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Endogenous sex hormones and colorectal cancer survival among men and women

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Abstract

Although previous studies have suggested a potential role of sex hormones in the etiology of colorectal cancer (CRC), no study has yet examined the associations between circulating sex

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Conflict of interest

Dr. Chan reports grants and personal fees from Bayer Pharma AG and personal fees from Pfizer Inc and Janssen. Dr. Fuchs reports grants and personal fees from Agios, Bain Capital, Bayer, Celgene, CytomX, CytomX, Dicerna, Eli Lilly, Entrinsic Health, Entrinsic Health, Five Prime Therapeutics, Genentech, Gilead Sciences, KEW, Merck, Merrimack, Pfizer, Roche, Sanofi, Taiho and Unum.

Additional Supporting Information may be found in the online version of this article.

hormones and survival among CRC patients. We prospectively assessed the associations of prediagnostic plasma concentrations of estrone, estradiol, free estradiol, testosterone, free testosterone and sex hormone-binding globulin (SHBG) with CRC-specific and overall mortality among 609 CRC patients (370 men and 239 postmenopausal women not taking hormone therapy at blood collection) from four U.S. cohorts. Multivariable hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using Cox proportional hazard regression. We identified 174 deaths (83 CRC-specific deaths) in men and 106 deaths (70 CRC-specific deaths) in women. In men, higher circulating level of free testosterone was associated with lower risk of overall (the highest *vs.* lowest tertiles, HR = 0.66, 95% CI, 0.45–0.99, p_{trend} = 0.04) and possibly CRC-specific mortality (HR = 0.73, 95% CI, 0.41–1.29, p_{trend} = 0.27). We generally observed nonsignificant inverse associations for other sex steroids, and a positive association for SHBG with CRC-specific mortality among male patients. In women, however, we found a suggestive positive association of estrone with overall (HR = 1.54, 95% CI, 0.92–2.60, p_{trend} = 0.11) and CRC-specific mortality (HR = 1.96, 95% CI, 1.01–3.84, p_{trend} = 0.06). Total estradiol, free estradiol and free testosterone were generally suggestively associated with higher risk of mortality among female patients, although not statistically significant. These findings implicated a potential role of endogenous sex hormones in CRC prognosis, which warrant further investigation.

Keywords

estrogen; estrone; testosterone; colorectal cancer; survival; cohort study

Introduction

Colorectal cancer (CRC) is one of the most common causes of cancer morbidity both in men and in women.[1] Although both sexes have the same 65% 5-year survival,[1] premenopausal women with CRC may have better survival than men of similar age (5-year survival of 77% in women *vs.* 57% in men).[2,3] This sex difference in survival was independent of tumor sites, grade, stage and treatment.[2-7] Female hormones[4,8] have been hypothesized to partly explain such a difference in CRC survival.

Consistent with a role of hormones on CRC survival, hormone therapy (HT) use before CRC diagnosis has been associated with improved survival in several[9-11] but not all observational studies.[12] In addition, studies addressing the association of plasma estrogens and androgens with CRC development have implicated a potential role of sex hormones in the etiology of CRC in both sexes, although the findings have not been entirely consistent.[13-18] Besides general benefits for facilitating defecation functions[19] and reducing bile acid synthesis,[20] estrogens may exert specific biological effects in the gastrointestinal tract through estrogen receptor (ER) β,[21] which then affect CRC development and/or progression. *In vitro*, expression of ERβ was shown to reduce the proliferation of normal and malignant colonic epithelium cells.[22] Also, ERβ expression is lower in CRC cancer cells compared to those in normal colon cells,[23] suggesting a possible role of estrogen in CRC progression. Indeed, higher ERβ expression was associated with better survival, and correlated with longer use of HT among CRC patients.[24,25] Similarly, lower androgenicity due to longer cytosine adenine guanine (CAG) repeats in androgen receptors (AR) gene, and decreased AR expression,

have been associated with a poor survival among male CRC patients.[26-28] Taken together, these observations begin to explain the potential role of estrogens in women and androgens in men in CRC prognosis.

To the best of our knowledge, no study has assessed the associations between circulating sex hormones and survival in CRC patients. We therefore comprehensively investigated prediagnostic plasma levels of sex steroids and sex hormone-binding globulin (SHBG) in relation to overall and CRC-specific mortality among patients with CRC. We utilized data from two male prospective cohorts, the Health Professionals Follow-up Study (HPFS) and the Physicians' Health Study II (PHSII), and two female prospective cohorts, the Nurses' Health Study (NHS), and the Women's Health Study (WHS). The present study extends our earlier report[15] regarding the association between sex hormones and risk of incident CRC in the same cohorts, which supported a potential role of sex hormones in CRC development.

