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A Prospective Evaluation of Endogenous Sex Hormone Levels and Colorectal Cancer Risk in Postmenopausal Women

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Abstract

Background: Postmenopausal hormone therapy use has been associated with lower colorectal cancer risk in observational studies. However, the role of endogenous sex hormones in colorectal cancer development in postmenopausal women is uncertain.

Methods: The relation of colorectal cancer risk with circulating levels of estradiol, estrone, free (bioactive) estradiol, progesterone and sex hormone–binding globulin (SHBG) was determined in a nested case-control study of 1203 postmenopausal women (401 case patients and 802 age and race/ethnicity–matched control patients) enrolled in the Women's Health Initiative Clinical Trial (WHI-CT) who were not assigned to the estrogen-alone or combined estrogen plus progestin intervention groups. We used multivariable-adjusted conditional logistic regression models that included established colorectal cancer risk factors. All statistical tests were two-sided.

Results: Comparing extreme quartiles, estrone (odds ratio [OR]$_{q4\text{-}q1}$ = 0.44, 95% confidence interval [CI] = 0.28 to 0.68, P_{trend} = .001), free estradiol (OR$_{q4\text{-}q1}$ = 0.43, 95% CI = 0.27 to 0.69, $P_{trend} \leq .0001$), and total estradiol (OR$_{q4\text{-}q1}$ = 0.58, 95% CI = 0.38 to 0.90, P_{trend} = .08) were inversely associated with colorectal cancer risk. SHBG levels were positively associated with colorectal cancer development (OR$_{[q4\text{-}q1]}$ = 2.30, 95% CI = 1.51 to 3.51, $P_{trend} \leq .0001$); this association strengthened after further adjustment for estradiol and estrone (OR$_{q4\text{-}q1}$ = 2.50, 95% CI = 1.59 to 3.92, $P_{trend} < .0001$). Progesterone was not associated with colorectal cancer risk.

Conclusion: Endogenous estrogen levels were inversely, and SHBG levels positively, associated with colorectal cancer risk, even after control for several colorectal cancer risk factors. These results suggest that endogenous estrogens may confer protection against colorectal tumorigenesis among postmenopausal women.

Colorectal cancer is the third most common cancer worldwide with more than one million new case patients diagnosed every year (1). Colorectal cancer incidence rates are lower among women compared with men across all age categories, and it has been hypothesized that higher exposure to estrogen among women may confer a protective role (2). Consistent with this hypothesis are findings from a substantial body of epidemiologic literature that report a 20% to 40% lower incidence of

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colorectal cancer among users than nonusers of early high-dose oral contraceptives (3) and of postmenopausal hormone therapy (HT) (4–9). However, in contrast to the results from observational studies of HT use and colorectal cancer, the Women's Health Initiative Clinical Trial (WHI-CT) reported no effect of estrogen-alone therapy on colorectal cancer risk (10). Further, while administration of estrogen plus progestin was initially found to yield a 44% reduction in risk of developing colorectal cancer compared with the placebo group (10), longer follow-up revealed this finding was a probable consequence of diagnostic delay (8).

Data from studies that have evaluated the association of endogenous, circulating estrogen on colorectal cancer incidence are limited and contradictory. We previously reported an unexpected, borderline statistically significant positive association between endogenous estradiol levels and colorectal cancer incidence among participants of the WHI Observational Study (WHI-OS) that was independent of related factors such as body habitus and hyperinsulinemia (11), although that study did not measure circulating levels of other sex hormones, such as estrone, progesterone, and free estradiol, or sex hormone–binding globulin (SHBG). Since the publication of the WHI-OS analysis, two follow-up studies conducted in the New York University Women's Health Study (NYUWHS) (12) and a joint Nurses' Health Study (NHS) and Women's Health Study (WHS) analysis reported no association between estradiol levels and colorectal cancer risk (13). Data on other components of the sex hormone axis, including estrone and sex hormone–binding globulin (SHBG), and their association with colorectal cancer are also sparse, and no studies have evaluated the association of endogenous progesterone levels with colorectal cancer.

Therefore, to further knowledge on the role of endogenous estradiol and other sex hormones on colorectal cancer development, we conducted a prospective evaluation of estradiol, estrone, free (unbound) estradiol, progesterone, and SHBG and colorectal cancer risk using the current gold standard sex hormone assays (14,15) among participants of the WHI-CT who were not assigned to the estrogen-alone or combined-estrogen-plus-progestin intervention arms and were therefore not using exogenous hormones at baseline. We also controlled for other serologic factors that are related to sex hormone levels and adiposity and have been linked to colorectal cancer incidence in some studies, namely fasting insulin, free insulin-like growth factor (IGF)-1, and C-reactive protein (CRP).

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Methods

Study Population and Collection of Blood Samples

At study baseline (1993–1998), 68 133 postmenopausal women (age 50–79 years) were enrolled into the WHI-CT, which had four components: 1) hormone therapy with estrogen-alone (E), 2) hormone therapy with combined estrogen plus progestin (E+P), 3) dietary modification (DM), and 4) calcium plus vitamin D (CaD) (16). Women were recruited from 40 clinical centers across the United States (US) using mass mailing to age-eligible women who were enumerated from voter registration, driver's licenses, and HCFA records (17). Fasting blood samples were collected from all participants at baseline and during the Year 1 clinic visits. Blood samples were labeled, centrifuged, and stored at -70°C within two hours of collection. All specimens were shipped to the central WHI biorepository for long-term storage.

