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Postmenopausal Levels of Endogenous Sex Hormones and Risk of Colorectal Cancer

Tess V. Clendenen¹, Karen L. Koenig^{1,2}, Roy E. Shore^{1,2,3}, Mortimer Levitz^{2,4}, Alan A. Arslan^{1,2,4}, and Anne Zeleniuch-Jacquotte^{1,2}

¹*Division of Epidemiology, Department of Environmental Medicine, New York University School of Medicine, New York*

²*New York University Cancer Institute, New York University School of Medicine, New York*

³*Radiation Effects Research Foundation, Hiroshima, Japan*

⁴*Department of Obstetrics and Gynecology, New York University School of Medicine, New York*

Abstract

Observational epidemiologic studies and randomized trials have reported a protective effect of oral hormonal replacement therapy on risk of colorectal cancer. Only one previous prospective study, the Women's Health Initiative Observational Study, has reported on the relationship between endogenous hormones and incident colorectal cancer. Contrary to expectation, the investigators found that women with higher circulating estradiol levels were at increased risk of developing colorectal cancer. We conducted a case-control study nested within the New York University Women's Health Study prospective cohort to evaluate the association between endogenous levels of estrone, estradiol, and sex hormone binding globulin (SHBG) with risk of colorectal cancer. We measured hormones and SHBG in serum samples collected at enrollment from a total of 148 women who subsequently developed colorectal cancer and 293 matched controls. Circulating estrone levels were positively associated with risk of colorectal cancer: the odds ratio for the highest versus lowest quartile of estrone was 1.8 (95% confidence interval = 1.0–3.3). We found a non-significant inverse association between SHBG and colorectal cancer, which disappeared after adjusting for body mass index. We did not find an association between estradiol and colorectal cancer risk, but we cannot rule out a potential association because of substantial laboratory error in the measurement. Our results suggest that endogenous estrone may be associated with increased risk of colorectal cancer in postmenopausal women.

Keywords

Colorectal Cancer; Estrone; Estradiol; SHBG; Progesterone; Postmenopausal

Introduction

Most observational studies have found that postmenopausal hormone replacement therapy (HRT) is associated with a reduced risk of colorectal cancer. A meta-analysis of 18 epidemiological studies reported that women who had ever used HRT had a 20% lower risk (relative risk = 0.80, 95% CI = 0.74–0.86) compared with women who had never taken such hormones (1). The risk reduction was observed in both case-control and prospective cohort

studies. Confirmation of an inverse association between use of hormonal therapy and risk of colorectal cancer was provided by the Women's Health Initiative (WHI) clinical trial in which women randomized to receive estrogen + progestin HRT had a 37% lower risk of colorectal cancer than women receiving placebo [hazard ratio (HR) = 0.63, 95%CI = 0.43–0.92] (2). In women who had had a hysterectomy, who were randomized to receive either estrogen alone (without progestin) or a placebo, a protective effect was not observed (HR = 0.93, 95%CI = 0.75–1.15) (3).

This protective effect of exogenous estrogen + progestin raises the possibility that higher levels of *endogenous* estrogen, and/or progesterone, may be associated with a reduced risk of colorectal cancer. To date, only one prospective study has reported on the association between endogenous circulating estrogen levels and subsequent colorectal cancer development. A recent study of 438 colorectal cancer cases and a subcohort of 816 women from the Women's Health Initiative Observational Study (WHI-OS), which includes women who were ineligible for, or not interested in the clinical trial portion of that study, found, contrary to expectation, an increased risk of colorectal cancer in women with higher levels of *endogenous* estradiol, despite the fact that women using oral HRT had a reduced risk of developing the disease (4).

In this study, we investigated the association between pre-diagnostic endogenous concentrations of estradiol, estrone, progesterone, and sex hormone binding globulin (SHBG) in postmenopausal women, and risk of subsequent colorectal cancer. We conducted a nested case-control study within the prospective New York University Women's Health Study cohort.

