Published in final edited form as:
Cancer Epidemiol Biomarkers Prev. 2011 May ; 20(5): 971–977. doi:10.1158/1055-9965.EPI-10-1222.

A Prospective Study of Inflammation Markers and Endometrial Cancer Risk in Postmenopausal Hormone Non-Users

Tao Wang[1], **Thomas E. Rohan**[1], **Marc J. Gunter**[1], **Xiaonan Xue**[1], **Jean Wactawski-Wende**[2], **Swapnil N. Rajpathak**[1], **Mary Cushman**[3], **Howard D. Strickler**[1], **Robert C. Kaplan**[1], **Sylvia Wassertheil-Smoller**[1], **Philipp E. Scherer**[4], and **Gloria Y.F. Ho**[1]

[1] Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, New.York

[2] Department of Social and Preventive Medicine, School of Public Health and Health Professions, University at Buffalo, SUNY, Buffalo, New York

[3] Departments of Medicine & Pathology, University of Vermont, Burlington, Vermont

[4] Touchstone Diabetes Center, University of Texas Southwestern Medical Center, Dallas, Texas

Abstract

BACKGROUND—It is hypothesized that inflammation may mediate the relationship between obesity and endometrial cancer risk. We examined the associations of three inflammation markers, C-reactive protein (CRP), interleukin (IL)-6, and tumor necrosis factor (TNF)-α, with risk of endometrial cancer.

METHODS—A case-cohort study was nested within the Women's Health Initiative, a cohort of postmenopausal women. Baseline plasma samples of 151 incident endometrial cancer cases and 301 subcohort subjects not using hormones were assayed.

RESULTS—CRP, but not IL-6 or TNF-α, was positively associated with endometrial cancer risk after adjusting for age and BMI [hazard ratio comparing extreme quartiles (HRq_4-q_1) = 2.29; 95% confidence interval (CI) = 1.13–4.65; p_{trend} = 0.012). After additional adjustment for estradiol and insulin, this association was attenuated (HRq_4-q_1 = 1.70;95% CI= 0.78–3.68; p_{trend} = 0.127). Obesity (BMI ≥ 30 kg/m^2) was associated with endometrial cancer risk in an age-adjusted model. The obesity effect was reduced by 48%, 67%, and 77% when either estradiol, CRP, or insulin, respectively, was included in the model, and it became null when all three factors were adjusted for simultaneously.

CONCLUSIONS—The association between inflammation, as indicated by a relatively high level of CRP, and endometrial cancer risk may partially be explained by hyperinsulinemia and elevated estradiol. Nevertheless, all three factors contribute to and mediate the link between obesity and endometrial cancer in postmenopausal women not using hormones.

IMPACT—The association between obesity and endometrial cancer risk in postmenopausal women may be attributed to inflammation, insulin resistance, and elevated estrogen.

Obesity is one of the strongest risk factors for endometrial cancer (1). There are several mechanisms that might account for this association. First, after menopause, adipose tissue is the primary site for estrogen production, due to aromatization of androgens to estrogens (2), and circulating estrogen levels are strongly associated with endometrial cancer risk (3).

Address reprint requests to Dr. Tao Wang at Division of Biostatistics, Department of Epidemiology and Population Health, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Belfer #1303, Bronx, NY 10461, or at tao.wang@einstein.yu.edu.

Second, obesity is associated with hyperinsulinemia and insulin resistance (2). Insulin has mitogenic and anti-apoptotic properties (4, 5), and it also decreases the synthesis of sex hormone-binding globulin and increases the bioavailability of estradiol (6). We have previously reported that high levels of fasting insulin are associated with increased risk of endometrial cancer in postmenopausal women (3). Third, obesity is associated with progressive adipose tissue infiltration by macrophages that secrete proinflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-6; the latter induces C-reactive protein (CRP), an acute-phase protein that is the most well established inflammation marker (7, 8). Laboratory studies have shown that IL-6 and TNF-α may have direct effects on carcinogenesis by promoting tumor invasion, progression, and metastasis (9, 10). They are also linked to the risk factors of endometrial cancer through their abilities to stimulate estrogen biosynthesis (11) (12) and induce insulin resistence (13). We therefore hypothesized that CRP, IL-6, and TNF-α may play a role in the etiology of endometrial cancer. We specifically studied these three inflammation markers, because IL-6 and TNF-α are potent proinflammatory cytokines with established carcinogenic bioactivities, and all three inflammation markers have detectable circulating levels even in individuals without clinical diseases (14, 15).

