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The effect of estrogen *vs.* combined estrogen-progestogen therapy on the risk of colorectal cancer

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Studies suggest that estrogen therapy (ET) and combined estrogen-progestogen therapy (EPT) may have different associations with colorectal cancer (CRC) risk, but data are conflicting. Prior meta-analyses did not distinguish between ET and EPT. We conducted a meta-analysis to summarize the relative risks (RR) of CRC due to ET versus EPT among peri- or postmenopausal women. From a total of 2,661 articles, four randomized controlled trials, eight cohort and eight case-control studies were included. Variables assessed included study characteristics, duration and recency of menopausal hormone therapy (HT) use, method of assessment of HT use, outcome definition and its ascertainment method. RRs were synthesized by random-effects models. We found that EPT ever use was associated with a decreased risk of CRC (RR 0.74, 95% CI 0.68-0.81), and so was ET ever use (RR 0.79, 95% CI 0.69-0.91). While current use of ET was associated with a significantly reduced risk of CRC (RR 0.70, 95% CI 0.57-0.85), former use was not (RR 0.86, 95%CI 0.67-1.11). Recency did not significantly modify the association between EPT and CRC risk. EPT former use was associated with a lower RR of CRC compared to ET former use ($p = 0.008$) but no such difference was observed between EPT and ET current use ($p = 0.12$). Overall, we found consistent evidence supporting the association between EPT and CRC risk reduction, regardless of recency. While literature for the association between ET and CRC risk is heterogeneous, our analyses suggest only current use of ET is associated with a decreased CRC risk.

Menopausal hormone therapy (HT) is indicated for short-term control of intolerable menopausal symptoms and it has a limited role in the treatment of osteoporosis for selected woman. While HT was found to increase the risks of venous thromboembolism, breast cancer and stroke, studies have also suggested that it may be associated with a reduced risk of colorectal cancer (CRC).[1] Grodstein et al. conducted a meta-analysis of 18 observational studies that summarized the effect of HT on CRC risk, and showed an overall inverse association, with a pooled relative risk (RR) of 0.80 (95% CI 0.74-0.86).[2] Another meta-analysis published by Nanda et al. revealed similar results.[3] The explanation for the observed association has been controversial, however, because selective prescribing of HT to healthier subjects (*i.e.*, healthy user bias) may have accounted for superior outcomes in the HT group. This concern was partly settled by the Women's Health Initiative (WHI) study, a large-scale randomized controlled trial (RCT), which found that the use of conjugated equine estrogens plus medroxyprogesterone acetate was associated with a reduction in CRC risk.[4] In contrast, one study did not find a significant association between the use of conjugated equine estrogen alone and CRC risk, although the small number of CRC cases may have limited its interpretation and generalizability.[5] In fact, the WHI study results were not directly comparable with previous observational studies and meta-analyses since the majority of these had investigated HT overall without differentiating between estrogen therapy (ET) *vs.* combined estrogen-progestogen therapy (EPT; progestogen encompasses both progesterone and progestin[6]). Therefore, we conducted a meta-analysis to summarize the available evidence and to compare the RR of CRC due to ET *vs.* EPT among peri- or postmenopausal women.

Key words: hormone replacement therapy, menopausal hormone therapy, colorectal cancer, estrogen, progesterone, progestogen

Abbreviations: CI: confidence interval; CRC: colorectal cancer; EPT: combined estrogen-progestogen therapy; ET: estrogen therapy; HT: menopausal hormone therapy; IGF-1: insulin-like growth factor-1; RCT: randomized controlled trial; RR: relative risks; WHI: Women's Health Initiative

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Material and Methods

Study selection

We searched PubMed and EMBASE for studies conducted up until September 2010 that investigated the association between ET or EPT and CRC risk among peri- or postmenopausal women. Articles were identified using the following key words: estrogen, hormone replacement therapy, menopausal hormone therapy, estradiol congeners, progestogen, progesterone and progestins in conjunction with colorectal neoplasm, such as colorectal, colon, sigmoid, or rectal cancer, tumor, or carcinoma. All of the above terms were entered as both MESH (exploded in EMBASE) terms and text words.

The search was restricted to human studies. We did not consider estimates published solely in the form of letters, commentaries, or abstracts. Case reports, reviews, or meta-analyses were also excluded. All entries retrieved by this strategy, plus references of eligible articles and key reviews, related to the effect of HT on CRC risk were examined to identify studies that satisfied the following predefined inclusion criteria: (*i*) Studies must have evaluated peri- or post-menopausal women. Studies investigating the association between oral contraceptives and CRC risk in young women were excluded; (*ii*) The primary exposure of interest was either ET or EPT. We did not consider those with nonspecific or mixed HT; (*iii*) Study endpoints were CRC, colon, or rectal cancer; and (*iv*) Studies were required to have reported or provided sufficient data to calculate the RR and derive its standard error.

Data extraction

Two independent investigators (KJL, JYCL) extracted the data from eligible articles, and a third investigator (WYC) validated the collected data for accuracy. If available, we extracted RRs, including ratios of cumulative incidence, incidence rates, and odds of developing the outcomes of interest, which were reported in the original articles. Information on study methods and quality-related characteristics were also documented. Variables that were evaluated included: year of publication and year of study, geographic region, inclusion and exclusion criteria of the study population, sample size, mean age of the study population, prospective *vs.* retrospective design, HT type, definitions for current *vs.* ever *vs.* never use, duration and recency of HT use, and duration of follow-up. Also, all articles were assessed for potential biases, including confounding, selection and information bias. For confounding bias, we extracted all the variables adjusted in each study and categorized the adjustment into: (*i*) no multivariate adjustment; (*ii*) with multivariate adjustment, including controlling for screening procedures, such as colonoscopy and sigmoidoscopy; (*iii*) with multivariate adjustment which did not include screening procedures. For selection bias, we extracted the participation rate if a study was questionnaire/interview-based and evaluated the characteristics among respondents and nonrespondents. Also, suboptimal (less than 70%) or differential participation rate among cases *vs.* controls, or significant loss to follow up (>10%) was considered a potential source of selection bias. For information bias, we evaluated each study for its methods of assessing HT exposure status and ascertaining cases of CRC. The methods of HT exposure assessment used by the selected studies can be categorized into three groups: (*i*) objective documents, such as medical charts, claims databases, or pharmacy records; (*ii*) HT allocation in a RCT; and (*iii*) patient self-report, such as interviews or questionnaires. The methods of case ascertainment used by the selected studies can be categorized into two groups: (*i*) cases ascertained by a cancer registry; (*ii*) cases identified by review of medical records. Of note, none of the included article ascertained cases by self-report methods (*i.e.*, questionnaire/interviews) alone.