Data Collection

All women enrolled in the WHI-CT completed a baseline clinic visit and detailed questionnaire that included information regarding medical and reproductive history, family medical history, a food frequency questionnaire, an inventory of currently used medications (including dietary supplements), and an assessment of psychosocial factors, quality-of-life, and health-related behaviors. During the baseline visit at the WHI Clinical Centre, height, weight, waist/hip circumferences, and blood pressure measurements were taken. Questionnaires were repeated annually thereafter. Incident cancers indicated in these questionnaires or by other self-report were subsequently confirmed through centralized review of all pathology reports, discharge and consultant summaries, operative and radiology reports, and tumor registry abstracts.

Selection of Case and Control Participants

Eligibility criteria for case and control participants were: 1) included within the WHI-CT, but not assigned to the E or E+P intervention arms; 2) no baseline use of hormones (pill, skin patch, cream, or shot) unless women underwent a washout period that ended prior to baseline blood draw; 3) no history of colorectal cancer at baseline; 4) availability of adequate serum sample (1.2 mL); 5) colorectal cancer diagnosed at least one year after random assignment (case patients only); 6) no history of diabetes at baseline; 7) no use of diabetes medication at baseline. Using these criteria, as of August 15, 2008, 401 colorectal cancer case patients were eligible for this analysis. Incident colorectal cancer was defined as the diagnosis of disease (International Classification of Diseases for Oncology site codes 153.3–153.4, 153.6–153.9, and 154.0–154.1) after more than one year of follow-up. Each case patient was matched with two control patients (n = 802 control patients) that exactly met the matching criteria of: age (±0 years), ethnicity (white, black, Hispanic, American Indian, Asian/Pacific Islander, or unknown), HT assignments (E placebo, P placebo, or not randomized (NR), DM assignments (intervention, control, or NR), and CaD assignments (intervention, control, or NR). Control selection was performed in a time-forward manner, selecting one control patient for each case patient first from the risk set at the time of the case patient's event, and this process was then repeated for the selection of the second control.

Laboratory Methods

All serologic assays were performed in the laboratory of Dr. Frank Stanczyk, University of Southern California, Los Angeles, CA. Serum levels of estradiol, estrone, and progesterone were quantified by validated, previously described radioimmunoassay (RIAs) (14,15). Prior to the RIA, the steroid hormones were extracted from serum with hexane:ethyl acetate (3:2 ratio). Estradiol, estrone, and progesterone were then separated from each other and from interfering steroids by Celite column partition chromatography using ethylene glycol as the stationary phase. Progesterone was eluted with trimethylpentane, and estrone and estradiol were eluted with 15% and 40% ethyl acetate in trimethylpentane, respectively. The sensitivities of the estradiol, estrone, and progesterone assays were 2 pg/mL, 4 pg/mL, and 10 pg/mL, respectively; all measured values were above the assay sensitivity lower limit. Assay specificity was achieved by undertaking organic solvent extraction and chromatographic steps prior to quantification of the analytes, and/or use

of highly specific anti-sera. Assay accuracy was established by demonstrating consistency between measured concentrations of a serially diluted analyte in serum and the corresponding standard curve. SHBG was quantified by a solid-phase, two-site chemiluminescent immunoassay using the Immulite Analyzer (Siemens Medical Solutions Diagnostics, Los Angeles, CA). The solid phase is a polystyrene bead with a monoclonal antibody specific for SHBG. Free estradiol levels were calculated using total estradiol concentrations, SHBG concentrations, and an assumed constant for albumin in a validated algorithm (18,19). This method does not distinguish between free estradiol and bioavailable (non-SHBG bound) estradiol. Insulin and free IGF-1 concentrations were determined by enzyme-linked immunosorbent assays (ELISAs) using commercially available immunoassay kits from Diagnostic Systems Laboratories (DSL, Webster, TX). CRP levels were determined using a solid-phase chemiluminescent immunometric assay on the Immulite analyzer (Siemens Medical Solutions Diagnostics, Los Angeles, CA). Case patients and matched control patients were assayed together in batches of 41 or less depending on where the blind duplicates fell in the random distribution within the pull—10 blind duplicates (5 pairs) were analyzed for every 100 participant samples. The mean intra-assay coefficients of variation from the duplicate samples were 11% for estradiol, 10% for estrone, 13% for progesterone, 4% for SHBG, 8% for insulin, 16% for free IGF-1, and 4% for CRP.

Statistical Analysis

Univariate differences between case patients and control patients were assessed using the Wilcoxon two-sample tests for continuous variables and χ^2 tests for categorical variables. Multivariable conditional logistic regression, stratified by case-control set, was used to compute odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between circulating levels of sex hormones and colorectal, colon, and rectal cancers. Participants were divided into quartiles (colorectal and colon cancers) or tertiles (rectal cancer) based on the distributions of circulating levels of sex hormones in the control group. Statistical tests for trend for a given analyte were calculated using the ordinal quartile entered into the models as a continuous variable. The multivariable models were adjusted for a set of a priori–determined colorectal cancer risk factors, namely waist circumference, alcohol consumption, family history of colorectal cancer, physical activity, smoking status, and nonsteroidal anti-inflammatory drug (NSAID) use. Additional adjustment for dietary intakes of fiber, calcium, and folate resulted in virtually unchanged OR estimates. Further adjustment for circulating levels of insulin and free IGF-1 were also made for the sex hormone, SHBG, and waist circumference models. The estrone and total estradiol models were additionally adjusted for SHBG, and vice versa. Possible nonlinear effects were modeled using restricted cubic spline models with five knots placed at the 10th, 25th, 50th, 75th, and 95th percentiles for estradiol, estrone, free estradiol, and SHBG models. Stratified analyses according to body mass index (BMI; <30 or ≥30 kg/m^2), waist circumference (above and below median based on distribution of the control group), and previous use of HT were also performed. Heterogeneity of associations for colon and rectal cancer subsites was assessed by calculating X^2 statistics using one degree of freedom from meta-analysing the odds ratios and 95% confidence intervals in the highest sex hormone and SHBG quantiles.