Methods

Study cohorts

Participants in our study were selected from four prospective U.S. cohorts including HPFS, PHSII, NHS and WHS. Details of the study design, scientific rationale and the collection of blood samples have been described elsewhere.[15] Briefly, the HPFS was established in 1986 with 51,529 U.S. male health professionals aged 40–75 years;[29] the PHSII is a randomized trial evaluating the benefits and risk of vitamin E, vitamin C, multivitamins and β-carotene, beginning in 1997, with 14,641 male physicians aged ≥50 years;[30] the NHS was established in 1976 with 121,700 female registered nurses aged 30–55 years;[31] and the WHS is a completed randomized trial of low-dose aspirin and vitamin E between 1992 and 1995, with 39,876 female health professionals aged ≥45 years.[32]

Ascertainment of CRC cases and deaths

The incident CRC cases were initially identified by self-report in all four cohorts.[29-32] In HPFS[29] and NHS,[31] participants were asked for written permission to obtain their medical records and pathological reports if they reported CRC on biennial questionnaires. For all deaths attributed to CRC, we requested permission from next-of-kin to review medical and pathological records. A study physician who was blinded to exposure data confirmed all possible cancer cases, and extracted information on stage, location and histology. We further searched state vital statistics records, the National Death Index for potential unreported cancer deaths, with this approach capturing >98% of overall deaths.[33] All-cause death was also confirmed by study physicians blinded to exposure status *via* review of all available sources including death certificates or medical records. For nonresponders who died of CRC, we contacted the next-of-kin to obtain permission to review medical records and confirm a diagnosis of CRC as described above. In each female cohort, cases were only selected from patients who were postmenopausal and were not currently using HT at blood collection. The study protocols were approved by the Institutional Review Board at the Brigham and Women's Hospital (BWH) and Harvard T.H. Chan School of Public Health, and those of participating registries as required.

Assessments of sex steroids, SHBG and C-peptide in the blood samples

In the HPFS, blood samples were collected in 1993 and 1994 among 18,025 men. In the PHSII, 11,120 men provided baseline blood samples. In the NHS, we collected blood samples among 32,826 women in 1989 and 1990 from women. In the WHS, blood samples were collected at baseline from 28,345 women. Baseline characteristics of participants who provided blood samples in each cohort were compared to those in the full cohort. Participants with blood samples in the four cohorts had similar baseline characteristics and shared similar methods of blood drawn.[15]

We assessed estrone and estradiol in men and women and testosterone in women at the Mayo Clinic (Rochester, MN) by using turbulent flow liquid chromatography-tandem mass spectrometry. SHBG and albumin in men and women and testosterone in men were assayed at the Boston Children's Hospital using a competitive electrochemiluminescence immunoassay (SHBG and testosterone) and a colorimetric assay (albumin). In the WHS and PHSII, the C-peptide samples were also measured at the Boston Children's Hospital using a competitive electrochemiluminescence immunoassay.[15] In the NHS and HPFS, the C-peptide samples were assessed with an enzyme-linked immunosorbent assay at the Boston Children's Hospital.[15] Free testosterone and estradiol were calculated using laws of mass action as suggested by Sodergard *et al.*[34]

The mean intra-assay coefficients of variation from our quality control samples ranged from 4% to 7% in men and from 3% to 7% in women for five biomarkers (i.e., estrone, estradiol, testosterone, C-peptide and SHBG).[15] The variation in plasma levels of testosterone, estradiol and SHBG within person over time have been validated in the NHS.[35] We found a strong correlation between the two blood measures collected 10 years apart, with intra-class correlation coefficients of 0.69 for estradiol, 0.71 for testosterone and 0.74 for SHBG.[35] Similar observations were also obtained in another investigation of sex hormones among 144 men in the HPFS.[36] The Spearman correlation coefficients for samples drawn 3 years apart were 0.68 for testosterone, 0.55 for estradiol and 0.74 for SHBG.[36] These results indicated that a single baseline plasma measure can reasonably capture long-term exposure.

Statistical analysis

Person-years of follow-up were calculated from the date of CRC diagnosis until the date of death or follow-up through 2012, if a patient was still alive. We categorized the plasma markers into tertiles within each cohort. To reduce departures from the normal distribution, all plasma biomarkers levels were natural logarithm ($\log_e$) transformed.[15] Cox regression models were fitted to calculate hazard ratios (HRs) and 95% confidence intervals (95% CIs) of overall and CRC-specific mortality. Trend tests were performed by assigning the median ($\log_e$-transformed plasma levels) of each tertile for each marker as a continuous variable in the models.

We presented risk estimates by sex in three multivariable models, because the associations between some selected hormones (i.e., estrone and free estradiol) and death among CRC patients statistically differed between sexes ($p_{\text{heterogenity}}$ for sex ≤0.05). In Model 1, we adjusted for age at diagnosis (years), location of primary tumor (proximal, distal, rectum or

unknown), grade of differentiation (well-differentiated, moderately differentiated, poorly differentiated or unknown), metastasis of the tumor (yes *vs.* no) and study cohort. Obesity is associated with increased levels of circulating estrogens and insulin in both sexes and reduced levels of testosterone in men.[37-39] In addition, both insulin[40] and obesity[41] have been suggested to worsen CRC survival. Therefore, in Model 2, we further adjusted for prediagnostic body mass index (BMI) and physical activity. In Model 3, we additionally adjusted for C-peptide, a plasma marker for endogenous insulin levels when we evaluated the associations between sex hormones and survival. We were unable to adjust for or conduct stratified analysis by tumor stage because data on tumor staging were not available in the PHS II and WHS. To test whether tumor stage is associated with levels of sex steroids and SHBG in the HPFS and NHS, one-way ANOVA were conducted. Spearman correlation coefficients between tumor stage and $\log_e$-transformed plasma biomarkers were also calculated. Trend tests were conducted using the median of each category as a continuous variable in each cohort, and *p*-value for trend was calculated using a Wald test.