Methods

The New York University Women's Health Study enrolled 14,274 women ages 35 to 65 who attended a mammography breast-screening clinic in New York City between 1985 and 1991. A total of 7054 women (49.4% of the cohort) were postmenopausal at enrollment. The cohort has been described in detail previously (5,6). Women who were pregnant or using exogenous hormones (oral contraceptives or HRT) within the 6 months prior to enrollment were not eligible for inclusion. At enrollment, participants answered a self-administered questionnaire with questions about their medical and reproductive history, use of medications, and demographic characteristics. Participants donated 30mL of peripheral venous blood at enrollment, and aliquots of serum were stored at -80°C .

Cases of incident malignant disease are identified through self-administered questionnaires mailed to participants every 2 to 4 years, with telephone follow-up of non-respondents. Medical records are retrieved for reported malignancies and reviewed to confirm colorectal cancer diagnoses. Active follow-up is supplemented by record linkages to state cancer registries in New York, New Jersey, and Florida. Ascertainment of vital status and cause of death is accomplished through record linkage with the National Death Index. A capture-recapture analysis estimated the cancer ascertainment rate to be 95% in the NYUWHS cohort (7).

Cases and Controls

Participants in this study of hormones and colorectal cancer were limited to women who were postmenopausal at the time of enrollment. Women were classified as postmenopausal if they reported not having a menstrual period in the six months prior to blood donation or having had a bilateral oophorectomy. Women who reported having had a hysterectomy without bilateral oophorectomy prior to natural menstrual period cessation were classified as postmenopausal if they were at least 52 years of age at the time of enrollment.

Incident cases of invasive colorectal cancer occurring after blood donation and before July 1, 2003 (the end of the last complete round of follow-up through questionnaires and state cancer

registry record linkages) were eligible for inclusion in this study. Women who had a previous cancer (other than non-melanoma skin cancer) were excluded. In total, 148 cases met the eligibility criteria. Two control participants per case were randomly selected from among the members of the cohort who were alive and free of cancer as of the date of diagnosis of the case and matched the case on age at enrollment (± 6 months) and date of blood donation (± 3 months). This study was approved by the Institutional Review Board at the New York University School of Medicine.

Laboratory Analyses

Laboratory assays for estrone, estradiol, and progesterone were conducted at the Quest Diagnostics Nichols Institute (San Juan Capistrano, CA). Estrone and estradiol were measured by radioimmunoassay following organic extraction and celite chromatography. The lower limit of quantitation (LLOQ) was 10 pg/mL for estrone and 2 pg/mL for estradiol. Progesterone was measured using liquid chromatography tandem mass spectrometry (LCMSMS assay, LLOQ 100 pg/mL). Sex hormone binding globulin (SHBG) was measured in the laboratory of Dr. Mortimer Levitz at the New York University School of Medicine, using a two-site immunometric chemiluminescent assay on an IMMULITE 2000 instrument (Diagnostic Products Corp., Los Angeles, CA). The SHBG assay has a LLOQ of 2 nmol/L.

Samples from all members of a case-control matched set were assayed together on the same well-plate. Duplicate samples were included for 10% of the subjects for quality control purposes. Samples were arranged and labeled in such a way that laboratory personnel were unaware of the case-control status of the samples and the identity of the duplicates. The mean intra- and inter-batch coefficients of variation were, respectively, 14.9% and 16.9% for estrone, 33.5% and 36.3% for estradiol, and 3.5% and 3.5% for SHBG. Since progesterone values were below the lower limit of quantitation for over 93% of our samples, data for this hormone could not be analyzed and the association between this hormone and risk of colorectal cancer could not be evaluated. The midpoint between the LLOQ and zero was used for 35 participants who had values below the LLOQ for estrone and 7 participants for estradiol. Measurements of estradiol were unavailable for 122 subjects (40 1:2 matched sets plus one 1:1 matched set) because of lack of sufficient serum volume to re-run the assay after a batch failure. Therefore, these 122 subjects, along with 4 subjects in other batches who did not have sufficient sample material for the assay are not included in the estradiol analyses.