In sensitivity analyses, to ensure that exogenous hormone use was not biasing our results, individuals with total estradiol levels greater than 30 pg/mL and current HT users (who underwent a washout period prior to study onset) were excluded and all models were rerun. Also in sensitivity analyses, the case-control match was broken and the associations were re-analyzed using unconditional logistic regression, plus additional adjustment for age and race/ethnicity. All analyses were also performed when cases diagnosed within the first three years of follow-up were excluded. Statistical tests used in the analysis were all two-sided and a P value of less than .05 was considered statistically significant. Analyses were conducted using Stata version 11.0.

Results

Descriptive Data Analysis

Compared with the control group participants, case patients had lower serum levels of estrone and free estradiol and higher levels of SHBG (Table 1). No other differences in baseline characteristics between case patients and control patients were found, with near identical medians found for BMI, waist circumference, and circulating levels of insulin. Estrone and estradiol had a moderate positive correlation with waist circumference and insulin (Supplementary Table 1, available online), whereas SHBG was inversely correlated with waist circumference and insulin.

Sex Hormone Levels and Risk of Colorectal Cancer

In the multivariable models, estrone (OR comparing quartile 4 and 1 $_{[q4\text{-}q1]}$ = 0.50, 95% CI = 0.33 to 0.75, P_{trend} = .002), free estradiol (OR $_{[q4\text{-}q1]}$ = 0.43, 95% CI = 0.28 to 0.67, $P_{trend} \leq .001$), and total estradiol (OR$_{[q4\text{-}q1]}$ = 0.64, 95% CI = 0.43 to 0.97, P_{trend} = .12) were inversely associated with colorectal cancer (Table 2). These associations were unaffected after adjusting simultaneously for serum levels of insulin, CRP, and free IGF-1 (estrone, OR$_{[q4\text{-}q1]}$ = 0.44, 95% CI = 0.28 to 0.68, P_{trend} = .001; free estradiol, $_{OR[q4\text{-}q1]}$ = 0.43, 95% CI = 0.27 to 0.69, $P_{trend} \leq .0001$; and total estradiol, OR$_{[q4\text{-}q1]}$ = 0.58, 95% CI = 0.38 to 0.90, P_{trend} = .08) (Table 3). In the restricted cubic spline models, no statistically significant deviations from linearity for the relationships between estrone ($P_{nonlinear}$ = .13), free estradiol ($P_{nonlinear}$ = .89), and total estradiol ($P_{nonlinear}$ = .87) and colorectal cancer were observed (Figure 1). Divergent associations were observed when analyzed by subsite, with strong inverse associations observed for colon cancer and statistically nonsignificant positive associations observed for rectal cancer; however, the differences between sites were statistically nonsignificant (estrone $P_{heterogeneity}$ = .15; total estradiol $P_{heterogeneity}$ = .13; free estradiol $P_{heterogeneity}$ = .09) (Table 4).

Levels of SHBG were positively associated with colorectal cancer risk in the multivariable model with elevations in risk evident from the second quartile upwards (OR$_{[q2\text{-}q1]}$ = 1.69, 95% CI = 1.16 to 2.45; OR$_{[q3\text{-}q1]}$ = 1.71, 95% CI = 1.16 to 2.51; OR$_{[q4\text{-}q1]}$ = 2.30, 95% CI = 1.51 to 3.51) ($P_{trend} \leq .0001$) (Table 2). Additional adjustments for estrone and estradiol, as well as insulin, CRP and free IGF-1, strengthened the positive association between SHBG and colorectal cancer (OR$_{[q4\text{-}q1]}$ = 2.50, 95% CI = 1.59 to 3.92, $P_{trend} \leq .0001$) (Table 3). In the restricted cubic spline model, no statistical significant deviation from linearity for the relationship between SHBG ($P_{nonlinear}$ = .08) and colorectal cancer was observed (Figure 1). The positive association of SHBG with colorectal cancer was consistent for both colon and rectal cancer case patients separately ($P_{heterogeneity}$ = .68) (Table 4). We observed no association between progesterone level and colorectal cancer in the multivariable model (OR$_{[q4\text{-}q1]}$ = 0.97, 95%

