

SUPPLEMENTARY MATERIAL

Supplementary Methods

Laboratory methods and assessment of sex hormone concentrations and SHBG

Plasma sex hormones and SHBG concentrations were measured at IARC (Lyon, France) using a validated analytical method adapted from previous publications (1, 2). Briefly for the sex hormones, 280 μ L of plasma (to which 20 μ L of a mixture of deuterated internal standards are added), were extracted with methyl tert-butyl ether, evaporated to dryness and reconstituted with a solution of 40% (v/v) methanol/water. Solutions were then injected into the liquid chromatography-mass spectrometry (LC-MS) system consisting of ultra-high performance LC (Agilent 1290, Agilent, Santa Clara, CA) and a QTRAP 5500 MS (SCIEX, Framingham, MA). Chromatographic conditions were as follows: a reversed phase C18 column (Waters Acquity UPLC CSH C18 1.7 μ m 2.1*100mm) at 50 °C with an 8-minute linear methanol/water gradient from 55% to 65% methanol and a flow rate of 0.4 mL/min. The general MS conditions were: APCI source in positive polarity for androstenedione, DHEA, testosterone, and progesterone, and negative polarity for estrone, and estradiol. Stable isotope labeled analogues of each analyte were used as internal standards and two MS/MS transitions were monitored for each compound, one for quantification and another to monitor specificity. Quantification was based on individual internal standards. Solid phase “sandwich” enzyme-linked immunoassay (DRG) was used for the measurement of SHBG concentrations. Lower limits of quantification (LLOQ) for each sex hormone was 7.5 pg/mL for androstenedione, 125 pg/mL for DHEA, 1.25 pg/mL for estradiol, and 1.25 pg/mL for estrone, 7.5 pg/mL for progesterone, 7.5 pg/mL for testosterone, and 4 nmol/l for SHBG. Three quality control samples at different concentration levels were measured in duplicate in each batch of analyses. Intra-batch coefficients of variation of sex hormone and SHBG concentrations ranged from 1.4 % and 8 %. Inter-batch coefficients of variations were <10 % for all analytes.

Plasma concentration of free estradiol was calculated using a validated algorithm (3) taking into consideration measured estradiol and SHBG concentrations, and an assumed constant for albumin. Free testosterone was also computed from previously validated mass action equation using absolute concentrations of testosterone and an assumed albumin constant of 43 g/L (4, 5). We also calculated the estradiol to testosterone ratio (by dividing the estradiol concentration by testosterone concentration) as a higher ratio indicates greater production of estradiol from aromatase conversion.

Meta-analysis

We performed a hand search up to July 2020 on PubMed using the keywords (“colorectal” OR “colorectum” OR “colon” OR “rectum”) and “cancer” and "sex hormone". We limited our search to studies published in English that prospectively evaluated the association between circulating estradiol and estrone with colorectal, colon, or rectal cancer risk.

The following information was extracted from each identified study: the first author’s last name, publication year, study design, country of origin, number of cases/controls, outcome measures, exposure level, relative risks (RRs) and 95% confidence intervals (CIs), and adjusted confounders from each study. Characteristics of the included studies are described in **Supplementary Table 5**.

We calculated summary RRs and 95% CIs for a 5 pg/ml increment in estradiol, and 10 pg/ml increment in estrone using a random effects model. In the primary analysis, we used the multivariable model from each study, which included greatest number of covariates. The average of the natural logarithm of the RRs was estimated and RR of each study was weighted using random effects weighting.

The dose-response analysis described by Greenland and Longnecker (6) was used to compute specific slopes (linear trends) and 95% CIs from the natural logs of the reported RRs and CIs across categories of estradiol and estrone concentrations. We contacted authors of two studies (7, 8) for additional data to be included in the dose-response analysis.

Heterogeneity in results across studies was also examined using Cochran Q and I^2 statistics.

Statistical analyses were performed with Stata (version 15.1; StataCorp, College Station, TX).

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Supplementary Tables

Supplementary Table 1. Baseline characteristics of colon cancer cases and controls.

