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Menopausal hormone therapy and colorectal cancer: a linkage between nationwide registries in Norway

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ABSTRACT

Objectives: With the present study, we aimed to investigate the association between menopausal hormone therapy (HT) and risk of colorectal cancer (CRC).

Setting: Cohort study based on the linkage of Norwegian population-based registries.

Participants: We selected 684,703 Norwegian women, aged 45-79, alive and residing in Norway as of January 1, 2004, and we followed them from 2004 to 2008. Each woman contributed person-years at risk as non-user, current user and/or past HT user.

Outcome measures: The outcome of interest was adenocarcinoma of the colorectal tract, overall, by anatomic site and stage at diagnosis. Incidence rate ratios (RR) with 95% confidence intervals (95% CI) were estimated by Poisson regression and were used to evaluate the association between HT and CRC incidence.

Results: During the median follow-up of 4.8 years, 178,309 (26%) women received HT and 4,137 (0.6%) incident CRCs occurred. Current, but not past, use of HT was associated with a lower risk of CRC (RR 0.85; 95% confidence interval (CI) 0.77-0.93). RRs for localized, regionally advanced and metastatic CRC were 1.15 (95% CI 0.93-1.41), 0.80 (0.70-0.92) and 0.70 (0.56-0.87), respectively. Current use of estrogen therapy (ET) was associated with a reduction of CRC risk (RR 0.82; 95% CI 0.72-0.93), both in oral (RR 0.81; 95% CI 0.66-0.99) and vaginal (RR 0.78; 95% CI 0.65-0.93) administrations, while current use of estrogen-progestin therapy (EPT) was not (RR 0.91; 95% CI 0.76-1.08). However, because of concern of confounding by menopausal status, we repeated the analysis in women of 55 years or more. In this group we obtained similar RRs for current use of ET and EPT versus non-use: 0.82 (95% CI 0.71-0.93) and 0.83 (95% CI 0.68-1.01), respectively.

Conclusions: In our nationwide cohort study, HT use lowered the risk of CRC, specifically the most advanced CRC.

SUMMARY BOX

Article summary

Strengths and limitations of this study

- Our cohort study, based on a linkage between nationwide registries in Norway, provided strong evidence showing that use of hormone therapy (HT) is associated with a reduced risk of colorectal cancer (CRC)
- HT had no impact on localized CRC but it protected against regionally advanced CRC and even more strongly against metastatic CRC. We therefore hypothesized that HT might play a key role in the inhibition of cancer progression
- For the first time, we showed that estrogens - in oral formulations - were associated with a decreased risk of CRC in a dose-response fashion
- The main strength of our study is that the registry linkages ensured detailed information on exposure of HT, including type of HT, with no risk of self-selection of women to participate.
- However, we did not have information on recognized risk factors for CRC (e.g. family history of CRC, body mass index, physical activity, diet, alcohol use and smoking) or information on aspirin use, so we could not adjust our estimates for those factors.

INTRODUCTION

Colorectal cancer (CRC) is the second most commonly diagnosed cancer in females and the third in males worldwide, with estimated 1.4 million cases and 700,000 deaths occurring globally in 2012 [1]. The detection and removal of precancerous lesions through CRC screening and the intervention on modifiable risk factors for CRC, such as diet, alcohol consumption, physical activity, and tobacco smoking, can reduce both CRC incidence and mortality [2,3]. Currently, new preventive strategies are being explored through different medications, aspirin being the most promising [4]. In addition to aspirin, menopausal hormone treatment (HT) has been suggested to reduce CRC risk. A 2012 meta-analysis of four clinical trials and sixteen observational studies found that use of estrogen therapy (ET) and combined estrogen-progestin therapy (EPT) was associated with a 20-30% lower risk of CRC [5]. Nevertheless, results from the Women's Health Initiative (WHI) clinical trial were not supportive of the protective effect of HT on CRC. Among women with no uterus, there was no difference in the risk of CRC between women who took ET and those who took the placebo [6]. Among women with an intact uterus, women who received EPT had a lower risk of colorectal cancer than women who took the placebo. However, the CRCs that occurred in the treatment group were more advanced at detection than those in the placebo group [7], suggesting that use of HT might simply delay CRC diagnosis.

Given these conflicting results, the association between use of menopausal HT and the risk of CRC remains controversial. With the present nationwide cohort study, based on the linkage of population-based registries, our aim was to supply new evidence on the association between HT and risk of CRC. We present results on the association between different types, routes of administration and doses of HT on the risk of CRC, overall, by anatomic site and stage at diagnosis.

PATIENTS AND METHODS

Cohort characteristics and definition of exposure to HT were described in details elsewhere [8]. Briefly, an 11-digit unique personal identification number allowed univocal linkage between different national Norwegian registries. We linked information about year and month of birth, immigration and emigration status, death, cause of death, education level and municipality of residence (Statistics Norway and the Population Registry), redeemed prescriptions (the Norwegian Prescription Database) and cancer cases (the Cancer Registry of Norway). The study was approved by the regional ethics committee in the South East region of Norway, and concession to data linkage was granted by the Norwegian Data Protection Authority.

We included data from 800,948 women born in Norway between 1925 and 1959, alive and residing in Norway as of January 1, 2004 (aged 45-79 years; Figure 1). We excluded women diagnosed with CRC or any other cancer diagnosis, as well as women who emigrated or died within the first three month of observation (n=54,516), to ensure a minimum of information on HT use before any disease occurrence. Observation started on April 1, 2004 for all women. We also excluded women receiving prescriptions of sex hormones other than ET, EPT or Tibolone, such as oral contraceptives and progestogen use only (n=33,299). Finally, we excluded women with only one prescription dated after June 2004 (n=28,430), because we assumed that such a short duration of use unlikely affected CRC risk. This left 684,703 women for analyses. Women were followed until December 31, 2008.

Exposure to HT

We retrieved data on use of menopausal hormone therapy (Anatomical Therapeutic Chemical (ATC) group G03) in the period 2004–2008. We did not have any data on prescriptions before 2004. Duration of HT use was estimated for each different type of drug as number of total treatment days, calculated from the package size multiplied by the number of packages prescribed regarding the dosing intervals recommended. Gaps between prescriptions of 4 months or less were assumed to represent continuous use, while gaps longer than 4 months were treated as stop in use, with eventual re-uptake. Women were included in the various type of HT preparation categories based on the specific product dispensed (Figure 2). Women who switched from one type of HT to another (e.g. from estradiol to estriol) contributed person-years at risk to the specific product dispensed. When studying the effect of the different hormone types on CRC incidence, women who redeemed at least two simultaneous prescriptions of different hormone types were classified in the "other" category. The same approach was used when studying the route of administration. Women were classified as ET users if they redeemed only ET prescriptions, and EPT users if they redeemed only EPT prescriptions during the follow-up. All combined regimens of estrogen–progestin available in Norway contain estradiol and norethisterone acetate. Use of other progestin types, such as medroxyprogesterone acetate or dienogest, is almost nonexistent in Norway.

All women in the study population contributed person-years at risk as a non-user, current user and/or past HT user (Figure 2). Person-years at risk were calculated from start of the study period, April 1, 2004, until event, censoring or end of follow-up. Women contributed person-years at risk as current users according to the accumulated duration of treatment for the type of HT dispensed. If there were gaps of more than 4 months between prescriptions, women contributed person-years at risk as a past user from the date that the estimated duration of HT use ended, until the next redeemed prescription date, if any, or end

of the study period. Non-users contributed person-years at risk from 1 April, 2004 until the date of the first redeemed prescription, if any, or end of follow-up.

Outcome

The outcome of interest was adenocarcinoma of the colorectal tract (topography codes C18-C20 according to the International Classification of Diseases, Tenth Revision, Clinical Modification). CRC with histology other than adenocarcinoma (i.e. small cell carcinoma, squamous cell carcinoma, carcinoid, sarcoma, gastrointestinal stromal tumor and lymphoma) were not analyzed as CRC cases and were censored at diagnosis.

Statistical analysis

Incidence rate ratios (RR) with 95% confidence intervals (95% CI) were estimated by Poisson regression. The number of incident CRCs was analyzed as a log-linear function of exposure time, HT use, and adjusting covariates. Women were censored at death, emigration, any tumor diagnosis or end of follow-up (December 31, 2008), whichever came first. We adjusted HT estimates for age in years calculated at the beginning of each exposure segment (Figure 2), number of births (nulliparous, 1, 2, 3, and ≥4)), highest level of education (elementary, high-school, university or higher, and missing) and marital status (not married, married or partnered, widowed, and divorced or separated) registered at the beginning of follow-up and use of antihypertensive drugs (ATC groups C02, C03, C07-C09), antidiabetic drugs (A10), statins (C10) and thyroid therapy (H03) registered anytime during follow-up. In each analysis, the reference group was non-users of HT. When analyzing the association of HT with CRC stage at diagnosis, only CRCs at a specific stage were analyzed as events, while CRCs at other stages were analyzed as censoring events. When analyzing the association of

HT with cancer diagnosed in a specific site of the colorectal tract (e.g. left colon), only cancer diagnosed in that specific site were analyzed as events, while others were analyzed as censoring events.

We evaluated the estrogen and progestin dose-response effect by limiting analyses to current oral ET and oral EPT users and non-users. The dose of estrogen and the dose of progestin were obtained from each prescription of oral ET and EPT. Doses of estrogens and progestins in non-users were set to zero. The dose of estrogen and the dose of progestins were entered simultaneously in the multivariable models as two continuous variables.

Menopausal status is a potential confounding variable when examining the effect of HT on CRC risk. Since we did not have information on menopausal status, we addressed this problem by repeating all analyses in the subgroup of women aged 55 years or older at entry. This excluded a large part of the pre- and peri-menopausal women, and ensured a predominantly post-menopausal population. All tests were two sided with a 5% significance level. Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC) and R software (http://cran.r-project.org/).

RESULTS

We followed 684,703 women born in Norway and with no previous history of cancer from 2004 to 2008. During the follow-up, which had a median duration of 4.8 years, 4,137 CRCs occurred. A total of 178,309 (26%) women used HT. Characteristics of the study population were not homogeneously distributed between HT users and non-users, and between ET users and EPT users (Table 1). Notably, ET users were substantially older than EPT users (median age was 61.3 and 56.9 years, respectively; P<0.001).

Table 1. Characteristics of the study population by hormone therapy use

		HT non-users No. (%)	HT users No. (%)	P	ET users No. (%)	EPT users No. (%)	P
All women		506,394	178,309		80,867	48,118	
Number of CRC		3,323 (0.66)	814 (0.46)		407 (0.50)	235 (0.49)	
Age [a]	**Median (IQR)**	60.0 (51-69)	59.0 (53-64)	<0.001	61.4 (55-68)	56.9 (52-61)	<0.001
Highest education [a]	**Elementary school**	167,960 (33.2)	48,001 (26.9)	<0.001	23,911 (29.6)	12,714 (26.4)	<0.001
	High school	225,635 (44.6)	88,408 (49.6)		39,429 (48.8)	24,498 (50.9)	
	University and higher	92,990 (18.4)	41,035 (23.0)		17,102 (21.2)	10,655 (22.1)	
	Missing	19,809 (3.9)	865 (0.5)		425 (0.5)	251 (0.5)	
Number of children [a]	**0**	68,724 (13.6)	15,335 (8.6)	<0.001	6,569 (8.1)	4,652 (9.7)	<0.001
	1	64,063 (12.7)	22,654 (12.7)		9,549 (11.8)	6,510 (13.5)	
	2	179,815 (35.5)	75,571 (42.4)		32,401 (40.1)	20,911 (43.5)	
	3	124,487 (24.6)	45,338 (25.4)		21,466 (26.5)	11,849 (24.6)	
	> 3	69,305 (13.7)	19,411 (10.9)		10,882 (13.5)	4,196 (8.7)	
Marital status [a]	**Single**	52,946 (10.5)	9,399 (5.3)	<0.001	3,591 (4.4)	3,307 (6.9)	<0.001
	Married / Partnered	261,627 (51.7)	106,761 (59.9)		48,107 (59.5)	27,748 (57.7)	
	Widow	109,720 (21.7)	29,253 (16.4)		17,349 (21.5)	6,006 (12.5)	
	Divorced / Separated	82,101 (16.2)	32,896 (18.5)		11,820 (14.6)	11,057 (23.0)	
Antihypertensives [b]	**User**	200,273 (39.6)	75,719 (42.5)	<0.001	38,149 (47.2)	18,426 (38.3)	<0.001
Antidiabetics [b]	**User**	29,166 (5.8)	7,975 (4.5)	<0.001	4,505 (5.6)	1,693 (3.5)	<0.001
Statins [b]	**User**	119,210 (23.5)	44,216 (24.8)	<0.001	24,694 (30.5)	9,048 (18.8)	<0.001
Thyroid therapy [b]	**User**	53,226 (10.5)	25,125 (14.1)	<0.001	12,069 (14.9)	6,048 (12.6)	<0.001

[a] Registered at baseline; [b] Prescribed anytime during the follow-up. HT: Hormone therapy. CRC: Colorectal cancer. IQR: Interquartile range. ET: Estrogen therapy. EPT: Estrogen-progestin therapy

Current use of HT was associated with a decreased risk of CRC compared to non-use, with a RR of 0.85 (95% CI 0.77-0.93; Table 2).