Statistical Analysis

Hormone concentrations were log₂-transformed to reduce departures from the normal distribution and to yield odds ratios associated with a doubling in hormone levels (8). We used the square root transformation for SHBG which resulted in a better approximation to the normal distribution than log-transformation and has been used by others (9,10). Conditional logistic regression, which takes into account the matched study design, was used to calculate the p-values comparing cases and controls. Spearman correlation coefficients, which do not require normality, were calculated between estrone, estradiol, SHBG, and BMI. Free estradiol concentrations were calculated from the total estradiol and SHBG values according to the mass action laws, assuming a constant serum albumin concentration of 43 g/L (11). Free estradiol was very highly correlated with total estradiol ($r=0.96$ in controls) and did not add any information to the analysis, so results for free estradiol are not presented in this manuscript.

Estrone and SHBG variables were divided into quartiles, while estradiol was divided into tertiles due to the frequency of missing data and the resultant smaller sample size. Quantile cutpoints were based on the distribution of values in the controls. Odds ratios and corresponding 95% confidence intervals for quantiles of estrone, estradiol, and SHBG were estimated using conditional logistic regression. Tests for trend across quantiles were conducted using the

median value of the quantile as the category score. The biomarkers were also analyzed as continuous variables. Potential confounding effects of body mass index (BMI) (kg/m^2) at enrollment, regular use of aspirin prior to index date (3 or more times per week for 6 months or longer), use of hormone replacement therapy (HRT) (ever/never up to index date), number of full term pregnancies, use of oral contraceptives (ever/never up to index date), smoking at baseline (current, former, never), education (high school, college, more than college), first degree family history of colorectal cancer (yes, no), alcohol use at baseline (none, less than 7 drinks/week, 7 or more drinks/week), and physical activity at baseline (tertiles of vigorous activity and of walking/light activity) were considered by including these variables in the conditional logistic regression models. To adequately compare the adjusted and unadjusted models, we restricted the study population to the subjects who did not have missing data for a given covariate and then compared models in this restricted population to observe the effect of the covariate on odds ratio estimates. BMI was the only covariate that appreciably altered the risk estimates and is included in the adjusted models along with the matching factors. The other potential confounders did not alter the odds ratios of colorectal cancer associated with hormone levels and are not included in the models.

Analyses were repeated within tumor location subgroups (colon, rectum) and after excluding women who were diagnosed within 5 years of blood donation to reduce potential effects of preclinical disease on any observed associations between hormones and colorectal cancer. The relationship between hormones and colorectal cancer risk was also evaluated within subgroups of BMI (<25 , ≥ 25 kg/m^2) and within never-users of HRT by breaking the matching and using unconditional logistic regression models adjusted for the matching factors and BMI. Because women using HRT within 6 months of cohort enrollment were not eligible for inclusion in the cohort, only 30 cases and 77 controls in this study had used HRT by diagnosis/index date, which limited our ability to meaningfully evaluate the details of HRT use, such as time of use and type of HRT. All analyses were conducted using SAS software (version 9.1, SAS Institute, Cary, NC).

Results

Descriptive characteristics of the cases and controls are shown in Table 1. The age range of both case and control subjects at blood donation was 53 to 64 years. Cases were less likely than controls to use aspirin on a regular basis (17 vs. 29%; $p = 0.002$) and were more likely to drink alcohol (16 vs. 8% consumed at least 7 drinks per week; $p = 0.02$). Cases were also more likely to have a first degree family member with colorectal cancer (30% vs. 17%; $p = 0.003$).

Although cases and controls did not differ significantly with regard to the other potential confounders shown in Table 1, differences were in the expected direction according to previous studies. Cases had higher BMI than controls (25.6 vs. 24.7 kg/m^2 respectively). Additionally, cases were less likely to have children than controls (77 vs. 80% ever pregnant) and cases who did have children were likely to have fewer than the controls (62% cases had 2 or more children vs. 68% of controls; $p = 0.15$). A lower proportion of cases used hormone replacement therapy (22 vs. 28% ever users, OR = 0.7; 95% CI = 0.4–1.1). Although average endogenous levels of SHBG and estradiol were not significantly different between cases and controls, estrone values were statistically significantly higher in cases (21 vs. 18 pg/mL ; $p = 0.02$) than in controls.