We conducted a case-cohort study within the Women's Health Initiative Observational Study (WHI-OS) to (1) investigate the associations of circulating levels of CRP, IL-6, and TNF-α with risk of endometrial cancer, (2) to assess if these associations are independent of other risk factors, including increased levels of estrogen and insulin, and (3) to examine whether these obesity-related factors (proinflammatory markers, hyperinsulinemia, and elevated estradiol) mediate the association between obesity and endometrial cancer.

METHODS

Study population

The WHI-OS is an ongoing prospective study with long-term follow-up of 93,676 postmenopausal women aged 50 to 79 years, who were enrolled at 40 clinical centers in the United States from 1993 to 1998, to examine the risk factors for subsequent development of several health outcomes.(16) At baseline, participants completed detailed epidemiologic questionnaires, and a physical examination was performed using standardized procedures to obtain various measurements, including height and weight. Morning, fasting blood samples were collected, centrifuged, frozen on-site at −80°C, and later shipped to the central specimen repository. Incident cancer was ascertained through annual self-administered questionnaires. Diagnosis of endometrial cancer was confirmed through centralized review of medical records.

Study Subjects

This endometrial cancer study was part of a case-cohort study in which three cancer outcomes (breast, colorectum, and endometrium) were examined, and a representative subcohort served as the comparison group.(3, 17, 18) By June 2004, there were 298 women who had an incident primary tumor of the endometrium diagnosed 12 months or more after the baseline visit in the WHI-OS (cases diagnosed during the first 12 months of follow-up were excluded). The subcohort was created by randomly sampling 892 subjects from the participants who had more than 12 months of follow-up and had no history of breast, colorectal, or endometrial cancer at 12 months. Subcohort subjects were selected regardless of their cancer outcome after more than 12 months of follow-up. Five of the selected subcohort subjects developed incident endometrial cancer subsequently, and these five subjects were included in both the subcohort and case group. This feature of the case-cohort design was taken into consideration in data analysis described later.(19) Since the subcohort

was selected as a comparison group for three cancer outcomes, no restriction on uterine status was made. As such, 373 of the 892 subcohort subjects had a hysterectomy and were ineligible for analyses related to endometrial cancer. Women who had diabetes treatment (15 cases and 25 subcohort subjects) or used hormone therapy (132 cases and 193 subcohort subjects) at baseline were further excluded, since these treatments may significantly alter levels of proinflammatory markers, estradiol, and insulin. The final sample size included 151 cases and 299 subcohort subjects (not counting two subjects who were also in the case group).

Laboratory Methods

EDTA plasma samples were assayed by the following methods: CRP by high-sensitivity latex-enhanced immunonephelometry (inter-assay correlation of variation [CV] = 4%; Behring Diagnostics, San Jose, CA), IL-6 by an ultra-sensitive solid-phase enzyme-linked immunosorbent assay (inter-assay CV = 9%; R&D Systems, Minneapolis, MN), and TNF-α by a multiplex assay (inter-assay CV of 18%; Milliplex Human Adipokine Panel B, Millipore, Billerica, MA). We previously reported that fasting levels of serum free IGF-1, insulin, and estradiol were significantly associated with endometrial cancer in multivariable analyses in this case-cohort study population, and the assay methods for these analytes were described previously.(3) Data for these three serum factors were included in data analysis reported here. The intraclass correlation coefficients (ICC) of the three inflammation markers, as well as those for free IGF-1, insulin, and estradiol, were published previously; they ranged from 0.4 for TNF-α to 0.8 for free IGF-1.(14, 20–22)

Statistical Analysis

In univariable analyses, we first compared the baseline characteristics of cases and the subcohort. To account for the features of the case-cohort design, these analyses were done by Cox proportional hazard regression with robust variance estimation using the Self-Prentice method.(19) We then examined the associations of the three inflammation markers with risk factors for endometrial cancer (e.g., age, BMI, serum levels of insulin and estradiol, etc.) in the subcohort subjects who did not have endometrial cancer (n =299) using Spearman rank correlations, and their 95% confidence limits were derived by Fisher's z transformation. In multivariable analyses, Cox regression models with robust variance estimation were used to estimate the associations of inflammation markers with risk of endometrial cancer.(19) To avoid assuming any linear effect, CRP, TNF-α and IL-6 levels were categorized based on quartile cut-points derived from the distribution of these variables in the subcohort. The models were adjusted for potential confounders, including age (continuous) and body mass index (BMI, <25, 25–29.9, ≥30 kg/m^2). In addition, serum levels of estradiol, insulin, and free IGF-1 expressed as quartiles were included in the multivariable models to examine whether the associations of CRP, IL-6, and TNF-α with endometrial cancer were independent of these endometrial cancer risk factors previously reported in this study population.(3) BMI was used as an adiposity indicator, because it was a more significant risk factor for endometrial cancer than waist circumference in this study population. Nevertheless, multivariable regression models adjusting for waist circumference yielded similar results for the three inflammation markers, and these results are not presented.