Table 1. Selected baseline characteristics of case patients and control patients

Variable	Case patients (n = 401)		Control patients (n = 802)		P*
Age, y, median (IQR)	66.0	(61.0 - 71.0)	66.0	(61.0 - 71.0)	.99
Ethnicity, No. (%)					.99
White	327	(81.6)	654	(81.6)	
Black	46	(11.5)	92	(11.5)	
Hispanic	10	(2.5)	20	(2.5)	
Other/unknown	18	(4.5)	36	(4.5)	
Weight, kg, median (IQR)	74.6	(65.4 - 85.7)	74.0	(64.4 - 87.5)	.95
Body mass index, kg/m^2, median (IQR)	28.5	(25.1 - 32.5)	28.4	(25.0 - 33.0)	.83
Waist circumference, cm, median (IQR)	89.0	(81.0 - 100.0)	89.0	(79.0 - 99.0)	.46
Waist-to-hip ratio, median (IQR)	0.82	(0.8 - 0.9)	0.82	(0.8 - 0.9)	.46
Past HT usage status, No. (%)					.11
Never	284	(70.8)	602	(75.1)	
Former	111	(27.7)	184	(22.9)	
Current	5	(1.3)	16	(2.0)	
Missing	1	(0.3)	0	(0.0)	
NSAID use, No. (%)					.20
No	277	(69.1)	524	(65.3)	
Yes	124	(30.9)	278	(34.7)	
Family history of colorectal cancer, No. (%)					.59
No	299	(74.6)	616	(76.8)	
Yes	68	(17.0)	118	(14.7)	
Missing	34	(8.5)	68	(8.5)	
Smoking status, No. (%)					.12
Never	185	(46.1)	420	(52.4)	
Former	181	(45.1)	304	(37.9)	
Current	29	(7.2)	63	(7.9)	
Missing	6	(1.5)	15	(1.9)	
Alcohol, servings/wk, No. (%)					.22
Nonconsumers	175	(43.7)	364	(45.4)	
0.1-<3.0	125	(31.2)	265	(33.0)	
≥3.0	97	(24.2)	171	(21.3)	
Missing	4	(1.0)	2	(0.3)	
Physical activity, MET-hours/wk, No. (%)					.71
<3.75	133	(33.2)	268	(33.4)	
3.75-<9.83	102	(25.4)	176	(22.0)	
9.83-<18.75	65	(16.2)	142	(17.7)	
≥18.75	67	(16.7)	147	(18.3)	
Missing	34	(8.5)	69	(8.6)	
Serological variables, median (IQR)					
Estradiol, pg/mL	9.4	(6.8 - 12.8)	9.5	(7.1 - 13.8)	.22
Estrone, pg/mL	40.7	(31.2 - 53.5)	43.0	(32.4 - 57.3)	.02
Progesterone, pg/mL	47.4	(36.1 - 62.0)	46.2	(34.9 - 62.9)	.68
Free estradiol, pg/mL	0.23	(0.2 - 0.3)	0.25	(0.2 - 0.4)	.04
SHBG, nmol/L	44.3	(33.5 - 64.4)	42.4	(29.6 - 60.3)	.02
Insulin, μIU/mL	5.6	(2.8 - 9.6)	5.7	(2.9 - 10.1)	.50
C-reactive protein, mg/L	2.8	(1.4 - 6.0)	3.0	(1.2 - 6.1)	.99
Free IGF-1, ng/mL	0.8	(0.4 - 1.1)	0.8	(0.5 - 1.1)	.43

* Calculated using Wilcoxon two-sample tests for continuous variables and $\chi 2$ tests for categorical variables. All statistical tests were two-sided. HR = hormone therapy; IGF-1 = insulin-like growth factor-1; IQR = interquartile range.

CI = 0.66 to 1.40, P_{trend} = .93) (Table 2), and this relationship was consistent when colon and rectal cancer were analyzed separately ($P_{heterogeneity}$ = .25) (further data not shown). Levels of insulin ($OR_{[q4-q1]}$ = 0.76, 95% CI = 0.50 to 1.14), CRP ($OR_{[q4-q1]}$ = 0.89, 95% CI = 0.60 to 1.34), and free IGF-1 ($OR_{[q4-q1]}$ = 0.70, 95% CI = 0.48 to 1.03) were not statistically significantly associated with colorectal cancer incidence (Table 2). Waist circumference was nonsignificantly positively associated with colorectal cancer in the multivariable model ($OR_{[q4-q1]}$ = 1.37, 95% CI = 0.93 to 2.01, P_{trend} = .32) (Table 2); however, this association strengthened and became statistically significant after adjusting for insulin, CRP, free IGF-1, estradiol, estrone, and SHBG ($OR_{[q4-q1]}$ = 2.24, 95% CI = 1.37 to 3.68, P_{trend} = .006) (Table 3). Divergent waist circumference associations were observed when analyzed by subsite, with stronger positive associations observed for colon cancer than rectal cancer; however, this difference was statistically nonsignificant ($P_{heterogeneity}$ = .21) (further data not shown). Similar strength nonstatistically significant positive associations were observed for BMI and colorectal, colon, and rectal cancers (data not shown).

Table 2. Association of circulating levels of sex hormones, SHBG, insulin, CRP, free IGF-1, and waist circumference with colorectal cancer in WHI-CT participants