Estimates of Spearman's correlation coefficients among hormones and BMI are reported in Table 2 for the control group. Briefly, estrone and estradiol were strongly positively correlated ($r = 0.55$). BMI was positively correlated with both estrogens ($0.34 \leq r \leq 0.42$); SHBG was negatively correlated with BMI ($r = -0.50$) and slightly less so with estrone and estradiol ($r \approx -0.26$). Correlations in cases were similar in direction and magnitude to correlations in controls (data not shown).

Odds ratio estimates from the conditional logistic regression models are shown in Table 3. The odds ratios for colorectal cancer were elevated in the highest quartiles of estrone (3rd quartile OR = 1.7, 95% CI = 0.96–3.0; 4th quartile OR = 1.8, 95% CI = 1.0–3.3, *p* for trend = 0.02 on the continuous scale). Adjustment for BMI at enrollment somewhat attenuated these odds ratios, but a positive association between estrone and colorectal cancer risk was still apparent (*p* for trend = 0.09 on the continuous scale). Adjusting for aspirin use, family history of colorectal cancer, and alcohol use (each considered individually and together) resulted in a less than 5% change in the odds ratios, and therefore we did not adjust for these covariates our final models. There were no statistically significant associations between estradiol or SHBG and risk of colorectal cancer; however, the estradiol measurements were missing for 29% of the population.

As shown in table 4, the positive association between estrone levels and risk of colorectal cancer persisted after the exclusion of women diagnosed within 5 years of blood donation (*n*=32 cases), suggesting that the association was not attributable to existing pre-clinical disease. Estrone analyses were repeated after stratifying on BMI. The positive association between estrone and risk in overweight and obese (BMI ≥ 25 kg/m²) women was somewhat stronger than the relationship for women with lower BMI but the test for interaction was not significant (*p* for interaction=0.90). When analyses were restricted to women who had never used HRT, the odds ratios still showed an elevated risk for women with higher estrone levels (OR₃₋₁ = 1.8; 95% CI = 0.9–3.6)(Table 4). Stratifying by colon (*n*=125 cases) versus rectal (*n*=23 cases) cancer did not yield statistically heterogeneous results (*p* for interaction = 0.83), and for colon cancer the odds ratios increased across tertiles of estrone with a 70% higher risk for the third tertile compared with the first tertile (OR = 1.7; 95% CI = 0.9–3.1; *p* for trend=0.10).

Discussion

In the early 1980's, McMichael and Potter (12) proposed the hypothesis that female sex hormones may be protective against colorectal cancer, citing the protective effect of parity, a potential protective effect of high-dose oral contraceptives, and biological evidence that sex hormones modify hepatic cholesterol metabolism and reduce bile acids. Since then, observational studies (1) and randomized controlled trials (2,13) have found a protective effect of exogenous hormonal therapy on risk of colorectal cancer. Exogenous and endogenous estrogens have been shown to stimulate cell proliferation and tumor development in the breast and uterus. These pro-tumorigenic effects are thought to be mediated through the estrogen receptor subtype, ER α , which is predominant in these tissues (14). It has been hypothesized that estrogens may be protective against colorectal cancer because the predominant estrogen receptor subtype in the colon, ER β , has been shown to inhibit transcription and induce apoptosis upon binding estrogen *in vitro* (14–16). The current study was designed to test the hypothesis that endogenous estrogen levels are associated with a reduced risk of colorectal cancer.

In this nested case-control study within the NYUWHS cohort, we evaluated the association between endogenous estrogens and colorectal cancer risk in postmenopausal women. Contrary to our initial hypothesis, we observed a *positive* association between levels of estrone and risk of colorectal cancer. These results were consistent in subgroups according to tumor site (colon/rectum), BMI, and lag time between blood donation and diagnosis. We were unable to reproduce the Women's Health Initiative Observational Study (WHI-OS) finding of an association between estradiol and risk of colorectal cancer. However, the lack of association in our study may be due to the large number of participants with missing data for estradiol and the substantial laboratory error in the measurements (average intra-batch coefficient of variation = 33.5%). We also found a non-significant inverse association between SHBG and risk of colorectal cancer. This result further argues against a protective effect of estrogens: according to the free hormone hypothesis, only non-SHBG bound estrogens are available to

enter target cells; if estrogens were protective, higher levels of SHBG, leading to reduced levels of bioavailable estrogens, would be expected to be positively associated with risk of colorectal cancer.