Table 2. Use of hormone therapy and risk of colorectal cancer

		All women			Women ≥ 55 years		
	HT use	PY	CRC cases	RR (95% CI)	PY	CRC cases	RR (95% CI)
Status	Non-use	2,451,056	3,323	Reference	1,552,615	2,965	Reference
	Current use	450,916	487	0.85 (0.77-0.93)	299,331	417	0.82 (0.74-0.92)
	Past use	240,932	327	0.94 (0.84-1.06)	171,720	296	0.95 (0.84-1.07)
	Ever use	691,848	814	0.88 (0.82-0.96)	471,051	713	0.87 (0.80-0.95)
HRT type	Non-use	2,451,056	3,323	Reference	1,552,615	2,965	Reference
	ET*	190,800	251	0.82 (0.72-0.93)	145,854	233	0.82 (0.71-0.93)
	*ET (Estradiol)**	*152,491*	*166*	*0.79 (0.67-0.92)*	*109,379*	*150*	*0.79 (0.67-0.93)*
	*ET (Estriol)**	*38,309*	*85*	*0.87 (0.70-1.08)*	*36,475*	*83*	*0.87 (0.70-1.09)*
	Tibolone*	29,705	23	0.80 (0.53-1.21)	18,898	19	0.78 (0.50-1.23)
	EPT*	140,205	132	0.91 (0.76-1.08)	87,350	103	0.83 (0.68-1.01)
	Other*	90,205	81	0.88 (0.71-1.10)	47,227	62	0.86 (0.67-1.11)
Route	Non-use	2,451,056	3,323	Reference	1,552,615	2,965	Reference
	ET Oral*	68,848	98	0.81 (0.66-0.99)	54,038	93	0.82 (0.66-1.00)
	ET Vaginal*	103,050	129	0.78 (0.65-0.93)	80,644	117	0.76 (0.63-0.91)
	ET Transdermal*	11,269	14	1.37 (0.81-2.32)	5,989	14	1.78 (1.05-3.01)
	EPT Oral*	137,068	132	0.92 (0.78-1.10)	85,910	103	0.85 (0.69-1.03)
	EPT Transdermal*	2,232	0	-	1,090	0	-
	Other	128,448	114	0.87 (0.72-1.05)	71,659	90	0.85 (0.69-1.05)
Oral dose Unit increase	Estrogen 1 mg / day*			0.87 (0.76-0.99)			0.86 (0.75-0.99)
	Progestin 10 mg / month*			1.04 (0.91-1.19)			1.00 (0.86-1.15)

Incidence rate ratios (RR) were adjusted for age, number of births, highest level of education, marital status, use of antihypertensives, antidiabetics, statins and thyroid therapy. ET: estrogen only therapy. EPT: combined estrogen-progestin therapy. PY: person-years. CRC: colorectal cancer. CI: confidence interval. *Current use.

The same figure for past and ever use (current or past users) was 0.94 (95% 0.84-1.06) and 0.88 (95% 0.82-0.96). While ET current use was associated with a statistically significant reduction of CRC risk (RR 0.82 (95% CI 0.72-0.93), both among oral users (RR 0.81; 95% CI 0.66-0.99) and vaginal users (RR 0.78; 95% CI 0.65-0.93), EPT use was not in the overall analyses (RR 0.91 (95% CI 0.76-1.08)). However, when we repeated the analysis in women aged ≥ 55 years at diagnosis, we obtained similar RRs for current use of HT, ET and EPT versus non-use: 0.82 (95% CI 0.74-0.92); 0.82 (95% CI 0.71-0.93) and 0.83 (95% CI 0.68-1.01), respectively.

From each prescription of oral ET and EPT, we retrieved the information on the administered dose of estrogens and progestins. Mean estrogen dose in oral ET and EPT treatments was 1.41 and 1.38 mg/day, respectively. Mean progestin dose in oral EPT users was 16.8 mg/month. We analyzed the dose effect of oral estrogen and progestin as continuous variables on CRC risk, and we found that estrogens were associated with a decreased risk of CRC in a dose-response fashion (RR 0.87 for each additional mg/day; 95% CI 0.76-0.99; Table 2), while progestins showed no effect. When removing EPT users, the RR estimate for each additional mg/day of estrogens was 0.88 (95% CI 0.76-1.01)

In Table 3 we reported the association between HT intake and CRC diagnosed at different stages: 729 localized, 2,179 regionally advanced and 860 metastatic CRCs.

Table 3. Use of hormone therapy and risk of colorectal cancer by colorectal cancer stage

HT use		PY	Localized CRC	RR (95% CI)	Regionally advanced CRC	RR (95% CI)	Metastatic CRC	RR (95% CI)
Status	**Non-use**	2,451,056	576	Reference	1,749	Reference	702	Reference
	Current use	450,916	109	1.15 (0.93-1.41)	241	0.80 (0.70-0.92)	89	0.70 (0.56-0.87)
	Past use	240,932	44	0.75 (0.55-1.01)	189	1.04 (0.90-1.21)	69	0.92 (0.71-1.16)
	Ever use	691,848	153	0.99 (0.83-1.19)	430	0.89 (0.80-0.99)	158	0.78 (0.65-0.93)
HRT type	**Non-use**	2,451,056	576	Reference	1,749	Reference	702	Reference
	ET*	190,800	66	1.22 (0.95-1.58)	128	0.80 (0.66-0.95)	33	0.51 (0.36-0.72)
	ET (Estradiol)*	*152,491*	*44*	*1.25 (0.92-1.70)*	*82*	*0.75 (0.60-0.94)*	*23*	*0.50 (0.33-0.75)*
	ET (Estriol)*	*38,309*	*22*	*1.18 (0.77-1.81)*	*46*	*0.89 (0.67-1.20)*	*10*	*0.55 (0.29-1.02)*
	Tibolone*	29,705	5	1.16 (0.48-2.81)	12	0.80 (0.45-1.42)	4	0.57 (0.21-1.53)
	EPT*	140,205	21	0.94 (0.61-1.46)	66	0.86 (0.67-1.11)	34	0.99 (0.70-1.40)
	Other*	90,205	17	1.17 (0.72-1.90)	35	0.73 (0.52-1.02)	18	0.85 (0.53-1.36)
Route	**Non-use**	2,451,056	576	Reference	1,749	Reference	702	Reference
	ET Oral*	68,848	25	1.15 (0.77-1.71)	51	0.80 (0.61-1.06)	13	0.53 (0.31-0.92)
	ET Vaginal*	103,050	34	1.18 (0.84-1.67)	67	0.78 (0.61-0.99)	16	0.45 (0.27-0.74)
	ET Transdermal*	11,269	6	3.95 (1.76-8.87)	6	1.13 (0.51-2.52)	1	0.41 (0.06-2.89)
	EPT Oral*	137,068	21	0.96 (0.62-1.49)	66	0.88 (0.69-1.13)	34	1.01 (0.71-1.43)
	EPT Transdermal*	2,232	0	-	0	-	0	-
	Other*	128,448	23	1.11 (0.73-1.69)	51	0.74 (0.56-0.98)	25	0.82 (0.55-1.22)
Oral dose Unit increase	**Estrogen 1 mg / day***			1.16 (0.90-1.49)		0.83 (0.68-1.00)		0.76 (0.54-1.06)
	Progestin 10 mg / month*			0.85 (0.63-1.14)		1.05 (0.87-1.28)		1.22 (0.90-1.65)

Incidence rate ratios (RR) were adjusted for age, number of births, highest level of education, marital status, use of antihypertensives, antidiabetics, statins and thyroid therapy. ET: estrogen only therapy. EPT: combined estrogen-progestin therapy. PY: person-years. CRC: colorectal cancer. CI: confidence interval. *Current use.

Compared to non-use, current use of HT was associated with a decreased risk of regionally advanced (RR 0.80; 95% CI 0.70-0.92) and metastatic CRC (RR 0.70; 95% CI 0.56-0.87), but not of localized CRC (RR 1.15; 95% CI 0.93-1.41). When performing the dose-response analysis on oral ET and EPT, the risk of regionally advanced CRC decreased significantly by 17% with each additional mg of estrogens per day. The same figure for metastatic CRC was 24% (not statistically significant). When limiting the analysis to women of 55 years or more, we obtained the following estimates for the association between ET current use and risk of localized, regionally advanced and metastatic CRC: 1.24 (95% CI 0.67-0.93), 0.77(0.64-0.93) and 0.51 (0.36-0.74). The same figures for EPT were: 0.87 (0.53-1.42), 0.81 (0.62-1.07), 0.86 (0.57-1.30).

In supplementary Table 1 we reported the association between HT and risk of CRC diagnosed in different sites of the colorectal tract. RRs for the association of current use of HT with colon cancer, right colon cancer, left colon cancer and rectal cancer were 0.83 (95% CI 0.74-0.94), 0.89 (0.77-1.04), 0.74 (0.60-0.90) and 0.88 (0.74-1.05), respectively. ET current use was associated with a significant decreased risk of cancer in the colon, both right and left, while EPT current use was associated with a significant decreased risk of cancer in the left side of the colon. The risk of rectal cancer was not statistically significantly associated with any type of HT use. When limiting the analysis to women of 55 years or more, we obtained the following RR estimates for the association between ET current use and risk of cancer in the colon, right colon, left colon and rectum: 0.79 (95% CI 0.67-0.93), 0.81(0.66-0.99), 0.73 (0.55-0.96) and 0.88 (0.69-1.12). The same figures for EPT were: 0.79 (95% CI 0.62-1.02), 0.90 (0.67-1.22), 0.60 (0.38-0.94) and 0.91 (0.65-1.27).

DISCUSSION

In this Norwegian nationwide cohort study, we evaluated the effect of menopausal HT on CRC incidence. Our results suggest that current use of HT is associated with a reduced risk of CRC, specifically the most advanced CRC. Current users of any HT had a 15% reduction of CRC, 20% reduction of regionally advanced CRC and 30% reduction of metastatic CRC. The effect was similar for ET and EPT in women aged 55 and older. Furthermore, we found that, in current users, the risk of CRC decreased with increasing doses of oral estrogens.

Colorectal polyps and tumors occur more frequently in men than in women, and many preclinical and clinical studies have provided evidence that female sex hormones, specifically estrogen, might form the basis for the protective effect in women [9]. Researchers have found that the estrogen receptor beta (ERβ) regulates DNA repair, increases apoptosis and reduces cell proliferation, and that ERβ activation can consequently reduce tumor occurrence and inhibit progression [10-13]. Consistent evidence showed an inverse relationship between ERβ expression in the colon and the presence and stage of colorectal polyps and tumors [14-18]. The possible protective effect of HT was evaluated in many observational studies and two clinical trials, with conflicting results. Current use of ET was associated with a 30% decreased CRC risk in a meta-analysis published in 2012 [5] and a 23% reduction of colon cancer and 17% reduction of rectal cancer in a recent nationwide registry-based study among one million Danish women [19]. In contrast to those findings, a lack of association was reported in 136,000 postmenopausal women in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort [20]. The only placebo-controlled clinical trial on the subject, the WHI, included 10,739 women with hysterectomy, showed no difference in either the risk of CRC or the stage of disease at diagnosis between women who took estrogen alone and those who took the placebo [6].

The effect of EPT use on CRC risk is also controversial. In the 2012 meta-analysis [5], current use of EPT was associated with a significant 20% reduction of CRC, and in the Danish study [19], it was associated with a significant 12% reduction of colon cancer and 11% reduction of rectal cancer. In the EPIC cohort, a non-significant 6% risk reduction due to EPT use was reported [20]. In the WHI, among the 16,608 postmenopausal women with intact uterus, authors reported that EPT was associated with a significant 28% reduction of CRC after 5.6 years of intervention (11.6 years of follow-up). However, EPT was associated with more advanced CRC, and the investigators concluded that their findings did not support a clinically meaningful benefit for EPT on CRC [7]. They hypothesized a potential CRC diagnostic delay due to EPT-related conditions such as vaginal bleeding.

Our study provides new evidence on the protective effect of HT use against CRC, both in ET and EPT users. In the overall study population, ET current use was associated with a significant 18% reduction of the risk of CRC, while EPT current use with a non-significant 9%. The lack of a significant effect of EPT use on CRC risk might be due to a potential confounding of menopausal status, unknown in our study. When we repeated the analyses in women aged 55 and older, the effect of HT use on CRC risk was similar in ET and EPT users (18% and 17%, respectively). These findings are in accordance to the 2012 meta-analysis, which showed a significant protection against CRC of both current use of ET and current use of EPT [5]. We also found that increasing doses of oral estrogens, and not progestins, were associated with decreasing risk of CRC. Altogether these results might indicate that estrogens reduce the risk of CRC, while progestins have no effect. In support of our findings, a recent study showed that the risk of CRC decreased with increasing levels of endogenous estrogen, while it did not depend on progesterone levels [21].

We found that current use of ET was associated with a reduction of CRC risk, even in women using vaginal formulations. In contrast to our findings, no significant impact of vaginal HT was reported in the study by Mørch et al [19]. An effect of vaginal ET could biologically be explained by intravaginal estrogen preparations affecting endogenous estrogen levels [22,23]. Nevertheless, women using vaginal HT were different from HT non-users (e.g. women using vaginal HT were 3 years older on average as compared to HT non-users) and the estimated impact of vaginal HT on CRC risk was possibly biased from residual confounding. Moreover, we cannot exclude the possibility that a significant number of vaginal HT users might have used HT systemic treatment before 2004, this possibly leading to an overestimation of the protective effect of vaginal HT on CRC risk in our analysis. More studies on the effect of intravaginal estrogen preparations on CRC risk are needed to clarify the association.

