A Prospective Evaluation of Insulin and Insulin-like Growth Factor-I as Risk Factors for Endometrial Cancer

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Abstract

Obesity is a major risk factor for endometrial cancer, a relationship thought to be largely explained by the prevalence of high estrogen levels in obese women. Obesity is also associated with high levels of insulin, a known mitogen. However, no prospective studies have directly assessed whether insulin and/or insulin-like growth factor-I (IGF-I), a related hormone, are associated with endometrial cancer while accounting for estrogen levels. We therefore conducted a case-cohort study of incident endometrial cancer in the Women's Health Initiative Observational Study, a prospective cohort of 93,676 postmenopausal women. The study involved all 250 incident cases and a random subcohort of 465 subjects for comparison. Insulin, total IGF-I, free IGF-I, IGF-binding protein-3, glucose, and estradiol levels were measured in fasting baseline serum specimens. Cox models were used to estimate associations with endometrial cancer, particularly endometrioid adenocarcinomas, the main histologic type (n = 205). Our data showed that insulin levels were positively associated with endometrioid adenocarcinoma [hazard ratio contrasting highest versus lowest quartile (HR_{q4-q1}), 2.33; 95% confidence interval (95% CI), 1.13-4.82] among women not using hormone therapy after adjustment for age and estradiol. Free IGF-I was inversely associated with endometrioid adenocarcinoma (HR_{q4-q1}, 0.53; 95% CI, 0.31-0.90) after adjustment for age, hormone therapy use, and estradiol. Both of these associations were stronger among overweight/obese women, especially the association between insulin and endometrioid adenocarcinoma (HR_{q4-q1}, 4.30; 95% CI, 1.62-11.43). These data indicate that hyperinsulinemia may represent a risk factor for endometrioid adenocarcinoma that is independent of estradiol. Free IGF-I levels were inversely associated with endometrioid adenocarcinoma, consistent with prior cross-sectional data.

Introduction

Endometrial cancer is the seventh most common malignancy worldwide (1). In the United States, ~ 39,080 new cases are expected during 2007 (2), and because obesity is a major risk factor for endometrial cancer, the incidence of these tumors may soon rise due to the epidemic of obesity in this country. The relationship between obesity and endometrial cancer, particularly endometrioid adenocarcinomas, is thought to largely, but not fully, be explained by the elevated estrogen levels in obese women (3-5). To better understand the relation of obesity with endometrial cancer, we sought to identify additional endocrinologic factors that are common in obese women and that, independent of estrogen, might be associated with the risk of endometrial tumors.

Recent hypotheses regarding the obesity–endometrial cancer relationship have focused particularly on hyperinsulinemia. Obesity is associated with high levels of insulin, a known mitogen with antiapoptotic activity, and endometrial cancer cell lines express high-affinity insulin receptors (6). Insulin-like growth factor-I (IGF-I), which shares extensive amino acid sequence homology and downstream signaling pathways with insulin, has similarly garnered research interest as a potential risk factor for endometrial cancer, especially because IGF-I has much stronger mitotic and antiapoptotic activity than insulin (7, 8). Most IGF-I in circulation is produced by the liver and circulates bound to IGF-binding proteins (IGFBP), with 75% bound specifically to IGFBP-3. Only 1% of IGF-I circulates free (unbound). However, as with estrogen, the free fraction may be the most biologically active (9).

Epidemiologic data regarding insulin and IGF-I as risk factors for endometrial cancer are limited, and no prospective study, to our knowledge, has directly assessed insulin and IGF-I in relation to endometrial cancer while accounting for endogenous estrogen levels.

This is critical, given the mutual association of hyperinsulinemia and high estrogen levels with obesity. Two recent prospective investigations of endometrial cancer that lacked fasting blood specimens reported positive associations between C-peptide levels (a marker of pancreatic insulin secretion) and endometrial cancer risk (10, 11), including one that also controlled for endogenous estrogen levels (10). In that study, adjustment for free estradiol levels attenuated the positive association of C-peptide with endometrial cancer. However, the assessment of C-peptide in nonfasting specimens has limitations; that is, C-peptide levels increase substantially postprandially (12) and there is an imperfect correlation between C-peptide and insulin (the putative carcinogenic agent), even when assessed in fasting specimens (13). Hence, further investigation of the insulin–endometrial cancer relationship is warranted. The current study therefore examined the associations of insulin, IGF-I, and estradiol with endometrial cancer incidence using specimens and data obtained from the Women's Health Initiative Observational Study (WHI-OS), a large prospective cohort investigation of postmenopausal women.

Materials and Methods

Study Population

Women's Health Initiative Observational Study. The WHI-OS is a longitudinal cohort of 93,676 postmenopausal women ages 50 to 79 years who were recruited at 40 different clinical centers across the United States between October 1, 1993, and December 31, 1998 (14, 15). At baseline, women gave informed consent and completed detailed questionnaires regarding medical and behavioral history, hormone and medication use, and demographic factors. A physical examination was conducted that included waist, hip, height, and weight measurements. Serum samples were obtained following an overnight fast of at least 8 h and were stored at −70°C. Incident cancer was ascertained through annual self-administered questionnaires or by self-report between the annual questionnaires. Case status and detailed diagnosis were confirmed through centralized review of all pathology reports, discharge and consultant summaries, operative and radiology reports, and tumor registry abstracts. Cases were coded according to National Cancer Institute Surveillance, Epidemiology and End-Results guidelines (16, 17). As of February 29, 2004 (the date when the participants of this endometrial cancer case-cohort study were selected), there was a mean follow-up of 77 months, 1.6% of the women had been lost to follow-up, and 4.7% were deceased.