Variables	Cases (n = 512)	Controls (n = 516)	<i>P</i> ^a
Mean age at blood collection (SD), y	62.0 (5.3)	61.9 (5.3)	0.66 ^a
Mean weight (SD), kg	69.0 (11.9)	67.6 (10.6)	0.18 ^a
Body mass index, No. (%)	26.7 (4.7)	26.5 (4.2)	0.79 ^b
Underweight (>18.5 kg/m ²)	5 (1.0)	6 (1.2)	0.95
Normal (18.5-24.9 kg/m ²)	204 (39.8)	202 (39.2)	
Overweight (25.0-29.9 kg/m ²)	201 (39.3)	210 (40.7)	
Obese (≥30 kg/m ²)	102 (19.9)	98 (19.0)	
Smoking status, No. (%)			0.73 ^b
Never	309 (60.4)	323 (62.6)	
Former	123 (24.0)	111 (21.5)	
Current	72 (14.1)	76 (14.7)	
Unknown	8 (1.6)	6 (1.2)	
Physical activity, No. (%)			0.99 ^b
Inactive	173 (33.8)	181 (35.1)	
Moderately inactive	157 (30.7)	156 (30.2)	
Moderately active	93 (18.2)	92 (17.8)	
Active	73 (14.3)	73 (14.2)	
Missing	16 (3.1)	14 (2.7)	
Ever menopausal hormone use, No. (%)			0.90 ^b
No	413 (80.7)	418 (81.0)	
Yes	70 (13.7)	72 (14.0)	
Unknown/missing	29 (5.7)	26 (5.0)	
Mean alcohol consumption (SD), g/day	5.6 (9.6)	5.7 (10.0)	0.80 ^a
Serologic variables, median (IQR)			
Estrone, pg/mL	18.3 (13.0-25.8)	18.1 (13.1-24.0)	0.27 ^a
Estradiol, pg/mL	4.0 (2.6-6.2)	4.1 (2.6-6.1)	0.92 ^a
Testosterone, pg/mL	187.3 (132.0-259.3)	185.0 (129.6-250.0)	0.52 ^a
Androstenedione, ng/mL	493.5 (350.0-681.3)	478.2 (353.9-646.4)	0.54 ^a
DHEA, ng/mL	1.9 (1.2-2.8)	1.8 (1.2-2.9)	0.99 ^a
Progesterone, pg/mL	52.7 (37.8-77.3)	53.8 (39.7-73.6)	0.79 ^a
SHBG, nmol/L	54.2 (38.4-73.6)	53.6 (41.0-70.7)	0.92 ^a
Free estradiol, pg/mL	88.3 (55.9-157.9)	94.9 (57.8-142.7)	0.91 ^a
Free testosterone, ng/mL	5.2 (3.3-7.9)	5.2 (3.3-7.4)	0.55 ^a

^a *P* value was calculated using a 2-sided Wilcoxon two-sample test. SD = standard deviation; IQR = interquartile range; DEHA = dehydroepiandrosterone; SHBG = sex hormone binding protein.

^b *P* value was calculated using a 2-sided χ^2 test.

Supplementary Table 2. Associations between circulating concentrations of sex hormones with colon cancer in postmenopausal women (n=1028) after imputing the concentration with <LLOQ values by multiple imputation.

Variables	Quartile 1	Quartile 2	Quartile 3	Quartile 4	<i>P</i> _{trend} ^a	Continuous model ^b
Estradiol						
Quartile cutpoints	<2.8	2.8-4.2	4.2-6.2	≥6.2		
No. (cases/controls)	132/135	124/127	118/127	138/127		
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	1.01 (0.71, 1.44)	0.99 (0.68, 1.44)	1.19 (0.81, 1.76)	0.43	1.11 (0.97, 1.27)
DHEA						
Quartile cutpoints	<1.2	1.2-1.8	1.8-2.9	≥2.9		
No. (cases/controls)	134/129	112/129	149/129	117/129		
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	0.82 (0.58, 1.17)	1.12 (0.79, 1.59)	0.85 (0.58, 1.23)	0.78	0.98 (0.85, 1.12)
Free estradiol						
Quartile cutpoints	<61.0	61.0-97.7	97.7-148.4	≥148.4		
No. (cases/controls)	137/134	126/128	101/127	148/127		
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	0.95 (0.68, 1.34)	0.79 (0.54, 1.15)	1.18 (0.80, 1.73)	0.56	1.09 (0.96, 1.24)
Estradiol to testosterone ratio						
Quartile cutpoints	<0.20	0.20-0.28	0.28-0.34	≥0.34		
No. (cases/controls)	125/135	145/127	99/126	143/128		
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	1.20 (0.85, 1.68)	0.85 (0.58, 1.25)	1.22 (0.82, 1.80)	0.62	2.10 (0.76, 5.79)

^a Statistical tests for trend (two-sided) were calculated using ordinal quartile variables entered into the model as a single continuous variable. CI = confidence intervals; DHEA = dehydroepiandrosterone; OR = odds ratio.