Our results could be interpreted to support the hypothesis that HT inhibits cancer progression, rather than formation. In our study, use of HT had no impact on localized CRC (RR=1.15) but it protected against regionally advanced CRC (RR=0.80) and even more strongly against metastatic CRC (RR=0.70). Similarly, in the Iowa Women's Health Study, the RR estimates for ever versus never use of HT by stage were 0.91 for localized, 0.78 for regional and 0.72 for distant disease [24]. In the California Teachers Study, current HT use versus baseline non-use was associated with these RRs: 0.99 for localized, 0.68 for regional and 0.33 for distant disease [25]. Results from the Danish study showed that HT had a stronger impact on metastatic rather than non-metastatic CRC [19], and other authors reported that HT users were significantly more likely to be diagnosed at an earlier disease stage as compared to HT non-users [26-27].

Our study has several strengths. The registry linkages ensured detailed information on exposure of HT, including type of HT. There was no self-selection of women to participate, and

the large size of the study population provided a large number of incident CRCs. However, our study has important limitations. First, we did not have information on menopausal status, recognized risk factors for CRC (e.g. family history of CRC, body mass index, physical activity, diet, alcohol use and smoking) or information on aspirin use. Some authors showed no significant effect of those factors on the association between HT and CRC risk [25,26,28], but in the California Teachers Study HT use was more strongly associated with CRC risk among women with a family history of CRC [25]. In addition, our estimates could be affected by the healthy user bias: it is probable that HT users were more concerned about their health than non-users and, for example, underwent more bowel examinations or had a better lifestyle. This bias could have resulted in overestimation of the HT protective effect. In fact we found that HT users had a higher education level than non-users, and education is positively associated with general good health and use of medical services [29]. Nevertheless, in an attempt to assess the magnitude of this potential bias, we stratified the study population into low, middle and high education level and we obtained similar estimates for the effect of current use of HT on CRC risk, respectively RR 0.80, 0.87 and 0.85. In addition, the fact that HT had no effect on risk of early stage CRC and strong effect on risk of advanced stage CRC indicates no healthy user bias, as more health conscious women are likely to have CRC detected in earlier rather than later stages.

In conclusion, we provided evidence that use of HT is associated with a reduced risk of CRC, in particular advanced CRC. The effect was similar for ET and EPT in women of age 55 years or older.

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Contributorship statement

Study concept and design: EB, SS, SGI, GU, SV, EW. Acquisition of data: SS, SGI, SH, GU, SV. Analysis of data: EB, SS, SGI. Statistical analysis of data: EB, VB. Interpretation of data: all. Drafting of the manuscript: EB. Critical revision of the manuscript for important intellectual content: SS, SGI, SH, TdL, GU, SV, EW.

Conflicting interests

The authors have declared no conflicts of interest.

Research reporting checklists

The present article follows the STROBE guidelines for research reporting of observational studies.

Data sharing statement

Authors are willing to share any data that are not published in the manuscript. Please contact the corresponding author.

Ethics approval

The study was approved by the regional ethics committee in the South East region of Norway, and concession to data linkage was granted by the Norwegian Data Protection Authority.

Figure 1. Selection of the study population

Figure 2. Follow-up of study participants

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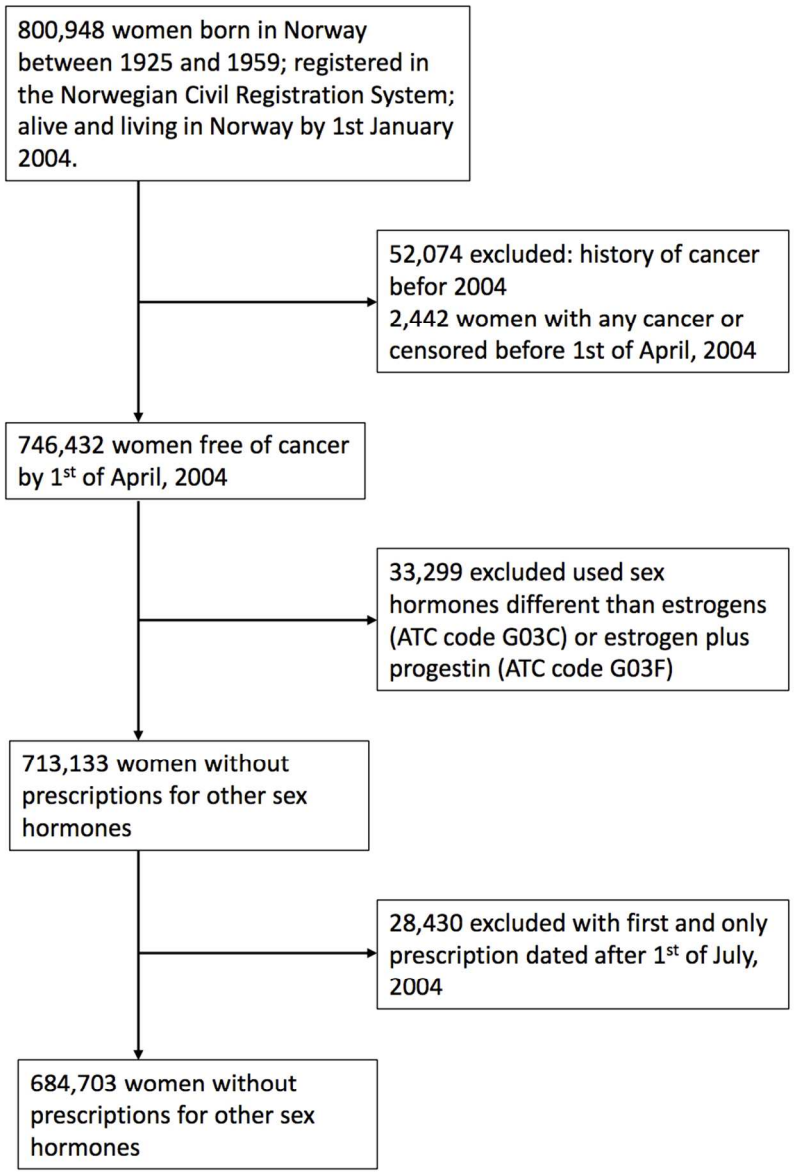

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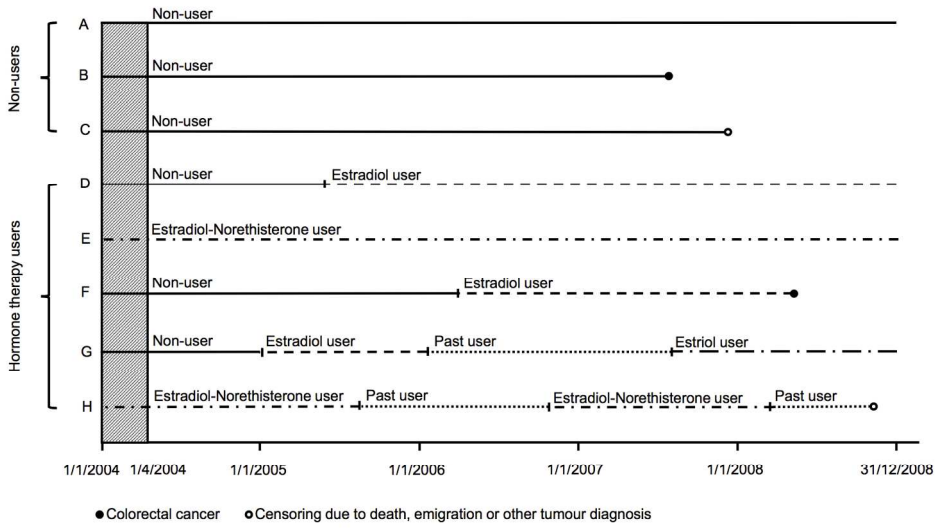

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Supplementary Table 1. Use of hormone therapy and risk of colorectal cancer by site

HT use	Category	PY	Colon	RR (95% CI)	Right colon	RR (95% CI)	Left Colon	RR (95% CI)	Rectum	RR (95% CI)
Status	**Non-use**	2,451,056	2,327	Reference	1,412	Reference	839	Reference	996	Reference
	Current use	450,916	330	0.83 (0.74-0.94)	209	0.89 (0.77-1.04)	109	0.74 (0.60-0.90)	157	0.88 (0.74-1.05)
	Past use	240,932	234	0.97 (0.85-1.11)	135	0.93 (0.78-1.12)	90	1.02 (0.82-1.26)	93	0.88 (0.71-1.09)
	Ever use	691,848	564	0.89 (0.81-0.97)	344	0.91 (0.81-1.03)	199	0.84 (0.72-0.98)	250	0.88 (0.77-1.01)
HRT type	**Non-use**	2,451,056	2327	Reference	1,412	Reference	839	Reference	996	Reference
	ET*	190,800	169	0.78 (0.67-0.91)	107	0.81 (0.67-0.99)	54	0.70 (0.53-0.92)	82	0.90 (0.72-1.13)
	ET (Estradiol)*	*152,491*	112	0.77 (0.64-0.93)	73	0.85 (0.67-1.08)	34	0.63 (0.45-0.89)	54	0.84 (0.64-1.10)
	ET (Estriol)*	*38,309*	57	0.80 (0.62-1.04)	34	0.75 (0.53-1.05)	20	0.85 (0.54-1.33)	28	1.06 (0.73-1.55)
	Tibolone*	29,705	17	0.89 (0.55-1.44)	8	0.75 (0.38-1.51)	9	1.17 (0.60-2.26)	6	0.63 (0.28-1.41)
	EPT*	140,205	83	0.85 (0.68-1.06)	54	0.97 (0.74-1.54)	26	0.68 (0.46-0.99)	49	1.02 (0.77-1.37)
	Other*	90,205	61	0.98 (0.76-1.27)	40	1.13 (0.82-1.54)	20	0.83 (0.53-1.29)	20	0.67 (0.43-1.05)
Route	**Non-use**	2,451,056	2327	Reference	1,412	Reference	839	Reference	996	Reference
	ET Oral*	68,848	66	0.77 (0.60-0.98)	40	0.75 (0.55-1.03)	24	0.80 (0.53-1.20)	32	0.92 (0.65-1.31)
	ET Vaginal*	103,050	86	0.74 (0.60-0.92)	61	0.87 (0.68-1.13)	21	0.50 (0.32-0.77)	43	0.87 (0.64-1.18)
	ET Transdermal*	11,269	9	1.34 (0.70-2.60)	2	0.53 (0.13-2.13)	7	2.58 (1.22-5.44)	5	1.45 (0.60-3.49)
	EPT Oral*	137,068	83	0.87 (0.70-1.08)	54	0.99 (0.75-1.31)	26	0.70 (0.47-1.02)	49	1.04 (0.78-1.39)
	EPT Transdermal*	2,232	0	-	0	-	0	-	0	-
	Other*	128,448	86	0.97 (0.78-1.21)	52	1.02 (0.77-1.35)	31	0.90 (0.62-1.28)	28	0.65 (0.45-0.95)
Oral dose Unit increase	**Estrogen 1 mg / day***			0.84 (0.71-0.99)		0.80 (0.64-0.99)		0.90 (0.69-1.17)		0.94 (0.75-1.19)
	Progestin 10 mg / month*			1.05 (0.89-1.24)		1.17 (0.95-1.45)		0.87 (0.65-1.16)		1.02 (0.81-1.29)

Incidence risk ratios (RR) were adjusted for age, number of births, highest level of education, marital status, use of antihypertensives, antidiabetics, statins and thyroid therapy. ET: estrogen only therapy. EPT: combined estrogen-progestin therapy. PY: person-years. CRC: colorectal cancer. CI: confidence interval. *Current use.

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Menopausal hormone therapy and colorectal cancer: a linkage between nationwide registries in Norway

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Menopausal hormone therapy and colorectal cancer: a linkage between nationwide registries in Norway

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ABSTRACT

Objectives: With the present study, we aimed to investigate the association between menopausal hormone therapy (HT) and risk of colorectal cancer (CRC).

Setting: Cohort study based on the linkage of Norwegian population-based registries.

Participants: We selected 466,822 Norwegian women, aged 55-79, alive and residing in Norway as of January 1, 2004, and we followed them from 2004 to 2008. Each woman contributed person-years at risk as non-user, current user and/or past HT user.

Outcome measures: The outcome of interest was adenocarcinoma of the colorectal tract, overall, by anatomic site and stage at diagnosis. Incidence rate ratios (RR) with 95% confidence intervals (95% CI) were estimated by Poisson regression and were used to evaluate the association between HT and CRC incidence.

Results: During the median follow-up of 4.8 years, 138,655 (30%) women received HT and 3,799 (0.8%) incident CRCs occurred. Current, but not past, use of HT was associated with a lower risk of CRC (RR 0.88; 95% confidence interval (CI) 0.80-0.98). RRs for localized, regionally advanced and metastatic CRC were 1.13 (95% CI 0.91-1.41), 0.81 (0.70-0.94) and 0.79 (0.62-1.00), respectively. RRs for current use of estrogen therapy (ET) was 0.91; 95% CI 0.80-1.04), while RR for current use of estrogen-progestin therapy (EPT) was 0.85 (0.70-1.03), as compared to no use of HT. The same figures for ET and EPT in oral formulations were 0.83 (95% CI 0.68-1.03) and 0.86 (0.71-1.05) respectively.

Conclusions: In our nationwide cohort study, HT use lowered the risk of CRC, specifically the most advanced CRC.