Study Subjects. Inclusion in the current study was limited to WHI-OS participants who were nondiabetic at baseline, remained free of endometrial cancer for at least 1 year following enrollment, and had not undergone hysterectomy. Patients with diabetes were identified through either self-report of clinical treatment for diabetes or were participants with a fasting glucose level of ≥125 mg/dL. Women who reported use of unopposed estrogen therapy at baseline (24 cases, 16 subcohort subjects) were excluded because use of unopposed estrogen is generally contraindicated in women with an intact uterus. Two individuals with missing data (both cases) were also excluded. All cases of incident endometrial cancer who met the above criteria ($n = 250$) were included in our study. A subcohort of 465 subjects randomly selected from all women in the WHI-OS at baseline and who met the same inclusion and exclusion criteria as cases was used as the comparison group. Those who underwent hysterectomy during follow-up ($n = 20$) were censored on the date of surgery.

Laboratory Methods. Serum insulin and glucose were measured by the designated laboratory for WHI, Medical Research Laboratories, Highland Heights, KY. Insulin resistance was estimated using the homeostasis model assessment–insulin resistance (HOMA-IR) index [fasting insulin (μIU/mL) × fasting glucose (mmol/L) / 22.5; refs. 18, 19]. Serum concentrations of total IGF-I, free IGF-I, and IGFBP-3 were determined using ELISA (Diagnostic Systems Laboratories), with which our laboratory has extensive experience (20-22). Testing of free IGF-I by ELISA, unlike with ultrafiltration, measures not only the fraction of IGF-I that is unbound to IGFBPs but also easily dissociable IGF-I, although these levels are highly correlated (23). Serum estradiol levels were measured using the Vitros-Eci Immunodiagnostic Assay (Ortho-Clinical Diagnostics) at the Esoterix Center for Clinical Trials (Calabasas Hills, CA). Estradiol, total IGF-I, free IGF-I, and IGFBP-3 tests were conducted in duplicate, and the mean value for each duplicate pair was used as the result for analysis. Insulin, glucose, total IGF-I, and IGFBP-3 tests with coefficients of variation of >10% were repeated. For free IGF-I, a coefficient of variation of >20% was used as the threshold for repeat testing because free IGF-I levels are low and, as mean values for a parameter approach zero, the coefficient of variation becomes mathematically sensitive to small changes in standard deviation. The Esoterix laboratory maintained its own quality control operations and any estradiol test with a coefficient of variation of >20% was repeated. Individual runs of any assay with quality control values outside of the expected range were also repeated. Approximately 5% of the WHI-OS samples were retested in a blinded fashion. The correlations of assay values determined in the replicates were very high (total IGF-I, $R^2 = 0.964$; free IGF-I, $R^2 = 0.903$; IGFBP-3, $R^2 = 0.895$; insulin, $R^2 = 0.984$; glucose, $R^2 = 0.947$; estradiol, $R^2 = 0.996$). In previous testing in our laboratory, the coefficients of variation within and between batches, respectively, were 3.6% and 4.9% for total IGF-I and 4.2% and 5.5% for IGFBP-3, based on masked specimens tested in 45 separate batches (data not shown).

The estradiol assays were completed in a single batch. All other assays were completed in two separate batches, with a relatively balanced number of cases (batch 1, $n = 152$; batch 2, $n = 98$) and subcohort specimens (batch 1, $n = 263$; batch 2, $n = 202$) in each batch (and on each assay plate) so that cases could be compared with noncases tested in the same batch. Insulin, glucose, total IGF-I, and IGFBP-3 had similar distributions in both batches. However, free IGF-I levels varied significantly ($P = 0.03$) by batch (batch 1: mean ± SD, 0.28 ± 0.21 ng/mL; batch 2: mean ± SD, 0.56 ± 0.41 ng/mL). The critical question was whether this difference, an apparent rightward shift in values measured by the assay in the second batch, might have affected our overall findings. Therefore, we tested the statistical equivalence of the