Variables	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}*
Estradiol					
Quartile cutpoints, pg/mL	<7.09	7.09-<9.46	9.46–13.90	≥13.90	
N (case patients/control patients)	110/201	91/200	121/201	79/200	
Unadjusted model OR (95% CI)	1.00	0.81 (0.57 to 1.15)	1.07 (0.77 to 1.49)	0.70 (0.48 to 1.01)	.23
Multivariable-adjusted OR† (95% CI)	1.00	0.79 (0.55 to 1.13)	0.99 (0.69 to 1.40)	0.64 (0.43 to 0.97)	.12
Estrone					
Quartile cutpoints, pg/mL	<32.50	32.50-<43.03	43.03-<57.28	≥57.28	
N (case patients/control patients)	114/201	115/200	103/201	69/200	
Unadjusted model OR (95% CI)	1.00	0.98 (0.71 to 1.37)	0.88 (0.63 to 1.23)	0.58 (0.39 to 0.84)	.005
Multivariable-adjusted OR† (95% CI)	1.00	0.94 (0.66 to 1.32)	0.87 (0.61 to 1.23)	0.50 (0.33 to 0.75)	.002
Progesterone					
Quartile cutpoints, pg/mL	<34.94	34.94-<46.24	46.24-<63.21	≥63.21	
N (case patients/control patients)	97/201	97/200	115/201	92/200	
Unadjusted model OR (95% CI)	1.00	1.01 (0.71 to 1.42)	1.20 (0.85 to 1.71)	0.95 (0.66 to 1.37)	.94
Multivariable-adjusted OR† (95% CI)	1.00	1.02 (0.72 to 1.46)	1.19 (0.83 to 1.72)	0.97 (0.66 to 1.40)	.93
SHBG					
Quartile cutpoints, nmol/L	<29.70	29.70-<42.50	42.50-<60.40	≥60.40	
N (case patients/control patients)	70/201	110/201	104/201	117/199	
Unadjusted model OR (95% CI)	1.00	1.59 (1.11 to 2.29)	1.52 (1.06 to 2.18)	1.75 (1.21 to 2.52)	.009
Multivariable-adjusted OR† (95% CI)	1.00	1.69 (1.16 to 2.45)	1.71 (1.16 to 2.51)	2.30 (1.51 to 3.51)	<.0001
Free estradiol					
Quartile cutpoints, pg/mL	<0.18	0.18-<0.26	0.26-<0.38	≥0.38	
N (case patients/control patients)	123/205	114/215	93/188	71/194	
Unadjusted model OR (95% CI)	1.00	0.85 (0.62 to 1.18)	0.80 (0.57 to 1.13)	0.58 (0.40 to 0.84)	.005
Multivariable-adjusted OR† (95% CI)	1.00	0.74 (0.52 to 1.05)	0.66 (0.45 to 0.96)	0.43 (0.28 to 0.67)	<.001
Insulin					
Quartile cutpoints, uIU/mL	<2.88	2.88-<5.73	5.73-<10.2	≥10.2	
N (case patients/control patients)	105/201	98/199	104/202	94/197	
Unadjusted model OR (95% CI)	1.00	0.94 (0.66 to 1.33)	0.98 (0.70 to 1.38)	0.91 (0.65 to 1.28)	.66
Multivariable-adjusted OR† (95% CI)	1.00	0.89 (0.61 to 1.29)	0.88 (0.60 to 1.29)	0.76 (0.50 to 1.14)	.21
CRP					
Quartile cutpoints, mg/L	<1.24	1.24-<2.96	2.96-<6.12	≥6.12	
N (case patients/control patients)	92/202	114/199	105/203	90/198	
Unadjusted model OR (95% CI)	1.00	1.25 (0.89 to 1.75)	1.14 (0.79 to 1.63)	1.00 (0.69 to 1.44)	.83
Multivariable-adjusted OR† (95% CI)	1.00	1.18 (0.83 to 1.67)	1.06 (0.72 to 1.56)	0.89 (0.60 to 1.34)	.47
Free IGF-1					
Quartile cutpoints, ng/mL	<0.47	0.47-<0.79	0.79-<1.15	≥1.15	
N (case patients/control patients)	117/205	89/198	108/199	87/200	
Unadjusted model OR (95% CI)	1.00	0.78 (0.55 to 1.10)	0.94 (0.67 to 1.31)	0.74 (0.51 to 1.06)	.21
Multivariable-adjusted OR† (95% CI)	1.00	0.78 (0.55 to 1.12)	0.93 (0.67 to 1.31)	0.70 (0.48 to 1.03)	.15
Waist circumference					
Quartile cutpoints, cm	<79.2	79.2-<89.4	89.4-<99.1	≥99.1	
N (case patients/control patients)	95/226	108/184	95/194	101/196	
Unadjusted model OR (95% CI)	1.00	1.58 (1.12 to 2.23)	1.28 (0.89 to 1.84)	1.40 (0.97 to 2.03)	.24
Multivariable-adjusted OR† (95% CI)	1.00	1.54 (1.09 to 2.19)	1.23 (0.85 to 1.78)	1.37 (0.93 to 2.01)	.32

* Statistical tests for trend (two-sided) were calculated using ordinal quartile variables (1–4) entered into the model as a single continuous variable. CI = confidence interval; CRP = C-reactive protein; IGF-1 = insulin-like growth factor-1; IQR = interquartile range; OR = odds ratio; SHBG = sex hormone–binding globulin; WHI-CT = Women's Health Initiative Clinical Trial.

† Multivariable model adjusted for waist circumference, alcohol consumption, family history of colorectal cancer, physical activity, smoking status, and NSAID use.

None of the associations of the sex hormones with colorectal cancer differed when stratified by waist circumference, BMI, or prior HT use, and we detected no significant heterogeneity between the sex hormones and other serologic factors (Supplementary Table 2, available online). In sensitivity analyses, when women with total estradiol levels over 30 pg/mL (n = 38) and current HT users (who had undergone washout period) (n = 20) were excluded from the analyses, the results were essentially unaltered. Similar relationships were also observed when participants who were part of the intervention groups of the DM and CaD study arms were excluded; the case-control match was broken, and all models were reanalyzed; the case patients diagnosed within the first three years of follow-up were excluded; the analyses were stratified by follow-up time (<5 years and ≥5 years) and when the analyses were limited to non-NSAID users only (data not shown).

Discussion

In this prospective study of postmenopausal women enrolled in the Women's Health Initiative, endogenous estradiol and estrone levels were inversely, and SHBG levels positively, associated

Table 3. Association of circulating levels of sex hormones, SHBG, and waist circumference with colorectal cancer after additional adjustment for insulin, free IGF-1, and CRP