Statistical analyses

All RRs extracted were the most finely adjusted estimates from the original studies. If one study presented estimates for multiple HT types (*e.g.*, ET and EPT) or multiple disease definitions (*e.g.*, CRC, colon cancer and rectal cancer), all of them were considered. If RRs were reported for different durations and recency of HT use, all of them were included since duration and recency were variables of interest. If RRs for the same population were reported more than once but in different articles, only the RRs from the most recent study were included.

Random effects models were applied to calculate pooled RRs and their 95% confidence intervals (CIs). In terms of HT exposure status, all included studies used "never use" as the referent group; and most studies used "ever use" as the primary exposure group. For studies reporting estimates regarding recency of use, ever HT use was subdivided into current *vs.* former use, as assigned by individual studies. For studies reporting estimates regarding duration of use, ever HT use was subdivided into short-term *vs.*. long-term use. To avoid ambiguity of intermediate duration, if a study reports estimates for duration of more than two categories, only the estimates for the shortest and longest duration were included in the analysis. For instance, if a study reports estimates for HT exposure with duration <5, 5–10 and >10 years, the estimate for <5 years is considered short-term use and the estimate for >10 years is long-term. Among the studies included in our analysis, 5 years is the most common cut-off used to define short-term *vs.* long-term use. Heterogeneity of effect estimates was assessed by the Cochrane Q test for heterogeneity[7] and the I^2 statistic described by Higgins and Thompson, which quantifies the proportion of total variability attributable to between-study variation.[8] To further determine study characteristics and their influence on RR estimates, we performed meta-regressions where we regressed the study-specific log-transformed RRs by the study characteristics variables, weighting the studies by the inverse of the sum of within-study and between-study variance.[9] Since data from different studies are only combinable when the study populations are homogenous, we performed subgroup analysis, pooling studies within the same category of the variables that are significant factors in the meta-regression

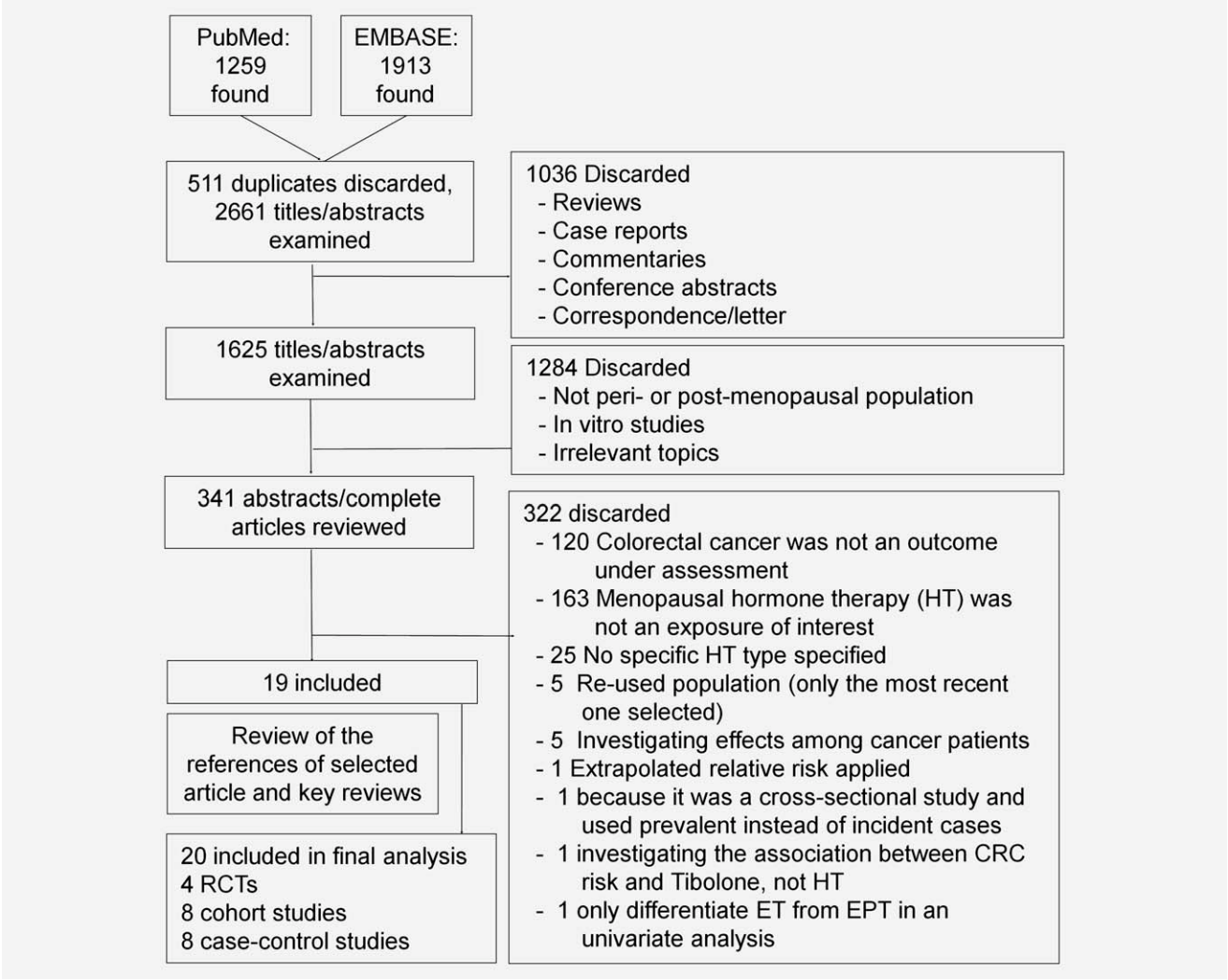

Figure 1. Flow chart of literature search for studies on the association between specific menopausal hormone therapy and colorectal cancer; CRC: colorectal cancer; RCT: randomized controlled trial; HT: menopausal hormone therapy.