We also conducted exploratory subgroup analyses according to age at diagnosis (<70 *vs.* ≥70 years) and time interval between blood drawn and a diagnosis of cancer (<5 *vs.* ≥5 years), despite the insufficient power given the limited sample size in the analysis. We only provided results for overall mortality because only few or no CRC-specific deaths in some stratifications among the aforementioned subgroup analysis. In secondary analysis, we adjusted for BMI after cancer diagnosis instead of prediagnostic BMI. Considering the potential reverse causality, we also repeated analysis after excluding patients diagnosed with CRC within 5 years of blood drawn. We used SAS 9.4 (SAS Institute, Cary, NC) for all analyses, and a two-sided *p*-value of 0.05 was considered statistically significant.

Data availability

The data that support the findings of our study are available from BWH and Harvard T.H. Chan School of Public Health. Restrictions apply to the availability of these data, which were used under license for our study. Data are available (https://sites.google.com/channing.harvard.edu/cohortdocs/)] with the permission of BWH and Harvard T.H. Chan School of Public Health.

Results

Among 609 CRC patients (370 men and 239 women), we documented 174 deaths (83 CRC deaths) in men and 106 deaths (70 CRC deaths) in women. Men with higher levels of testosterone had older age at cancer diagnosis, lower BMI and C-peptide levels, and less physical activity (Table 1 and Supporting Information Table S1). Women with higher estradiol levels tended to have higher BMI and plasma C-peptide levels, and were less physically active (Table 2 and Supporting Information Table S2).

In men, the circulating levels of testosterone, which was strongly and positively correlated with SHBG, was moderately positively correlated with estradiol levels, and slightly inversely correlated with BMI and C-peptide. The ratio of total estradiol to testosterone was moderately positively correlated with BMI but inversely correlated with SHBG. In women, free estradiol was highly positively correlated with total estradiol, estrone and BMI. SHBG

was moderately inversely correlated with free estradiol in women, but slightly positively correlated with free testosterone in men (Supporting Information Table S3).

In men, higher circulating level of free testosterone was associated with lower risk of overall mortality (the highest *vs.* lowest tertiles, HR = 0.66, 95% CI: 0.45–0.99, p_{trend} = 0.04) and possibly CRC-specific mortality (HR = 0.73, 95% CI, 0.41–1.29, p_{trend} = 0.27). We generally observed inverse but nonsignificant associations for other sex steroids including total testosterone and estradiol, and a suggestive positive association for SHBG with CRC-specific mortality (HR = 1.49, 95% CI: 0.82–2.72, p_{trend} = 0.25) among male patients (Table 3).

In women, higher levels of estrone were suggestively associated with higher risk of overall mortality (the highest *vs.* lowest tertiles, HR = 1.54, 95% CI: 0.92–2.60, p_{trend} = 0.11) and CRC-specific mortality (highest *vs.* lowest tertiles, HR = 1.96, 95% CI: 1.01–3.84, p_{trend} = 0.06). Similar positive but nonsignificant associations were observed comparing the highest *versus* lowest tertiles of total estradiol (overall mortality: HR = 1.45, 95% CI: 0.79–2.66, p_{trend} = 0.20; CRC-specific mortality: HR = 2.00, 95% CI: 0.92–4.35, p_{trend} = 0.10), free estradiol (overall mortality: HR = 1.43, 95% CI: 0.77–2.67, p_{trend} = 0.22; CRC-specific mortality: HR = 1.67, 95% CI: 0.76–3.64, p_{trend} = 0.18) and free testosterone (overall mortality: HR = 1.23, 95% CI: 0.72–2.11, p_{trend} = 0.49; CRC-specific mortality: HR = 1.50, 95% CI: 0.79–2.85, p_{trend} = 0.19) (Table 4). We found nonsignificant association between the ratio of estradiol to testosterone and mortality in either men or women.

In exploratory subgroup analysis, the association of sex hormones with overall deaths among CRC survivors were generally not appreciably altered in each stratification (Supporting Information Tables S4 and S5). However, the association of free testosterone in men and the associations of several sex hormones in women with risk of overall death appeared slightly stronger in younger patients (<70 years), despite limited statistical power. The results were also similar after excluding participants diagnosed with CRC within 5 years of blood drawn. When we adjusted for BMI after cancer diagnosis instead of prediagnostic BMI, the results did not materially change. There were no correlations between tumor stage and circulating levels of sex steroids and SHBG (Supporting Information Table S6). In addition, we found no difference in circulating levels of these biomarkers according to tumor stage (All *p* values for one-way ANOVA were greater than 0.10).

Discussion

To the best of our knowledge, this is the first prospective study to examine prediagnostic endogenous sex hormones and long-term mortality among patients diagnosed with CRC. We found that in men, higher prediagnostic levels of free testosterone were associated with lower risk of overall mortality, even after adjustment for tumor characteristics, BMI, physical activity and plasma C-peptide levels. In postmenopausal women not taking HT, we observed that higher levels of prediagnostic estrone, estradiol, free estradiol and testosterone were generally associated with higher risk of mortality among CRC patients, although only the association of estradiol with CRC-specific mortality was statistically significant.

In men, we found a suggestive beneficial association between circulating prediagnostic free testosterone and CRC survival. This association appeared to be independent of BMI and plasma C-peptide, although obese men generally have low levels of testosterone and high levels of insulin.[37-39] Similarly, we previously reported that men with higher levels of testosterone have lower risk of developing CRC in the same cohorts.[15] In addition, men with lower androgenicity due to longer CAG repeats in the *AR* gene,[26-28] or undergoing androgen deprivation therapy,[42] were at a higher risk of CRC development. Our findings were also in agreement with findings from *in vitro* and *in vivo* studies[43-45] showing antiproliferative, apoptotic and antimigration effects of testosterone in colonic cancer cells *via* membrane AR activation. In addition, we found a nonsignificant positive association between higher SHBG levels and CRC-specific mortality after cancer diagnosis. This result further supports a possible protective association with free testosterone, given that higher levels of SHBG lead to lower levels of non-SHBG-bound (free or bioactive) testosterone.