Table 1. Selected baseline characteristics of the study population

Variable	Cases ($n = 250$)	Subcohort* ($n = 465$)	$P^\dagger$
Age (y)	63.8 (6.78)	62.4 (7.55)	0.01
Ethnicity, n (%)			0.34
White	229 (92.71)	410 (88.36)	
Black	7 (2.83)	21 (4.53)	
Hispanic	5 (2.02)	16 (3.45)	
Asian/other	7 (2.36)	17 (3.66)	
Weight (kg)	72.9 (18.8)	70.1 (16.1)	0.26
BMI (kg/m^2)	27.6 (7.01)	26.7 (5.44)	0.76
Waist (cm)	85.0 (15.3)	83.15 (12.5)	0.41
Waist-to-hip ratio	0.80 (0.08)	0.80 (0.08)	0.60
Parity			0.40
Never pregnant	32 (12.80)	59 (12.83)	
Never had term pregnancy	8 (3.20)	13 (2.83)	
1	27 (10.80)	42 (9.13)	
2+	193 (73.20)	346 (75.21)	
Age at first live birth (y)			0.34
<20	15 (7.98)	27 (7.61)	
20-24	88 (46.81)	175 (49.30)	
25-29	66 (35.11)	104 (29.30)	
30-34	17 (9.04)	34 (9.58)	
35-39	2 (1.06)	12 (3.38)	
40-44	0 (0)	3 (0.85)	
Age at menarche (y)			0.53
≤10	12 (4.80)	31 (6.70)	
11-12	106 (42.40)	175 (37.80)	
>13	132 (52.80)	257 (55.50)	
Age at onset of menopause (y)	51.4 (6.20)	50.1 (4.95)	0.02
Use of oral contraceptives, n (%)	116 (46.40)	194 (41.72)	0.23
Current use of HT, n (%)	111 (44.00)	177 (38.00)	0.10
Duration of HT use (y)	9.32 (6.91)	6.59 (6.39)	<0.001
Current use of NSAIDs, n (%)	95 (38.00)	155 (33.33)	0.21
Family history of endometrial cancer, n (%)	14 (11.67)	23 (10.70)	0.96
Smoking status, n (%)			0.09
Never	130 (53.06)	246 (53.48)	
Former	106 (43.27)	179 (38.91)	
Current	9 (3.67)	35 (7.61)	
Alcohol (servings per wk)	3.79 (9.54)	2.81 (5.04)	0.32
Physical activity (MET)	15.6 (15.1)	14.1 (13.5)	0.21

NOTE: All values are means (SD) unless otherwise stated.

Abbreviations: HT, hormone therapy; NSAID, nonsteroidal anti-inflammatory drugs; MET, metabolic equivalent tasks (defined as the caloric need per kilogram of body weight per hour of activity divided by the caloric need per kilogram of body weight per hour at rest) per hour per week.

*For this comparison, five endometrial cancer cases that arose in the subcohort were excluded.

$^\dagger P$ values derived from Wilcoxon rank sum test for continuous data and Pearson's χ^2 for categorical data.

age-adjusted hazard ratio estimates for the association of free IGF-I and endometrial cancer between batches and confirmed that the results did not significantly differ ($P = 0.35$).

Statistical Analysis. The baseline characteristics of cases and the subcohort were compared using the Wilcoxon rank sum test (for continuous data) or Pearson's χ^2 (for categorical data). Serologic assay results were expressed as quartiles or tertiles based on the distribution of values in the subcohort. For those assays conducted in two separate batches, we determined the quartiles separately for each batch. This was done to minimize the possibility that even unrecognized variations in laboratory results across batches might affect our findings. Correlations between these serologic data, age, and body mass index (BMI) were assessed in the subcohort using Spearman's correlation coefficient. To assess the effects of hormone therapy on each of the measured serologic factors, we determined their mean values by hormone therapy stratum categorized as (a) users of combined estrogen and progesterone or (b) nonusers of estrogen and progesterone and compared

these values using the Wilcoxon rank sum test. Hazard ratios measuring the associations of our serologic data, as well as other risk factors, with risk for endometrial cancer were estimated using multivariable Cox proportional hazard regression models that used the Self-Prentice method for robust standard error estimates (to account for the case-cohort design), with time to event as the underlying time scale. Our main models were adjusted for (a) age, categorized as 50-54 (reference), 55-59, 60-64, 65-69, 70-74, or 75-79 years of age; (b) BMI, categorized as <18.5, 18.5 to <25 (reference), 25.0 to <30, 30 to <34, or ≥34 kg/m^2; and (c) hormone therapy use or endogenous estradiol levels. Estradiol data were assessed in non–hormone therapy users only because hormone therapy complicates estradiol measurement. This created four nonoverlapping groups, namely, non–hormone therapy users with (a) low (reference), (b) moderate or (c) high estradiol tertile levels, and (d) hormone therapy users. We then parameterized these groups as separate dummy variables with low estradiol as the common reference, permitting potentially different effects of hormone therapy use and high estradiol (among non–hormone therapy

Table 2. Spearman's correlation coefficients for the associations between all measured serologic factors, age, and BMI (kg/m^2) in the representative subcohort

	Age	BMI	Total IGF-I	Free IGF-I	IGFBP-3	Insulin	Glucose	HOMA-IR
Age	—							
BMI	0.08	—						
Total IGF-I	−0.09*	0.06	—					
Free IGF-I	0.01	0.10	0.38 †	—				
IGFBP-3	−0.07	0.21 †	0.49 †	0.07	—			
Insulin	−0.01	0.53 †	0.10*	0.10*	0.20 †	—		
Glucose	0.10*	0.21 †	0.07	0.11*	0.15 †	0.37 †	—	
HOMA-IR index	0.01	0.52 †	0.10*	0.11*	0.20 †	0.99 †	0.49 †	—
Estradiol	−0.05	0.25 †	0.05	−0.21 †	−0.11	0.18 †	0.05	0.17 †

NOTE: Among nonusers of hormone therapy only.
*$P < 0.05$.
† $P < 0.001$.

users) to be modeled. Reported epidemiologic risk factors for endometrial cancer (that is, parity, age at first live birth, age at menopause, oral contraceptive use, physical activity, smoking, and alcohol consumption) were tested as potential confounding variables by stepwise inclusion in these multivariable models, and variables that altered the hazard ratio by 10% or more were retained in the final model. Analyses stratified by hormone therapy use (user/nonuser) and BMI (<25.0 or ≥25.0 kg/m^2) were also performed, and tests of interaction were conducted by including in each model a term that is the product of the variable of interest and either hormone therapy use or BMI. All P values are two-sided, and $P < 0.05$ was considered statistically significant.