Our finding of a positive association between *endogenous* estrone and colorectal cancer was unexpected given the substantial evidence that *exogenous* estrogens are protective. Despite this apparent contradiction, the odds ratios for estrone and risk of colorectal cancer in our study were in the same direction and of similar magnitude to the hazard ratios reported in the WHI-OS for risk of colorectal cancer by tertile of estradiol ($HR_{t2-t1} = 1.63$; 95%CI = 1.14–2.46 and $HR_{t3-t1} = 1.43$; 95%CI = 0.95–2.16) (4). Our observation of the effect of estrone on risk of colorectal cancer is consistent with a linear trend of increasing risk with increasing levels of estrone (p for trend = 0.02), yet the similarity of the odds ratios for the two highest quartiles versus the lowest quartile of estrone are also compatible with a plateau effect, similar to what was observed in the WHI-OS, where the risk was elevated in the second tertile but did not increase further in the highest tertile of estradiol.

After controlling for the effects of BMI, the effect of estrone on risk was reduced and only marginally significant (p for trend on the continuous scale = 0.09). We adjusted for BMI because it may influence colorectal cancer risk through biological pathways other than hormonal, including the insulin and IGF-I pathways. It could be argued, though, that adjusting for BMI results in overadjustment since adipose tissue is the location of aromatization of androgens to estrogens in postmenopausal women and therefore BMI is antecedent to estrone on the causal pathway to colorectal cancer. For this reason (17–19), results from both BMI-adjusted and unadjusted models are presented.

Gunter et al. (WHI-OS) provided two possible explanations for why oral hormonal therapy may be protective for colorectal cancer while endogenous estrogen has an adverse effect: 1) Oral administration of hormonal therapy exposes the liver to a large bolus of estrogen, which results in reduced synthesis of potentially harmful hepatic proteins, such as IGF-I, IGFBP-3, insulin, and low-density lipoprotein, thereby reducing the risk of colorectal cancer development; or 2) Since the most commonly prescribed HRT preparations contain estrone rather than estradiol, risk reduction by use of hormonal therapy may be due to anti-proliferative effects of estrone seen on colonic cells *in vitro* (4). Endogenous estrone levels were not measured in the WHI-OS. However, our findings support an adverse effect of estrone similar to the adverse effect of estradiol observed by Gunter et al.

The other possible explanation for the discrepant results proposed by Gunter et al. is that oral estrogens affect the hepatic synthesis of various proteins (“first pass” effect). In particular, the production of IGF-1 and insulin, which are associated with an increased risk of colorectal cancer (20), is reduced by oral estrogens. Only a few studies have compared the effects of oral and transdermal (where there is no first pass effect) hormonal therapy on colorectal cancer risk, and results have been conflicting (21–23). Future comparisons of the effects of oral and transdermal hormone therapies may shed light on the relevance of the first pass effect to explain the reduced risk of colorectal cancer observed with HRT.

Another potential explanation for the opposite effects of endogenous and exogenous estrogens is that, since the WHI clinical trial only found a protective effect of hormonal therapy composed of estrogen + progestin and found no effect for estrogen-only therapy, progestin is the protective agent (2,3). In the current study we planned to evaluate the hypothesis that endogenous progesterone is associated with a reduced risk of colorectal cancer, but 93% of our study subjects had progesterone values below the lower limit of quantitation for the LC/MS/MS assay (100pg/ml). Other investigators have used a radioimmunoassay which has a lower LLOQ to measure progesterone in postmenopausal women (24). It should be noted, though, that the

radioimmunoassay is less specific than the LC/MS/MS method and therefore that the levels measured by radioimmunoassay are likely to be overestimated. Although we were not able to examine the association with progesterone levels below 100 pg/mL, our results confirm that progesterone levels in postmenopausal women are very low, well below levels observed in women taking estrogen + progestin preparations. In light of this and findings from *in vitro* studies that progesterone does not influence growth of colon cells (25), it is unlikely that endogenous levels of progesterone in postmenopausal women contribute strongly to preventing CRC risk. However, we cannot discount a possible role for exogenous progestins, which may interact with other steroid receptors, including the androgen receptor, to influence colorectal cancer risk (26).