^b Serologic variables were log₂-transformed in continuous models

^c ORs and 95% CIs were estimated by conditional logistic regression model. Adjusted for body mass index (underweight, normal, overweight, or obese), smoking status (never, former, current or unknown), physical activity (inactive, moderately inactive, moderately active, active, or unknown), ever hormone use (yes, no, or unknown/missing), alcohol consumption (continuous), and estrone, estradiol and testosterone were adjusted for SHBG and vice versa.

Supplementary Table 3. Association between circulating concentrations of sex hormones and SHBG with colon cancer in postmenopausal women after excluding participants who were diagnosed with colon cancer in the first 2 years of follow-up (n=1009)

Variables	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}^a	Continuous model ^b
Estrone						
Quartile cutpoints	<13.1	13.1-18.1	18.1-24.0	≥ 24.0		
No. (cases/controls)	122/129	114/129	106/129	151/129		
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	0.90 (0.62, 1.32)	0.88 (0.60, 1.30)	1.32 (0.88, 1.97)	0.22	1.16 (0.98, 1.36)
Estradiol						
Quartile cutpoints	<2.6	2.6-4.1	4.1-6.1	≥ 6.1		
No. (cases/controls)	121/129	128/129	113/129	131/129		
Multivariable-adjusted OR (95% CI) ^c	1	1.04 (0.73, 1.51)	0.92 (0.63, 1.36)	1.10 (0.73, 1.65)	0.78	1.04 (0.92, 1.18)
Testosterone						
Quartile cutpoints	<129.3	129.3-184.5	184.5-249.9	≥ 249.9		
No. (cases/controls)	121/129	115/129	114/129	143/129		
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	0.93 (0.65, 1.34)	0.91 (0.62, 1.32)	1.11 (0.77, 1.62)	0.58	1.00 (0.83, 1.19)
Androstenedione						
Quartile cutpoints	<353.8	353.8-477.7	477.7-645.5	≥ 645.5		
No. (cases/controls)	129/129	103/129	117/129	144/129		
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	0.80 (0.55, 1.17)	0.90 (0.63, 1.30)	1.08 (0.74, 1.58)	0.58	1.00 (0.82, 1.22)
DHEA						
Quartile cutpoints	<1.2	1.2-1.8	1.8-2.9	≥ 2.9		
No. (cases/controls)	129/129	106/129	145/129	113/129		
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	0.80 (0.56, 1.14)	1.07 (0.75, 1.53)	0.81 (0.55, 1.18)	0.60	0.97 (0.84, 1.11)
Progesterone						
Quartile cutpoints	<39.6	39.6-53.6	53.6-73.6	≥ 73.6		
No. (cases/controls)	135/129	115/128	105/130	138/129		
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	0.85 (0.59, 1.22)	0.78 (0.53, 1.12)	0.99 (0.68, 1.42)	0.85	0.94 (0.79, 1.12)
SHBG						
Quartile cutpoints	<40.8	40.8-53.5	53.5-70.7	≥ 70.7		
No. (cases/controls)	143/128	102/130	180/129	140/129		
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	0.74 (0.51, 1.08)	0.74 (0.51, 1.08)	1.00 (0.69, 1.46)	1.00	0.99 (0.81, 1.20)

Free estradiol						
Quartile cutpoints	<57.7	57.7-94.7	94.7-142.3	≥142.3		
No. (cases/controls)	128/129	132/129	90/129	143/129		
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	0.98 (0.69, 1.40)	0.67 (0.44, 1.00)	1.08 (0.72, 1.61)	1.00	1.03 (0.91, 1.15)
Free testosterone						
Quartile cutpoints	<3.3	3.3-5.2	5.2-7.4	≥7.4		
No. (cases/controls)	121/129	117/129	104/129	151/129		
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	0.97 (0.68, 1.39)	0.84 (0.58, 1.22)	1.23 (0.85, 1.78)	0.37	1.03 (0.91, 1.15)
Estradiol to testosterone ratio						
Quartile cutpoints	<0.19	0.19-0.27	0.27-0.33	≥0.33		
No. (cases/controls)	116/129	142/129	103/129	132/129		
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	1.19 (0.84, 1.69)	0.86 (0.58, 1.28)	1.10 (0.73, 1.66)	0.95	1.24 (0.48, 3.18)

^a Statistical tests for trend (two-sided) were calculated using ordinal quartile variables entered into the model as a single continuous variable. CI = confidence intervals; DHEA = dehydroepiandrosterone; OR = odds ratio; SHBG = sex hormone-binding globulin.