SUMMARY BOX

Article summary

Strengths and limitations of this study

- Our cohort study, based on a linkage between nationwide registries in Norway, provided strong evidence showing that use of hormone therapy (HT) is associated with a reduced risk of colorectal cancer (CRC)
- HT had no impact on localized CRC but it protected against regionally advanced CRC and even more strongly against metastatic CRC. We therefore hypothesized that HT might play a key role in the inhibition of cancer progression
- For the first time, we showed that estrogens - in oral formulations - were associated with a decreased risk of CRC in a dose-response fashion
- The main strength of our study is that the registry linkages ensured detailed information on exposure of HT, including type of HT, with no risk of self-selection of women to participate.
- However, we did not have information on recognized risk factors for CRC (e.g. family history of CRC, body mass index, physical activity, diet, alcohol use and smoking) or information on aspirin use, so we could not adjust our estimates for those factors.

INTRODUCTION

Colorectal cancer (CRC) is the second most commonly diagnosed cancer in females and the third in males worldwide, with estimated 1.4 million cases and 700,000 deaths occurring globally in 2012 [1]. The detection and removal of precancerous lesions through CRC screening and the intervention on modifiable risk factors for CRC, such as diet, alcohol consumption, physical activity, and tobacco smoking, can reduce both CRC incidence and mortality [2,3]. Currently, new preventive strategies are being explored through different medications, aspirin being the most promising [4]. In addition to aspirin, menopausal hormone treatment (HT) has been suggested to reduce CRC risk. A 2012 meta-analysis of four clinical trials and sixteen observational studies found that use of HT was associated with a 20-30% lower risk of CRC [5]. Moreover, a 2016 Danish nationwide cohort study involving 1 million women showed that use of HT was associated with approximately a 15% reduction in CRC risk [6]. Nevertheless, results from the Women's Health Initiative (WHI) clinical trial were not supportive of the protective effect of HT on CRC. Among women with no uterus, there was no difference in the risk of CRC between women who took estrogen therapy (ET) and those who took the placebo [7]. Among women with an intact uterus, women who received combined estrogen-progestin therapy (EPT) had a lower risk of colorectal cancer than women who took the placebo. However, the CRCs that occurred in the treatment group were more advanced at detection than those in the placebo group [8], suggesting that use of HT might simply delay CRC diagnosis.

Given these conflicting results, the association between use of menopausal HT and the risk of CRC remains controversial. With the present nationwide cohort study, based on the linkage of population-based registries, our aim was to supply new evidence on the association between HT and risk of CRC. We present results on the association between different types,

routes of administration and doses of HT on the risk of CRC, overall, by anatomic site and stage at diagnosis.

PATIENTS AND METHODS

Cohort characteristics and definition of exposure to HT were described in details elsewhere [9]. Briefly, an 11-digit unique personal identification number allowed univocal linkage between different national Norwegian registries. We linked information about year and month of birth, immigration and emigration status, death, cause of death, education level and municipality of residence (Statistics Norway and the Population Registry), redeemed prescriptions (the Norwegian Prescription Database) and cancer cases (the Cancer Registry of Norway). The study was approved by the regional ethics committee in the South East region of Norway, and concession to data linkage was granted by the Norwegian Data Protection Authority.

We included data from 466,822 women born in Norway between 1925 and 1949, alive and residing in Norway as of January 1, 2004 (aged 55-79 years), who did not have a CRC or any other cancer diagnosis before January 1, 2004. Women were followed until December 31, 2008.

Exposure to HT

We retrieved data on use of menopausal hormone therapy (Anatomical Therapeutic Chemical (ATC) group G03) in the period 2004–2008. We did not have any data on prescriptions before 2004. Duration of HT use was estimated for each different type of drug as number of total treatment days, calculated from the package size multiplied by the number of packages prescribed regarding the dosing intervals recommended. The estimated duration of HT use was extended by 4 months to account for prolonged HT use beyond the treatment days prescribed. If there were gaps of more than 4 months between HT exposures, women contributed person-years at risk as a previous user from the date that the estimated duration of HT use ended, until the next redeemed prescription date if any, or end of the study period. Women receiving prescriptions of sex hormones other than ET, EPT or Tibolone, such as oral contraceptives and progestogen only, were censored at the date of prescription

Women were included in the various type of HT preparation categories based on the specific product dispensed (Figure 1). Women who switched from one type of HT to another (e.g. from estradiol to estriol) contributed person-years at risk to the specific product dispensed. When studying the effect of the different hormone types on CRC incidence, women who redeemed at least two simultaneous prescriptions of different hormone types were classified in the "other" category. The same approach was used when studying the route of administration. Women were classified as ET users if they redeemed only ET prescriptions, and EPT users if they redeemed only EPT prescriptions during the follow-up. All combined regimens of estrogen–progestin available in Norway contain estradiol and norethisterone acetate. Use of other progestin types, such as medroxyprogesterone acetate or dienogest, is almost nonexistent in Norway.

All women in the study population contributed person-years at risk as a non-user, current user and/or past HT user (Figure 1). Person-years at risk were calculated from start of

the study period, January 1, 2004, until event, censoring or end of follow-up. Women contributed person-years at risk as current users according to the accumulated duration of treatment for the type of HT dispensed. If there were gaps of more than 4 months between prescriptions, women contributed person-years at risk as a past user from the date that the estimated duration of HT use ended, until the next redeemed prescription date, if any, or end of the study period. Non-users contributed person-years at risk from January 1, 2004 until the date of the first redeemed prescription, if any, event, censoring or end of follow-up.

Outcome

The outcome of interest was adenocarcinoma of the colorectal tract (topography codes C18-C20 according to the International Classification of Diseases, Tenth Revision, Clinical Modification). CRC with histology other than adenocarcinoma (i.e. small cell carcinoma, squamous cell carcinoma, carcinoid, sarcoma, gastrointestinal stromal tumor and lymphoma) were not analyzed as CRC cases and were censored at diagnosis.

Statistical analysis

Incidence rate ratios (RR) with 95% confidence intervals (95% CI) were estimated by Poisson regression. The number of incident CRCs was analyzed as a log-linear function of exposure time, HT use, analyzed as a time-dependent variable (Figure 1), and adjusting covariates. Women were censored at death, emigration, any tumor diagnosis, prescription of sex hormones other than ET, EPT or Tibolone, or end of follow-up (December 31, 2008), whichever came first. We adjusted HT estimates for age in years, number of births (nulliparous, 1, 2, 3, and ≥4)), highest level of education (elementary, high-school, university or higher, and missing) and marital status (not married, married or partnered, widowed, and

divorced or separated) registered at the beginning of follow-up and use of antihypertensive drugs (ATC groups C02, C03, C07-C09), antidiabetic drugs (A10), statins (C10) and thyroid therapy (H03) registered anytime during follow-up. Time on study was used as timescale in the Poisson regression and split into 1-year time intervals assuming a constant risk of CRC within each interval. At the beginning of each interval age at diagnosis was updated. In each analysis, the reference group was non-users of HT. When analyzing the association of HT with CRC stage at diagnosis, only CRCs at a specific stage were analyzed as events, while CRCs at other stages were analyzed as censoring events. When analyzing the association of HT with cancer diagnosed in a specific site of the colorectal tract (e.g. left colon), only cancer diagnosed in that specific site were analyzed as events, while others were analyzed as censoring events.

We evaluated the estrogen and progestin dose-response effect by limiting analyses to current oral ET and oral EPT users and non-users. The dose of estrogen and the dose of progestin were obtained from each prescription of oral ET and EPT. Doses of estrogens and progestins in non-users were set to zero. The dose of estrogen and the dose of progestins were entered simultaneously in the multivariable models as two continuous variables.

All tests were two sided with a 5% significance level. Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC) and R software (http://cran.r-project.org/).

RESULTS

We followed 466,822 women born in Norway and with no previous history of cancer from 2004 to 2008. During the follow-up, which had a median duration of 4.8 years, 3,799 CRCs occurred. A total of 138,655 (30%) women used HT. Characteristics of the study population were not homogeneously distributed between HT users and non-users, and between ET users and EPT users (Table 1). Notably, ET users were substantially older than EPT users (median age was 64.0 and 60.0 years, respectively; P<0.001).

Table 1. Characteristics of the study population by hormone therapy use

		HT non-users No. (%)	HT users[b] No. (%)	P	ET users[b] No. (%)	EPT users[b] No. (%)	P
All women		328,167	138,655		79,195	30,455	
Number of CRC		3,020 (0.92)	779 (0.56)		434 (0.55)	202 (0.66)	
Age [a]	**Median (IQR)**	65.0 (59-72)	62.0 (57-67)	<0.001	64.0 (58-70)	60.0 (57-64)	<0.001
Highest education [a]	**Elementary school**	127,238 (38.8)	42,317 (30.5)	<0.001	26,455 (33.4)	8,592 (28.2)	<0.001
	High school	143,564 (43.7)	68,401 (49.3)		38,197 (48.2)	15,684 (51.5)	
	University and higher	40,899(12.5)	27,189 (19.6)		14,094 (17.8)	6,013 (19.7)	
	Missing	16,466 (5.0)	748 (0.5)		449 (0.6)	166 (0.5)	
Number of children [a]	**0**	45,536 (13.9)	10,857 (7.8)	0.004	5,984 (7.6)	2,715 (8.9)	<0.001
	1	39,595 (12.1)	15,761 (11.4)		8,731 (11.0)	3,685 (12.1)	
	2	106,742 (32.5)	55,416 (40.0)		29,982 (37.9)	12,795 (42.0)	
	3	81,622 (24.9)	37,495 (27.0)		21,784 (27.5)	8,059 (26.5)	
	> 3	54,672 (16.7)	19,126 (13.8)		12,714 (16.1)	3,201 (10.5)	
Marital status [a]	**Single**	27,218 (8.3)	5,129 (3.7)	<0.001	2,770 (3.5)	1,427 (4.7)	<0.001
	Married / Partnered	154,016 (46.9)	80,077 (57.8)		44,774 (56.5)	17,361 (57.0)	
	Widow	103,202 (31.4)	31,982 (23.1)		21,460 (27.1)	5,400 (17.7)	
	Divorced / Separated	43,731 (13.3)	21,467 (15.5)		10,191 (12.9)	6,267 (20.6)	
Antihypertensives [b]	**User**	163,131 (49.7)	69,572 (50.2)	0.004	42,166 (53.2)	13,688 (45.5)	<0.001
Antidiabetics [b]	**User**	23,988 (7.3)	7,748 (5.6)	<0.001	5,207 (6.6)	1,274 (4.2)	<0.001
Statins [b]	**User**	100,863 (30.7)	42,646 (30.8)	0.886	27,821 (35.1)	7,036 (23.1)	<0.001
Thyroid therapy [b]	**User**	38,511 (11.7)	20,948 (15.1)	<0.001	12,334 (15.6)	4,160 (13.7)	<0.001

[a] Registered at baseline; [b] Prescribed anytime during the follow-up. HT: Hormone therapy. CRC: Colorectal cancer. IQR: Interquartile range. ET: Estrogen therapy. EPT: Estrogen-progestin therapy

Current use of HT was associated with a decreased risk of CRC compared to non-use, with a RR of 0.88 (95% CI 0.80-0.98; Table 2).

Table 2. Use of hormone therapy and risk of colorectal cancer

	HT use	PY	CRC cases	RR (95% CI)
Status	**Non-use**	2,126,753	3,020	Reference
	Current use	320,202	441	0.88 (0.80-0.98)
	Past use	203,759	338	0.98 (0.87-1.09)
	Ever use	523,961	779	0.92 (0.85-1.00)
HRT type	**Non-use**	2,126,753	3,020	Reference
	ET*	159,495	252	0.91 (0.80-1.04)
	ET (Estradiol)*	*118,910*	*159*	*0.87 (0.74-1.03)*
	ET (Estriol)*	*40,585*	*93*	*0.98 (0.79-1.21)*
	Tibolone*	20,043	21	0.86 (0.56-1.32)
	EPT*	91,654	106	0.85 (0.70-1.03)
	Other*	49,010	62	0.86 (0.67-1.10)
Route	**Non-use**	2,126,753	3,020	Reference
	ET Oral*	57,031	94	0.83 (0.68-1.03)
	ET Vaginal*	89,719	134	0.92 (0.77-1.09)
	ET Transdermal*	7,246	15	1.63 (0.98-2.71)
	EPT Oral*	90,126	106	0.86 (0.71-1.05)
	EPT Transdermal*	1,163	0	-
	Other	74,917	92	0.86 (0.70-1.06)
Oral dose Unit increase	**Estrogen 1 mg / day***			0.87 (0.73-1.04)
	Progestin 10 mg / month*			1.01 (0.86-1.19)

Incidence rate ratios (RR) were adjusted for age, number of births, highest level of education, marital status, use of antihypertensives, antidiabetics, statins and thyroid therapy. ET: estrogen only therapy. EPT: combined estrogen-progestin therapy. PY: person-years. CRC: colorectal cancer. CI: confidence interval. *Current use.

The same figure for past and ever use (current or past users) was 0.98 (95% 0.87-1.09) and 0.92 (95% 0.85-1.00). RRs for current use of ET and EPT versus non-use were 0.91 (95% CI 0.80-1.04) and 0.85 (95% CI 0.70-1.03), respectively.

From each prescription of oral ET and EPT, we retrieved the information on the administered dose of estrogens and progestins. Mean estrogen dose in oral ET and EPT treatments was 1.40 and 1.36 mg/day, respectively. Mean progestin dose in oral EPT users was 18.3 mg/month. We analyzed the dose effect of oral estrogen and progestin as continuous variables on CRC risk, and we found that estrogens were associated with a decreased risk of CRC in a dose-response fashion, even if the result was not statistically significant (RR 0.87 for each additional mg/day; 95% CI 0.73-1.04; Table 2), while progestins showed no effect. We then repeated the analysis to estimate the dose effect of estrogens on CRC risk after removing EPT users from the analysis, to avoid a possible interference of progestins, and the RR estimate for each additional mg/day of estrogens was 0.88 (95% CI 0.74-1.04).