Results

Patient Characteristics. We compared the characteristics of cases and the subcohort at baseline. As shown in Table 1, cases were older than the subcohort (63.8 versus 62.4 years, $P = 0.01$), had a slightly higher mean age at onset of menopause (51.4 versus 50.1 years, $P = 0.02$), and reported longer mean duration of hormone therapy use (9.32 versus 6.59 years, $P < 0.001$). Correlations between the several serologic factors measured in this study, age, and BMI are presented in Table 2. Briefly, total IGF-I was strongly correlated with free IGF-I and IGFBP-3. Insulin was strongly correlated with BMI and was moderately correlated with total IGF-I and IGFBP-3 levels. Estradiol had a moderate inverse correlation with free IGF-I and was positively correlated with insulin and BMI. We also examined the effects of hormone therapy use on levels of insulin, IGF-I, and IGFBP-3. Mean insulin levels were 7.4 μIU/mL in women not using hormone therapy but significantly lower in women using hormone therapy (5.9 μIU/mL; $P < 0.001$). Similarly, total IGF-I, free IGF-I, and IGFBP-3 levels varied by use of hormone therapy. For example, free IGF-I levels were 0.49 ng/mL in nonusers and 0.39 ng/mL in women using hormone therapy ($P < 0.001$).

All Endometrial Cancers. The level of free IGF-I was inversely associated with risk for endometrial cancer in an age-adjusted model (hazard ratio for the highest versus the lowest quartile [HR$_{q4\text{-}q1}$], 0.55; 95% confidence interval (CI); 0.34-0.88; Table 3). This association remained statistically significant after inclusion of BMI

in the age-adjusted model (HR$_{q4\text{-}q1}$, 0.60; 95% CI, 0.36-0.98) but was of borderline significance after the inclusion of hormone therapy use and endogenous estradiol (among non–hormone therapy users; HR$_{q4\text{-}q1}$, 0.62; 95% CI, 0.38-1.01). Estradiol level itself was positively associated with endometrial cancer risk, even after adjustment for free IGF-I, BMI, and age (HR$_{t3\text{-}t1}$, 3.16; 95% CI, 1.71-5.81). None of the other serologic factors measured, including total IGF-I, IGFBP-3, IGF-I/IGFBP-3 ratio (data not shown), or insulin was significantly associated with the risk for endometrial cancer. None of the other endometrial cancer risk factors meaningfully altered the associations of the serologic factors with endometrial cancer and were therefore not considered in the final multivariate models.

Endometrioid Adenocarcinoma. The relation of free IGF-I with endometrial cancer incidence was strengthened when analysis was restricted to endometrioid adenocarcinoma, which accounted for 82% of the tumors in this study (age-adjusted HR$_{q4\text{-}q1}$, 0.46; 95% CI, 0.28-0.77; Table 4). This association remained largely unchanged following additional adjustment for BMI (HR$_{q4\text{-}q1}$, 0.50; 95% CI, 0.29-0.86), hormone therapy use and endogenous estradiol (HR$_{q4\text{-}q1}$, 0.53; 95% CI, 0.31-0.90), and insulin (HR$_{q4\text{-}q1}$, 0.43; 95% CI, 0.25-0.74), although it was of borderline statistical significance when all four covariates were added concurrently to the model (HR$_{q4\text{-}q1}$, 0.57; 95% CI, 0.32-1.02). For insulin, there was a strong positive association with the risk for endometrioid adenocarcinoma among non–hormone therapy users (age-adjusted HR$_{q4\text{-}q1}$, 2.79; 95% CI, 1.39-5.60) that was not evident among hormone therapy users ($P_{\text{interaction}} = 0.07$). The positive association between insulin and endometrioid adenocarcinoma among women not using hormone therapy remained significant following separate adjustment for BMI (HR$_{q4\text{-}q1}$, 2.44; 95% CI, 1.09-5.45), endogenous estradiol levels (HR$_{q4\text{-}q1}$, 2.33; 95% CI, 1.13-4.82), and free IGF-I (HR$_{q4\text{-}q1}$, 2.73; 95% CI, 1.35-5.52), although as with free IGF-I, the association was somewhat attenuated when all four covariates were added to the model (HR$_{q4\text{-}q1}$, 2.03; 95% CI, 0.87-4.78; $P_{\text{trend}} = 0.10$). HOMA-IR index had associations with endometrioid adenocarcinoma similar to those of insulin itself (data not shown).

None of the other serologic factors were associated with endometrioid adenocarcinoma, and apart from the association between insulin and endometrioid

adenocarcinoma, we detected no significant heterogeneity in the results stratified by hormone therapy use. There was no association between any of the serologic factors and non-endometrioid adenocarcinomas (data not shown).