models. If any of the RRs in the meta-regression model were extracted from the same studies for different HT types or multiple outcome definitions, we would use a generalized linear model to account for the correlation between observations. We explored potential publication bias using both Begg's and Egger's test.[10,11] Stata 10.1 (Stata Corp., College Station, TX) was used for pooling the RRs and SAS 9.2 (SAS Institute Inc., Cary, NC) was used for meta-regression models. All reported *p*-values were based on two-sided tests with a significant level of 0.05.

Results

We identified 1259 relevant titles from Pubmed and 1913 from EMBASE. We discarded 511 duplicates that were identified by both databases. We rejected 1036 because they were not original articles and 1284 because they were either in vitro studies or those evaluating populations other than peri- or post-menopausal women. Of the remaining articles, we excluded 120 because CRC was not an outcome; 163 because HT was not an exposure; 25 because no specific HT type was specified; 5 because they used a repeated population, 5 because they investigated effects only among cancer patients; 1 because it used extrapolated RR to calculate risk difference, 1 because it was a cross-sectional study and used prevalent instead of incident cases.[12] Review of references among eligible studies and key reviews identified one additional article.[13] One study investigated the association between tibolone and CRC risk.[14] Because tibolone has androgenic effect in addition to estrogenenic and progestogenic effect and is different from conventional HT regimens, we only included it in an exploratory analysis and treated tibolone as one kind of EPT. Another study only differentiated ET from EPT in an univariate analysis,[15] so we excluded it in our primary analysis. Of note, including it in an exploratory analysis did not materially change our results. Therefore, 20 articles were included in our primary analysis (Fig. 1).[4,5,13,16–32] Table 1 summarizes their characteristics.

Table 1. Summary of included studies

Refs.	Study design	First author	Published year	Study period	Geographical area	Sample size (No. of subjects enrolled)	Outcome definition[1]	Total no. of cases[2]	Follow-up period
4	RCT	Heiss	2008	1993–2005	USA	16,608[3]	CRC	189	8
5		Anderson	2004	1993–Feb 2004	USA	10,739	CRC	119	6.8
24		Hulley	2002	1993 and 2000	USA	2,763[4]	Colon	47	6.8
13		Nachtigall	1979	1965–1979	New York, USA	168	Colon	2	10
29	Cohort study	Henderson	2010	1995–2006	Calfornia	56,864	Colon	442	11
30		Hildebrand	2009	1992–2005	USA	67,412	CRC, colon, rectum	776	13.2
16		Johnson	2009	1979–1998	USA	56,733	CRC	717	14
26		Tannen	2007	1990–Feb 2001	UK	18,462	CRC	N/A	5.5
21		Tannen	2007	1990–Apr 1999	UK	51,388	CRC	N/A	3.64
23		Pukkala	2001	Jan 1994–Dec 1997	Finland	94,505	Colon, rectum	N/A	3.2
28		Persson	1996	1977–1991	Sweden	22,597	Colon, rectum	153 colon & 80 rectum	13
20		Risch	1995	1976–1990	Saskatchewan	32,973	Colon, rectum	298 colon & 166 rectum	15
32	Case-control study	Wu	2010	Jan 1998–Dec 2002	Los Angeles	1,586	Colon	831	N/A
31		Hoffmeister	2009	2003–2006	Germany	886	CRC	288	N/A
17		Newcomb	2007	Oct 1998–Feb 2002	Washington State, USA	2,066	CRC	881	N/A
25		Prihartono	2000	Jul 1992–Dec 1994	Massachusetts, USA	808	Colon	247	N/A
18		Jacobs	1999	1984–1993	Washington state, USA	2,020	Colon	341	N/A
22		Newcomb	1995	1990–1991	Wisconsin, US	2,316	Colon, rectum	3059 CRC, 2123 colon & 936 rectum	N/A
19		Campbell	2007	Jan 1997–Apr 2006	Ontario and Newfoundland & Labrador, Canada	2,607	CRC	1,035	N/A
27		Csizmadi	2004	Jan 1981–Jun 1998	Saskatchewan	15,175[5]	CRC, colon, rectum	3,019 CRC, 2,094 colon, 925 rectal	N/A