There are several biologically plausible reasons why endogenous estrogens may be associated with increased risk of colorectal cancer. *In vitro* studies, although somewhat inconsistent, have reported mitogenic and tumorigenic actions of estrogens on colorectal cancer cells (25,27–30). Furthermore, reduced enzyme-mediated inactivation of estradiol has been observed in colorectal cancer tissues as compared with normal tissues (31,32) and may indicate that malignant colorectal cancer cells are exposed to higher levels of endogenous estradiol. Although epidemiologic studies are not entirely consistent, several have shown that obesity, which is associated with higher levels of endogenous estrogen, is associated with moderately elevated colorectal cancer risk (19).

A limitation of this study is that estrogen and SHBG levels were measured in samples collected at a single point in time and may not reflect changes occurring between blood collection and diagnosis. However, in a temporal reproducibility study using repeated blood samples from a subset of the NYUWHS postmenopausal participants, we found the within-subject variation over a 2–3 year period to be substantially lower than the between-subject variation (intra-class correlations were 0.64 for estradiol, 0.65 for estrone, and 0.88 for SHBG), indicating that a single measurement is a reasonably reliable representation of an individual's average long-term level, relative to other individuals (33).

The estradiol batch failure was the result of an instrumentation malfunction and unrelated to sample quality or quantity. Matched sets were randomly allocated into batches, therefore decreasing the likelihood that the samples in the failed batch were systematically different from those in successful batches. To evaluate how the exclusion of participants with missing estradiol measurements may have influenced the results, we repeated the main analyses for estrone in the subsample of women for whom estradiol measurements were available and found no differences in the results for estrone in this subgroup vs. the entire study sample. This suggests that the exclusion of samples from the failed batch may not have substantially influenced the estradiol results. However, the high degree of laboratory error (CV=33.5%) in the estradiol measurements decreases our confidence in the estradiol results and prevents us from ruling out an association between estradiol and risk of colorectal cancer.

The NYUWHS cohort study was initiated in a breast cancer screening setting. Participants in the cohort are mostly Caucasian, middle-class, and more likely to be health conscious than the general population, which should be taken into consideration when generalizing the results of this study. Information collected from the participants at baseline was primarily related to breast cancer, and thus, for some colorectal cancer risk factors (e.g. family history) data was collected only through follow-up questionnaires. Biases that are common in retrospective studies (i.e. those that collect information about participants after disease diagnosis) may have influenced the measurement of these covariates in participants who had already been diagnosed with disease when they completed follow-up questionnaires. However, inclusion of the potential confounders as covariates in the regression models did not appreciably affect risk estimates (except for BMI, which was calculated at enrollment for all participants).

One of the strengths of this study is that all participants included in the NYUWHS were free of cancer and not using any exogenous hormones for at least 6 months prior to blood donation, ensuring that measurements of endogenous hormones were not influenced by hormonal medications or existing clinical disease. The latter was further confirmed by an analysis limited to cases that were diagnosed at least five years after blood donation.

In conclusion, we found some evidence of an association between SHBG levels in postmenopausal women and risk of colorectal cancer; however this association was attenuated by adjustment for BMI. We did not find an association between estradiol and risk, though the large amount of missing data and measurement error for estradiol prevents us from ruling out a potential association. Our results support a positive association between endogenous estrone and risk of colorectal cancer.