Stratification by BMI. The age-adjusted association of insulin with endometrioid adenocarcinoma was much stronger among overweight/obese women (HR_{q4-q1}, 4.38; 95% CI, 1.64-11.64) than among leaner women (HR_{q4-q1}, 0.90; 95% CI, 0.32-2.53), albeit the formal test for interaction was not significant ($P_{interaction}$ = 0.11). Among overweight/obese women, additional adjustment for estradiol and hormone therapy use did not meaningfully affect the association of insulin (HR_{q4-q1}, 4.30; 95% CI, 1.62-11.43) with endometrioid adenocarcinoma. Thus, among overweight and obese women, it was not necessary to stratify the data by hormone therapy use to observe the strong relation of insulin with endometrioid adenocarcinoma. The limited number of cases in this analysis, however, already stratified by histologic type and BMI, precluded our exploring the matter further with additional stratification by hormone therapy. Similarly, the association of free IGF-I was undiminished by additional adjustment for hormone therapy and estradiol (HR_{q4-q1}, 0.43; 95% CI, 0.20-0.97) and was similar, with a borderline level of statistical significance (HR_{q4-q1}, 0.46; 95% CI, 0.20-1.04) when insulin was included in the model.

Statistical Independence of the Association between Insulin and Endometrioid Adenocarcinoma. Despite stratification by BMI ($\geq$25 kg/m^2, as above), residual confounding by BMI remained a possible explanation for the observed relationship between high insulin levels and endometrioid adenocarcinoma (Table 5). To further address this concern, we limited analysis to the $\geq$25 kg/m^2 BMI stratum and additionally adjusted for BMI expressed

Table 3. Adjusted hazard ratios (95% CI) for the associations of incident endometrial cancer with baseline IGF-I, free IGF-I, insulin, IGFBP-3, glucose, HOMA index, and endogenous estradiol levels

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}*
Total IGF-I					
Quartile cut points (ng/mL)	<94.2	94.2 to <119.6	119.6 to <151.0	$\geq$151.0	
Cases (n)	54	69	54	73	
Age adjusted	1.00 [†]	1.14 (0.72-1.82)	0.73 (0.45-1.18)	1.01 (0.63-1.59)	0.60
Age + estradiol + HT	1.00 [†]	1.26 (0.78-2.05)	0.81 (0.50-1.32)	1.27 (0.78-2.06)	0.73
Age + BMI	1.00 [†]	1.14 (0.70-1.86)	0.71 (0.43-1.17)	1.10 (0.67-1.79)	0.63
Free IGF-I					
Quartile cut points (ng/mL)	<0.21	0.21 to <0.36	0.36 to <0.55	$\geq$0.55	
Cases (n)	70	63	65	46	
Age adjusted	1.00 [†]	0.75 (0.48-1.18)	0.76 (0.49-1.20)	0.55 (0.34-0.88)	0.02
Age + estradiol + HT	1.00 [†]	0.82 (0.52-1.31)	0.90 (0.56-1.44)	0.62 (0.38-1.01)	0.09
Age + BMI	1.00 [†]	0.72 (0.45-1.16)	0.78 (0.49-1.26)	0.60 (0.36-0.98)	0.04
Insulin					
Quartile cut points (μIU/mL)	<3.6	3.6 to <5.7	5.7 to <9.5	$\geq$9.5	
Cases (n)	59	54	61	68	
Age adjusted	1.00 [†]	0.88 (0.56-1.39)	1.11 (0.70-1.74)	1.37 (0.87-2.16)	0.11
Age + estradiol + HT	1.00 [†]	0.87 (0.54-1.38)	1.13 (0.71-1.81)	1.27 (0.79-2.03)	0.21
Age + BMI	1.00 [†]	0.95 (0.59-1.52)	1.25 (0.75-2.07)	1.54 (0.88-2.67)	0.05
IGFBP-3					
Quartile cut points (ng/mL)	<3604.0	3604.0 to <4053.6	4053.6 to <4583.5	$\geq$4583.5	
Cases (n)	65	52	57	76	
Age adjusted	1.00 [†]	0.72 (0.45-1.15)	0.71 (0.45-1.12)	0.88 (0.57-1.37)	0.65
Age + estradiol + HT	1.00 [†]	0.74 (0.46-1.20)	0.74 (0.47-1.18)	0.87 (0.55-1.38)	0.63
Age + BMI	1.00 [†]	0.70 (0.43-1.15)	0.67 (0.42-1.08)	0.86 (0.53-1.38)	0.48
Glucose					
Quartile cut points (mg/dL)	<86.0	86.0 to <91.5	91.5 to <100	$\geq$100	
Cases (n)	60	70	54	65	
Age adjusted	1.00 [†]	1.13 (0.72-1.78)	0.77 (0.48-1.22)	0.92 (0.58-1.45)	0.38
Age + estradiol + HT	1.00 [†]	1.13 (0.71-1.80)	0.75 (0.46-1.21)	0.89 (0.55-1.44)	0.33
Age + BMI	1.00 [†]	1.08 (0.68-1.74)	0.72 (0.44-1.18)	0.76 (0.46-1.26)	0.22
HOMA-IR index					
Quartile cut points	<0.71	0.71 to <1.14	1.14 to <1.93	$\geq$1.93	
Cases (n)	58	59	62	63	
Age adjusted	1.00 [†]	0.83 (0.53-1.30)	1.11 (0.70-1.75)	1.24 (0.77-1.98)	0.21
Age + estradiol + HT	1.00 [†]	0.86 (0.54-1.37)	1.12 (0.70-1.79)	1.20 (0.74-1.95)	0.31
Age + BMI	1.00 [†]	0.85 (0.53-1.35)	1.18 (0.72-1.95)	1.29 (0.73-2.29)	0.32
Estradiol [‡]					
Tertile cut points (pg/mL)	<8.0	8.0 to <14.0	$\geq$14.0	N/A	
Cases (n)	23	54	62	N/A	
Age adjusted	1.00 [†]	2.05 (1.16-3.64)	3.61 (2.02-6.43)	N/A	<0.001
Age + BMI	1.00 [†]	1.89 (1.06-3.38)	3.16 (1.71-5.81)	N/A	<0.001