In Table 3 we reported the association between HT intake and CRC diagnosed at different stages: 698 localized, 2,023 regionally advanced and 737 metastatic CRCs.

Table 3. Use of hormone therapy and risk of colorectal cancer by colorectal cancer stage

HT use		PY	Localized CRC	RR (95% CI)	Regionally advanced CRC	RR (95% CI)	Metastatic CRC	RR (95% CI)
Status	**Non-use**	2,126,753	548	Reference	1,607	Reference	598	Reference
	Current use	320,202	101	1.13 (0.91-1.41)	216	0.81 (0.70-0.94)	78	0.79 (0.62-1.00)
	Past use	203,759	49	0.79 (0.59-1.06)	200	1.08 (0.93-1.26)	61	0.90 (0.69-1.18)
	Ever use	523,961	150	0.99 (0.82-1.20)	416	0.92 (0.83-1.03)	139	0.83 (0.69-1.01)
HRT type	**Non-use**	2,126,753	548	Reference	1,607	Reference	598	Reference
	ET*	159,495	67	1.33 (1.03-1.72)	125	0.84 (0.70-1.02)	34	0.64 (0.45-0.91)
	ET (Estradiol)*	*118,910*	*44*	*1.36 (0.99-1.85)*	*75*	*0.78 (0.62-0.98)*	*22*	*0.60 (0.39-0.93)*
	ET (Estriol)*	*40,585*	*23*	*1.27 (0.83-1.94)*	*50*	*0.97 (0.73-1.30)*	*12*	*0.73 (0.41-1.29)*
	Tibolone*	20,043	5	1.20 (0.50-2.90)	12	0.93 (0.52-1.64)	4	0.75 (0.28-2.01)
	EPT*	91,654	17	0.79 (0.49-1.29)	56	0.84 (0.64-1.10)	24	0.91 (0.60-1.37)
	Other*	49,010	12	0.94 (0.53-1.66)	23	0.60 (0.40-0.90)	16	1.09 (0.67-1.80)
Route	**Non-use**	2,126,753	548	Reference	1,607	Reference	598	Reference
	ET Oral*	57,031	24	1.15 (0.76-1.73)	48	0.79 (0.59-1.06)	13	0.63 (0.36-1.10)
	ET Vaginal*	89,719	36	1.36 (0.97-1.91)	67	0.86 (0.68-1.10)	17	0.59 (0.37-0.96)
	ET Transdermal*	7,246	6	3.80 (1.70-8.50)	7	1.44 (0.69-3.04)	1	0.51 (0.07-3.65)
	EPT Oral*	90,126	17	0.81 (0.50-1.31)	56	0.86 (0.65-1.12)	24	0.86 (0.61-1.39)
	EPT Transdermal*	1,163	0	-	0	-	0	-
	Other*	74,917	18	0.96 (0.60-1.54)	38	0.67 (0.49-0.93)	23	1.05 (0.69-1.60)
Oral dose Unit increase	**Estrogen 1 mg / day***			1.13 (0.82-1.57)		0.75 (0.57-0.98)		0.97 (0.67-1.39)
	Progestin 10 mg / month*			0.80 (0.57-1.13)		1.11 (0.88-1.41)		1.02 (0.74-1.42)

Incidence rate ratios (RR) were adjusted for age, number of births, highest level of education, marital status, use of antihypertensives, antidiabetics, statins and thyroid therapy. ET: estrogen only therapy. EPT: combined estrogen-progestin therapy. PY: person-years. CRC: colorectal cancer. CI: confidence interval. *Current use.

Compared to non-use, current use of HT was associated with a decreased risk of regionally advanced (RR 0.81; 95% CI 0.70-0.94) and metastatic CRC (RR 0.79; 95% CI 0.62-1.00), but not of localized CRC (RR 1.13; 95% CI 0.91-1.41).

In supplementary Table 1 we reported the association between HT and risk of CRC diagnosed in different sites of the colorectal tract. RRs for the association of current use of HT with colon cancer, right colon cancer, left colon cancer and rectal cancer were 0.88 (95% CI 0.78-0.99), 0.89 (0.77-1.04), 0.85 (0.69-1.04) and 0.90 (0.75-1.09), respectively.

We repeated the main analyses after censoring the CRC cases that occurred in the first year of follow-up (2014), and results were stronger than in the main analysis (supplementary Table 2). RR for use of HT, ET, EPT, oral ET and oral EPT were 0.83 (95% CI 0.74-0.93), 0.86 (0.75-1.00), 0.74 (0.59-0.92), 0.72 (0.57-0.92) and 0.75 (0.60-0.94), respectively, compared to no use. Finally, estrogens were significantly associated with a decreased risk of CRC in a dose-response fashion (RR 0.79 for each additional mg/day; 95% CI 0.64-0.96).

DISCUSSION

In this Norwegian nationwide cohort study, we evaluated the effect of menopausal HT on CRC incidence. Our results suggest that current use of HT is associated with a reduced risk of CRC, specifically the most advanced CRC. Current users of any HT had a 12% reduction of CRC, 19% reduction of regionally advanced CRC and 21% reduction of metastatic CRC. Furthermore, we found that, in current users, the risk of CRC decreased with increasing doses of oral estrogens.

Colorectal polyps and tumors occur more frequently in men than in women, and many preclinical and clinical studies have provided evidence that female sex hormones, specifically estrogen, might form the basis for the protective effect in women [10]. Researchers have found that the estrogen receptor beta (ERβ) regulates DNA repair, increases apoptosis and reduces cell proliferation, and that ERβ activation can consequently reduce tumor occurrence and inhibit progression [11-14]. Consistent evidence showed an inverse relationship between ERβ expression in the colon and the presence and stage of colorectal polyps and tumors [15-19]. The possible protective effect of HT was evaluated in many observational studies and two clinical trials, with conflicting results. Current use of ET was associated with a 30% decreased CRC risk in a meta-analysis published in 2012 [5] and a 23% reduction of colon cancer and 17% reduction of rectal cancer in a recent nationwide registry-based study among one million Danish women [6]. In contrast to those findings, a lack of association was reported in 136,000 postmenopausal women in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort [20]. The only placebo-controlled clinical trial on the subject, the WHI, included 10,739 women with

hysterectomy, showed no difference in either the risk of CRC or the stage of disease at diagnosis between women who took estrogen alone and those who took the placebo [7]. The effect of EPT use on CRC risk is also controversial. In the 2012 meta-analysis [5], current use of EPT was associated with a significant 20% reduction of CRC, and in the Danish study [6], it was associated with a significant 12% reduction of colon cancer and 11% reduction of rectal cancer. In the EPIC cohort, a non-significant 6% risk reduction due to EPT use was reported [20]. In the WHI, among the 16,608 postmenopausal women with intact uterus, authors reported that EPT was associated with a significant 28% reduction of CRC after 5.6 years of intervention (11.6 years of follow-up). However, EPT was associated with more advanced CRC, and the investigators concluded that their findings did not support a clinically meaningful benefit for EPT on CRC [8]. They hypothesized a potential CRC diagnostic delay due to EPT-related conditions such as vaginal bleeding. The discrepancies observed in the literature might be explained by several factors, including the different designs (clinical trials, case-control studies and cohort studies) and methods of HT exposure assessment (e.g. self-reported versus registry-based) used in the different studies [5].

Our study provides new evidence on the protective effect of HT use against CRC. For the first time, we also found that increasing doses of oral estrogens, and not progestins, were associated with decreasing risk of CRC. Altogether these results might indicate that estrogens reduce the risk of CRC, while progestins have no effect. In support of our findings, a recent study showed that the risk of CRC decreased with increasing levels of endogenous estrogen, while it did not depend on progesterone levels [21].

Our results could be interpreted to support the hypothesis that HT inhibits cancer progression, rather than formation. In our study, use of HT had no impact on localized CRC

(RR=1.13) but it protected against regionally advanced CRC (RR=0.81) and metastatic CRC (RR=0.79). Similarly, in the Iowa Women's Health Study, the RR estimates for ever versus never use of HT by stage were 0.91 for localized, 0.78 for regional and 0.72 for distant disease [22]. In the California Teachers Study, current HT use versus baseline non-use was associated with these RRs: 0.99 for localized, 0.68 for regional and 0.33 for distant disease [23]. Results from the Danish study showed that HT had a stronger impact on metastatic rather than non-metastatic CRC [6], and other authors reported that HT users were significantly more likely to be diagnosed at an earlier disease stage as compared to HT non-users [24-25].

In the 2012 meta-analysis [5] and the 2016 Danish study [6], HT was associated with lower risk of colon cancer but less so with rectal cancer. In our study, we found similar estimated for colon and rectal cancer. Within the colon tract, we found similar estimates for left and right colon cancer. More studies are warranted to understand whether HT has different effects in CRC depending on the anatomical location.

Our study has several strengths. The registry linkages ensured detailed information on exposure of HT, including type of HT. There was no self-selection of women to participate, and the large size of the study population provided a large number of incident CRCs. However, our study has important limitations. First, we did not have information on recognized risk factors for CRC (e.g. family history of CRC, body mass index, physical activity, diet, alcohol use and smoking) or information on aspirin use. Some authors showed no significant effect of those factors on the association between HT and CRC risk [23,24,26], but in the California Teachers Study HT use was more strongly associated with CRC risk among women with a family history of CRC [23]. In addition, our estimates could be affected by the healthy user bias: it is probable that HT users were more concerned about their health than non-users and, for example,

underwent more bowel examinations or had a better lifestyle. This bias could have resulted in overestimation of the HT protective effect. In fact we found that HT users had a higher education level than non-users, and education is positively associated with general good health and use of medical services [27]. However, the fact that HT had no effect on risk of early stage CRC and strong effect on risk of advanced stage CRC indicates no healthy user bias, as more health conscious women are likely to have CRC detected in earlier rather than later stages. Finally, given the relatively short follow-up of our study, we were not able to evaluate the influence of duration of HT use on CRC risk, as other authors did [6].

In conclusion, we provided evidence that use of HT is associated with a reduced risk of CRC, in particular advanced CRC. The effect was similar for ET and EPT in women of age 55 years or older.

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Contributorship statement

Study concept and design: EB, SS, SGI, GU, SV, EW. Acquisition of data: SS, SGI, SH, GU, SV. Analysis of data: EB, SS, SGI. Statistical analysis of data: EB, VB. Interpretation of data: all. Drafting of the manuscript: EB. Critical revision of the manuscript for important intellectual content: SS, SGI, SH, TdL, GU, SV, EW.

Conflicting interests

The authors have declared no conflicts of interest.

Research reporting checklists

The present article follows the STROBE guidelines for research reporting of observational studies.

Data sharing statement

Authors are willing to share any data that are not published in the manuscript. Please contact the corresponding author.

Ethics approval

The study was approved by the regional ethics committee in the South East region of Norway, and concession to data linkage was granted by the Norwegian Data Protection Authority.

Figure 1. Follow-up of study participants

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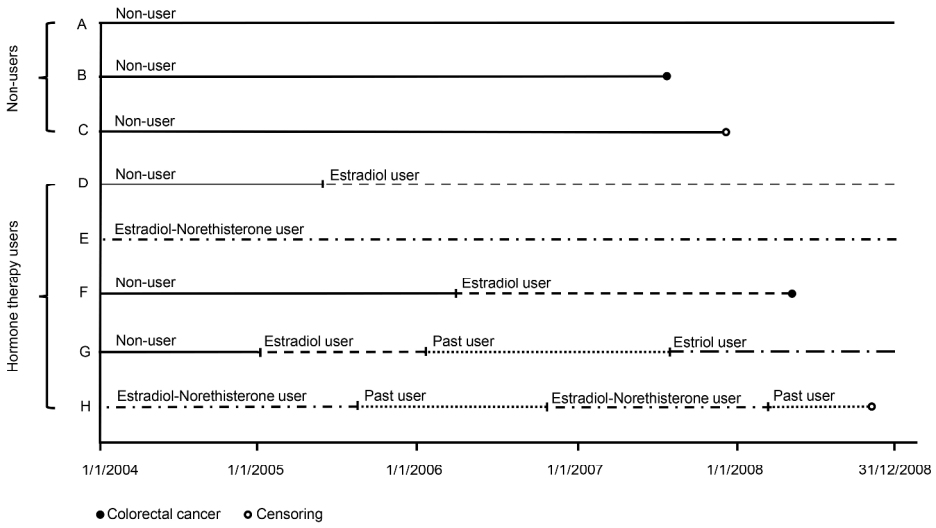

Follow-up of study participants

338x190mm (300 x 300 DPI)

Supplementary Table 1. Use of hormone therapy and risk of colorectal cancer by site