Table 1. Summary of included studies

Refs	HT type	Exposure categories	Age	Variables adjusted
4	EPT	Allocation and intention to treat in a RCT	50–79	Randomization, adjustment for participation in the calcium and vitamin D trial
5	ET	Allocation and intention to treat in a RCT	50–79	Randomization, and also stratified by age, prior disease, and randomization status in the dietary modification trial.
24	EPT	Allocation and intention to treat in a RCT	67	Randomization
13	EPT	Allocation and intention to treat in a RCT	N/A	Randomization
29	ET, EPT	Ever, recent use, duration <5, 5 to 15, >15 years	62.7	Race , body mass index, and physical activity, and stratified by age at cohort entry
30	ET, EPT	Current, former use, duration <5, 5 to <10, 10 to <15, 15 to <20, >20	50–74	Age, BMI, smoking, education, race, physical activity, current use of NSAIDs, vitamins, family history of CRC, hysterectomy, type of menopause, red and processed meat intake, history of CRC endoscopy, age at menopause, age at menarche, age at first live birth, parity, OC use, vegetable intake total calcium intake, energy intake, alcohol use
16	ET, EPT, P	Ever use, with duration <2, 2–5, and >5 years, current use, time since last use <5 , and > = 5 years	>55	Age, calendar time, NSAID use, cigarette smoking, family history of colorectal cancer, and red meat intake, percent calories from fat.
26	ET	Simulating WHI: intention to treat	55–79	Age, blood pressure, BMI , smoking, previous MI, CAD, TIA, stroke, heart failure, and DM
21	EPT	Simulating WHI: intention to treat	50–79	Age, blood pressure, BMI , smoking, previous MI, CAD, TIA, stroke, heart failure, and DM
23	EPT	Ever use	N/A	Age
28	ET, EPT	Ever use	Mean 54.5	Age
20	ET, EPT, P	Ever use	43–49	Age
32	ET, EPT	Current use with duration >1–5, >5–10, >10, former use with duration >1–5, >5–10, >10	55–74	Age, race/ethnicity ,education, type of menopause, age at menopause, screening tests, BMI, smoking years , alcohol years , parity, family history of colon cancer, years of OCs use, years of aspirin use and years of physical activity
31	ET, EPT	Ever use	45–75	Age, county of residence, previous colorectal endoscopy, previous general-health screening examination, diabetes, average alcohol consumption, regular use of NSAIDs, and body mass index
17	ET, EPT	Ever use with duration <5 and >5 years, current use, time since last use < 5 and > 5 years	50–74	Age, BMI, DM, smoking, regular NSAID use, colorectal screening sigmoidoscopy history, physical activity, and family history of CRC
25	ET, EPT	Recent use (time since last use<1 year), long term use (>5 years)	40–69	Cases and controls were matched on 7-year age group, gender, and town precinct; in model adjusted for fat, fruit, and vegetable intake, vigorous leisure time physical activity, BMI, and history of screening for CRC
18	ET, EPT	Ever use with duration <2.5 vs. >2.5 years for ET, and <1.5 vs. >1.5 years for EPT	55–79	Smoking, height, weight, BMI, oral contraceptive use, parity, age at first birth, age at menopause, and hysterectomy status
22	ET, EPT	Recent use (interval from last use<1 year)	30–74	Age, use of sigmoidoscopy screening, family history of large-bowel cancer, BMI, and intake of beer or hard liquor

Table 1. Summary of included studies (Continued)

Refs	HT type	Exposure categories	Age	Variables adjusted
19	ET, EPT	Ever use with duration <5 and >5 years, current and former use	20–74	Age, province of residence, education, ever use of OCs, colorectal screening endoscopy, physical activity, BMI, and reason for menopause
27	ET, EPT	Ever use with duration <5 and >5 years	>50	Cases and controls were matched on month and year of birth, and living in Saskatchewan at the time the colorectal cancer case, to whom she was matched having had a sigmiodoscopy 3–5 years prior to index dates

(1) CRC: colorectal cancer, colon: colon cancer, rectum: rectal cancer. (2) The number of cases included in the analysis for the effect of specific menopausal hormone therapy on CRC. (3) 16608 in the trial phase, 15730 in the post-interventional phase. (4) 2763 in HERS; 2321 HERS II. (5) 15175 for CRC, 10534 for colon cancer, 4641 for rectal cancer.
Abbreviations: RCT: randomized controlled trial; ET: estrogen therapy; EPT: combined estrogen-progestogen therapy; P: progestogen alone; BMI: body mass index; MI: myocardial infarction; CAD: coronary artery disease; TIA: transient ischemic attack; DM: or diabetes mellitus; BP: blood pressure.

Figure 2 shows the RR estimates reported by individual studies as well as the random effects pooled estimates for both ET and EPT. The pooled RRs of CRC were 0.79 (0.69-0.91) for ET ever use and 0.74 (0.68-0.81) for EPT ever use. Among former users, EPT was associated with a significantly lower RR of CRC compared to ET ($p = 0.008$); in contrast, among current users, the RR was not significantly different for ET *vs.* EPT ($p = 0.12$). When pooled together, there was no apparent difference in the overall estimate for ET and EPT ever use ($p = 0.43$, Table 2). Substantial heterogeneity was found among studies reporting RRs for ET (Cochrane Q test: $p < 0.001$). Test for heterogeneity among EPT studies were not significant (Cochrane Q test $p = 0.88$).

Table 2 shows the results from the stratified analyses. For ET studies, the inverse association was more prominent in case-control studies than in RCTs and cohort studies. The pooled RRs of CRC for ET studies were 0.65 (0.51-0.82) from case-control studies, 0.89 (0.75-1.04) from cohort studies, and 1.08 (0.75-1.55) from the RCT. This difference was significant in the meta-regression model where case-control studies gave a significantly smaller RR than cohort studies ($p = 0.018$) and RCTs ($p = 0.0023$). On the other hand, EPT studies reported similar RRs regardless of study design. In both ET and EPT studies, there was a trend that pooled estimates for colon cancer were lower than those for rectal cancer. The pooled RR associated with ET ever use was 0.74 (0.61-0.89) for colon cancer, 0.86 (0.75-0.99) for rectal cancer and 0.84 (0.73-0.97) for CRC. Similarly, the RR associated with EPT ever use was 0.78 (0.69-0.89) for colon cancer, 0.87 (0.70-1.08) for rectal cancer and 0.74 (0.66-0.82) for CRC.

There was no apparent difference in pooled RRs of CRC for either ET or EPT use of different duration. For recency of HT use, however, differences in the risks of CRC were evident among ET *vs.* EPT users. In the ET group, the RR of CRC associated with current use was 0.70 (0.57-0.85), which was significantly lower than that associated with former use (RR 0.86, 0.67-1.11; p-value for difference between current and former use <0.0001). For EPT, however, there was no significant difference between current use and former use ($p = 0.25$).