RESULTS

Table 1 shows baseline characteristics of the study population. As compared to the subcohort, cases were older, had higher BMI and waist circumference, and had higher endogenous estradiol levels. Cases had significantly higher mean levels of insulin, but

tended to have lower levels of free IGF-I than the subcohort. Mean levels of CRP, IL-6, and TNF-α did not differ between the cases and the subcohort members.

Spearman rank correlations of the three inflammation markers with insulin, free IGF-1, and statistically significant risk factors for endometrial cancer (as identified in Table 1) were examined in the subcohort women who did not have endometrial cancer (Table 2). CRP is induced by IL-6, and as expected, these two markers were correlated with each other (r = 0.58). There was a modest correlation between IL-6 and TNF-α (r = 0.23) and a weak correlation between CRP and TNF-α (r=0.14). Both CRP and IL-6 were positively correlated with adiposity, insulin, and estradiol. TNF-α was positively, but modestly, correlated with age, BMI, and free IGF-1.

Table 3 shows the multivariable associations between the three inflammation markers and endometrial cancer risk. Individuals with an IL-6 level in the second quartile or above tended to have a decreased risk for endometrial cancer, whereas relatively high levels of TNF-α tended to be associated with an increased risk. However, the associations for both IL-6 and TNF-α were not statistically significant and did not show any linear trend. On the other hand, levels of CRP were positively associated with risk of endometrial cancer in the age adjusted model [hazard ratio comparing the highest versus lowest quartiles (HRq_4-q_1) =2.47; 95% confidence interval (CI) = 1.34–4.54; $p_{trend} < 0.001$]. BMI was then added into the model to assess whether CRP was simply a marker for obesity, and hence whether obesity could account for the CRP and endometrial cancer association. CRP remained significant after adjusting for BMI (HRq_4-q_1 = 2.29; 95% CI= 1.13–4.65; p_{trend} = 0.012). Keeping BMI in the model and further adjusting for free IGF-1 also did not change the relationship between CRP and endometrial cancer risk. However, CRP became borderline significant after adjustment for insulin (HRq_4-q_1 = 2.02;95% CI= 0.96–4.25; p_{trend} = 0.053) or estradiol (HRq_4-q_1 = 1.82;95% CI= 0.87–3.79; p_{trend} = 0.050) separately. The association between CRP and endometrial cancer risk was further attenuated when both insulin and estradiol were entered into the model simultaneously (HRq_4-q_1 = 1.70;95% CI= 0.78–3.68; p_{trend} = 0.127). In contrast, with CRP in the model, insulin and estradiol remained significantly associated with endometrial cancer (HRq_4-q_1 for insulin = 2.45;95% CI= 1.13–5.32; p_{trend} = 0.007; HRq_4-q_1 for estradiol = 4.38;95% CI= 2.15–8.92; p_{trend} <0.001).

There was evidence that estradiol, CRP, and insulin might mediate the association between obesity and endometrial cancer risk in hormone non-users. Obesity itself (BMI ≥ 30 kg/m^2) was positively associated with risk of endometrial cancer in an age-adjusted model without other covariates ($HR_{BMI \geq 30 \text{ versus } <25}$ = 1.85; 95% CI = 1.13–3.04, p = 0.015). The obesity effect was no longer significant after further adjustment for estradiol ($HR_{BMI \geq 30 \text{ versus } <25}$ = 1.38; 95% CI = 0.80–2.39, p = 0.253), CRP ($HR_{BMI \geq 30 \text{ versus } <25}$ = 1.23; 95% CI = 0.66–2.29, p = 0.517), or insulin ($HR_{BMI \geq 30 \text{ versus } <25}$ = 1.15; 95% CI = 0.63–2.11, p = 0.642). The attenuated HRs corresponded, respectively, to 48%, 67%, and 77% reductions in the β coefficient for BMI in the Cox regression model with estradiol, CRP, or insulin entered into the model, as compared to that from the model adjusted for age only. When estradiol, CRP, and insulin were included in the model simultaneously, the β coefficient for BMI was reduced by 117% ($HR_{BMI \geq 30 \text{ versus } <25}$ = 0.90; 95% CI = 0.46–1.78, p = 0.764).