HT use	Category	PY	Colon	RR (95% CI)	Right colon	RR (95% CI)	Left Colon	RR (95% CI)	Rectum	RR (95% CI)
Status	Non-use	2,126,753	2,138	Reference	1,322	Reference	756	Reference	882	Reference
	Current use	320,202	312	0.88 (0.78-0.99)	198	0.89 (0.77-1.04)	104	0.85 (0.69-1.04)	129	0.90 (0.75-1.09)
	Past use	203,759	244	0.98 (0.86-1.12)	143	0.91 (0.76-1.08)	91	1.09 (0.87-1.36)	94	0.96 (0.77-1.19)
	Ever use	523,961	556	0.92 (0.83-1.01)	341	0.90 (0.79-1.01)	195	0.94 (0.80-1.11)	223	0.92 (0.79-1.07)
HRT type	Non-use	2,126,753	2,138	Reference	1,322	Reference	756	Reference	882	Reference
	ET*	159,495	176	0.88 (0.75-1.03)	110	0.86 (0.71-1.05)	60	0.90 (0.69-1.18)	82	0.99 (0.78-1.25)
	ET (Estradiol) *	*118,910*	112	0.86 (0.71-1.05)	71	0.88 (0.69-1.12)	37	0.82 (0.59-1.15)	47	0.89 (0.67-1.20)
	ET (Estriol) *	*40,585*	64	0.90 (0.70-1.16)	39	0.82 (0.59-1.13)	23	1.07 (0.70-1.63)	29	1.19 (0.82-1.74)
	Tibolone*	20,043	18	1.06 (0.67-1.69)	9	0.90 (0.47-1.74)	9	1.40 (0.72-2.71)	3	0.40 (0.13-1.23)
	EPT*	91,654	70	0.80 (0.63-1.02)	46	0.88 (0.65-1.18)	21	0.66 (0.43-1.01)	36	0.96 (0.69-1.34)
	Other*	49,010	48	0.94 (0.70-1.25)	33	1.04 (0.74-1.47)	14	0.78 (0.46-1.32)	14	0.67 (0.39-1.13)
Route	Non-use	2,126,753	2,138	Reference	1,322	Reference	756	Reference	882	Reference
	ET Oral*	57,031	64	0.77 (0.60-0.99)	40	0.74 (0.54-1.01)	23	0.87 (0.58-1.33)	30	1.00 (0.69-1.44)
	ET Vaginal*	89,719	95	0.91 (0.74-1.12)	65	0.99 (0.77-1.27)	27	0.76 (0.51-1.11)	39	0.94 (0.68-1.30)
	ET Transdermal*	7,246	10	1.57 (0.84-2.92)	2	0.53 (0.13-2.10)	8	3.38 (1.68-6.79)	5	1.79 (0.74-4.31)
	EPT Oral*	90,126	70	0.81 (0.64-1.03)	46	0.89 (0.66-1.19)	21	0.67 (0.43-1.03)	36	0.98 (0.70-1.36)
	EPT Transdermal*	1,163	0	-	0	-	0	-	0	-
	Other*	74,917	73	0.97 (0.77-1.22)	45	0.97 (0.72-1.31)	25	0.93 (0.62-1.39)	19	0.61 (0.38-0.96)
Oral dose Unit increase	Estrogen 1 mg / day*			0.84 (0.69-1.04)		0.78 (0.59-1.03)		0.98 (0.71-1.35)		0.94 (0.69-1.29)
	Progestin 10 mg / month*			1.02 (0.85-1.24)		1.13 (0.88-1.45)		0.81 (0.59-1.13)		0.99 (0.74-1.31)

Incidence risk ratios (RR) were adjusted for age, number of births, highest level of education, marital status, use of antihypertensives, antidiabetics, statins and thyroid therapy. ET: estrogen only therapy. EPT: combined estrogen-progestin therapy. PY: person-years. CRC: colorectal cancer. CI: confidence interval. *Current use.

Supplementary Table 2. Use of hormone therapy and risk of colorectal cancer. Sensitivity analysis where CRC cases diagnosed in 2014 were censored.

	HT use	PY	CRC cases	RR (95% CI)
Status	**Non-use**	2,126,753	2,580	Reference
	Current use	320,202	357	0.83 (0.74-0.93)
	Past use	203,759	332	1.10 (0.98-1.24)
	Ever use	523,961	689	0.94 (0.86-1.03)
HRT type	**Non-use**	2,126,753	2,580	Reference
	ET*	159,495	209	0.86 (0.75-1.00)
	ET (Estradiol)*	*118,910*	*137*	*0.87 (0.73-1.04)*
	ET (Estriol)*	*40,585*	*72*	*0.85 (0.67-1.08)*
	Tibolone*	20,043	15	0.72 (0.43-1.20)
	EPT*	91,654	78	0.74 (0.59-0.92)
	Other*	49,010	55	0.88 (0.68-1.15)
Route	**Non-use**	2,126,753	2,580	Reference
	ET Oral*	57,031	72	0.72 (0.57-0.92)
	ET Vaginal*	89,719	119	0.94 (0.78-1.13)
	ET Transdermal*	7,246	10	1.28 (0.69-2.38)
	EPT Oral*	90,126	78	0.75 (0.60-0.94)
	EPT Transdermal*	1,163	0	-
	Other	74,917	78	0.85 (0.68-1.07)
Oral dose Unit increase	**Estrogen 1 mg / day***			0.79 (0.64-0.96)
	Progestin 10 mg / month*			1.02 (0.85-1.23)

Incidence risk ratios (RR) were adjusted for age, number of births, highest level of education, marital status, use of antihypertensives, antidiabetics, statins and thyroid therapy. ET: estrogen only therapy. EPT: combined estrogen-progestin therapy. PY: person-years. CRC: colorectal cancer. CI: confidence interval. *Current use.

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Menopausal hormone therapy and colorectal cancer: a linkage between nationwide registries in Norway

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ABSTRACT

Objectives: With the present study, we aimed to investigate the association between menopausal hormone therapy (HT) and risk of colorectal cancer (CRC).

Setting: Cohort study based on the linkage of Norwegian population-based registries.

Participants: We selected 466,822 Norwegian women, aged 55-79, alive and residing in Norway as of January 1, 2004, and we followed them from 2004 to 2008. Each woman contributed person-years at risk as non-user, current user and/or past HT user.

Outcome measures: The outcome of interest was adenocarcinoma of the colorectal tract, overall, by anatomic site and stage at diagnosis. Incidence rate ratios (RR) with 95% confidence intervals (95% CI) were estimated by Poisson regression and were used to evaluate the association between HT and CRC incidence.

Results: During the median follow-up of 4.8 years, 138,655 (30%) women received HT and 3,799 (0.8%) incident CRCs occurred. Current, but not past, use of HT was associated with a lower risk of CRC (RR 0.88; 95% confidence interval (CI) 0.80-0.98). RRs for localized, regionally advanced and metastatic CRC were 1.13 (95% CI 0.91-1.41), 0.81 (0.70-0.94) and 0.79 (0.62-1.00), respectively. RRs for current use of estrogen therapy (ET) was 0.91; 95% CI 0.80-1.04), while RR for current use of estrogen-progestin therapy (EPT) was 0.85 (0.70-1.03), as compared to no use of HT. The same figures for ET and EPT in oral formulations were 0.83 (95% CI 0.68-1.03) and 0.86 (0.71-1.05) respectively.

Conclusions: In our nationwide cohort study, HT use lowered the risk of CRC, specifically the most advanced CRC.

SUMMARY BOX

Article summary

Strengths and limitations of this study

- Our cohort study, based on a linkage between nationwide registries in Norway, provided strong evidence showing that use of hormone therapy (HT) is associated with a reduced risk of colorectal cancer (CRC)
- HT had no impact on localized CRC but it protected against regionally advanced CRC and even more strongly against metastatic CRC. We therefore hypothesized that HT might play a key role in the inhibition of cancer progression
- For the first time, we showed that estrogens - in oral formulations - were associated with a decreased risk of CRC in a dose-response fashion
- The main strength of our study is that the registry linkages ensured detailed information on exposure of HT, including type of HT, with no risk of self-selection of women to participate.
- However, we did not have information on recognized risk factors for CRC (e.g. family history of CRC, body mass index, physical activity, diet, alcohol use and smoking) or information on aspirin use, so we could not adjust our estimates for those factors.

INTRODUCTION

Colorectal cancer (CRC) is the second most commonly diagnosed cancer in females and the third in males worldwide, with estimated 1.4 million cases and 700,000 deaths occurring globally in 2012 [1]. The detection and removal of precancerous lesions through CRC screening and the intervention on modifiable risk factors for CRC, such as diet, alcohol consumption, physical activity, and tobacco smoking, can reduce both CRC incidence and mortality [2,3]. Currently, new preventive strategies are being explored through different medications, aspirin being the most promising [4]. In addition to aspirin, menopausal hormone treatment (HT) has been suggested to reduce CRC risk. A 2012 meta-analysis of four clinical trials and sixteen observational studies found that use of HT was associated with a 20-30% lower risk of CRC [5]. Moreover, a 2016 Danish nationwide cohort study involving 1 million women showed that use of HT was associated with approximately a 15% reduction in CRC risk [6]. Nevertheless, results from the Women's Health Initiative (WHI) clinical trial were not supportive of the protective effect of HT on CRC. Among women with no uterus, there was no difference in the risk of CRC between women who took estrogen therapy (ET) and those who took the placebo [7]. Among women with an intact uterus, women who received combined estrogen-progestin therapy (EPT) had a lower risk of colorectal cancer than women who took the placebo. However, the CRCs that occurred in the treatment group were more advanced at detection than those in the placebo group [8], suggesting that use of HT might simply delay CRC diagnosis.

Given these conflicting results, the association between use of menopausal HT and the risk of CRC remains controversial. With the present nationwide cohort study, based on the linkage of population-based registries, our aim was to supply new evidence on the association between HT and risk of CRC. We present results on the association between different types,

routes of administration and doses of HT on the risk of CRC, overall, by anatomic site and stage at diagnosis.

PATIENTS AND METHODS

Cohort characteristics and definition of exposure to HT were described in details elsewhere [9]. Briefly, an 11-digit unique personal identification number allowed univocal linkage between different national Norwegian registries. We linked information about year and month of birth, immigration and emigration status, death, cause of death, education level and municipality of residence (Statistics Norway and the Population Registry), redeemed prescriptions (the Norwegian Prescription Database) and cancer cases (the Cancer Registry of Norway). The study was approved by the regional ethics committee in the South East region of Norway, and concession to data linkage was granted by the Norwegian Data Protection Authority.

We included data from 466,822 women born in Norway between 1925 and 1949, alive and residing in Norway as of January 1, 2004 (aged 55-79 years), who did not have a CRC or any other cancer diagnosis before January 1, 2004. Women were followed until December 31, 2008.

Exposure to HT

We retrieved data on use of menopausal hormone therapy (Anatomical Therapeutic Chemical (ATC) group G03) in the period 2004–2008. We did not have any data on prescriptions before 2004. Duration of HT use was estimated for each different type of drug as number of total treatment days, calculated from the package size multiplied by the number of packages prescribed regarding the dosing intervals recommended. The estimated duration of HT use was extended by 4 months to account for prolonged HT use beyond the treatment days prescribed. If there were gaps of more than 4 months between HT exposures, women contributed person-years at risk as a previous user from the date that the estimated duration of HT use ended, until the next redeemed prescription date if any, or end of the study period. Women receiving prescriptions of sex hormones other than ET, EPT or Tibolone, such as oral contraceptives and progestogen only, were censored at the date of prescription

Women were included in the various type of HT preparation categories based on the specific product dispensed (Figure 1). Women who switched from one type of HT to another (e.g. from estradiol to estriol) contributed person-years at risk to the specific product dispensed. When studying the effect of the different hormone types on CRC incidence, women who redeemed at least two simultaneous prescriptions of different hormone types were classified in the "other" category. The same approach was used when studying the route of administration. Women were classified as ET users if they redeemed only ET prescriptions, and EPT users if they redeemed only EPT prescriptions during the follow-up. All combined regimens of estrogen–progestin available in Norway contain estradiol and norethisterone acetate. Use of other progestin types, such as medroxyprogesterone acetate or dienogest, is almost nonexistent in Norway.

All women in the study population contributed person-years at risk as a non-user, current user and/or past HT user (Figure 1). Person-years at risk were calculated from start of

the study period, January 1, 2004, until event, censoring or end of follow-up. Women contributed person-years at risk as current users according to the accumulated duration of treatment for the type of HT dispensed. If there were gaps of more than 4 months between prescriptions, women contributed person-years at risk as a past user from the date that the estimated duration of HT use ended, until the next redeemed prescription date, if any, or end of the study period. Non-users contributed person-years at risk from January 1, 2004 until the date of the first redeemed prescription, if any, event, censoring or end of follow-up.

Outcome

The outcome of interest was adenocarcinoma of the colorectal tract (topography codes C18-C20 according to the International Classification of Diseases, Tenth Revision, Clinical Modification). CRC with histology other than adenocarcinoma (i.e. small cell carcinoma, squamous cell carcinoma, carcinoid, sarcoma, gastrointestinal stromal tumor and lymphoma) were not analyzed as CRC cases and were censored at diagnosis.

Statistical analysis

Incidence rate ratios (RR) with 95% confidence intervals (95% CI) were estimated by Poisson regression. The number of incident CRCs was analyzed as a log-linear function of exposure time, HT use, analyzed as a time-dependent variable (Figure 1), and adjusting covariates. Women were censored at death, emigration, any tumor diagnosis, prescription of sex hormones other than ET, EPT or Tibolone, or end of follow-up (December 31, 2008), whichever came first. We adjusted HT estimates for age in years, number of births (nulliparous, 1, 2, 3, and ≥4)), highest level of education (elementary, high-school, university or higher, and missing) and marital status (not married, married or partnered, widowed, and

divorced or separated) registered at the beginning of follow-up and use of antihypertensive drugs (ATC groups C02, C03, C07-C09), antidiabetic drugs (A10), statins (C10) and thyroid therapy (H03) registered anytime during follow-up. Time on study was used as timescale in the Poisson regression and split into 1-year time intervals assuming a constant risk of CRC within each interval. At the beginning of each interval, age of all women was updated. In each analysis, the reference group was non-users of HT. When analyzing the association of HT with CRC stage at diagnosis, only CRCs at a specific stage were analyzed as events, while CRCs at other stages were analyzed as censoring events. When analyzing the association of HT with cancer diagnosed in a specific site of the colorectal tract (e.g. left colon), only cancer diagnosed in that specific site were analyzed as events, while others were analyzed as censoring events.