Abbreviation: N/A, not applicable.

*Significance tests for trend were calculated using ordinal quantile variables (1-4 for quartile and 1-3 for tertile) entered into the model as a single continuous variable.

[†] Reference category.

[‡] Among non–hormone therapy users only.

Table 4. Adjusted hazard ratios (95% CI) for the associations of incident endometrioid adenocarcinoma with free IGF-I and insulin (stratified by hormone therapy use)

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}*
Free IGF-I					
Cases (*n*)	61	51	55	34	
Age adjusted	1.00 [†]	0.70 (0.43-1.12)	0.74 (0.46-1.19)	0.46 (0.28-0.77)	0.007
Age + HT + estradiol	1.00 [†]	0.77 (0.47-1.26)	0.89 (0.54-1.45)	0.53 (0.31-0.90)	0.05
Age + BMI	1.00 [†]	0.66 (0.40-1.08)	0.74 (0.45-1.23)	0.50 (0.29-0.86)	0.01
Age + insulin	1.00 [†]	0.69 (0.42-1.13)	0.73 (0.45-1.18)	0.43 (0.25-0.74)	0.004
Full model [‡]	1.00 [†]	0.77 (0.43-1.26)	0.93 (0.54-1.59)	0.57 (0.32-1.02)	0.07
Insulin					
Non–HT users					
Cases (*n*)	16	20	27	44	
Age adjusted	1.00 [†]	1.05 (0.50-2.22)	1.35 (0.67-2.73)	2.79 (1.39-5.60)	0.002
Age + estradiol	1.00 [†]	0.97 (0.45-2.08)	1.21 (0.58-2.51)	2.33 (1.13-4.82)	0.02
Age + BMI	1.00 [†]	1.11 (0.51-2.40)	1.38 (0.63-3.02)	2.44 (1.09-5.45)	0.06
Age + free IGF-I	1.00 [†]	1.03 (0.48-2.20)	1.36 (0.66-2.80)	2.73 (1.35-5.52)	0.002
Full model [§]	1.00 [†]	0.92 (0.41-2.10)	1.21 (0.53-2.72)	2.03 (0.87-4.78)	0.10
HT users					
Cases (*n*)	27	24	24	16	
Age adjusted	1.00 [†]	1.01 (0.49-2.06)	1.50 (0.68-3.30)	0.79 (0.35-1.79)	0.86
Age + BMI	1.00 [†]	0.90 (0.43-1.91)	1.65 (0.64-4.22)	1.01 (0.37-2.79)	0.70
Age + free IGF-I	1.00 [†]	0.89 (0.42-1.90)	1.72 (0.74-4.00)	0.91 (0.39-2.12)	0.73
Full model [‖]	1.00 [†]	0.83 (0.37-1.87)	1.77 (0.65-4.79)	1.10 (0.41-2.98)	0.44

NOTE: No statistical interaction was detected between hormone therapy use and the effects of free IGF-I on the risk for endometrioid adenocarcinoma (data not shown).

*Significance tests for trend were calculated using ordinal quantile variables (1-4 for quartile and 1-3 for tertile) entered into the model as a single continuous variable.

[†] Reference category.

[‡] Age, hormone therapy and estradiol, BMI, and insulin.

[§] Age, estradiol, BMI, and free IGF-I.

[‖] Age, BMI, and free IGF-I.

as a continuous variable. Because of the limited sample size within this stratum, we viewed this analysis as exploratory. Nonetheless, in a model that included age, hormone therapy use and estradiol, free IGF-I, and BMI as a continuous variable, the risk for endometrioid adenocarcinoma associated with the highest quartile of insulin remained strong (HR_{q4-q1}, 2.66; 95% CI, 0.95-7.44; $P_{trend} = 0.04$), if somewhat attenuated from those above, and the trend across the insulin quartiles was statistically significant.

Discussion

In this prospective study of postmenopausal women, fasting insulin levels were found to have a positive association with incident endometrioid adenocarcinoma, whereas high circulating levels of free IGF-I were associated with decreased risk for these tumors. Both associations were statistically independent of estradiol levels and hormone therapy use and were strongest among overweight and obese women. In contrast, total IGF-I, IGFBP-3, and glucose were not associated with endometrial cancer.