DISCUSSION

In this study of postmenopausal women not using hormones, we found that a relatively high level of CRP, but not IL-6 or TNF-α, was associated with increased risk of endometrial cancer after adjusting for BMI, but that this association was attenuated by adjustment for insulin and estradiol. It is important for data interpretation to recognize that CRP is a biomarker for inflammation and may not necessarily have tumorigenic potential. CRP is an

acute-phase protein synthesized primarily by the liver in response to IL-6 (23). The well known property of CRP is its ability to activate the classical complement cascade, but recent studies have shown that CRP has proatherogenic and prothrombotic potential (24). Consistent with these laboratory data, a high CRP level is a well established risk marker for coronary heart disease (25, 26). However, two recent large scale studies identified genetic variants that were significantly related to circulating CRP levels and yet failed to directly link these genotypes with risk of cancer or coronary heart disease (27, 28). These data suggest that CRP is merely an inflammation marker and that it is not a causal agent in cancer or coronary heart disease.

One of the objectives of this study was to examine if inflammation could mediate the association between obesity and endometrial cancer. Of the three inflammation markers under study, levels of IL-6 and TNF-α were not related to endometrial cancer risk, and therefore could not explain the obesity effect. On the other hand, the obesity effect virtually became null in multivariable models adjusting for CRP, estradiol, or insulin individually as well as simultaneously. This supports the notion that in addition to inflammation, hyperinsulinemia and elevated estradiol are part of the obesity pathway and may provide the link between obesity and endometrial cancer risk.

These obesity related factors – inflammation, hyperinsulinemia, and elevated estradiol – are interconnected. In our study, CRP was associated with increased endometrial cancer risk after adjusting for BMI, but the HR was attenuated from 2.29 to 1.70 and did not reach statistical significance following further adjustment for estradiol and insulin; yet, the associations of estradiol and insulin with endometrial cancer remained significant. These observations may have either or both of two explanations. First, the observed CRP association may have been partially confounded by insulin and estradiol, and our relatively small sample size did not provide enough power to detect the moderate independent relationship between CRP and endometrial cancer risk, if it exists. Second, our observation raises the possibility that estradiol and insulin lie downstream of inflammation in the obesity pathway leading to endometrial cancer and therefore partially explain the CRP and endometrial cancer association. This notion is supported by laboratory data that demonstrate proinflammatory cytokines can induce insulin resistance (29), stimulate the activities of enzymes involved in estrogen biosynthesis, and increase the levels of estrogens (11). Therefore, inflammation could contribute to increased levels of insulin and estradiol and indirectly lead to development of endometrial cancer via these two factors.

As demonstrated in other laboratory studies, inflammation may also have its own direct effects on cancer risk through the tumorigenic bioactivities of proinflammatory cytokines on cell proliferation, cell survival, angiogenesis, and the immune response (9, 10). Yet, our data did not demonstrate any association between endometrial cancer and the proinflammatory cytokines IL-6 and TNF-α. Given that IL-6 induces CRP, it is puzzling why CRP, but not IL-6, was associated with endometrial cancer risk in hormone non-users. Several reasons may explain the null results. First, tissue levels of IL-6 and TNF-α may be more relevant than circulating levels. Second, there may be less misclassification in measuring and classifying the exposure status of CRP than IL-6 and TNF-α. CRP is a stable biomarker that can be assayed reliably and has an ICC of about 0.6 to 0.8 (30, 31). In contrast, the reported ICCs for IL-6 and TNF-α are about 0.4 to 0.5; the CVs of multiplex assays for measuring cytokines are generally higher than those for standard ELISA (14, 32). Finally, other proinflammatory cytokines, that were not measured in this study, may contribute to disease. Given these caveats, future epidemiological studies are needed to further examine if proinflammatory cytokines, other than those studied here, have any direct and independent effects on endometrial cancer risk after adjusting for insulin and estradiol.

Prospective data regarding the associations between inflammation markers and endometrial cancer risk are limited (33, 34). A recent case-control study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) found CRP and IL-6 to be associated with endometrial cancer only in a univariable analysis, but they were no longer significant after adjusting for BMI(35). Although both the EPIC and our study involved only hormone non-users, about a quarter of the EPIC study subjects were premenopausal women. The relative importance of inflammation versus other unmeasured obesity-related factors in the etiology of endometrial cancer could be different between premenopausal and postmenopausal women. The two studies also used different assays for inflammation markers. Nevertheless, data from both studies suggest that inflammation as well as high levels of estrogen and insulin (or C-peptide) mediate the link between obesity and endometrial cancer.