Variables	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}*
Estradiol					
Quartile cutpoints, pg/mL	<7.09	7.09-<9.46	9.46–13.90	≥13.90	
N (case patients/control patients)	110/201	91/200	121/201	79/200	
Multivariable-adjusted OR† (95% CI)	1.00	0.79 (0.55 to 1.13)	0.99 (0.69 to 1.40)	0.64 (0.43 to 0.97)	.12
Multivariable-adjusted + insulin, IGF-1, CRP, and SHBG OR† (95% CI)	1.00	0.83 (0.57 to 1.20)	1.03 (0.72 to 1.48)	0.58 (0.38 to 0.90)	.08
Estrone					
Quartile cutpoints, pg/mL	<32.50	32.50-<43.03	43.03-<57.28	≥57.28	
N (case patients/control patients)	114/201	115/200	103/201	69/200	
Multivariable-adjusted OR† (95% CI)	1.00	0.94 (0.66 to 1.32)	0.87 (0.61 to 1.23)	0.50 (0.33 to 0.75)	.002
Multivariable-adjusted + insulin, IGF-1, CRP, and SHBG OR† (95% CI)	1.00	0.95 (0.67 to 1.34)	0.89 (0.62 to 1.27)	0.44 (0.28 to 0.68)	.001
SHBG					
Quartile cutpoints, nmol/L	<29.70	29.70-<42.50	42.50-<60.40	≥60.40	
N (case patients/control patients)	70/201	110/201	104/201	117/199	
Multivariable-adjusted OR† (95% CI)	1.00	1.69 (1.16 to 2.45)	1.71 (1.16 to 2.51)	2.30 (1.51 to 3.51)	<.0001
Multivariable-adjusted + insulin, IGF-1, CRP, estradiol, and estrone OR† (95% CI)	1.00	1.71 (1.17 to 2.49)	1.69 (1.13 to 2.52)	2.50 (1.59 to 3.92)	<.0001
Free estradiol					
Quartile cutpoints, pg/mL	<0.18	0.18-<0.26	0.26-<0.38	≥0.38	
N (case patients/control patients)	123/205	114/215	93/188	71/194	
Multivariable-adjusted OR† (95% CI)	1.00	0.74 (0.52 to 1.05)	0.66 (0.45 to 0.96)	0.43 (0.28 to 0.67)	<.0001
Multivariable-adjusted + insulin, IGF-1, and CRP OR† (95% CI)	1.00	0.75 (0.53 to 1.08)	0.67 (0.45 to 0.99)	0.43 (0.27 to 0.69)	<.0001
Waist circumference					
Quartile cutpoints, cm	<79.2	79.2-<89.4	89.4-<99.1	≥99.1	
N (case patients/control patients)	95/226	108/184	95/194	101/196	
Multivariable-adjusted OR† (95% CI)	1.00	1.54 (1.09 to 2.19)	1.23 (0.85 to 1.78)	1.37 (0.93 to 2.01)	.32
Multivariable-adjusted + insulin, IGF-1, CRP, estradiol, estrone, and SHBG OR† (95% CI)	1.00	1.87 (1.28 to 2.73)	1.77 (1.14 to 2.76)	2.24 (1.37 to 3.68)	.006

* Statistical tests for trend (two-sided) were calculated using ordinal quartile variables (1–4) entered into the model as a single continuous variable. CI = confidence interval; CRP = C-reactive protein; IGF-1 = insulin-like growth factor-1; IQR = interquartile range; OR = odds ratio; SHBG = sex hormone–binding globulin; WHI-CT = Women's Health Initiative Clinical Trial.

† Multivariable model adjusted for waist circumference, alcohol consumption, family history of colorectal cancer, physical activity, smoking status, and NSAID use.

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with colorectal cancer risk, even after control for a number of relevant established colorectal cancer risk factors. Each of these associations showed a statistically significant biologic gradient. These collective data suggest that endogenous estrogens may be biologically related to one or more molecular pathways that are protective against colorectal cancer development.

To our knowledge, this study is the first to report a statistically significant inverse relationship between circulating endogenous estrogen levels and colorectal cancer risk. It is noteworthy, therefore, that several sources of experimental data also suggest that estrogen may have protective biologic effects on colorectal cancer development. In vitro studies have shown that expression of the β estrogen receptor (ERβ) results in the inhibition of proliferation and G1 phase cell cycle arrest in colon cancer cells (20), and in xenograft mouse studies ERβ expression has been shown to inhibit cMyc expression and tumor growth (20). Further, expression of ERβ is low in human colorectal cancer cells (21) and is inversely associated with stage of colon cancer (22), suggesting a possible role in disease progression. Consistent with this, it has been reported that there is high CpG island methylation of the estrogen receptor gene within colorectal tumors (23).

The current study also found a robust positive association between circulating SHBG levels and colorectal cancer risk that was independent of estrogen. SHBG is a hepatically synthesized glycoprotein that binds circulating estradiol and testosterone and is therefore an important regulator of their bioactivity. In the current analysis, the associations of estrone and estradiol with colorectal cancer were unaffected by control for SHBG, and, similarly, the SHBG–colorectal cancer relation was not modified by adjustment for estrone or estradiol. This may suggest a novel pathway for SHBG in elevating colorectal cancer risk that is independent of estrogen and other related factors, such as hyperinsulinemia. Adjustment for circulating testosterone (which was unmeasured in our study) would need to be made to fully confirm this hypothesis. To date, the SHBG receptor has yet to be cloned, meaning that biological roles beyond sex hormone regulation and transportation are poorly understood. Further research on the potential role of SHBG activity in colorectal tumorigenesis is warranted.