In women, there was a suggestive positive association between prediagnostic plasma estrogen and the risk of total mortality, although current evidence suggests that exogenous estrogens and/or progestin use may predict better survival (~30% risk reduction for all-cause and ~40% for CRC-specific mortality) among patients with CRC in women.[9-11] Interestingly, a similar pattern (i.e., opposite effects of endogenous and exogenous estrogens) was also observed for CRC incidence from the New York University Women's Health Study (NYUWHS)[16] and the Women's Health Initiative-Observational study (WHI-OS).[17] In NYUWHS,[16] Clendenen *et al.* found a positive association between endogenous estrone and the risk of incident CRC (RR = 1.8; 95%CI: 1.0–3.3) among 148 postmenopausal women who did not use exogenous hormones at baseline, and 293 matched controls nested within the cohort. Similarly, higher plasma estradiol levels were positively associated with the risk of CRC (RR = 1.53; 95% CI: 1.05–2.22) among postmenopausal women not taking HT within WHI-OS, although the assay of estrone and estradiol (radioimmunoassay) in our study is relatively insensitive among postmenopausal women.[17] In contrast to endogenous estrogens, a meta-analysis of 18 observational studies suggested a ~20% risk reduction of both colon and rectal cancers among women using postmenopausal HT.[46] These findings indicated a possible differential role between endogenous and exogenous estrogens in CRC incidence and prognosis, although the WHI-Clinical Trial (WHI-CT) found that both endogenous estrone and estradiol were significantly inversely associated with risk of CRC development in postmenopausal women.[13] A cohort consortium project to pool multiple cohorts with CRC patients is needed to validate the findings.

One possible reason why prediagnostic endogenous estrogens might be associated with poor CRC survival in postmenopausal women is a change in the ratio of ERα to ERβ expression during colorectal cancer progression. In normal colonic tissue, ERα enhances cell growth and is expressed at low levels, while the antiproliferative ERβ is abundant. In neoplastic colonic tissue, this ratio is reversed.[23,47] Given the change from predominantly ERβ expression in the healthy colon to ERα in neoplastic tissue, it is reasonable to hypothesize that endogenous estrogens may afford protection in the healthy colon, but promote tumorgenesis once neoplastic changes occur.[48]

However, HT use among postmenopausal women may exert beneficial effects on CRC survival through preventing the loss of ERβ expression.[24,25] This may also partly explain the opposite effects of endogenous and exogenous estrogens in women from our cohorts. In NHS, we previously suggested that postmenopausal HT use within 5 years of CRC diagnosis was associated with improved survival; while this benefit was lost among HT past users,[10] suggesting timing of start of HT (shorter interval to cancer diagnosis) may play a more important role, as ERβ expression decreases throughout the process of carcinogenesis.[36]

We did not find any significant association between the ratio of estradiol to testosterone and mortality either in men or in women, although the higher ratio of total estradiol over testosterone, which reflects greater aromatase enzyme activity and, as a result, higher estradiol synthesis, was suggested to be associated with a lower risk of CRC development in women, but with a higher risk in men, in our previous report.[15] We observed that the association between several sex hormones and death risk among CRC survivors seemed slightly stronger in younger age groups. This finding could be due to chance. Alternatively, it is plausible because testosterone and estrogen levels fall with age in both sexes.

There are several limitations in the study. First, we cannot rule out chance findings due to the limited number of deaths and multiple comparisons, although this is a hypothesis-driven study and multiple testing is therefore less of a concern. Second, residual confounding might exist. For example, we cannot adjust for tumor stage in all analysis. However, the levels of sex hormones appeared not to correlate with (or differ according to) tumor stage in the NHS and HPFS, indicating that our findings were unlikely to be confounded by tumor stage. Further, previous studies from our cohorts generally showed no interaction between risk factors and tumor stage in the association with mortality among CRC patients.[49,50] We did not control for C-reactive protein (CRP) because BMI that has been adjusted in the analysis moderately correlated with CRP levels among US adults.[13] We were unable to adjust for other inflammatory biomarkers and insulin-like growth factor (IGF)-1 since we have no such data in all four cohorts, although a previous study suggested that further adjustments for CRP and free IGF-I did not alter the association between sex hormones and CRC risk.[13]

Third, having only a single measure of prediagnostic sex hormones at baseline may reduce our ability to assess associations between long-term circulating levels of these exposures and survival. However, our validation studies have suggested that one-time blood measure captures long-term exposure reasonably well.[35,36] In particular, there is a strong correlation between the two blood measures collected 10 years apart in our cohorts.[35] Finally, our results might be influenced by a possible reverse causality. However, after excluding cancer case diagnosed within 5 years of blood drawn, the results were not appreciably altered.