Figure 3 shows funnel plots by HT types. There was no evidence of publication bias for either group. For ET, the p-value of Egger's test was 0.48 and Begg's test was 0.41. For EPT, the p-value of Egger's test was 0.65 and Begg's test was 0.54.

We examined the included studies for potential information, selection and confounding bias. We found misclassification of the outcome unlikely in all included studies since all of the cancer cases were identified through either registries or chart review which usually requires verification with pathology reports. As for misclassification of HT status, we found that ET studies where HT exposure was assessed by self-report showed a pooled RR of 0.68 (0.57-0.81), which was significantly lower than that obtained by objective methods with a pooled RR of 0.99 (0.84-1.16, p-value for the

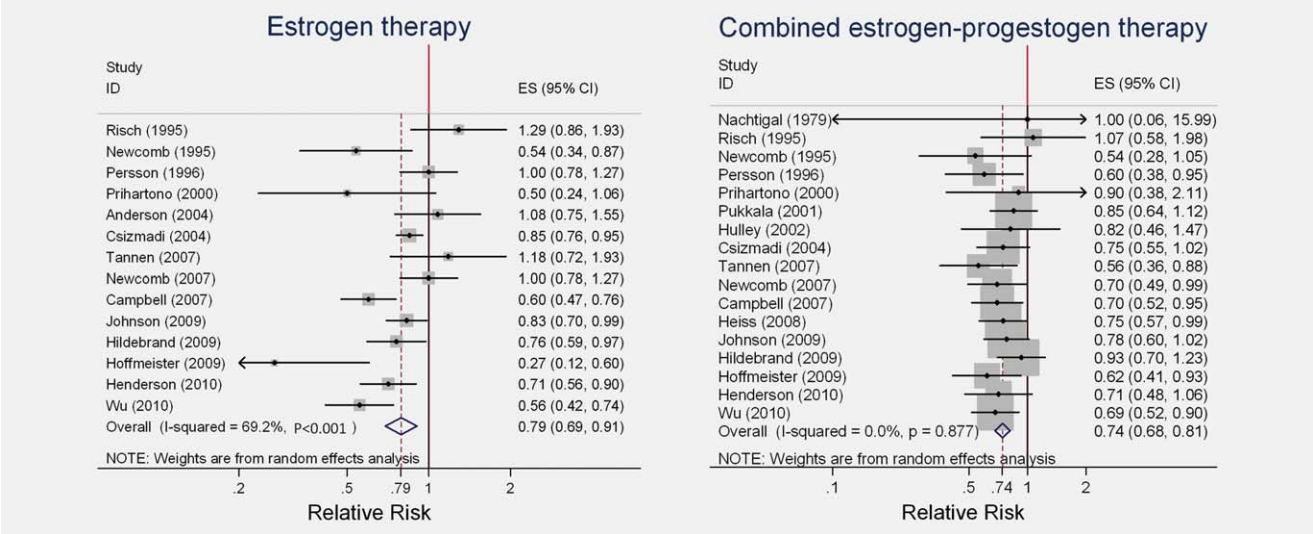

Figure 2. Forest plots by menopausal hormone therapy types; on the left are the first author and the publication year of the study reporting the estimate. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

difference <0.0001). However, this difference was not observed among EPT studies. In our meta-regression models, we did not find significant differences in studies with *vs.* without other quality-related characteristics, including multivariate adjustment ($p = 0.74$ for ET and 0.53 for EPT studies), adjustment for screening procedures ($p = 0.10$ for ET and 0.30 for EPT studies) and potential for selection bias ($p = 0.28$ for ET and 0.26 for EPT studies).

Sensitivity analysis was done by omitting one study at a time to evaluate the influence of individual studies. Figure 4 demonstrates how the pooled estimate changed when a certain study was omitted. Overall, no single study was identified as extremely influential for either HT type. For ET, omitting the most influential study by Wu et al. resulted in a pooled RR of 0.82 which was close to the overall pooled estimate of 0.79 and within its 95% CI of 0.69-0.91. No single influential study was identified among EPT studies.

Discussion

Based on our meta-analysis, there is consistent evidence supporting the association between EPT and CRC risk reduction. However, we found significant heterogeneity among the ET studies. Several factors were identified as potential explanation for this heterogeneity, including: recency of HT use, study design, and method of HT exposure assessment.

The WHI study showed a significant inverse association with CRC risk for EPT, but not for ET. While our results for EPT are consistent with WHI findings, we found that current use of ET was also associated with a reduced risk of CRC. This difference may be explained by the low adherence rates in the ET arm of the WHI: 53.8% of women had stopped taking the study medication by the end the study. Nondifferential misclassification of the exposure could bias the results towards the null. Furthermore, women assigned to receive ET who dropped out early during follow-up may very well represent former rather than current users of ET, which is consistent with our analysis showing a lack of association between former use and CRC risk. Grodstein et al. conducted a meta-analysis summarizing the effect of HT on CRC risk from 18 observational studies and showed an overall inverse association, with a pooled RR of 0.80 (0.74-0.86).[2] In the current study, 13 articles have provided estimates for both ET and EPT use. By combining the two arms, we found the pooled RR of CRC associated with mixed HT ever use to be 0.81 (95% CI 0.74-0.89), which is very similar to the results from the prior study. Another meta-analysis published by Nanda et al. found that this association was limited to recent use (RR 0.67, 0.59-0.77); ever use of HT was not significantly associated with colon cancer risk reduction (RR 0.92, 0.79-1.08).[3] Since the majority of the articles included in these two prior meta-analyses did not differentiate between ET and EPT use, their pooled estimates cannot be directly compared with our results.