^b Serologic variables were log₂-transformed in continuous models.

^c ORs and 95% CIs were estimated by conditional logistic regression model. Adjusted for body mass index (underweight, normal, overweight, or obese), smoking status (never, former, current or unknown), physical activity (inactive, moderately inactive, moderately active, active, or unknown), ever hormone use (yes, no, or unknown/missing), alcohol consumption (continuous), and estrone, estradiol and testosterone were adjusted for SHBG and vice versa.

Supplementary Table 4. Association between circulating concentrations of sex hormones, and SHBG with risks of proximal colon and distal colon cancer in postmenopausal women (n= 1028)

Variables	Tertile 1	Tertile 2	Tertile 3	P_{trend}^a	Continuous model ^b
Estrone					
Tertile cutpoints	<14.5	14.5-21.7	≥ 21.7		
Proximal colon (n = 278)					
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	1.29 (0.83, 2.02)	1.58 (0.98, 2.53)	0.06	1.22 (1.00, 1.48)
Distal colon (n = 176)					
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	0.86 (0.50, 1.49)	0.80 (0.43, 1.50)	0.48	1.00 (0.71, 1.41)
Estradiol					
Tertile cutpoints	<3.0	3.0-5.2	≥ 5.2		
Proximal colon (n = 278)					
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	1.30 (0.85, 2.00)	1.20 (0.75, 1.93)	0.45	1.11 (0.95, 1.30)
Distal colon (n = 176)					
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	0.74 (0.44, 1.24)	0.74 (0.39, 1.41)	0.33	0.92 (0.73, 1.17)
Testosterone					
Tertile cutpoints	<149.5	149.5-221.1	≥ 221.1		
Proximal colon (n = 278)					
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	0.92 (0.61, 1.38)	1.33 (0.87, 2.03)	0.20	1.08 (0.86, 1.36)
Distal colon (n = 176)					
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	0.68 (0.38, 1.21)	0.92 (0.51, 1.68)	0.90	0.97 (0.69, 1.37)
Androstenedione					
Tertile cutpoints	<394.7	394.7-589.2	≥ 589.2		
Proximal colon (n = 289)					
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	1.10 (0.73, 1.66)	1.12 (0.74, 1.68)	0.59	1.05 (0.83, 1.34)
Distal colon (n = 188)					
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	0.97 (0.55, 1.69)	0.85 (0.48, 1.51)	0.57	0.85 (0.58, 1.25)
DHEA					
Tertile cutpoints	<1414.3	1414.3-2482.3	≥ 2482.3		
Proximal colon (n = 289)					

Multivariable-adjusted OR (95% CI) ^c	1 (reference)	0.93 (0.61, 1.40)	1.07 (0.69, 1.66)	0.79	1.01 (0.85, 1.21)
Distal colon (n = 188)					
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	1.35 (0.75, 2.42)	0.74 (0.42, 1.30)	0.29	0.87 (0.68, 1.11)
Progesterone					
Tertile cutpoints	<44.1	44.1-65.4	≥65.4		
Proximal colon (n = 289)					
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	1.24 (0.82, 1.86)	1.17 (0.77, 1.80)	0.46	0.99 (0.80, 1.23)
Distal colon (n = 188)					
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	0.95 (0.52, 1.72)	0.94 (0.53, 1.66)	0.82	0.88 (0.64, 1.21)
SHBG					
Tertile cutpoints					
Proximal colon (n = 289)	<46.0	46.0-63.7	≥63.7		
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	0.61 (0.40, 0.94)	1.02 (0.65, 1.60)	0.93	0.98 (0.75, 1.28)
Distal colon (n = 188)					
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	0.67 (0.37, 1.20)	0.82 (0.47, 1.44)	0.57	0.97 (0.69, 1.37)
Free estradiol					
Tertile cutpoints	<69.5	69.5-123.4	≥123.4		
Proximal colon (n = 289)					
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	0.98 (0.64, 1.48)	1.08 (0.67, 1.73)	0.75	1.08 (0.93, 1.25)
Distal colon (n = 188)					
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	0.53 (0.31, 0.91)	0.68 (0.36, 1.27)	0.17	0.93 (0.74, 1.17)
Free testosterone					
Tertile cutpoints	<3.9	3.9-6.6	≥6.6		
Proximal colon (n = 289)					
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	0.95 (0.63, 1.43)	1.35 (0.89, 2.06)	0.16	1.08 (0.93, 1.25)
Distal colon (n = 188)					
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	1.11 (0.64, 1.93)	1.17 (0.65, 2.11)	0.61	0.93 (0.74, 1.17)
Estradiol to testosterone ratio					
Tertile cutpoints	<0.22	0.22-0.31	≥0.31		
Proximal colon (n = 289)					
Multivariable-adjusted OR (95% CI) ^c	1 (reference)	1.25 (0.82, 1.91)	1.23 (0.75, 2.02)	0.40	1.99 (0.63, 6.32)