To our knowledge, this is the first prospective study of endometrial cancer to directly measure fasting insulin and to simultaneously control for estradiol and IGF-I levels. A recent study conducted among both premenopausal and postmenopausal women from 10 European countries prospectively investigated the association of C-peptide levels with endometrial cancer risk in 286 endometrial cancer cases and 555 matched controls, with adjustment for estradiol and several other sex hormones

(10). Consistent with the positive association observed between fasting insulin levels and endometrioid adenocarcinoma in the current study, C-peptide levels were positively associated with endometrial cancer incidence [relative risk contrasting the highest and the lowest quartile (RR_{q4-q1}), 2.13; 95% CI, 1.33-3.41]. However, the results from the European study differed from ours in that the association of C-peptide with endometrial cancer was attenuated following adjustment for free estradiol levels (RR_{q4-q1}, 1.28; 95% CI, 0.67-2.45). The direct measurement of fasting levels of insulin (the putative carcinogenic agent) was an advantage to the current investigation, as was the ability to focus the analysis on endometrioid adenocarcinomas, because only endometrioid tumors are known to be associated with obesity and estrogen exposure. On the other hand, we measured only total estradiol levels and not free (presumably bioactive) estradiol, as assessed in the European study.

Although further studies are indicated, we note that, in the current data, a positive association between insulin and incident endometrioid adenocarcinoma was observed among non–hormone therapy users in multivariate statistical models that controlled for age, BMI, free IGF-I, and estradiol, and that adjustment for additional potential confounders did not alter the findings. Furthermore, the insulin effect remained strong even after stratifying by high BMI and concurrently adjusting for BMI expressed as a continuous variable. The reasons why a positive association between insulin and endometrioid adenocarcinoma was only detected among non–hormone therapy users are unclear. However, a similar interaction was detected by Weiderpass et al. (24); that is, they observed a positive association between insulin

and endometrial cancer only among non–hormone therapy users (albeit the effect was not significant). Such an interaction may reflect the well-known impact of oral estrogens on various proteins produced by the liver, termed the "first pass effect." In the current study, for example, we report that use of oral hormone therapy was associated with significantly lower insulin, total and free IGF-I, and IGFBP-3—data consistent with numerous other reports. The full range of proteins that are altered by the first pass effect is unknown. Therefore, among hormone therapy users, the interpretation of insulin/IGF-axis data are complicated, and it may require a greater understanding of the hepatic first-pass effect to determine the true insulin–endometrial cancer association in hormone therapy users. A major alternative interpretation is that the association between insulin and endometrial cancer risk is difficult to detect among hormone therapy users because uniformly high circulating estrogen levels in hormone therapy users obscure the insulin–endometrial cancer association.

A direct, causal role for insulin in endometrial carcinogenesis is well supported by laboratory data. For example, insulin has mitogenic and antiapoptotic activity, and endometrial cancer cell lines express high-affinity insulin receptors, consistent with there being a direct biological effect of insulin on the growth of endometrial cancer cells (6). The insulin signaling pathway is also known to exhibit biological cross-talk with obesity-related inflammatory mechanisms, and elevated adipokine levels can interfere with insulin signal transduction leading to insulin resistance (25). Thus, the interactions of the insulin signaling pathway with other biological mechanisms that are affected by adiposity, such as the sex hormone and inflammatory pathways, may partly explain why we observed stronger associations of insulin with endometrioid adenocarcinoma among overweight and obese women (an interaction also reported in prior studies; ref. 26). However, despite strong mechanistic evidence to support a direct role for insulin in endometrial tumorigenesis, it is also possible that insulin is simply a marker of a strong etiologic risk factor related to endometrial cancer development.

The inverse association between free IGF-I and endometrial cancer was surprising given our a priori hypothesis that predicted a positive relationship, in keeping with the mitogenic effects of IGF-I in endometrial tissue. However, five cross-sectional studies, representing the majority of published data, in fact, observed an inverse, albeit not significant, association between serum total IGF-I levels and endometrial cancer (24, 27-30). In the current study, only free IGF-I, not total IGF-I, was associated with endometrial cancer. The one cross-sectional study that actually measured free IGF-I reported a nonsignificant 50% reduction in endometrial cancer risk among women with the highest free IGF-I levels and no association with total IGF-I (29). Furthermore, two recent studies that directly measured IGF-I mRNA levels in human endometrial cancer specimens (rather than in cell lines) found IGF-I mRNA levels to be low (31, 32). Taken as a whole, the inverse association of free IGF-I with the risk for endometrial cancer, although initially unexpected by us, is in fact supported by prior epidemiologic studies and is not inconsistent with recent laboratory data.