In our analysis, recency played an important role for the association between ET and CRC risk. Specifically, current users of ET, but not former users, had a significant CRC risk reduction. For EPT users, however, both current and former users had a significant reduction in CRC risk. As a result, EPT was associated with a significantly lower RR of CRC than that conferred by ET ($p = 0.008$) among former users, but not among current users ($p = 0.12$). When pooled together, there was no apparent difference in the overall estimate for ET and EPT use ($p = 0.43$).This finding suggests that the inclusion of progestogen in HT not only strengthens its effect against CRC, but makes this effect persist despite discontinuing the medication. On the other hand, duration of use did not appear to modify CRC risk in either ET or EPT significantly. The fact that even short-term use reduced CRC

Table 2. Subgroup analysis

Hormone type[1]	Variable	Subgroups	No. of studies	Pooled estimates	*p*-values for significant association	I-squared[2], % (95%CI)	*p*-values for hetero-geneity[3]	*p*-values from meta-regression for variables of the strata[4]
Hormone type		ET	14	0.79 (0.69–0.91)	0.001	69 (47–82)	0.001	0.43[5]
		EPT	17	0.74 (0.68–0.81)	<0.001	0 (0–51)	0.88	
ET	Design	RCT	1	1.08 (0.75–1.55)	0.68	–	-	0.0023[6]
		Cohort	6	0.89 (0.75–1.04)	0.136	53 (0–81)	0.061	0.018[6]
		Case-control	7	0.65 (0.51–0.82)	<0.001	78 (53–89)	<0.001	Ref
	Outcome definition	CRC	9	0.84 (0.73–0.97)	0.017	67 (32–83)	0.002	Ref
		Colon	7	0.74 (0.61–0.89)	0.002	62 (14–83)	0.05	0.74[7]
		Rectal	5	0.86 (0.75–0.99)	0.029	0 (0–79)	0.55	0.28[7]
	Duration	Short-term	9	0.88 (0.80–0.96)	0.004	0 (0–65)	0.46	0.08
		Long-term	9	0.74 (0.61–0.91)	0.004	65 (29–83)	0.003	
	Recency	Current use	5	0.70 (0.57–0.85)	<0.001	61 (0–85)	0.036	<0.0001
		Former use	5	0.86 (0.67–1.11)	0.25	68 (18–88)	0.013	
	HT assessed by self-reported methods	Yes	9	0.68 (0.57–0.81)	0.008	66 (30–83)	0.003	<0.0001
		No	5	0.99 (0.84–1.16)	0.90	41 (0–78)	0.15	
EPT	Design	RCT	3	0.77 (0.59–0.98)	0.037	0 (0–90)	0.95	0.15[6]
		Cohort	7	0.79 (0.69–0.90)	0.001	6 (0–72)	0.38	0.03[6]
		Case-control	7	0.69 (0.60–0.80)	<0.001	0 (0–71)	0.96	Ref
	Outcome definition	CRC	10	0.74 (0.66–0.82)	<0.001	0 (0–62)	0.75	Ref
		Colon	9	0.78 (0.69–0.89)	<0.001	0 (0–65)	0.81	0.27[7]
		Rectal	6	0.87 (0.70–1.08)	0.41	0 (0–75)	0.90	0.77[7]
	Duration	Short-term	9	0.77 (0.66–0.91)	0.001	0 (0–65)	0.85	0.92
		Long-term	9	0.81 (0.70–0.94)	0.005	0 (0–65)	0.50	
	Recency	Current use	5	0.80 (0.69–0.93)	0.003	6 (0–80)	0.37	0.25
		Former use	5	0.70 (0.56–0.89)	0.003	0 (0–79)	0.59	
	HT assessed by self-reported methods	Yes	9	0.74 (0.66–0.83)	<0.001	0 (0–65)	0.75	0.84
		No	8	0.76 (0.66–0.87)	<0.001	0 (0–68)	0.70	

[1]ET: estrogen therapy; EPT: combined estrogen-progestogen therapy. [2]I^2 statistic described by Higgins et al to assess heterogeneity. [3]Cochrane Q test for heterogeneity. [4]Results of meta-regression models, regressing log-transformed relative risks by the variable of the stratum. [5]$p = 0.12$ among current users; $p = 0.008$ among former users. [6]p value of the test that pooled estimate of studies of the stratum is significantly different from that of case-control studies. [7]p value of the test that pooled estimate of studies of the stratum is significantly different from those with CRC as the outcome. Abbreviations: CRC: colorectal cancer; Colon: colon cancer; rectal: rectal cancer, Ref: referent category.