We evaluated the estrogen and progestin dose-response effect by limiting analyses to current oral ET and oral EPT users and non-users. The dose of estrogen and the dose of progestin were obtained from each prescription of oral ET and EPT. Doses of estrogens and progestins in non-users were set to zero. The dose of estrogen and the dose of progestins were entered simultaneously in the multivariable models as two continuous variables.

All tests were two sided with a 5% significance level. Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC) and R software (http://cran.r-project.org/).

RESULTS

We followed 466,822 women born in Norway and with no previous history of cancer from 2004 to 2008. During the follow-up, which had a median duration of 4.8 years, 3,799 CRCs occurred. A total of 138,655 (30%) women used HT. Characteristics of the study population were not homogeneously distributed between HT users and non-users, and between ET users and EPT users (Table 1). Notably, ET users were substantially older than EPT users (median age was 64.0 and 60.0 years, respectively; P<0.001).

Table 1. Characteristics of the study population by hormone therapy use

		HT non-users No. (%)	HT users[b] No. (%)	P	ET users[b] No. (%)	EPT users[b] No. (%)	P
All women		328,167	138,655		79,195	30,455	
Number of CRC		3,020 (0.92)	779 (0.56)		434 (0.55)	202 (0.66)	
Age [a]	**Median (IQR)**	65.0 (59-72)	62.0 (57-67)	<0.001	64.0 (58-70)	60.0 (57-64)	<0.001
Highest education [a]	**Elementary school**	127,238 (38.8)	42,317 (30.5)	<0.001	26,455 (33.4)	8,592 (28.2)	<0.001
	High school	143,564 (43.7)	68,401 (49.3)		38,197 (48.2)	15,684 (51.5)	
	University and higher	40,899(12.5)	27,189 (19.6)		14,094 (17.8)	6,013 (19.7)	
	Missing	16,466 (5.0)	748 (0.5)		449 (0.6)	166 (0.5)	
Number of children [a]	**0**	45,536 (13.9)	10,857 (7.8)	0.004	5,984 (7.6)	2,715 (8.9)	<0.001
	1	39,595 (12.1)	15,761 (11.4)		8,731 (11.0)	3,685 (12.1)	
	2	106,742 (32.5)	55,416 (40.0)		29,982 (37.9)	12,795 (42.0)	
	3	81,622 (24.9)	37,495 (27.0)		21,784 (27.5)	8,059 (26.5)	
	> 3	54,672 (16.7)	19,126 (13.8)		12,714 (16.1)	3,201 (10.5)	
Marital status [a]	**Single**	27,218 (8.3)	5,129 (3.7)	<0.001	2,770 (3.5)	1,427 (4.7)	<0.001
	Married / Partnered	154,016 (46.9)	80,077 (57.8)		44,774 (56.5)	17,361 (57.0)	
	Widow	103,202 (31.4)	31,982 (23.1)		21,460 (27.1)	5,400 (17.7)	
	Divorced / Separated	43,731 (13.3)	21,467 (15.5)		10,191 (12.9)	6,267 (20.6)	
Antihypertensives [b]	**User**	163,131 (49.7)	69,572 (50.2)	0.004	42,166 (53.2)	13,688 (45.5)	<0.001
Antidiabetics [b]	**User**	23,988 (7.3)	7,748 (5.6)	<0.001	5,207 (6.6)	1,274 (4.2)	<0.001
Statins [b]	**User**	100,863 (30.7)	42,646 (30.8)	0.886	27,821 (35.1)	7,036 (23.1)	<0.001
Thyroid therapy [b]	**User**	38,511 (11.7)	20,948 (15.1)	<0.001	12,334 (15.6)	4,160 (13.7)	<0.001

[a] Registered at baseline; [b] Prescribed anytime during the follow-up. HT: Hormone therapy. CRC: Colorectal cancer. IQR: Interquartile range. ET: Estrogen therapy. EPT: Estrogen-progestin therapy

Current use of HT was associated with a decreased risk of CRC compared to non-use, with a RR of 0.88 (95% CI 0.80-0.98; Table 2).

Table 2. Use of hormone therapy and risk of colorectal cancer

	HT use	PY	CRC cases	RR (95% CI)
Status	**Non-use**	2,126,753	3,020	Reference
	Current use	320,202	441	0.88 (0.80-0.98)
	Past use	203,759	338	0.98 (0.87-1.09)
	Ever use	523,961	779	0.92 (0.85-1.00)
HRT type	**Non-use**	2,126,753	3,020	Reference
	ET*	159,495	252	0.91 (0.80-1.04)
	ET (Estradiol)*	*118,910*	*159*	*0.87 (0.74-1.03)*
	ET (Estriol)*	*40,585*	*93*	*0.98 (0.79-1.21)*
	Tibolone*	20,043	21	0.86 (0.56-1.32)
	EPT*	91,654	106	0.85 (0.70-1.03)
	Other*	49,010	62	0.86 (0.67-1.10)
Route	**Non-use**	2,126,753	3,020	Reference
	ET Oral*	57,031	94	0.83 (0.68-1.03)
	ET Vaginal*	89,719	134	0.92 (0.77-1.09)
	ET Transdermal*	7,246	15	1.63 (0.98-2.71)
	EPT Oral*	90,126	106	0.86 (0.71-1.05)
	EPT Transdermal*	1,163	0	-
	Other	74,917	92	0.86 (0.70-1.06)
Oral dose Unit increase	**Estrogen 1 mg / day***			0.87 (0.73-1.04)
	Progestin 10 mg / month*			1.01 (0.86-1.19)

Incidence rate ratios (RR) were adjusted for age, number of births, highest level of education, marital status, use of antihypertensives, antidiabetics, statins and thyroid therapy. ET: estrogen only therapy. EPT: combined estrogen-progestin therapy. PY: person-years. CRC: colorectal cancer. CI: confidence interval. *Current use.

The same figure for past and ever use (current or past users) was 0.98 (95% 0.87-1.09) and 0.92 (95% 0.85-1.00). RRs for current use of ET and EPT versus non-use were 0.91 (95% CI 0.80-1.04) and 0.85 (95% CI 0.70-1.03), respectively.

From each prescription of oral ET and EPT, we retrieved the information on the administered dose of estrogens and progestins. Mean estrogen dose in oral ET and EPT treatments was 1.40 and 1.36 mg/day, respectively. Mean progestin dose in oral EPT users was 18.3 mg/month. We analyzed the dose effect of oral estrogen and progestin as continuous variables on CRC risk, and we found that estrogens were associated with a decreased risk of CRC in a dose-response fashion, even if the result was not statistically significant (RR 0.87 for each additional mg/day; 95% CI 0.73-1.04; Table 2), while progestins showed no effect. We then repeated the analysis to estimate the dose effect of estrogens on CRC risk after censoring EPT users at time of a first use of EPT, to avoid a possible interference of progestins, and the RR estimate for each additional mg/day of estrogens was 0.88 (95% CI 0.74-1.04).

In Table 3 we reported the association between HT intake and CRC diagnosed at different stages: 698 localized, 2,023 regionally advanced and 737 metastatic CRCs.

Table 3. Use of hormone therapy and risk of colorectal cancer by colorectal cancer stage

HT use		PY	Localized CRC	RR (95% CI)	Regionally advanced CRC	RR (95% CI)	Metastatic CRC	RR (95% CI)
Status	**Non-use**	2,126,753	548	Reference	1,607	Reference	598	Reference
	Current use	320,202	101	1.13 (0.91-1.41)	216	0.81 (0.70-0.94)	78	0.79 (0.62-1.00)
	Past use	203,759	49	0.79 (0.59-1.06)	200	1.08 (0.93-1.26)	61	0.90 (0.69-1.18)
	Ever use	523,961	150	0.99 (0.82-1.20)	416	0.92 (0.83-1.03)	139	0.83 (0.69-1.01)
HRT type	**Non-use**	2,126,753	548	Reference	1,607	Reference	598	Reference
	ET*	159,495	67	1.33 (1.03-1.72)	125	0.84 (0.70-1.02)	34	0.64 (0.45-0.91)
	ET (Estradiol)*	*118,910*	*44*	*1.36 (0.99-1.85)*	*75*	*0.78 (0.62-0.98)*	*22*	*0.60 (0.39-0.93)*
	ET (Estriol)*	*40,585*	*23*	*1.27 (0.83-1.94)*	*50*	*0.97 (0.73-1.30)*	*12*	*0.73 (0.41-1.29)*
	Tibolone*	20,043	5	1.20 (0.50-2.90)	12	0.93 (0.52-1.64)	4	0.75 (0.28-2.01)
	EPT*	91,654	17	0.79 (0.49-1.29)	56	0.84 (0.64-1.10)	24	0.91 (0.60-1.37)
	Other*	49,010	12	0.94 (0.53-1.66)	23	0.60 (0.40-0.90)	16	1.09 (0.67-1.80)
Route	**Non-use**	2,126,753	548	Reference	1,607	Reference	598	Reference
	ET Oral*	57,031	24	1.15 (0.76-1.73)	48	0.79 (0.59-1.06)	13	0.63 (0.36-1.10)
	ET Vaginal*	89,719	36	1.36 (0.97-1.91)	67	0.86 (0.68-1.10)	17	0.59 (0.37-0.96)
	ET Transdermal*	7,246	6	3.80 (1.70-8.50)	7	1.44 (0.69-3.04)	1	0.51 (0.07-3.65)
	EPT Oral*	90,126	17	0.81 (0.50-1.31)	56	0.86 (0.65-1.12)	24	0.86 (0.61-1.39)
	EPT Transdermal*	1,163	0	-	0	-	0	-
	Other*	74,917	18	0.96 (0.60-1.54)	38	0.67 (0.49-0.93)	23	1.05 (0.69-1.60)
Oral dose Unit increase	**Estrogen 1 mg / day***			1.13 (0.82-1.57)		0.75 (0.57-0.98)		0.97 (0.67-1.39)
	Progestin 10 mg / month*			0.80 (0.57-1.13)		1.11 (0.88-1.41)		1.02 (0.74-1.42)

Incidence rate ratios (RR) were adjusted for age, number of births, highest level of education, marital status, use of antihypertensives, antidiabetics, statins and thyroid therapy. ET: estrogen only therapy. EPT: combined estrogen-progestin therapy. PY: person-years. CRC: colorectal cancer. CI: confidence interval. *Current use.

Compared to non-use, current use of HT was associated with a decreased risk of regionally advanced (RR 0.81; 95% CI 0.70-0.94) and metastatic CRC (RR 0.79; 95% CI 0.62-1.00), but not of localized CRC (RR 1.13; 95% CI 0.91-1.41).

In supplementary Table 1 we reported the association between HT and risk of CRC diagnosed in different sites of the colorectal tract. RRs for the association of current use of HT with colon cancer, right colon cancer, left colon cancer and rectal cancer were 0.88 (95% CI 0.78-0.99), 0.89 (0.77-1.04), 0.85 (0.69-1.04) and 0.90 (0.75-1.09), respectively.

We repeated the main analyses after censoring the CRC cases that occurred in the first year of follow-up (2014), and results were stronger than in the main analysis (supplementary Table 2). RR for use of HT, ET, EPT, oral ET and oral EPT were 0.83 (95% CI 0.74-0.93), 0.86 (0.75-1.00), 0.74 (0.59-0.92), 0.72 (0.57-0.92) and 0.75 (0.60-0.94), respectively, compared to no use. Finally, estrogens were significantly associated with a decreased risk of CRC in a dose-response fashion (RR 0.79 for each additional mg/day; 95% CI 0.64-0.96).

DISCUSSION

In this Norwegian nationwide cohort study, we evaluated the effect of menopausal HT on CRC incidence. Our results suggest that current use of HT is associated with a reduced risk of CRC, specifically the most advanced CRC. Current users of any HT had a 12% reduction of CRC, 19% reduction of regionally advanced CRC and 21% reduction of metastatic CRC. Furthermore, we found that, in current users, the risk of CRC decreased with increasing doses of oral estrogens.

Colorectal polyps and tumors occur more frequently in men than in women, and many preclinical and clinical studies have provided evidence that female sex hormones, specifically estrogen, might form the basis for the protective effect in women [10]. Researchers have found that the estrogen receptor beta (ERβ) regulates DNA repair, increases apoptosis and reduces cell proliferation, and that ERβ activation can consequently reduce tumor occurrence and inhibit progression [11-14]. Consistent evidence showed an inverse relationship between ERβ expression in the colon and the presence and stage of colorectal polyps and tumors [15-19]. The possible protective effect of HT was evaluated in many observational studies and two clinical trials, with conflicting results. Current use of ET was associated with a 30% decreased CRC risk in a meta-analysis published in 2012 [5] and a 23% reduction of colon cancer and 17% reduction of rectal cancer in a recent nationwide registry-based study among one million Danish women [6]. In contrast to those findings, a lack of association was reported in 136,000 postmenopausal women in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort [20]. The only placebo-controlled clinical trial on the subject, the WHI, included 10,739 women with

hysterectomy, showed no difference in either the risk of CRC or the stage of disease at diagnosis between women who took estrogen alone and those who took the placebo [7]. The effect of EPT use on CRC risk is also controversial. In the 2012 meta-analysis [5], current use of EPT was associated with a significant 20% reduction of CRC, and in the Danish study [6], it was associated with a significant 12% reduction of colon cancer and 11% reduction of rectal cancer. In the EPIC cohort, a non-significant 6% risk reduction due to EPT use was reported [20]. In the WHI, among the 16,608 postmenopausal women with intact uterus, authors reported that EPT was associated with a significant 28% reduction of CRC after 5.6 years of intervention (11.6 years of follow-up). However, EPT was associated with more advanced CRC, and the investigators concluded that their findings did not support a clinically meaningful benefit for EPT on CRC [8]. They hypothesized a potential CRC diagnostic delay due to EPT-related conditions such as vaginal bleeding. The discrepancies observed in the literature might be explained by several factors, including the different designs (clinical trials, case-control studies and cohort studies) and methods of HT exposure assessment (e.g. self-reported versus registry-based) used in the different studies [5].