Table 5. Adjusted hazard ratios (95% CI) for the associations of free IGF-I and insulin with endometrioid adenocarcinoma, with stratification by BMI

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}*
BMI <25 kg/m²					
Free IGF-I					
Cases (n)	32	26	22	17	
Age adjusted	1.00[†]	0.79 (0.40-1.55)	0.72 (0.35-1.46)	0.59 (0.27-1.26)	0.16
Age + HT + estradiol	1.00[†]	0.89 (0.44-1.83)	1.03 (0.48-2.17)	0.72 (0.33-1.61)	0.54
Age + insulin	1.00[†]	0.72 (0.35-1.50)	0.71 (0.34-1.50)	0.54 (0.24-1.21)	0.15
Insulin[‡]					
Cases (n)	38	27	25	7	
Age adjusted	1.00[†]	0.89 (0.47-1.65)	1.66 (0.86-3.19)	0.90 (0.32-2.53)	0.44
Age + HT + estradiol	1.00[†]	0.79 (0.41-1.52)	1.69 (0.84-3.40)	0.79 (0.26-2.38)	0.57
Age + free IGF-I	1.00[†]	0.89 (0.47-1.70)	1.73 (0.86-3.46)	0.94 (0.32-2.74)	0.37
BMI ≥ 25 kg/m²					
Free IGF-I					
Cases (n)	29	25	32	17	
Age adjusted	1.00[†]	0.64 (0.32-1.26)	0.78 (0.48-1.47)	0.39 (0.19-0.80)	0.03
Age + HT + estradiol	1.00[†]	0.69 (0.34-1.41)	0.87 (0.44-1.72)	0.43 (0.20-0.97)	0.07
Age + insulin	1.00[†]	0.67 (0.32-1.40)	0.85 (0.43-1.67)	0.40 (0.18-0.88)	0.05
Full model[§]	1.00[†]	0.76 (0.35-1.65)	0.97 (0.47-1.99)	0.46 (0.20-1.04)	0.12
Insulin[‡]					
Cases (n)	5	17	25	53	
Age adjusted	1.00[†]	1.92 (0.67-5.54)	2.25 (0.79-6.37)	4.38 (1.64-11.64)	<0.001
Age + HT + estradiol	1.00[†]	2.06 (0.71-6.02)	2.29 (0.80-6.56)	4.30 (1.62-11.43)	0.001
Age + free IGF-I	1.00[†]	1.80 (0.62-5.22)	2.20 (0.36-6.31)	4.40 (1.65-11.71)	<0.001
Full model[‖]	1.00[†]	1.77 (0.61-5.14)	1.96 (0.68-5.68)	3.95 (1.49-10.47)	0.001

*Significance tests for trend were calculated using ordinal quantile variables (1-4 for quartile and 1-3 for tertile) entered into the model as a single continuous variable.
[†] Reference category.
[‡] Concurrent stratification by hormone therapy use was precluded by sample size.
[§] Age, hormone therapy and estradiol, and insulin.
[‖] Age, hormone therapy and estradiol, and free IGF-I.

The seemingly paradoxical inverse association of free IGF-I and endometrioid adenocarcinoma has several potential explanations. Notably, there is thought to be a disconnect between local uterine IGF-I levels and those in circulation. Unlike many other tissues, the uterus is insensitive to growth hormone. Instead, uterine IGF-I levels are largely regulated by estrogen (33, 34). In fact, IGF-I produced by uterine stromal cells is believed to be the primary mediator of the proliferative effects of estrogen on endometrial tissue during normal menses (35-37). Circulating IGF-I, in contrast, is mainly produced by the liver and regulated by growth hormone. A protective effect of circulating IGF-I levels could also, in this context, be explained by the anti-inflammatory effects of IGF-I; serum IGF-I is inversely correlated with C-reactive protein and levels of several inflammatory cytokines (38). Consistent with this hypothesis, a recent study showed that high C-reactive protein levels are associated with increased risk of endometrial cancer (39). Alternatively, free IGF-I levels could simply be a biomarker of high inflammatory cytokine levels in circulation. Additional studies involving concurrent measurement of these several factors in blood, as well as in healthy and neoplastic endometrium, are needed.

There are several additional limitations to this study that warrant discussion. First, we studied only baseline serum measures, whereas repeated measurements over time would have provided more precise classification of subjects. On the other hand, in adults, within-individual levels of total IGF-I, free IGF-I, IGFBP-3, insulin, and estradiol are known be stable over several years (40-42), and failure to have optimally addressed variations in serum values would most likely have caused bias toward the null. Second, although we controlled for hormone therapy use and estradiol levels in our analysis, we did not measure sex hormone–binding globulin, estrone, or free estradiol levels. Among the subset of women who were overweight or obese, the findings indicated that stratification by hormone therapy use was not necessary. However, the limited number of cases in our analysis precluded concurrent stratification by histologic type, high BMI, and hormone therapy use, which would be necessary to fully explore their potentially interactive effects. Noting that the current investigation is already among the largest prospective cohort studies of endometrial cancer, it may require the coordinated efforts of several large cohort studies to fully examine these issues.

Third, although we excluded women who developed endometrial cancer within the first 12 months of follow-up, we cannot entirely exclude the possibility that some cases had subclinical disease at baseline and that the associations we observed might be partly due to reverse causality; too few endometrial cases were available in this study to permit meaningful sensitivity analysis by time to diagnosis while addressing the additional necessary factors.

In summary, our data suggest that hyperinsulinemia is a risk factor for endometrioid adenocarcinoma that may be independent of estradiol levels as well as body habitus, whereas free IGF-I was inversely associated with endometrioid adenocarcinoma. If correct, hyperinsulinemia, low free IGF-I, and high estradiol levels may together account for a substantial proportion of endometrial cancers because they are each common exposures with moderate to strong associations with the risk for endometrial cancer.

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