Distal colon (n = 188)

Multivariable-adjusted OR (95% CI) ^c	1 (reference)	0.75 (0.45, 1.26)	0.69 (0.37, 1.28)	0.22	0.48 (0.07, 3.19)
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^a Statistical tests for trend (two-sided) were calculated using ordinal quartile variables entered into the model as a single continuous variable. OR = odds ratio; CI = confidence intervals; DHEA = dehydroepiandrosterone; SHBG = sex hormone-binding globulin.

^b Serologic variables were log₂-transformed in continuous models.

^c ORs and 95% CIs were estimated by conditional logistic regression model. Adjusted for body mass index (continuous), smoking status (never, former, current or unknown), physical activity (inactive, moderately inactive, moderately active, active, or unknown), ever hormone use (yes, no or unknown/missing), alcohol consumption (continuous), and estrone, estradiol and testosterone were adjusted for SHBG and vice versa.

Supplementary Table 5. Association between circulating concentrations of sex hormones and SHBG with colon cancer by follow-up time in postmenopausal women (n=1120)

Variables	Tertile 1	Tertile 2	Tertile 3	$P_{\text{trend}}^{\text{a}}$	Continuous model ^b	$P_{\text{interaction}}^{\text{c}}$
Estrone						
Tertile cutpoints	<14.5	14.5-21.7	≥ 21.7			
No. (cases/controls)	161/171	161/173	190/172			
Follow-up time						0.17
<13.9 years, multivariable-adjusted OR (95% CI) ^d	1 (reference)	0.97 (0.45, 2.12)	0.63 (0.28, 1.43)	0.24	0.93 (0.62, 1.38)	
≥ 13.9 years, multivariable-adjusted OR (95% CI) ^d	1 (reference)	1.79 (0.78, 4.11)	1.78 (0.68, 4.67)	0.21	1.16 (0.81, 1.67)	
Estradiol						
Tertile cutpoints	<3.0	3.0-5.2	≥ 5.2			
No. (cases/controls)	169/171	177/173	166/172			
Follow-up time						0.80
<13.9 years, multivariable-adjusted OR (95% CI) ^d	1 (reference)	0.79 (0.35, 1.80)	0.67 (0.26, 1.76)	0.42	1.03 (0.76, 1.39)	
≥ 13.9 years, multivariable-adjusted OR (95% CI) ^d	1 (reference)	0.72 (0.34, 1.53)	0.52 (0.19, 1.42)	0.19	0.96 (0.69, 1.33)	
Testosterone						
Tertile cutpoints	<149.5	149.5-221.1	≥ 221.1			
No. (cases/controls)	170/171	147/172	195/173			
Follow-up time						0.94
<13.9 years, multivariable-adjusted OR (95% CI) ^d	1 (reference)	0.45 (0.21, 0.97)	0.92 (0.40, 2.11)	0.87	0.97 (0.62, 1.51)	
≥ 13.9 years, multivariable-adjusted OR (95% CI) ^d	1 (reference)	0.56 (0.26, 1.22)	0.99 (0.39, 2.49)	0.83	0.97 (0.60, 1.58)	
Androstenedione						
Tertile cutpoints	<394.7	394.7-589.2	≥ 589.2			
No. (cases/controls)	165/171	166/171	181/173			
Follow-up time						0.37
<13.9 years, multivariable-adjusted OR (95% CI) ^d	1 (reference)	0.70 (0.34, 1.43)	0.86 (0.42, 1.76)	0.69	0.77 (0.50, 1.20)	
≥ 13.9 years, multivariable-adjusted OR (95% CI) ^d	1 (reference)	1.65 (0.70, 3.90)	1.02 (0.41, 2.57)	0.87	1.21 (0.74, 2.00)	
DHEA						
Tertile cutpoints	<1414.3	1414.3-2482.3	≥ 2482.3			
No. (cases/controls)	171/171	173/172	168/173			
Follow-up time						0.07