The findings of the current investigation are inconsistent with three prior prospective studies that assessed the relationships between endogenous estrogen levels and colorectal cancer, including two studies with null results and one that found a positive estrogen–colorectal cancer relationship. The latter study was a case-cohort investigation in the WHI-OS that included 273 women with colorectal cancer who were not using HT at baseline and observed a hazard ratio of 1.53 (95% CI = 1.05 to 2.22) for the highest tertile of estradiol after control for insulin, free-IGF-1, and waist circumference, as well as other colorectal cancer risk factors (11). Two other prospective studies reported

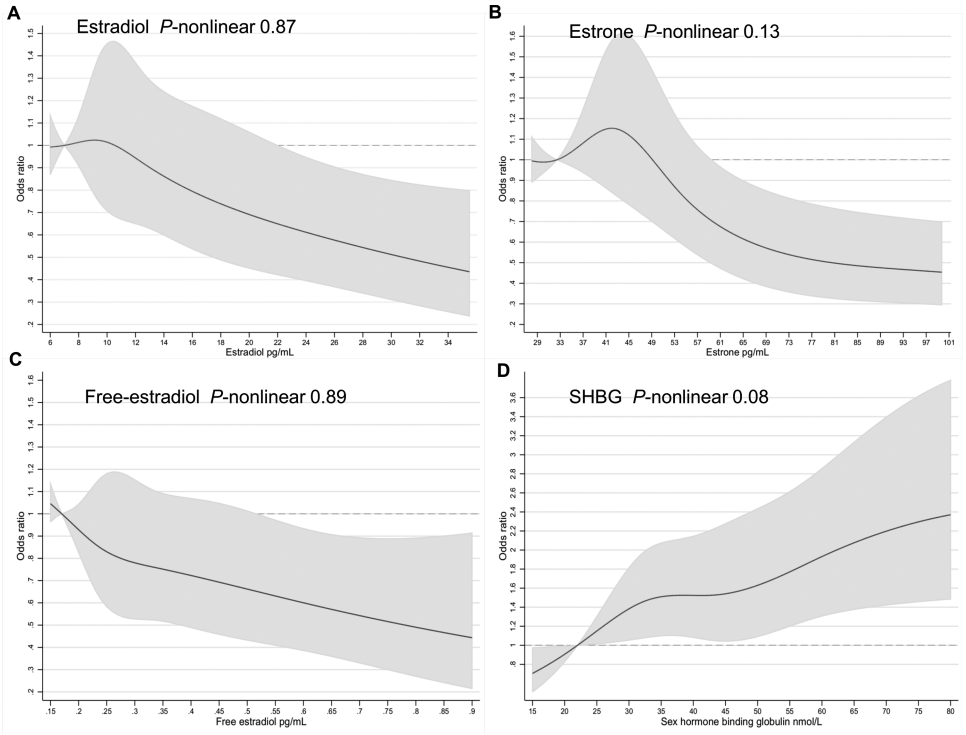

Figure 1. Association between circulating **(A)** estradiol, **(B)** estrone, **(C)** free estradiol, and **(D)** sex hormone–binding globulin (SHBG) with colorectal cancer allowing for nonlinear effects (restricted cubic spline). **Solid lines** indicate the odds ratio, and **shaded gray areas** indicate the 95% confidence intervals. Multivariable models only—adjusted for waist circumference, alcohol consumption, family history of colorectal cancer, physical activity, smoking status, and NSAID use. Estradiol and estrone models additionally adjusted for insulin, insulin-like growth factor-1 (IGF-1), C-reactive protein (CRP), and SHBG. Free estradiol model additionally adjusted for insulin, IGF-1, and CRP. SHBG model additionally adjusted for insulin, IGF-1, CRP, estradiol, and estrone. The references for these restricted cubic spline plots (with five knots placed at the 10th, 25th, 50th, 75th, and 95th percentiles) were estradiol 7 pg/mL, estrone 32.4 pg/mL, free estradiol 0.17 pg/mL, and SHBG 22 nmol/L.

no association between circulating sex hormones and colorectal cancer. A case-control study nested in the NYUWHS found no relation of circulating levels of estradiol, estrone, and SHBG with colorectal cancer risk (12). A joint NHS and WHS nested case-control analysis with 270 case patients also reported no statistically significant relationships for colorectal cancer risk with these same serologic measures (13). However, the NHS/WHS study did observe an inverse association for colorectal cancer risk with the ratio of total estradiol to testosterone, a finding that the authors hypothesized reflected greater aromatase expression and, as a consequence, higher estradiol synthesis (13).

Reasons for the difference in the results from the current analysis compared with the prior investigations are not entirely clear. Possible explanations for the divergent results include that our analysis is considerably larger than the previous studies, we used the current gold standard sex hormone assays, we performed individual matching for important risk factors, and we had the most thorough covariate information available of any study to date investigating these relationships. Of note, circulating estradiol levels were relatively similar between our analysis and the previous studies (11–13). However, estrone levels were substantially higher in the current study (quartile 1 to quartile 4 <32.5 to ≥57.28 pg/mL) compared with the joint NHS/WHS (quartile 1 to quartile 4 range: 6–16 to ≥32 pg/mL; quartile 1 to quartile 4 range: 5–19 to ≥37 pg/mL) (13) and NYUWHS (quartile 1 to quartile 4 range: ≤13 to ≥26 pg/mL) analyses (12). Further, our analyses of nonlinear effects showed that statistically significant lower colorectal cancer risks were only observed at estrone levels over approximately 60 pg/mL when compared with the reference level (32.4 pg/mL). Thus, it is possible that estrone, and not estradiol, is driving the inverse relationships we observed, and the null results found in the NHS/WHS and NYUWHS studies are the consequence of lower measured estrone levels. Unfortunately, because of the high correlation between estradiol and estrone (r = 0.82), we could not disentangle these relationships and further studies are warranted to investigate which estrogenic components are most relevant for colorectal cancer in postmenopausal women.

Interestingly, the results of the current study may also enhance understanding of the established positive association between adiposity and colorectal cancer (24,25). It has been consistently shown that the positive relationships between obesity and colorectal cancer are weaker among women than men (24). One proposed explanation for this sex difference is that higher circulating estrogens in women may mitigate the potential tumorigenic effects of excess adiposity on the colorectum (26). In our analysis, the waist circumference and colorectal cancer relationship strengthened and became statistically significant after the multivariable models were additionally adjusted for estrone, estradiol, and SHBG. This suggests that the estrogen–colorectal cancer association may indeed mask the adiposity–colorectal cancer relation in women, and future studies that investigate this hypothesis should incorporate estrogen measurements to limit the effects of this confounding bias.