In summary, we found a suggestive positive association between prediagnostic levels of estrogen as well as other sex steroids and death risk among colorectal cancer survivors in women. In men, prediagnostic free testosterone was inversely associated with a lower risk of total mortality. These findings add evidence at a population level that endogenous sex hormones may play a complex role in CRC prognosis. Future study that leverages data from multiple cohorts is warranted to validate our findings. In addition, the underlying

mechanisms linking sex hormones with colorectal cancer survival need to be elucidated in animal or *in vitro* studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

AR	androgen receptors
BMI	body mass index
CAG	cytosine adenine guanine
CI	confidence interval
CRC	colorectal cancer
ER	estrogen receptor
FFQ	food frequency questionnaire
HBV	hepatitis B virus
HPFS	Health Professionals Follow-Up Study
HR	hazard ratio
HT	hormone therapy
IGF	insulin-like growth factor
NHS	Nurses' Health Study
NYUWHS	New York University Women's Health Study
PHSII	Physicians' Health Study II
SHBG	sex hormone-binding globulin

WHI-CT	Women's Health Initiative-Clinical Trial
WHI-OS	Women's Health Initiative-Observational study
WHS	Women's Health Study

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What's new?

Premenopausal women with colorectal cancer have a higher 5-year survival rate than men of the same age, suggesting that female hormones may boost survival. In this prospective study, the authors compared the levels of various sex hormones to mortality rates among 609 CRC patients. In men, they found a beneficial relationship between higher levels of circulating testosterone and lower mortality. In women, though it was not statistically significant, they found a suggestive positive association between higher levels of circulating estrogens and mortality. This is the first study to investigate prediagnostic sex hormone levels as they correlate with CRC mortality.

Table 1.

Age-adjusted characteristics according to tertiles of selected hormones and SHBG in men

	Estradiol			Estrone			Testosterone			SHBG		
	Tertile 1	**Tertile 2**	**Tertile 3**	**Tertile 1**	**Tertile 2**	**Tertile 3**	**Tertile 1**	**Tertile 2**	**Tertile 3**	**Tertile 1**	**Tertile 2**	**Tertile 3**
Number of cases	125	126	119	124	131	115	125	126	119	124	124	122
Age at diagnosis[1], years	71.2 (9.1)	72.1 (9.0)	73.7 (8.4)	71.0 (8.2)	72.1 (9.7)	74.0 (8.5)	71.2 (9.1)	72.1 (9.0)	73.7 (8.4)	69.9 (9.6)	72.6 (8.2)	74.5 (8.2)
Body mass index[2], kg/m^2	25.9 (3.2)	26.4 (3.2)	26.7 (3.8)	26.1 (3.5)	26.1 (3.4)	26.7 (4.0)	26.8 (3.3)	26.2 (3.7)	25.4 (3.1)	26.9 (3.6)	26.5 (3.2)	25.2 (3.1)
Being physically active[3], %	77	75	75	82	77	71	77	75	75	71	78	80
C-peptide, ng/ml	3.4 (2.7)	3.0 (2.1)	2.7 (2.2)	3.3 (2.3)	3.0 (2.7)	2.9 (2.4)	3.4 (2.7)	3.0 (2.1)	2.7 (2.2)	3.2 (2.6)	2.6 (1.9)	3.0 (2.5)
Tumor location, %												
Proximal colon	67	69	68	65	71	65	67	69	68	71	68	59
Distal colon	23	24	23	24	20	29	23	24	23	20	28	29
Rectum	10	7	9	11	9	6	10	7	9	9	5	12
Tumor differentiation grade, %												
Well	8	10	9	11	8	10	8	10	9	9	8	17
Moderate	67	72	67	65	73	67	67	72	67	67	78	53
Poor	25	18	24	24	19	23	25	18	24	24	14	30
Tumor stage %												
Stage 1	7	10	13	7	10	9	7	10	13	13	8	8
Stage 2	12	15	11	13	13	12	12	15	11	6	15	12
Stage 3	22	21	22	25	21	24	22	21	22	22	25	24
Stage 4	2	1	1	2	3	1	2	1	1	3	1	1
Unknown[4]	57	52	54	53	53	55	57	52	54	56	52	55
Tumor metastasized, %	20	23	19	25	18	27	20	23	19	22	25	21
Years between blood drawn and cancer diagnosis	5.5 (3.4)	5.1 (3.0)	6.0 (3.5)	5.7 (3.2)	5.8 (3.3)	5.3 (3.7)	5.5 (3.4)	5.1 (3.0)	6.0 (3.5)	5.6 (3.6)	5.9 (3.5)	5.4 (3.3)

Values were means (SD) or percentages and were age-adjusted.

[1] Age at diagnosis is not age-adjusted. We created the tertile categories for estradiol (<24, 24–30, ≥30 pg/ml), estrone (<31, 31–42, ≥42 pg/ml), testosterone (<376, 376–501, ≥501 ng/dl) and SHBG (<20, 20–27, ≥27 ng/dl).

[2] Body mass index before colorectal cancer diagnosis was calculated as weight in kilograms divided by the square of height in meters.

[3]Being physically active is defined as at least 3 METS-hours/week (approximately equal 1 hr brisk walking per week) in the Nurse's Health Study, the Women's Health Study and the Health Professionals Follow-up Study; and engage in a regular program of exercise vigorous enough to work up a sweat with the frequency of less than 1 day per week in the Physician's Health Study II.

[4]Stage information was not available in the Physician's Health Study II.

Abbreviation: SHBG, sex hormone-binding globulin.

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Table 2.