<13.9 years, multivariable-adjusted OR (95% CI) ^d	1 (reference)	0.58 (0.27, 1.24)	0.81 (0.38, 1.74)	0.57	0.74 (0.53, 1.02)	
≥13.9 years, multivariable-adjusted OR (95% CI) ^d	1 (reference)	1.10 (0.44, 2.72)	2.02 (0.75, 5.39)	0.18	1.19 (0.81, 1.76)	
Progesterone						
Tertile cutpoints	<44.1	44.1-65.4	≥65.4			
No. (cases/controls)	170/172	168/172	174/172			
Follow-up time						0.26
<13.9 years, multivariable-adjusted OR (95% CI) ^d	1 (reference)	1.80 (0.79, 4.11)	2.36 (1.16, 4.83)	0.02	1.12 (0.75, 1.67)	
≥13.9 years, multivariable-adjusted OR (95% CI) ^d	1 (reference)	1.11 (0.46, 2.70)	1.17 (0.49, 2.79)	0.73	0.98 (0.60, 1.59)	
SHBG						
Tertile cutpoints	<46.0	46.0-63.7	≥63.7			
No. (cases/controls)	195/172	131/172	186/172			
Follow-up time						0.35
<13.9 years, multivariable-adjusted OR (95% CI) ^d	1 (reference)	0.80 (0.36, 1.77)	1.02 (0.47, 2.21)	0.93	1.04 (0.65, 1.66)	
≥13.9 years, multivariable-adjusted OR (95% CI) ^d	1 (reference)	0.16 (0.05, 0.50)	0.55 (0.20, 1.49)	0.37	0.82 (0.46, 1.47)	
Free estradiol						
Tertile cutpoints	<69.5	69.5-123.4	≥123.4			
No. (cases/controls)	185/171	152/172	175/173			
Follow-up time						0.71
<13.9 years, multivariable-adjusted OR (95% CI) ^d	1 (reference)	0.42 (0.19, 0.92)	0.57 (0.24, 1.38)	0.33	0.99 (0.76, 1.30)	
≥13.9 years, multivariable-adjusted OR (95% CI) ^d	1 (reference)	0.95 (0.42, 2.13)	0.89 (0.33, 2.37)	0.81	0.97 (0.71, 1.33)	
Free testosterone						
Tertile cutpoints	<3.9	3.9-6.6	≥6.6			
No. (cases/controls)	157/171	161/172	194/173			
Follow-up time						0.76
<13.9 years, multivariable-adjusted OR (95% CI) ^d	1 (reference)	0.59 (0.28, 1.26)	0.98 (0.44, 2.16)	0.96	0.99 (0.76, 1.30)	
≥13.9 years, multivariable-adjusted OR (95% CI) ^d	1 (reference)	1.11 (0.51, 2.44)	1.30 (0.49, 3.42)	0.60	0.97 (0.71, 1.33)	
Estradiol to testosterone ratio						
Tertile cutpoints	<0.22	0.22-0.31	≥0.31			
No. (cases/controls)	168/171	175/172	169/173			
Follow-up time						0.96
<13.9 years, multivariable-adjusted OR (95% CI) ^d	1 (reference)	0.81 (0.36, 1.82)	0.70 (0.30, 1.65)	0.42	0.79 (0.08, 8.12)	

≥13.9 years, multivariable-adjusted OR (95% CI)^d 1 (reference) 0.85 (0.41, 1.77) 0.60 (0.21, 1.73) 0.37 1.02 (0.10, 10.74)

^a Statistical tests for trend (two-sided) were calculated using ordinal quartile variables entered into the model as a single continuous variable. CI = confidence intervals; DHEA = dehydroepiandrosterone; OR = odds ratio; SHBG = sex hormone-binding globulin.