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Table 1

Characteristics of Colorectal Cancer Cases and Controls NYU Women's Health Study, 1985–2003

Characteristics	Cases (n=148)	Controls (n=293)
Age (years) at enrollment, median (10th–90th percentiles)	60.4 (53.7–64.3)	60.4 (53.8–64.2)
Age (years) at diagnosis, median (10th–90th percentiles)	69.9 (61.2–77.7)	
Lag time (years) to diagnosis, median (10th–90th percentiles)	10.7 (2.3–16.7)	
Body mass index (kg/m ²), median (10th–90th percentiles)	25.6 (21.1–33.3)	24.7 (20.3–31.6)
Age (years) at menarche, median (10th–90th percentiles)	12.5 (11–14)	12.0 (11–15)
Age (years) at menopause, median (10th–90th percentiles)	50 (42–54)	51 (41–55)
Ever use of aspirin 3 or more times/week for 6 months or longer (N, %) ^{†‡}	22 (16.9)	76 (28.6)
First-degree family history of colorectal cancer (N, %) ^{†§}	33 (30.3)	41 (16.5)
Ever use of hormone replacement therapy (N, %) [‡]	30 (22.7)	77 (28.1)
Ever use of oral contraceptives (N, %) [‡]	19 (14.7)	36 (13.5)
Ever pregnant (N, %)	114 (77.0)	235 (80.2)
Age (years) at first pregnancy [*] , median (10th–90th percentiles)	25 (20–31)	25 (19–31)
Smoking at enrollment [§]		
Non-smokers (N, %)	59 (44.4)	118 (45.6)
Current smokers (N, %)	21 (15.8)	46 (17.8)
Former smokers (N, %)	53 (39.8)	95 (36.7)
Education [§]		
Completed high school or less (N, %)	54 (47.0)	103 (41.5)
Completed college or less (N, %)	38 (33.0)	101 (40.7)
Completed graduate school or less (N, %)	23 (20.0)	44 (17.7)
Hysterectomy (N, %)	39 (26.7)	78 (26.8)
Complete Bilateral Oophorectomy (N, %)	27 (18.7)	43 (14.4)
Ethnicity [§]		

Characteristics	Cases (n=148)	Controls (n=293)
European (N, %)	112 (84.3)	221 (88.2)
African American (N, %)	15 (11.9)	18 (7.1)
Hispanic (N, %)	2 (1.5)	10 (3.9)
Asian (N, %)	3 (2.2)	1 (0.4)
Alcohol intake ^{†‡}		
Non-drinker (N, %)	80 (60.2)	183 (69.3)
Less than 7 drinks/week (N, %)	32 (24.1)	59 (22.3)
7 or more drinks/week (N, %)	21 (15.8)	22 (8.3)
Physical activity		
Vigorous exercise at baseline (MET $\geq$ 5.5)		
No vigorous exercise (N, %)	91 (61.5)	183 (62.5)
$\leq$ 11 met hours/week (N, %)	20 (13.5)	54 (18.4)
>11 met hours/week (N, %)	37 (25.0)	56 (19.1)
Light activity at baseline (MET<5.5) [‡]		
< 2.5 met hours/week (N, %)	54 (41.5)	92 (34.6)
< 7.5 met hours/week (N, %)	35 (26.9)	91 (34.2)
$\geq$ 7.5 met hours/week (N, %)	41 (31.5)	83 (31.2)
Estrone (pg/mL), median (10th–90th percentiles) [†]	21 (11–41)	18 (10–33)
Estradiol (pg/mL), median, (10th–90th percentiles) ^{//}	7 (4–16)	7 (4–16)
SHBG (nmol/L), median (10th–90th percentiles)	47.2 (22.0–83.8)	49.6 (25.6–88.7)

* In parous women only

[†] P-value for differences between cases and controls <0.05 for ever use of aspirin (p=0.002), family history of colorectal cancer (p=0.003), alcohol intake (p=0.02), and estrone (p=0.02)

[‡] Missing for less than 10% of subjects

[§] Missing for less than 20% of subjects

^{//} Missing for 28% of subjects

Note: MET=metabolic equivalents defined as the metabolic rate associated with an activity divided by the metabolic rate at rest.