Age-adjusted characteristics according to tertiles of selected hormones and SHBG in women

	Estradiol			Estrone			Testosterone			SHBG		
	Tertile 1	**Tertile 2**	**Tertile 3**	**Tertile 1**	**Tertile 2**	**Tertile 3**	**Tertile 1**	**Tertile 2**	**Tertile 3**	**Tertile 1**	**Tertile 2**	**Tertile 3**
Number of cases	80	80	79	88	74	77	91	71	77	85	79	75
Age at diagnosis[1], years	68.9 (6.8)	71.4 (5. 7)	69.8 (6.6)	69.4 (6.1)	70.4 (6.7)	70.4 (6.6)	69.5 (6.3)	69.6 (5.87)	71.0 (7.0)	69.1 (6.5)	71.2 (6.5)	69.8 (6.2)
Body mass index[2], kg/m^2	23.8 (3.9)	25.5 (4.2)	30.8 (5.4)	25.2 (4.9)	26.1 (4.6)	30.1 (6.1)	26.6 (5.8)	26.8 (4.8)	26.6 (6.2)	29.5 (5.9)	26.0 (4.8)	23.9 (3.7)
Being physically active[3], %	64	64	61	66	58	66	64	73	57	42	67	80
C-peptide, ng/mL	2.2 (1.4)	2.4 (1.2)	3.2 (1.5)	2.5 (1.4)	2.4 (1.2)	3.1 (1.5)	2.7 (1.7)	2.5 (1.2)	2.3 (1.1)	3.1 (1.6)	2.4 (1.1)	1.8 (1.1)
Tumor location, %												
Proximal colon	71	78	76	73	77	74	72	76	78	74	80	73
Distal colon	21	12	12	17	16	12	22	12	14	19	10	16
Rectum	8	10	12	10	7	14	6	12	8	7	9	11
Tumor differentiation grade, %												
Well	8	12	7	9	9	12	5	15	11	4	13	7
Moderate	80	74	85	78	79	79	79	76	83	85	82	77
Poor	12	14	8	13	12	8	16	9	6	11	6	15
Tumor stage, %												
Stage 1	11	5	11	6	7	10	10	10	8	1	9	12
Stage 2	10	12	9	12	8	10	5	19	13	6	12	14
Stage 3	24	33	28	29	25	30	25	34	18	15	31	45
Stage 4	5	2	4	3	2	3	3	1	4	1	1	5
Unknown[4]	50	47	47	50	57	47	57	37	58	76	47	24
Tumor metastasized, %	20	29	21	28	20	21	21	21	35	33	21	13
Years between blood drawn and cancer diagnosis	8.1 (3.8)	7.4 (4.0)	7.9 (4. 1)	8.0 (4.0)	7.4 (3.9)	8.2 (4.1)	7.3 (4.0)	7.9 (4.4)	8.0 (3.9)	7.4 (3.6)	8.0 (4.2)	7.8 (3.8)

Values were means (SD) or percentages and were age-adjusted.

[1] Age at diagnosis is not age-adjusted. We created the tertile categories for estradiol (<4.2, 4.2–7.6, ≥7.6 pg/ml), estrone (<21, 21–31, ≥31 pg/ml), testosterone (<18, 18–26, ≥26 ng/dl) and SHBG (<20, 20–27, ≥27 ng/dl).

[2] Body mass index before colorectal cancer diagnosis was calculated as weight in kilograms divided by the square of height in meters.

[3] Being physically active is defined as at least 3 METS-hours/week (approximately equal 1 hr brisk walking per week) in the Nurse's Health Study, the Women's Health Study and the Health Professionals Follow-up Study; and engage in a regular program of exercise vigorous enough to work up a sweat with the frequency of less than 1 day per week in the Physician's Health Study II.

[4] Stage information was not available in the Women's Health Study.

Abbreviation: SHBG, sex hormone-binding globulin.

Table 3.

Multivariable hazard ratios of death according to tertiles of prediagnostic hormone levels in men

