin, are strong candidates in these processes, as adiponectin levels have been shown to predict insulin resistance and have been suggested to reduce endometrial cancer risk by decreasing circulating insulin and glucose levels. The potential mechanisms underlying the obesity-endometrial cancer associations are complex, but our findings suggest that these adipokines may operate independent of circulating estradiol.

Strengths of this study include the use of pre-diagnostic serum, prospective follow-up of approximately 10 years, the inclusion of multiple adipokines and adjustment for circulating C-peptide and estradiol, important biomarkers of the insulin and sex steroid pathways. Cases and controls were matched on age and time of blood draw, thus minimizing the potential for these factors to have influenced any observed differences in disease status. Despite these strengths, the limited sample size of this analysis may have hindered our ability to detect significant differences between the circulating markers and endometrial case status, particularly adiponectin and HMW adiponectin. Additionally, we did not have detailed information on histology for the endometrial cases, which precluded us from evaluating potential differences between type I and type II tumors. Adipokine levels were measured at baseline and may not be reflective of exposure over the duration of the follow-up period, a common limitation with prospective biomarker studies. However, Kaplan et al. found that both adiponectin and leptin appear to be relatively stable during a three year period (ICC=0.73 and 0.58, respectively) (Kaplan, et al. 2007).

In summary, findings from this prospective study support the notion that higher leptin levels may be predictive of endometrial cancer risk among postmenopausal women and further, that the ratio of adiponectin (total and HMW) to leptin may also be informative in studies of endometrial cancer. Continued exploration of these adipokines in larger prospective studies may help elucidate mechanisms underlying observed obesity-endometrial cancer associations.

Declaration of Interest: Dr. Chia is currently an employee and shareholder of Amgen, Inc. The authors declare no conflict of interest.

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Table 1. Baseline characteristics of incident endometrial cases and controls

Characteristic	Incident Endometrial Cases (n=62)	Matched Controls (n=124)	*P*-value[1]
	N (%)	N (%)	
	mean ± SD (range: 10, 90%)		
Age at blood draw (y)[3]	67.4 ± 5.5 (59, 74)	67.5 ± 5.1 (60, 74)	0.94
Age at diagnosis (y)	73.8 ± 5.7 (66, 80)		
Years since menopause	17.3 ± 6.2 (11.3, 23.4)	18.6 ± 8.0 (9.2, 29.0)	0.20
Years between blood draw and diagnosis	6.4 ± 3.2 (1.8, 10.3)		
BMI (kg/m^2)	29.5 ± 6.9	26.8 ± 4.7	0.006
Waist (cm)[4]	100.5 ± 18.5	96.1 ± 14.1	0.13
Waist-to-hip ratio[4]	0.92 ± 0.1	0.92 ± 0.09	0.93
		N (%)	
BMI (kg/m^2)			
< 25	19 (30.7)	50 (40.3)	
25-29.9	18 (29.0)	47 (37.9)	
30-34.9	11 (17.7)	19 (15.3)	
35+	14 (22.6)	8 (6.5)	0.01
Trial participation status[3]			
Screenee-only	55 (88.7)	89 (71.8)	
FIT	6 (9.7)	23 (18.5)	
FIT/FLEX	1 (1.6)	12 (9.7)	0.02
History of diabetes	8 (12.9)	3 (2.4)	0.006[2]
History of hypertension	32 (51.6)	36 (29.0)	0.003
Measured hypertension	8 (12.9)	13 (10.5)	0.65
Smoking status			
Never	38 (61.3)	63 (50.8)	
Former	23 (37.1)	48 (38.7)	
Current	-	12 (9.7)	0.03
Ever pregnant			
Yes	51 (82.3)	116 (93.5)	
No	11 (17.7)	8 (6.5)	0.02
Ever used postmenopausal estrogen pills			
Yes	23 (37.1)	32 (25.8)	
No	39 (62.9)	92 (74.2)	0.11
Ever used postmenopausal progestin pills			
Yes	12 (19.4)	11 (8.9)	
No	50 (80.6)	111 (89.5)	0.05

[1]*P*-values calculated using t-tests for continuous variables and X^2 for categorical; [2]exact test

[3]Age at blood draw and trial participation status were matching factors. FIT=participant in the Fracture Intervention Trial; FIT/FLEX=participant in FIT and the Long Term Extension of the FIT Trial (FLEX)

[4]Waist circumference missing for 24 subjects (6 cases, 18 controls)

Note: Missing values included in the denominator for calculation of above percentages.

Table 2. Distribution of serum adipokines and c-peptide by case-control status

Analyte	Incident Endometrial Cases (n=62)	Matched Controls (n=124)	*P*-value[1]
	Median (10^{th}, 90^{th})		
Leptin (ng/ml)	42.4 (12.5, 92.7)	25.1 (8.3, 65.7)	0.005
Total Adiponectin (ug/ml)	14.3 (6.7, 26.0)	14.6 (11.0, 30.6)	0.47
HMWAdiponectin (ug/ml)	8.3 (3.5, 17.3)	8.8 (3.7, 20.1)	0.37
C-peptide (ng/ml)	2.3 (1.1, 5.2)	2.0 (0.96, 4.1)	0.14

[1]Wilcoxon rank sum test for differences in medians

Table 3. Spearman partial[1] correlations of serum adipokines, C-peptide, estrogens, and anthropometrics among controls (n=106)[2]