Table 2Spearman Correlations between Estrogens, SHBG, and BMI in controls (n = 293[†])

Hormone	Estrone	Estradiol	SHBG	BMI
Estrone	1.0			
Estradiol	0.55 [*]	1.0		
SHBG	-0.27 [*]	-0.25 [*]	1.0	
BMI	0.42 [*]	0.34 [*]	-0.50 [*]	1.0

*
p<0.001[†]
Estradiol missing for 84 controls

Table 3

Odds Ratios for Associations of Estrogens and SHBG with Incident Colorectal Cancer

Hormone	Cases (n=148) [*]	Controls (n=293) [*]	Univariate Odds Ratio (95% CI) [†]	BMI-Adjusted Odds Ratio (95% CI) [‡]
Estrone Quartiles				
≤13 pg/mL (%)	27 (18.2)	78 (26.6)	1.0 (reference)	1.0 (reference)
14–18 pg/mL (%)	32 (21.6)	72 (24.6)	1.3 (0.7–2.4)	1.2 (0.7–2.2)
19–25 pg/mL (%)	45 (30.4)	75 (25.6)	1.7 (0.96–3.0)	1.5 (0.8–2.7)
≥26 pg/mL (%)	44 (29.7)	68 (23.2)	1.8 (1.0–3.3)	1.6 (0.8–3.0)
			p-trend=0.03	p-trend=0.15
Continuous scale (log ₂)			1.4 (1.1–1.9)	1.3 (0.96–1.8)
			p-trend =0.02	p-trend =0.09
Estradiol Tertiles				
≤6 pg/mL (%)	31 (29.2)	66 (31.6)	1.0 (reference)	1.0 (reference)
7–9 pg/mL (%)	44 (41.5)	81 (38.8)	1.2 (0.7–2.2)	1.0 (0.6–1.9)
≥10 pg/mL (%)	31 (29.2)	62 (29.7)	1.1 (0.6–2.2)	0.8 (0.4–1.7)
			p-trend=0.81	p-trend=0.43
Continuous scale (log ₂)			0.9 (0.6–1.3)	0.7 (0.5–1.2)
			p-trend =0.70	p-trend =0.17
SHBG Quartiles				
≤37 nmol/L (%)	49 (33.1)	79 (27.0)	1.0 (reference)	1.0 (reference)
38–50 nmol/L (%)	34 (23.0)	70 (23.9)	0.8 (0.5–1.3)	0.8 (0.5–1.3)
51–66 nmol/L (%)	36 (24.3)	69 (23.5)	0.9 (0.5–1.5)	1.0 (0.5–1.8)
≥67 nmol/L (%)	29 (19.6)	75 (25.6)	0.6 (0.4–1.1)	0.8 (0.4–1.4)
			p-trend=0.13	p-trend=0.48
Continuous scale (square root)			0.9 (0.8–1.1)	1.0 (0.8–1.1)
			p-trend =0.18	p-trend =0.60

* All analyses are based on 148 cases and 293 controls except for estradiol which includes 106 cases and 209 controls

[†] Adjusted for matching factors only

[‡] Adjusted for matching factors and BMI

Table 4
Odds Ratios Relating Estrone to Colorectal Cancer in Subgroups

OR (95% CI) for Tertiles of Estrone [*]				
Subgroups	1	2	3	p-trend
All Cases and Controls	1.0 (reference)	1.6 (0.9–2.7)	1.7 (0.99–3.0)	0.08
Body Mass Index [†]				
<25 kg/m ² (n = 65 cases/151 controls)	1.0 (reference)	1.2 (0.6–2.4)	1.7 (0.7–3.9)	0.18
≥25 kg/m ² (n = 81 cases/142 controls)	1.0 (reference)	2.6 (1.1–5.9)	2.2 (0.98–5.0)	0.15
Tumor Location				
Colon (n = 125 cases/247 controls)	1.0 (reference)	1.5 (0.9–2.6)	1.7 (0.9–3.1)	0.10
Rectum (n = 23 cases/46 controls)	1.0 (reference)	2.3 (0.5–10.7)	2.0 (0.5–8.8)	0.49
Diagnosed ≥5 Years After Blood Donation (n = 116 cases/ 229 controls)	1.0 (reference)	1.9 (1.0–3.3)	2.0 (1.1–3.9)	0.05
Never Users of HRT (n=102 cases/ 197 controls)	1.0 (reference)	1.7 (0.9–3.2)	1.8 (0.9–3.6)	0.10

^{*} Adjusted for matching factors and BMI

[†] P-value for interaction between BMI and estrone was 0.90 with estrone on the continuous log₂ scale and BMI categorized as <25kg/m² and ≥25 kg/m².