	Overall mortality (*n* = 174)				CRC mortality (*n* = 83)			
	Tertile 1	**Tertile 2**	**Tertile 3**	p_{trend}	**Tertile 1**	**Tertile 2**	**Tertile 3**	p_{trend}
Estradiol								
Model 1[1]	Referent	0.89 (0.62–1.29)	0.80 (0.55–1.16)	0.23	Referent	0.94 (0.56–1.57)	0.71 (0.41–1.23)	0.22
Model 1 + BMI+PA[2]	Referent	0.90 (0.62–1.30)	0.79 (0.54–1.15)	0.22	Referent	0.96 (0.57–1.60)	0.79 (0.45–1.39)	0.41
Model 1 + BMI + PA + C-peptide[3]	Referent	0.88 (0.60–1.28)	0.80 (0.55–1.18)	0.27	Referent	0.91 (0.54–1.54)	0.79 (0.45–1.38)	0.40
Estrone								
Model 1[1]	Referent	0.74 (0.50–1.07)	0.94 (0.65–1.37)	0.83	Referent	0.64 (0.36–1.12)	1.06 (0.63–1.80)	0.78
Model 1 + BMI + PA[2]	Referent	0.73 (0.50–1.08)	0.92 (0.62–1.37)	0.77	Referent	0.69 (0.39–1.23)	1.25 (0.72–2.17)	0.42
Model 1 + BMI + PA + C-peptide[3]	Referent	0.75 (0.51–1.10)	0.95 (0.64–1.42)	0.90	Referent	0.69 (0.39–1.22)	1.25 (0.72–2.18)	0.42
Free estradiol								
Model 1[1]	Referent	1.07 (0.74–1.55)	0.84 (0.58–1.21)	0.33	Referent	0.88 (0.52–1.49)	0.71 (0.42–1.22)	0.21
Model 1 + BMI+PA[2]	Referent	1.05 (0.72–1.54)	0.83 (0.57–1.21)	0.30	Referent	0.93 (0.55–1.58)	0.80 (0.46–1.39)	0.43
Model 1 + BMI + PA + C-peptide[3]	Referent	1.04 (0.72–1.52)	0.84 (0.58–1.23)	0.34	Referent	0.91 (0.54–1.56)	0.79 (0.46–1.37)	0.40
Testosterone								
Model 1[1]	Referent	0.87 (0.60–1.27)	0.84 (0.57–1.22)	0.37	Referent	0.68 (0.39–1.20)	1.15 (0.68–1.95)	0.56
Model 1 + BMI + PA[2]	Referent	0.86 (0.59–1.25)	0.82 (0.56–1.22)	0.35	Referent	0.69 (0.39–1.20)	1.05 (0.61–1.81)	0.81
Model 1 + BMI + PA + C-peptide[3]	Referent	0.86 (0.59–1.27)	0.87 (0.58–1.31)	0.52	Referent	0.68 (0.39–1.20)	1.05 (0.60–1.82)	0.84
Free testosterone								
Model 1[1]	Referent	0.84 (0.57–1.22)	0.64 (0.44–0.94)	0.02	Referent	0.65 (0.37–1.13)	0.77 (0.44–1.33)	0.35
Model 1 + BMI + PA[2]	Referent	0.82 (0.56–1.20)	0.64 (0.43–0.94)	0.02	Referent	0.63 (0.36–1.11)	0.71 (0.40–1.24)	0.23
Model 1 + BMI + PA + C-peptide[3]	Referent	0.84 (0.57–1.23)	0.66 (0.45–0.99)	0.04	Referent	0.64 (0.36–1.12)	0.73 (0.41–1.29)	0.27
The ratio of estradiol to testosterone								
Model 1[1]	Referent	1.49 (1.01–2.18)	1.14 (0.78–1.68)	0.72	Referent	1.50 (0.89–2.55)	0.78 (0.43–1.39)	0.24

	Overall mortality (*n* = 174)				CRC mortality (*n* = 83)			
	Tertile 1	**Tertile 2**	**Tertile 3**	p_{trend}	**Tertile 1**	**Tertile 2**	**Tertile 3**	p_{trend}
Model 1 + BMI + PA[2]	Referent	1.50 (1.02–2.21)	1.16 (0.77–1.74)	0.70	Referent	1.58 (0.93–2.70)	0.90 (0.49–1.65)	0.56
Model 1 + BMI + PA + C-peptide[3]	Referent	1.46 (0.98–2.15)	1.10 (0.73–1.67)	0.92	Referent	1.58 (0.92–2.73)	0.87 (0.46–1.62)	0.45
SHBG								
Model 1[1]	Referent	1.04 (0.70–1.54)	1.07 (0.72–1.59)	0.74	Referent	1.45 (0.81–2.60)	1.54 (0.86–2.77)	0.18
Model 1 + BMI + PA[2]	Referent	1.06 (0.72–1.58)	1.08 (0.72–1.61)	0.73	Referent	1.53 (0.86–2.73)	1.48 (0.81–2.67)	0.27
Model 1 + BMI + PA + C-peptide[3]	Referent	1.07 (0.72–1.60)	1.09 (0.73–1.63)	0.70	Referent	1.54 (0.86–2.75)	1.49 (0.82–2.72)	0.25

Fifty-nine patients had missing value for plasma C-peptide levels. The values were replaced by the median. The tertile categories for estradiol, estrone, testosterone, and SHBG in men were shown in Table 1 footnote. The tertile categories for free estradiol, free testosterone and the ratio of estradiol to testosterone in men were <0.41, 0.41–0.51, ≥0.51 pg/ml for free estradiol, <6.98, 6.98–9.22, ≥9.22 ng/dl for free testosterone and <0.005, 0.005–0.006, ≥0.006 (pg/ml)/(ng/dl) for the ratio of estradiol to testosterone.

[1]Adjusted for age at diagnosis (years), location of primary tumor (proximal, distal, rectum or unknown), grade of differentiation (well-differentiated, moderately differentiated, poorly differentiated or unknown), metastasis of the tumor (yes *vs.* no) and study cohort.

[2]Adjusted for covariates included in Model 1 plus body mass index and physical activity before colorectal cancer diagnosis.

[3]Adjusted for covariates included in Model 2 plus prediagnostic plasma C-peptide levels.

Abbreviations: BMI, body mass index; PA, physical activity; SHBG, sex hormone-binding globulin.

Table 4.

Multivariable hazard ratios of death according to tertiles of prediagnostic hormone levels in women