A strength of our analysis is that virtually all women were non-HT users (98.8% of case patients and 98% of control patients) at baseline, and the remaining current users underwent a washout period. Analysis with this latter small group

Table 4. Association of circulating levels of sex hormones and SHBG with colon and rectal cancers after additional adjustment for insulin, free IGF-1, and CRP

Variables	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}†	$P_{heterogeneity}$ colon vs rectal
Estradiol						.13
Colon cancer (n = 303)						
Multivariable-adjusted + insulin, IGF-1, CRP, and SHBG OR‡ (95% CI)	1.00	0.71 (0.46 to 1.09)	0.90 (0.59 to 1.37)	0.39 (0.23 to 0.64)	.003	
Rectal cancer (n = 93)	Tertile 1	Tertile 2	Tertile 3			
Multivariable-adjusted + insulin, IGF-1, CRP, and SHBG OR‡ (95% CI)	1.00	1.11 (0.54 to 2.27)	1.44 (0.63 to 3.28)		.39	
Estrone						
Colon cancer (n = 303)						.15
Multivariable-adjusted + insulin, IGF-1, CRP, and SHBG OR‡ (95% CI)	1.00	0.79 (0.52 to 1.19)	0.70 (0.46 to 1.07)	0.34 (0.21 to 0.57)	<.001	
Rectal cancer (n = 93)	Tertile 1	Tertile 2	Tertile 3			
Multivariable-adjusted + insulin, IGF-1, CRP, and SHBG OR‡ (95% CI)	1.00	1.78 (0.85 to 3.74)	1.13 (0.49 to 2.62)		.79	
SHBG						
Colon cancer (n = 303)						.68
Multivariable-adjusted + insulin, IGF-1, CRP, estradiol, and estrone OR‡ (95% CI)	1.00	1.72 (1.08 to 2.72)	1.66 (1.02 to 2.71)	2.35 (1.36 to 4.07)	.006	
Rectal cancer (n = 93)	Tertile 1	Tertile 2	Tertile 3			
Multivariable-adjusted + insulin, IGF-1, CRP, estradiol, and estrone OR‡ (95% CI)	1.00	2.03 (0.94 to 4.37)	3.02 (1.31–6.98)		.01	
Free estradiol						.09
Colon cancer (n = 303)						
Multivariable-adjusted + insulin, IGF-1, and CRP OR‡ (95% CI)	1.00	0.69 (0.46 to 1.05)	0.65 (0.41 to 1.03)	0.28 (0.16 to 0.49)	<.001	
Rectal cancer (n = 93)	Tertile 1	Tertile 2	Tertile 3			
Multivariable-adjusted + insulin, IGF-1, and CRP OR‡ (95% CI)	1.00	0.82 (0.41 to 1.66)	1.40 (0.62 to 3.18)		.43	

* Quartile cutpoints: estradiol (<7.09, 7.09-<9.46, 9.46-<13.90, ≥13.90 pg/mL), estrone (<32.50, 32.50-<43.03, 43.03-<57.28, ≥57.28 pg/mL), SHBG (<29.70, 29.70-<42.50, 42.50-<60.40, ≥60.40 nmol/L), and free estradiol (<0.18, 0.18-<0.26, 0.26-<0.38, ≥0.38 pg/mL). Tertile cutpoints: estradiol (<7.85, 7.85-<11.97, ≥11.97 pg/mL), estrone (<35.73, 35.73-<52.06, ≥52.06 pg/mL), SHBG (<34.60, 34.60-<52.90, ≥52.90 nmol/L), and free estradiol (<0.20, 0.20-<0.32, ≥0.32 pg/mL).
† Statistical tests for trend (two-sided) were calculated using ordinal quartile (1–4) or tertile (1–3) variable entered into the model as a single continuous variable.
‡ Multivariable model adjusted for waist circumference, alcohol consumption, family history of colorectal cancer, physical activity, smoking status, and NSAID use.

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of women excluded produced essentially unchanged findings. The vast majority of women were never users of HT (70.8% of case patients and 75.1% of control patients). A limitation of our analysis is that sex hormone levels were measured only once at baseline, and it is possible that these measurements may not reflect exposure levels across time. However, a previous analysis of postmenopausal women reported that the within-person correlation coefficients for free estradiol, estrone, and SHBG over a two- to three-year period were 0.73, 0.74, and 0.92 respectively (27), indicating that single measurements provide good estimates of longer-term exposures. A further possible explanation for our results was that preclinical disease at baseline may have introduced bias (reverse causality) into our analyses. However, all of the 401 colorectal cancer case patients were diagnosed after more than one year of follow-up. Furthermore, our results remained essentially unaltered when case patients diagnosed within the first three years of follow-up were excluded and when the analyses were stratified by follow-up time (<5 years and ≥5 years). Finally, our study lacked data on testosterone and other hormones related to the estrogen and SHBG pathway. Future studies should incorporate testosterone and other androgen measurements into analyses to further inform on the role of the sex hormone axis in colorectal tumorigenesis

In conclusion, in this prospective analysis of postmenopausal women, endogenous levels of estrogens were inversely, and SHBG levels positively, associated with colorectal cancer, and, in the case of estrogens, the association was confined to colon cancer. These associations were independent of other colorectal cancer risk factors and are consistent with mechanistic data and observational studies of exogenous hormone use and colorectal cancer risk. While further studies of the relationships between endogenous sex hormone levels, SHBG, and colorectal cancer are warranted, these findings suggest that endogenous estrogen may confer a protective effect on colorectal cancer development in postmenopausal women.

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