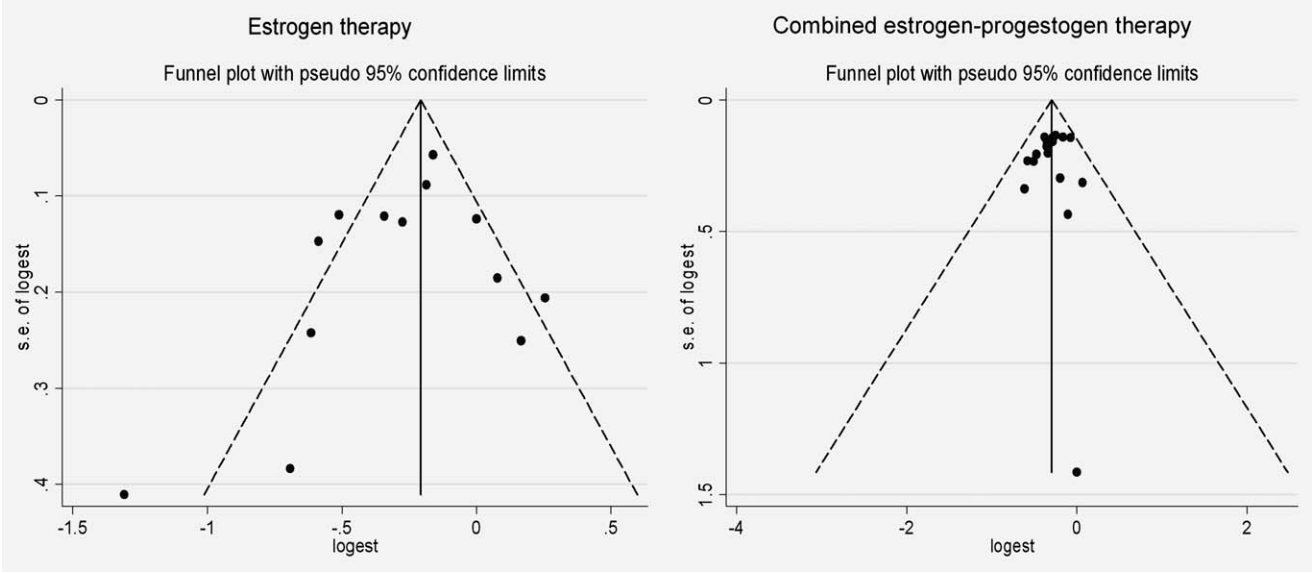

Figure 3. Funnel plots by menopausal hormone therapy types.

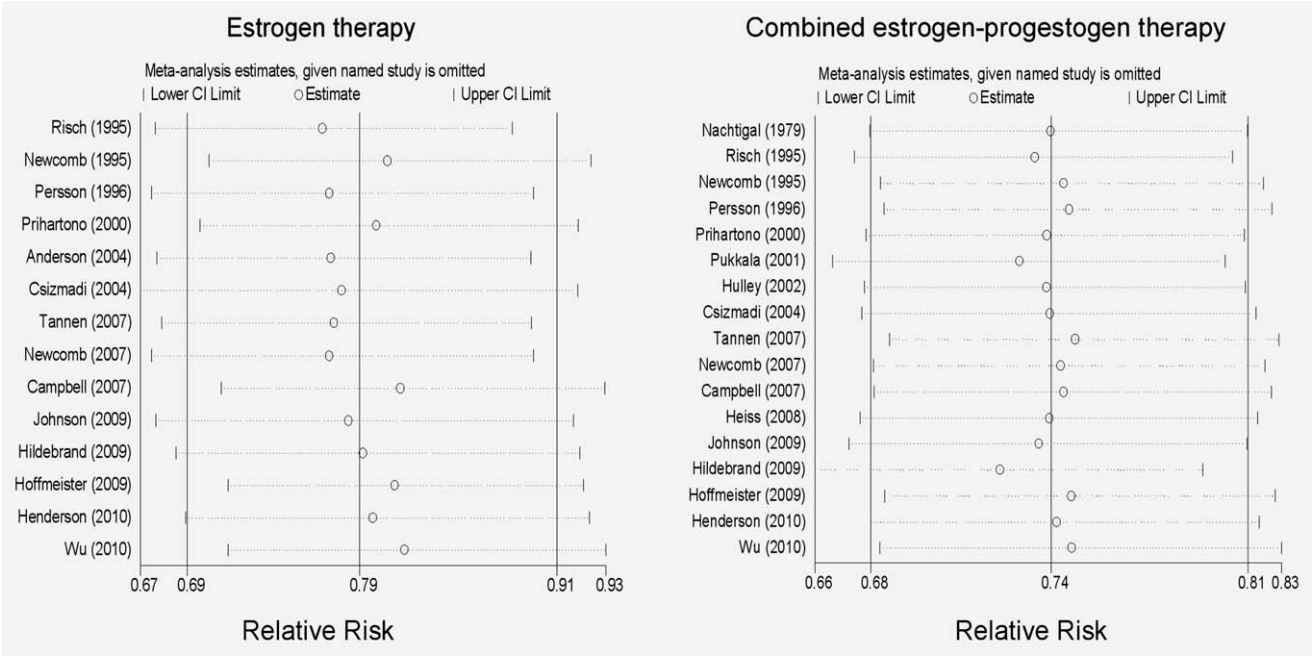

Figure 4. Sensitivity analysis.

risk suggests that estrogens may intervene on carcinogenesis in the late stages of disease by effectively halting tumor progression. The immediacy of this effect makes it biologically implausible for HT to have acted on tumor formation, since a time lag between exposure and effect would be expected in this setting.

ET was associated with lower CRC risk in case-control studies only. This finding based on study design was not observed in the EPT group. It is important to note that case-control studies included more CRC cases than in RCTs and cohort studies. Among the selected articles in the current meta-analysis, the mean total number of cases was 89 (standard error [s.e.] 6-166) for RCTs, 361 (s.e. 229-493) for cohort and 969 (s.e. 496-1442) for case-control studies. Because CRC is a relatively rare disease, smaller studies may not be sufficiently powered to detect the full effect of HT. However, this factor can only explain the difference in the degree of significance, but not in the magnitude of association.

Another potential explanation for a significant association only in case-control studies is the method of HT exposure assessment. When compared to other study designs, case-control studies were more likely to use self-report to identify

HT exposure status (Fisher's exact $p = 0.009$). Self-report is subject to recall inaccuracies whereby former users are likely to recall their use incorrectly. Csizmadi et al. compared self-reported data with objective dispensing data for a subsample of women and found former users tended to forget their HT use and thus be misclassified as nonusers.[27] As a result, the investigators of case-control studies might be ascertaining current or recent users despite that they attempted to identify ever users (encompassing both current/recent and former users). Indeed, the significant association between "ever use" of ET and CRC risk observed in case-control studies was similar to that for current use of ET regardless of study design. In a subanalysis, we also found that, even among case-control studies, HT assessment by self-report showed a lower pooled RR of 0.59 (95% CI 0.44-0.80) when compared to that using other methods (RR 0.85, 0.76-0.95). Taken together, it is likely that only current use of ET was associated with a CRC risk reduction and case-control studies tended to find this specific association if the HT exposure was assessed by self-report. Incorporating former use, studies of other designs found mostly nonsignificant association between ever use of ET and CRC risk. Interestingly, recency appeared to play a less important role for the effect of EPT on CRC risk. Therefore, case-control design and HT assessment by self-report did not make a significant difference in the association between EPT and CRC risk.