	Overall mortality (*n* = 106)				CRC mortality (*n* = 70)			
	Tertile 1	**Tertile 2**	**Tertile 3**	p_{trend}	**Tertile 1**	**Tertile 2**	**Tertile 3**	p_{trend}
Estradiol								
Model 1[1]	Referent	1.03 (0.63–1.68)	1.20 (0.75–1.94)	0.42	Referent	1.37 (0.75–2.51)	1.53 (0.83–2.81)	0.21
Model 1 + BMI + PA[2]	Referent	1.08 (0.66–1.77)	1.49 (0.81–2.73)	0.18	Referent	1.48 (0.80–2.76)	2.04 (0.96–4.37)	0.08
Model 1 + BMI + PA + C-peptide[3]	Referent	1.03 (0.62–1.71)	1.45 (0.79–2.66)	0.20	Referent	1.49 (0.78–2.83)	2.00 (0.92–4.35)	0.10
Estrone								
Model 1[1]	Referent	1.22 (0.74–2.03)	1.35 (0.85–2.15)	0.22	Referent	2.12 (1.11–4.05)	1.76 (0.97–3.21)	0.09
Model 1 + BMI+PA[2]	Referent	1.29 (0.78–2.16)	1.54 (0.92–2.61)	0.10	Referent	2.18 (1.14–4.20)	2.00 (1.03–3.91)	0.048
Model 1 + BMI + PA + C-peptide[3]	Referent	1.30 (0.78–2.18)	1.54 (0.92–2.60)	0.11	Referent	2.26 (1.17–4.37)	1.96 (1.01–3.84)	0.06
Free estradiol								
Model 1[1]	Referent	0.98 (0.60–1.60)	1.19 (0.74–1.92)	0.41	Referent	1.03 (0.57–1.88)	1.31 (0.72–2.38)	0.35
Model 1 + BMI + PA[2]	Referent	1.05 (0.63–1.75)	1.49 (0.80–2.77)	0.19	Referent	1.11 (0.59–2.07)	1.69 (0.79–3.60)	0.16
Model 1 + BMI + PA + C-peptide[3]	Referent	1.01 (0.60–1.70)	1.43 (0.77–2.67)	0.22	Referent	1.11 (0.58–2.10)	1.67 (0.76–3.64)	0.18
Testosterone								
Model 1[1]	Referent	1.04 (0.64–1.69)	0.96 (0.60–1.53)	0.85	Referent	1.35 (0.73–2.49)	1.27 (0.71–2.27)	0.47
Model 1 + BMI + PA[2]	Referent	1.08 (0.66–1.77)	1.01 (0.63–1.61)	0.99	Referent	1.37 (0.74–2.55)	1.27 (0.71–2.30)	0.47
Model 1 + BMI + PA + C-peptide[3]	Referent	1.08 (0.66–1.77)	1.02 (0.64–1.64)	0.94	Referent	1.41 (0.76–2.62)	1.29 (0.71–2.35)	0.45
Free testosterone								
Model 1[1]	Referent	1.17 (0.72–1.90)	1.20 (0.72–2.01)	0.52	Referent	1.02 (0.55–1.91)	1.41 (0.76–2.61)	0.25
Model 1 + BMI + PA[2]	Referent	1.21 (0.75–1.97)	1.24 (0.73–2.11)	0.47	Referent	1.04 (0.56–1.95)	1.47 (0.78–2.75)	0.21
Model 1 + BMI + PA + C-peptide[3]	Referent	1.19 (0.72–1.95)	1.23 (0.72–2.11)	0.49	Referent	1.09 (0.57–2.07)	1.50 (0.79–2.85)	0.19
The ratio of estradiol to testosterone								
Model 1[1]	Referent	0.88 (0.54–1.44)	1.06 (0.67–1.70)	0.71	Referent	0.71 (0.39–1.30)	0.96 (0.54–1.69)	0.99

	Overall mortality (*n* = 106)				CRC mortality (*n* = 70)			
	Tertile 1	**Tertile 2**	**Tertile 3**	p_{trend}	**Tertile 1**	**Tertile 2**	**Tertile 3**	p_{trend}
Model 1 + BMI + PA[2]	Referent	0.94 (0.56–1.56)	1.16 (0.65–2.05)	0.57	Referent	0.74 (0.39–1.40)	1.05 (0.51–2.20)	0.82
Model 1 + BMI + PA + C-peptide[3]	Referent	0.92 (0.55–1.53)	1.11 (0.62–1.99)	0.68	Referent	0.74 (0.39–1.41)	0.97 (0.46–2.06)	0.99
SHBG								
Model 1[1]	Referent	1.42 (0.85–2.37)	1.15 (0.65–2.03)	0.88	Referent	1.52 (0.80–2.89)	1.45 (0.73–2.90)	0.40
Model 1 + BMI + PA[2]	Referent	1.44 (0.85–2.43)	1.13 (0.60–2.14)	0.98	Referent	1.54 (0.79–3.00)	1.50 (0.70–3.21)	0.43
Model 1 + BMI + PA + C-peptide[3]	Referent	1.45 (0.86–2.45)	1.20 (0.63–2.28)	0.80	Referent	1.57 (0.81–3.07)	1.62 (0.75–3.52)	0.31

Twenty-three patients had missing value for plasma C-peptide levels. The values were replaced by the median. The tertile categories for estradiol, estrone, testosterone and SHBG in women were shown in Table 1 footnote. The tertile categories in women were <0.05, 0.05–0.11, ≥0.11 pg/ml for free estradiol, <0.17, 0.17–0.29, ≥0.29 ng/dl for free testosterone and <0.022, 0.022–0.035, ≥0.035 (pg/ml)/(ng/dl) for the ratio of estradiol to testosterone.

[1]Adjusted for age at diagnosis (years), location of primary tumor (proximal, distal, rectum or unknown), grade of differentiation (well-differentiated, moderately differentiated, poorly differentiated or unknown), metastasis of the tumor (yes *vs.* no) and study cohort.

[2]Adjusted for covariates included in Model 1 plus body mass index and physical activity before colorectal cancer diagnosis.

[3]Adjusted for covariates included in Model 2 plus prediagnostic plasma C-peptide levels.

Abbreviations: BMI, body mass index; PA, physical activity; SHBG, sex hormone-binding globulin.