Analytes[3]	Leptin	Total Adiponectin	HMW Adiponectin	C-peptide	BMI (kg/m^2)	Waist (cm)
			r (p-value)			
Leptin	—	-0.26 (0.008)	-0.31 (0.002)	0.38 (<.0001)	0.73 (<.0001)	0.69 (<.0001)
Total Adiponectin	—	—	0.95 (<.0001)	-0.44 (<.0001)	-0.24 (0.005)	-0.35 (0.0002)
HMW Adiponectin	—	—	—	-0.44 (<.0001)	-0.25 (0.009)	-0.36 (0.0002)
C-peptide	—	—	—	—	0.26 (0.007)	0.31 (0.002)
BMI (kg/m^2)	—	—	—	—	—	0.84 (<.0001)
Estrone	0.55 (<.0001)	-0.40 (<.0001)	-0.43 (<.0001)	0.30 (0.002)	0.48 (<.0001)	0.51 (<.0001)
Estradiol	0.47 (<.0001)	-0.41 (<.0001)	-0.42 (<.0001)	0.17 (0.08)	0.42 (<.0001)	0.45 (<.0001)
Estriol	0.53 (<.0001)	-0.44 (<.0001)	-0.47 (<.0001)	0.27 (0.005)	0.46 (<.0001)	0.54 (<.0001)

[1]Spearman partial correlation coefficients adjusted for age at blood draw
[2]Waist circumference missing for 18 controls
[3]Leptin and c-peptide (ng/ml); Total adiponectin and HMW adiponectin (ug/ml); Estrone, estradiol and estriol

Table 4. Odds ratios[1] and 95% confidence limits from conditional logistic regression models estimating the association between BMI, circulating adipokine levels and endometrial cancer risk among postmenopausal women

	Cases/ Controls	OR (95% CI)	Estradiol Adj. OR (95% CI)	Estradiol & C-peptide Adj. OR (95% CI)	BMI adjusted OR (95% CI)
BMI (kg/m^2)					
< 25	19/50	1.00	1.00	1.00	
25-29.9	18/47	1.06 (0.46, 2.48)	1.03 (0.44, 2.42)	1.05 (0.45, 2.46)	
≥ 30	25/27	2.44 (1.14, 5.21)	2.30 (1.05, 5.04)	2.19 (0.99, 4.83)	
P-trend		0.02	0.03	0.05	
Leptin (ng/ml)					
≤ 18.29	13/41	1.00	1.00	1.00	1.00
18.30-35.0	15/41	1.33 (0.54, 3.25)	1.30 (0.53, 3.19)	1.29 (0.53, 3.18)	1.13 (0.44, 2.88)
≥ 35.10	34/42	3.29 (1.41, 7.69)	3.13 (1.31, 7.48)	2.96 (1.21, 7.25)	2.11 (0.69, 6.44)
P-trend		0.004	0.007	0.01	0.18
Total Adiponectin (ug/ml)					
≤ 11.67	19/41	1.00	1.00	1.00	1.00
11.68-18.0	24/40	1.37 (0.65, 2.89)	1.48 (0.69, 3.18)	1.99 (0.85, 4.67)	1.55 (0.70, 3.43)
≥ 18.1	19/43	0.87 (0.39, 1.94)	1.00 (0.44, 2.31)	1.45 (0.56, 3.72)	1.31 (0.55, 3.12)
P-trend		0.78	0.96	0.47	0.51
HMW Adiponectin (ug/ml)					
≤ 6.64	23/41	1.00	1.00	1.00	1.00
6.65-11.49	21/40	0.95 (0.44, 2.04)	0.92 (0.42, 1.98)	1.07 (0.47, 2.43)	0.94 (0.43, 2.08)
≥ 11.50	18/43	0.62 (0.27, 1.41)	0.66 (0.28, 1.52)	0.82 (0.32, 2.08)	0.87 (0.37, 2.09)
P-trend		0.26	0.33	0.67	0.76
Ratio of Total adiponectin to Leptin (ug/ng)					
≤ 0.34	31/42	1.00	1.00	1.00	1.00
0.35-0.83	15/40	0.56 (0.27, 1.18)	0.57 (0.27, 1.21)	0.61 (0.28, 1.33)	0.85 (0.36, 2.02)
≥ 0.84	16/42	0.40 (0.17, 0.90)	0.43 (0.18, 1.01)	0.46 (0.18, 1.16)	0.76 (0.26, 2.24)
P-trend		0.02	0.03	0.08	0.61
Ratio of HMW Adiponectin to Leptin (ug/ng)					
≤ 0.22	34/40	1.00	1.00	1.00	1.00
0.22-0.51	12/41	0.42 (0.19, 0.91)	0.42 (0.19, 0.93)	0.43 (0.19, 1.00)	0.54 (0.23, 1.27)
> 0.52	16/43	0.35 (0.16, 0.79)	0.37 (0.16, 0.87)	0.38 (0.15, 0.94)	0.56 (0.20, 1.58)
P-trend		0.005	0.009	0.02	0.21

[1] Cases matched to controls on age at baseline/blood draw ± 5 years, clinic site, time of blood draw ± 2 hours, trial participation status; controls selected from non-cases alive and disease free at the time of diagnosis of the case and with an intact uterus at FIT baseline**.** Odds ratios and 95% confidence limits estimated by conditional logistic