^b Serologic variables were log₂-transformed in continuous models.

^c Heterogeneity by colon subsites were tested using χ^2 tests. The test was 2-sided.

^d ORs and 95% CIs were estimated by conditional logistic regression models. Models were adjusted for body mass index (continuous), smoking status (never, former, current or unknown), physical activity (inactive, moderately inactive, moderately active, active, or unknown), ever hormone use (yes, no or unknown/missing), alcohol consumption (continuous), and estrone, estradiol and testosterone were adjusted for SHBG and vice versa.

Supplementary Table 6. Characteristics of studies included in the meta-analysis.

Reference	Study design	Country of origin	Total no. of cases/controls	Method of sex hormone measurement	Outcome	Categories (highest vs. lowest)	OR (95%CI)	Matched/Adjusted factors
Lin, et al. 2013 (9)	NCCS	United States	293/437	Turbulent flow liquid chromatography tandem mass spectrometry	CRC	Estrone: Q1 vs. Q4 Estradiol: Q1 vs. Q4	Estrone: 1.30 (0.74–2.26) Estradiol: 1.12 (0.62–2.03)	Age at blood draw, fasting status, hour at blood draw, smoking, current alcohol intake, family history, physical activity, history of polyps, screening exam; BMI, and C-peptide.
Gunter, et al. 2008 (10)	NCCS	United States	438/809	Vitros-Eci Immunodiagnostic Assay	CRC	Estradiol: Q1 vs. Q2	Estradiol: 1.53 (1.02–2.27)	Age, smoking, ethnicity, family history of colorectal cancer, history of colonoscopy, physical activity, use of NSAIDs, and alcohol consumption.
Clendenen, et al. 2009 (7)	NCCS	United States	148/293	Radioimmunoassay following organic extraction and celite chromatography	CRC	Estrone: Q1 vs. Q4 Estradiol: Q1 vs. Q3	Estrone: 1.6 (0.8–3.0) Estradiol: 0.8 (0.4–1.7)	Matching factors and BMI.
			65/151		CC	Estrone: Q1 vs. Q3	Estrone: 1.7 (0.9–3.1)	Matching factors and BMI.
			81/142		RC	Estrone: Q1 vs. Q3	Estrone: 2.0 (0.5–8.8)	Matching factors and BMI.
Falk, et al. 2015 (8)	NCCS	United States	182/475	Stable isotope dilution liquid chromatography–tandem mass spectrometry	CRC	Estrone: Q1 vs. Q4 Estradiol: Q1 vs. Q4	Estrone: 1.15 (0.69–1.93) Estradiol: 0.98 (0.58–1.64)	BMI, parity, smoking, postmenopausal hormone use, and ages at menarche, birth of the first child, and menopause.
Murphy, et al. 2015 (11)	NCCS	United States	401/802	Radioimmunoassay	CRC	Estrone: Q1 vs. Q4 Estradiol: Q1 vs. Q4	Estrone: 0.64 (0.43–0.97) Estradiol: 0.50 (0.33–0.75)	Waist circumference, alcohol consumption, family history of colorectal cancer, physical activity, smoking status, and NSAID use.
			303/605		CC	Estrone: Q1 vs. Q4 Estradiol: Q1 vs. Q4	Estrone: 0.70 (0.46–1.07) Estradiol: 0.90 (0.59–1.37)	Waist circumference, alcohol consumption, family history of colorectal cancer, physical activity, smoking status, and NSAID use

			93/185		RC	Estrone: Q1 vs. Q3	Estrone: 1.13 (0.49–2.62)	Waist circumference, alcohol consumption, family history of colorectal cancer, physical activity, smoking status, and NSAID use
						Estradiol: Q1 vs. Q3	Estradiol: 1.14 (0.63–3.28)	
Mori, et al. 2019 (12)	NCCS	Japan	185/361	Electrochemiluminescence	CRC	Estradiol: Q1 vs. Q3	Estradiol: 2.28 (0.77–6.78)	BMI, smoking status, alcohol consumption, and physical activity.

^aNCCS = nested case-control study; CRC = colorectal cancer; CC = colon cancer; RC = rectal cancer; OR = odds ratio; CI = confidence interval; Q = quantile; NSAID = non-steroidal anti-inflammatory drugs BMI = body mass index