We have already discussed a few limitations of this study, including a relatively small sample size, assessing circulating versus tissue levels of inflammation markers, and a relatively high inter-assay CV of the TNF-α measurement. Moreover, our data may not be generalizable to premenopausal women or current users of hormone therapy. Reverse causality, however, was unlikely to contribute to our study results. In sensitivity analysis where we excluded endometrial cancer cases diagnosed within the first 3 years of follow-up (97 cases remained), we obtained similar results for the association between CRP and endometrial cancer.

In conclusion, our data suggest that inflammation, as indicated by a relatively high level of CRP, hyperinsulinemia, and increased endogenous estrogen, may link obesity and endometrial cancer in postmenopausal women not using hormones. The latter two obesity-related factors may, in turn, mediate the effects of inflammation on endometrial cancer risk.

Acknowledgments

FUNDING

This work was supported by contract N01WH74310 (Dr. Ho) with the National Heart, Lung, and Blood Institute (NHLBI). The WHI program is funded by the NHLBI through contracts N01WH22110, 24152, 32100-2, 32105-6, 32108-9, 32111-13, 32115, 32118-32119, 32122, 42107-26, 42129-32, and 44221. The complete list of WHI centers and investigators can be found online at http://www.whiscience.org/publications/WHI_investigators_shortlist.pdf.

The authors thank Elaine Cornell and Danielle Parent for assistance in laboratory measurements.

References

1. Kaaks R, Lukanova A, Kurzer MS. Obesity, endogenous hormones, and endometrial cancer risk: a synthetic review. Cancer Epidemiol Biomarkers Prev. 2002; 11:1531–43. [PubMed: 12496040]
2. Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nature Reviews. 2004; 4:579–91.
3. Gunter MJ, Hoover DR, Yu H, Wassertheil-Smoller S, Manson JE, Li J, et al. A prospective evaluation of insulin and insulin-like growth factor-I as risk factors for endometrial cancer. Cancer Epidemiol Biomarkers Prev. 2008; 17:921–9. [PubMed: 18398032]
4. Park D, Pandey SK, Maksimova E, Kole S, Bernier M. Akt-dependent antiapoptotic action of insulin is sensitive to farnesyltransferase inhibitor. Biochemistry. 2000; 39:12513–21. [PubMed: 11027130]
5. Straus DS. Growth-stimulatory actions of insulin *in vitro* and *in vivo*. Endocrine Reviews. 1984; 5:356–69. [PubMed: 6376081]
6. Tchernof A, Despres JP. Sex steroid hormones, sex hormone-binding globulin, and obesity in men and women. Horm Metab Res. 2000; 32:526–36. [PubMed: 11246820]