Our study provides new evidence on the protective effect of HT use against CRC. For the first time, we also found that increasing doses of oral estrogens, and not progestins, were associated with decreasing risk of CRC. Altogether these results might indicate that estrogens reduce the risk of CRC, while progestins have no effect. In support of our findings, a recent study showed that the risk of CRC decreased with increasing levels of endogenous estrogen, while it did not depend on progesterone levels [21].

Our results could be interpreted to support the hypothesis that HT inhibits cancer progression, rather than formation. In our study, use of HT had no impact on localized CRC

(RR=1.13) but it protected against regionally advanced CRC (RR=0.81) and metastatic CRC (RR=0.79). Similarly, in the Iowa Women's Health Study, the RR estimates for ever versus never use of HT by stage were 0.91 for localized, 0.78 for regional and 0.72 for distant disease [22]. In the California Teachers Study, current HT use versus baseline non-use was associated with these RRs: 0.99 for localized, 0.68 for regional and 0.33 for distant disease [23]. Results from the Danish study showed that HT had a stronger impact on metastatic rather than non-metastatic CRC [6], and other authors reported that HT users were significantly more likely to be diagnosed at an earlier disease stage as compared to HT non-users [24-25].

In the 2012 meta-analysis [5] and the 2016 Danish study [6], HT was associated with lower risk of colon cancer but less so with rectal cancer. In our study, we found similar estimated for colon and rectal cancer. Within the colon tract, we found similar estimates for left and right colon cancer. More studies are warranted to understand whether HT has different effects in CRC depending on the anatomical location.

Our study has several strengths. The registry linkages ensured detailed information on exposure of HT, including type of HT. There was no self-selection of women to participate, and the large size of the study population provided a large number of incident CRCs. However, our study has important limitations. First, we did not have information on recognized risk factors for CRC (e.g. family history of CRC, body mass index, physical activity, diet, alcohol use and smoking) or information on aspirin use. Some authors showed no significant effect of those factors on the association between HT and CRC risk [23,24,26], but in the California Teachers Study HT use was more strongly associated with CRC risk among women with a family history of CRC [23]. In addition, our estimates could be affected by the healthy user bias: it is probable that HT users were more concerned about their health than non-users and, for example,

underwent more bowel examinations or had a better lifestyle. This bias could have resulted in overestimation of the HT protective effect. In fact we found that HT users had a higher education level than non-users, and education is positively associated with general good health and use of medical services [27]. However, the fact that HT had no effect on risk of early stage CRC and strong effect on risk of advanced stage CRC indicates no healthy user bias, as more health conscious women are likely to have CRC detected in earlier rather than later stages. Finally, given the relatively short follow-up of our study, we were not able to evaluate the influence of duration of HT use on CRC risk, as other authors did [6].

In conclusion, we provided evidence that use of HT is associated with a reduced risk of CRC, in particular advanced CRC. The effect was similar for ET and EPT in women of age 55 years or older.

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Contributorship statement

Study concept and design: EB, SS, NCS, SGI, GU, SV, EW. Acquisition of data: SS, SGI, SH, GU, SV. Analysis of data: EB, NCS, SS, SGI. Statistical analysis of data: EB, NCS, VB. Interpretation of data: all. Drafting of the manuscript: EB. Critical revision of the manuscript for important intellectual content: SS, SGI, SH, TdL, GU, SV, EW.

Conflicting interests

The authors have declared no conflicts of interest.

Research reporting checklists

The present article follows the STROBE guidelines for research reporting of observational studies.

Data sharing statement

Authors are willing to share any data that are not published in the manuscript. Please contact the corresponding author.

Ethics approval

The study was approved by the regional ethics committee in the South East region of Norway, and concession to data linkage was granted by the Norwegian Data Protection Authority.

Figure 1. Follow-up of study participants

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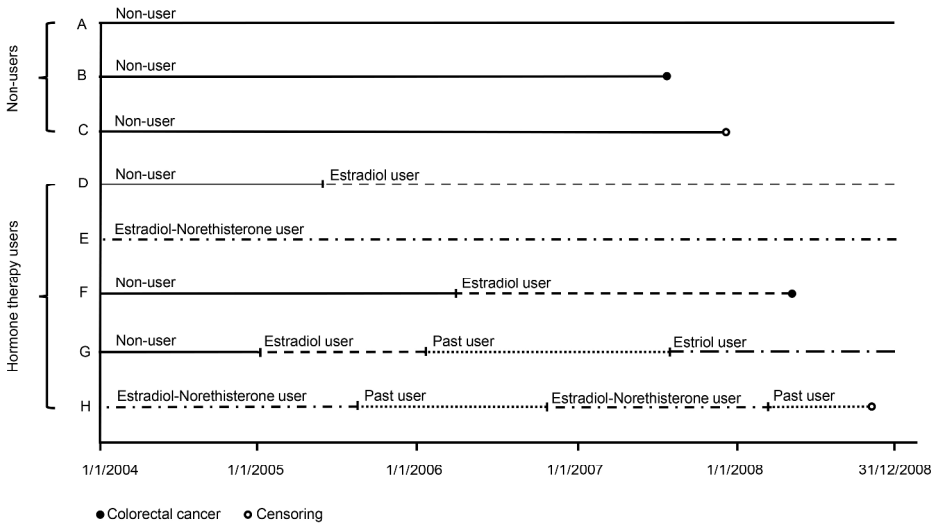

Follow-up of study participants

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Supplementary Table 1. Use of hormone therapy and risk of colorectal cancer by site

HT use	Category	PY	Colon	RR (95% CI)	Right colon	RR (95% CI)	Left Colon	RR (95% CI)	Rectum	RR (95% CI)
Status	Non-use	2,126,753	2,138	Reference	1,322	Reference	756	Reference	882	Reference
	Current use	320,202	312	0.88 (0.78-0.99)	198	0.89 (0.77-1.04)	104	0.85 (0.69-1.04)	129	0.90 (0.75-1.09)
	Past use	203,759	244	0.98 (0.86-1.12)	143	0.91 (0.76-1.08)	91	1.09 (0.87-1.36)	94	0.96 (0.77-1.19)
	Ever use	523,961	556	0.92 (0.83-1.01)	341	0.90 (0.79-1.01)	195	0.94 (0.80-1.11)	223	0.92 (0.79-1.07)
HRT type	Non-use	2,126,753	2,138	Reference	1,322	Reference	756	Reference	882	Reference
	ET*	159,495	176	0.88 (0.75-1.03)	110	0.86 (0.71-1.05)	60	0.90 (0.69-1.18)	82	0.99 (0.78-1.25)
	ET (Estradiol) *	*118,910*	112	0.86 (0.71-1.05)	71	0.88 (0.69-1.12)	37	0.82 (0.59-1.15)	47	0.89 (0.67-1.20)
	ET (Estriol) *	*40,585*	64	0.90 (0.70-1.16)	39	0.82 (0.59-1.13)	23	1.07 (0.70-1.63)	29	1.19 (0.82-1.74)
	Tibolone*	20,043	18	1.06 (0.67-1.69)	9	0.90 (0.47-1.74)	9	1.40 (0.72-2.71)	3	0.40 (0.13-1.23)
	EPT*	91,654	70	0.80 (0.63-1.02)	46	0.88 (0.65-1.18)	21	0.66 (0.43-1.01)	36	0.96 (0.69-1.34)
	Other*	49,010	48	0.94 (0.70-1.25)	33	1.04 (0.74-1.47)	14	0.78 (0.46-1.32)	14	0.67 (0.39-1.13)
Route	Non-use	2,126,753	2,138	Reference	1,322	Reference	756	Reference	882	Reference
	ET Oral*	57,031	64	0.77 (0.60-0.99)	40	0.74 (0.54-1.01)	23	0.87 (0.58-1.33)	30	1.00 (0.69-1.44)
	ET Vaginal*	89,719	95	0.91 (0.74-1.12)	65	0.99 (0.77-1.27)	27	0.76 (0.51-1.11)	39	0.94 (0.68-1.30)
	ET Transdermal*	7,246	10	1.57 (0.84-2.92)	2	0.53 (0.13-2.10)	8	3.38 (1.68-6.79)	5	1.79 (0.74-4.31)
	EPT Oral*	90,126	70	0.81 (0.64-1.03)	46	0.89 (0.66-1.19)	21	0.67 (0.43-1.03)	36	0.98 (0.70-1.36)
	EPT Transdermal*	1,163	0	-	0	-	0	-	0	-
	Other*	74,917	73	0.97 (0.77-1.22)	45	0.97 (0.72-1.31)	25	0.93 (0.62-1.39)	19	0.61 (0.38-0.96)
Oral dose Unit increase	Estrogen 1 mg / day*			0.84 (0.69-1.04)		0.78 (0.59-1.03)		0.98 (0.71-1.35)		0.94 (0.69-1.29)
	Progestin 10 mg / month*			1.02 (0.85-1.24)		1.13 (0.88-1.45)		0.81 (0.59-1.13)		0.99 (0.74-1.31)

Incidence risk ratios (RR) were adjusted for age, number of births, highest level of education, marital status, use of antihypertensives, antidiabetics, statins and thyroid therapy. ET: estrogen only therapy. EPT: combined estrogen-progestin therapy. PY: person-years. CRC: colorectal cancer. CI: confidence interval. *Current use.

Supplementary Table 2. Use of hormone therapy and risk of colorectal cancer. Sensitivity analysis where CRC cases diagnosed in 2014 were censored.

	HT use	PY	CRC cases	RR (95% CI)
Status	**Non-use**	2,126,753	2,580	Reference
	Current use	320,202	357	0.83 (0.74-0.93)
	Past use	203,759	332	1.10 (0.98-1.24)
	Ever use	523,961	689	0.94 (0.86-1.03)
HRT type	**Non-use**	2,126,753	2,580	Reference
	ET*	159,495	209	0.86 (0.75-1.00)
	ET (Estradiol)*	*118,910*	*137*	*0.87 (0.73-1.04)*
	ET (Estriol)*	*40,585*	*72*	*0.85 (0.67-1.08)*
	Tibolone*	20,043	15	0.72 (0.43-1.20)
	EPT*	91,654	78	0.74 (0.59-0.92)
	Other*	49,010	55	0.88 (0.68-1.15)
Route	**Non-use**	2,126,753	2,580	Reference
	ET Oral*	57,031	72	0.72 (0.57-0.92)
	ET Vaginal*	89,719	119	0.94 (0.78-1.13)
	ET Transdermal*	7,246	10	1.28 (0.69-2.38)
	EPT Oral*	90,126	78	0.75 (0.60-0.94)
	EPT Transdermal*	1,163	0	-
	Other	74,917	78	0.85 (0.68-1.07)
Oral dose Unit increase	**Estrogen 1 mg / day***			0.79 (0.64-0.96)
	Progestin 10 mg / month*			1.02 (0.85-1.23)

Incidence risk ratios (RR) were adjusted for age, number of births, highest level of education, marital status, use of antihypertensives, antidiabetics, statins and thyroid therapy. ET: estrogen only therapy. EPT: combined estrogen-progestin therapy. PY: person-years. CRC: colorectal cancer. CI: confidence interval. *Current use.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract **PAG 2**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found **PAG 2**
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported **PAG 4, 5**
Objectives	3	State specific objectives, including any prespecified hypotheses **PAG 4, 5**
Methods		
Study design	4	Present key elements of study design early in the paper **PAG 6, 7, 8**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection **PAG 6, 7, 8**
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up **PAG 6, 7, 8** *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed **Not applicable** *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable **PAG 6, 7, 8, 9**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group **PAG 6, 7, 8, 9**
Bias	9	Describe any efforts to address potential sources of bias **PAG 7, 8, 9**
Study size	10	Explain how the study size was arrived at **Pag 6**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why **PAG 8,9**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding **PAG 8,9**
		(*b*) Describe any methods used to examine subgroups and interactions **PAG 8,9**
		(*c*) Explain how missing data were addressed **PAG 8,9**
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed **8,9** *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed **NOT APPLICABLE- NATIONWIDE STUDY**
		(b) Give reasons for non-participation at each stage **NOT APPLICABLE- NATIONWIDE STUDY**
		(c) Consider use of a flow diagram **OK**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders **pagg 10-16**
		(b) Indicate number of participants with missing data for each variable of interest **pagg 10-16**
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) **pagg 10-16**
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time **pagg 10-16**
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included **pagg 12-16**
		(*b*) Report category boundaries when continuous variables were categorized **pagg 10-16**
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period **Not relevant**
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses **pagg 14,16**
Discussion		
Key results	18	Summarise key results with reference to study objectives **pagg 17-20**
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias **pagg 19-20**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence **pagg 17-20**
Generalisability	21	Discuss the generalisability (external validity) of the study results **pagg 17-20**
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based **OK**

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.