7. Black S, Kushner I, Samols D. C-reactive Protein. J Biol Chem. 2004; 279:48487–90. [PubMed: 15337754]
8. Austin H, Austin JM Jr, Partridge EE, Hatch KD, Shingleton HM. Endometrial cancer, obesity, and body fat distribution. Cancer Res. 1991; 51:568–72. [PubMed: 1985774]
9. Philip M, Rowley DA, Schreiber H. Inflammation as a tumor promoter in cancer induction. Semin Cancer Biol. 2004; 14:433–9. [PubMed: 15489136]
10. Shacter E, Weitzman SA. Chronic inflammation and cancer. Oncology (Williston Park). 2002; 16:217–26. 29. [PubMed: 11866137]
11. Simard J, Gingras S. Crucial role of cytokines in sex steroid formation in normal and tumoral tissues. Molecular and Cellular Endocrinology. 2001; 171:25–40. [PubMed: 11165008]
12. Purohit A, Newman SP, Reed MJ. The role of cytokines in regulating estrogen synthesis: implications for the etiology of breast cancer. Breast Cancer Res. 2002; 4:65–9. [PubMed: 11879566]
13. Olefsky JM, Glass CK. Macrophages, inflammation, and insulin resistance. Annu Rev Physiol. 2010; 72:219–46. [PubMed: 20148674]
14. Kaplan RC, Ho GY, Xue X, Rajpathak S, Cushman M, Rohan TE, et al. Within-individual stability of obesity-related biomarkers among women. Cancer Epidemiol Biomarkers Prev. 2007; 16:1291–3. [PubMed: 17548700]
15. Rohde LE, Arroyo LH, Rifai N, Creager MA, Libby P, Ridker PM, et al. Plasma concentrations of interleukin-6 and abdominal aortic diameter among subjects without aortic dilatation. Arterioscler Thromb Vasc Biol. 1999; 19:1695–9. [PubMed: 10397687]
16. Anderson G, Cummings S, Freedman LS, Furberg C, Henderson M, Johnson SR, et al. Design of the Women's Health Initiative Clinical Trial and Observational Study. Control Clin Trials. 1998; 19:61–109. [PubMed: 9492970]
17. Gunter MJ, Hoover DR, Yu H, Wassertheil-Smoller S, Rohan TE, Manson JE, et al. Insulin, insulin-like growth factor-I, and risk of breast cancer in postmenopausal women. J Natl Cancer Inst. 2009; 101:48–60. [PubMed: 19116382]
18. Gunter MJ, Hoover DR, Yu H, Wassertheil-Smoller S, Rohan TE, Manson JE, et al. Insulin, insulin-like growth factor-I, endogenous estradiol, and risk of colorectal cancer in postmenopausal women. Cancer Res. 2008; 68:329–37. [PubMed: 18172327]
19. Barlow WE, Ichikawa L, Rosner D, Izumi S. Analysis of case-cohort designs. J Clin Epidemiol. 1999; 52:1165–72. [PubMed: 10580779]
20. Ridker PM, Rifai N, Pfeffer MA, Sacks F, Braunwald E. Long-term effects of pravastatin on plasma concentration of C-reactive protein. The Cholesterol and Recurrent Events (CARE) Investigators. Circulation. 1999; 100:230–5. [PubMed: 10411845]
21. Muti P, Quattrin T, Grant BJ, Krogh V, Micheli A, Schunemann HJ, et al. Fasting glucose is a risk factor for breast cancer: a prospective study. Cancer Epidemiol Biomarkers Prev. 2002; 11:1361–8. [PubMed: 12433712]
22. Hankinson SE, Manson JE, Spiegelman D, Willett WC, Longcope C, Speizer FE. Reproducibility of plasma hormone levels in postmenopausal women over a 2–3-year period. Cancer Epidemiol Biomarkers Prev. 1995; 4:649–54. [PubMed: 8547832]
23. Marnell L, Mold C, Du Clos TW. C-reactive protein: ligands, receptors and role in inflammation. Clin Immunol. 2005; 117:104–11. [PubMed: 16214080]
24. Venugopal SK, Devaraj S, Jialal I. Effect of C-reactive protein on vascular cells: evidence for a proinflammatory, proatherogenic role. Curr Opin Nephrol Hypertens. 2005; 14:33–7. [PubMed: 15586013]
25. Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A, et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N Engl J Med. 2004; 350:1387–97. [PubMed: 15070788]
26. Shah T, Casas JP, Cooper JA, Tzoulaki I, Sofat R, McCormack V, et al. Critical appraisal of CRP measurement for the prediction of coronary heart disease events: new data and systematic review of 31 prospective cohorts. Int J Epidemiol. 2009; 38:217–31. [PubMed: 18930961]

27. Allin KH, Nordestgaard BG, Zacho J, Tybjaerg-Hansen A, Bojesen SE. C-reactive protein and the risk of cancer: a mendelian randomization study. J Natl Cancer Inst. 2010; 102:202–6. [PubMed: 20056955]
28. Elliott P, Chambers JC, Zhang W, Clarke R, Hopewell JC, Peden JF, et al. Genetic Loci associated with C-reactive protein levels and risk of coronary heart disease. JAMA. 2009; 302:37–48. [PubMed: 19567438]
29. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-α-and obesity-induced insulin resistance. Science. 1996; 271:665–8. [PubMed: 8571133]
30. Aziz N, Fahey JL, Detels R, Butch AW. Analytical performance of a highly sensitive C-reactive protein-based immunoassay and the effects of laboratory variables on levels of protein in blood. Clinical and Diagnostic Laboratory Immunology. 2003; 10:652–7. [PubMed: 12853400]
31. Giltay EJ, Geleijnse JM, Schouten EG, Katan MB, Kromhout D. High stability of markers of cardiovascular risk in blood samples. Clin Chem. 2003; 49:652–5. [PubMed: 12651820]
32. Wong HL, Pfeiffer RM, Fears TR, Vermeulen R, Ji S, Rabkin CS. Reproducibility and correlations of multiplex cytokine levels in asymptomatic persons. Cancer Epidemiol Biomarkers Prev. 2008; 17:3450–6. [PubMed: 19064561]
33. Trichopoulos D, Psaltopoulou T, Orfanos P, Trichopoulou A, Boffetta P. Plasma C-reactive protein and risk of cancer: a prospective study from Greece. Cancer Epidemiol Biomarkers Prev. 2006; 15:381–4. [PubMed: 16492932]
34. Wen W, Cai Q, Xiang YB, Xu WH, Ruan ZX, Cheng J, et al. The modifying effect of C-reactive protein gene polymorphisms on the association between central obesity and endometrial cancer risk. Cancer. 2008; 112:2409–16. [PubMed: 18383516]
35. Dossus L, Rinaldi S, Becker S, Lukanova A, Tjonneland A, Olsen A, et al. Obesity, inflammatory markers, and endometrial cancer risk: a prospective case-control study. Endocrine-related cancer. 2010; 17:1007–19. [PubMed: 20843938]

Table 1

Selected baseline characteristics of the study population

Characteristic	Cases	Subcohort	p-value*
Total number of women	151	301	
Age (years), mean (SD)	65.2 (7.1)	63.5 (7.5)	0.021
Ethnicity, n (%)			0.805
White	134 (90.5)	265 (88.3)	
Black	6 (4.1)	18 (6.0)	
Other	8 (5.4)	17 (5.7)	
Anthropometric measures			
BMI (kg/m^2), mean (SD)	29.7 (7.8)	27.5 (5.8)	0.002
Waist circumference (cm), mean (SD)	90.0 (16.4)	85.5 (13.2)	0.003
Parity, n(%)			0.527
Never pregnant/No term pregnancy	22 (14.8)	44 (14.8)	
1	16 (10.7)	25 (8.4)	
2–3	73 (49.0)	143 (48.2)	
≥4	38 (25.5)	85 (28.6)	
Age at first pregnancy in parous women, n(%)			0.678
<25	69 (61.1)	134(58.0)	
≥25	44 (39.9)	97 (42.0)	
Years of menstrual cycle, n (%)			0.128
<=36	48 (34.0)	111 (40.1)	
37–39	40 (28.4)	81 (29.2)	
≥ 40	53 (37.6)	85 (30.7)	
Ever used hormone therapy, n (%)			0.161
Never	105 (70.4)	233 (77.4)	
Former user	44 (29.5)	68 (22.6)	
Estradiol (pg/mL), n (%)			
<8	23 (15.9)	98 (33.1)	<0.001
8–13.9	53 (36.6)	97 (32.8)	
≥14	69 (47.6)	102 (43.1)	
Oral contraceptives use, n (%)	58 (39.9)	109 (36.2)	0.717
Smoking status n (%)			0.140
Never	74 (50.3)	150 (50.2)	
Former	70 (47.6)	127 (42.5)	
Current	3 (2.0)	22 (7.4)	
Alcohol (servings per wk), n (%)			0.769
<0.01	60 (40.5)	120 (40.0)	
0.01–1.56	33 (22.3)	77 (25.7)	
≥1.57	55 (37.2)	103 (34.3)	
Physical activity (MET), n (%)			0.876
<3.75	37 (25.0)	71 (23.8)	

Characteristic	Cases	Subcohort	p-value*
3.75–9.99	36 (24.3)	88 (29.5)	
10–19.99	40 (27.0)	66 (22.2)	
≥20	35 (23.7)	73 (24.5)	
Diagnosed with inflammation-related diseases†	86 (57.7)	153 (51.0)	0.089
Free IGF-1 (ng/mL), mean (SD)	0.40 (0.29)	0.45 (0.36)	0.087
Insulin (uIU/mL), mean (SD)	8.6 (6.6)	6.9 (5.1)	0.005
CRP (ug/mL), mean (SD)	3.9 (5.6)	3.1 (6.0)	0.284
IL-6 (pg/mL), mean (SD)	2.2 (2.0)	2.1 (2.0)	0.594
TNF-α (pg/mL), mean (SD)	3.6 (5.2)	3.3 (5.4)	0.791

*P-value for trend was used for ordinal variables.

†Ever been diagnosed with one of the following inflammation-related diseases: asthma, emphysema/chronic bronchitis, stomach or duodenal ulcer, diverticulitis, ulcerative colitis/Crohn's disease, systemic erythematosus, pancreatitis, multiple sclerosis, cardiovascular disease, or rheumatoid arthritis.

Table 2

Spearman rank correlation coefficients (95% CI) between inflammation markers and endometrial cancer risk factors among subcohort subjects who were not hormone users and did not have endometrial cancer

	CRP	IL-6	TNF-α
Age	−0.06 (−0.18—0.05)	0.01 (−0.10–0.13)	0.14 (0.03–0.25)
BMI	0.54 (0.45–0.61)	0.44 (0.34–0.53)	0.15 (0.03–0.26)
Waist	0.53 (0.44–0.61)	0.44 (0.35–0.53)	0.11 (−0.00–0.23)
Estradiol	0.26 (0.15–0.37)	0.21 (0.09–0.32)	−0.03 (−0.16—0.09)
Insulin	0.39 (0.28–0.48)	0.32 (0.21–0.42)	0.078 (−0.04–0.19)
Free IGF-1	0.05 (−0.06–0.17)	0.03 (−0.09–0.15)	0.16 (0.04–0.27)
CRP	NA	0.58 (0.50–0.65)	0.14 (0.02–0.25)
IL-6	0.58 (0.50–0.65)	NA	0.23 (0.11–0.33)
TNF-α	0.14 (0.02–0.25)	0.23 (0.11–0.33)	NA

Table 3

Adjusted hazard ratios (95% CI) for the associations of plasma levels of CRP, IL-6, and TNF-α and endometrial cancer risk

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}
CRP					
Cut-point (μg/mL)	<0.64	0.64–1.37	1.38–3.33	>3.33	
# cases/subcohort	21/72	26/75	39/72	56/75	
Adjusted for					
Age	1	1.09 (0.56, 2.11)	1.89 (1.00, 3.56)	2.47 (1.34,4.54)	<0.001
Age + BMI	1	1.17 (0.59,2.31)	1.88 (0.89, 3.94)	2.29 (1.13, 4.65)	0.012
Age + BMI + Free IGF-1	1	1.25 (0.61, 2.54)	1.88 (0.87, 4.06)	2.35 (1.14, 4.82)	0.013
Age + BMI + Estradiol	1	0.94 (0.46, 1.94)	1.56 (0.72, 3.39)	1.82 (0.87, 3.79)	0.050
Age + BMI + Insulin	1	1.27 (0.63, 2.58)	1.87 (0.96, 4.07)	2.02 (0.96,4.25)	0.053
Age + BMI + Free IGF-1 + Estradiol + Insulin	1	1.08 (0.50, 2.36)	1.55 (0.67, 3.63)	1.70 (0.78, 3.68)	0.127
IL-6					
Cut-point (pg/mL)	<0.98	0.98–1.52	1.53–2.36	>2.36	
# cases/subcohort	30/72	35/73	34/73	43/73	
Adjusted for					
Age	1	1.07 (0.60, 1.93)	1.07 (0.59, 1.94)	1.40 (0.79, 2.48)	0.266
Age + BMI	1	0.96 (0.49, 1.87)	0.87 (0.44. 1.71)	1.03 (0.52, 2.03)	0.970
Age + BMI + Free IGF-1	1	0.87 (0.49, 1.89)	0.87 (0.43, 1.76)	1.07 (0.54, 2.13)	0.860
Age + BMI + Estradiol	1	0.96 (0.48, 1.93)	0.76 (0.38, 1.55)	0.86 (0.41, 1.84)	0.604
Age + BMI + Insulin	1	0.90 (0.43,1.86)	0.75 (0.36, 1.57)	0.84 (0.38, 1.86)	0.623
Age + BMI + Free IGF-1 + Estradiol + Insulin	1	0.91 (0.43, 1.92)	0.64 (0.29, 1.40)	0.70 (0.29, 1.68)	0.328
TNF-α					
Cut-point (pg/mL)	<1.76	1.77–2.67	2.68–3.61	>3.61	
# cases/subcohort	25/73	38/74	31/74	48/74	
Adjusted for					
Age	1	1.45 (0.79, 2.67)	1.27 (0.68, 2.37)	1.73 (0.95, 3.17)	0.121
Age + BMI	1	1.48 (0.79, 2.75)	1.26 (0.66, 2.40)	1.52 (0.82, 2.82)	0.297
Age + BMI + Free IGF-1	1	1.65 (0.87, 3.15)	1.34 (0.69, 2.63)	1.64 (0.83, 3.22)	0.265
Age + BMI + Estradiol	1	1.58 (0.81, 3.10)	1.27 (0.63, 2.56)	1.80 (0.93, 3.48)	0.149
Age + BMI + Insulin	1	1.27 (0.64, 2.50)	1.12 (0.55, 2.29)	1.38 (0.71, 2.70)	0.433

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}
Age + BMI + Free IGF-1 + Estradiol + Insulin	1	1.56 (0.73, 3.30)	1.08 (0.49, 2.39)	1.65 (0.77, 3.54)	0.350