It is also possible that the stronger association between ET use and CRC risk in studies using self-report HT status may be due to misclassification of EPT as ET use, assuming EPT was associated with a lower RR compared to that of ET. If this were the case, we would expect that EPT studies using self-report to find a weaker association between EPT use and CRC risk (due to mixing of EPT and ET, the latter which has a weaker association with CRC risk). We did not observe this pattern (RR was 0.74 *vs.* 0.76 in self-report *vs.* non-self-report EPT studies). Moreover, current use of ET was associated with a lower RR compared to former use in our analysis. Theoretically, the possibility that women incorrectly reported using ET when they actually used EPT is higher in former than in current users. Consequently, we should have observed stronger association between CRC risk and ET former use compared to that with ET current use, which is not apparent in our findings.

Our results are consistent with the WHI[33] and prior meta-analysis[3] that HT is associated with a lower risk of CRC or colon cancer, but less so with rectal cancer. This trend was observed for both ET and EPT studies. One possible explanation is that colon and rectal cancers behave differently. First, there is strong evidence supporting the histological differences between the two diseases. Second, the anatomical location of rectal tumors allows for more rapid progression of disease. As it is not surrounded by peritoneum, neoplasms in the rectum encounter fewer barriers for spread and growth. If the effect of HT actually comes from its effect on the late stages of carcinogenesis, the aggressiveness of rectal cancer leaves less opportunity for HT to intervene on cancer development.

Several mechanisms have been postulated to explain the effect of HT on CRC risk. One theory involves carcinogenic bile acid of which the synthesis is reduced for woman on ET.[34–36] Based on in vitro studies, others postulate that estrogen may have a direct effect on steroid hormone receptors found in colonic epithelium. Activation of the receptors can pose a downstream effect that likely leads to inhibition of cell growth.[37–39] Furthermore, some prior research suggests that estrogen itself is tumor suppressive and HT may enhance this relationship. For instance, methylation of the promotor region of estrogen receptor gene has been linked to human colon cancer.[40] There is also evidence to indicate that use of estrogen can reduce the serum insulin-like growth factor-1 (IGF-1) levels for postmenopausal women.[41,42] Increased endogenous IGF-1 secretion has been linked with higher risk of CRC.[43,44] Estrogen may also be tumor suppressive by inducing apoptosis in premalignant cells caused by p53 and p21 protein expression.[45] Moreover, progestogen could accelerate the conversion of the less potent estrone to the more potent estradiol by inducing the key enzyme, isozyme of 17h-hydroxy steroid dehydrogenase.[46,47]

Although our results support the association between EPT and CRC risk reduction, some have raised doubts about this association. It is observed that only early-stage colon cancers were reduced by EPT in WHI,[33] and the risk reduction for colon cancer of both early and advanced stage was not observed in the postintervention phase. Since WHI was a large-scale RCT, it is unlikely that the observed association was due to confounding. An alternative explanation is that EPT delays the diagnosis of early-stage colon cancer through a biologic effect (*e.g.*, decrease in tumor bleeding).[33,48] While results from WHI postinterventional phase did not show any mortality benefit from the lower incidence rate among EPT users compared to nonusers, this is compatible with the explanation of delayed diagnosis caused by EPT or simply the inadequate length of follow-up to reveal any demonstrable mortality benefit.[48] In contrast, Newcomb et al. found that EPT was associated with a risk reduction not only for localized, but also for advanced CRC.[17] We believe further studies on the association between specific stages of CRC and ET *vs.* EPT as well as on the potential biologic mechanism for the effect of EPT on delaying CRC diagnosis are needed to confirm the causal relationship.

In an exploratory analysis, we included an RCT investigating the association between tibolone and CRC risk.[14] Tibolone is approved in 90 countries to treat menopausal symptoms and its metabolites have estrogenic, progestogenic and androgenic activities.[49,50] Adding our study to the EPT group yielded a pooled RR of CRC 0.74 (0.68-0.81), which is essentially unchanged compared to our primary analysis. However, the magnitude in risk reduction of colon cancer associated with tibolone use reported by this additional study (RR 0.31, 0.10-0.96) was greater than conventional EPT (pooled RR of

colon cancer: 0.80, 0.65-0.99). This difference can be explained by different biologic effects of tibolone *vs.* conventional EPT; or simply by chance since this comparison was based on only one study for tibolone with 17 colon cancer cases.

We need to acknowledge several limitations. For meta-analyses to combine and integrate results of independent studies together, the studies should be similar and comparable. However, this can be a strong assumption when dealing with a pool of RCTs and observational studies that are based on different populations, different diseases and different methods of exposure and outcome assessment. Indeed, among ET studies, we observed significant heterogeneity, mainly by study design. Further stratification of the analysis according to some of the study characteristics provided more homogeneous subgroups, but some subgroup analyses were based on relatively few studies (*e.g.*, the analyses for duration and recency of HT) and thus the results should be interpreted with caution. In addition, despite our attempt for a thorough search, it is possible that eligible articles may have been missed. This may reduce the power of our study and lead to a more conservative pooled estimate and it may also result in biased pooled estimates if we selectively missed studies reporting extreme estimates. To avoid this bias, the decisions regarding inclusion or exclusion of a study had been made independently of their results. It is reassuring that our sensitivity analysis did not identify any influential studies. In addition, our meta-analysis involves 16 observational studies that are vulnerable to confounding. Nonetheless, the "multivariate adjustment for confounding" was not a significant factor affecting RR estimates in the meta-regression model. Furthermore, because of the small number of selected studies in our analysis, we were not able to categorize HT more finely than ET *vs.* EPT; and thus cannot clarify if different formulations of ET or EPT have differential effects on CRC risk. Finally, given the complex risk and benefit profile of HT regarding other health outcomes, the current study is meant to investigate the relationship between specific HT and CRC in order to better understand this disease, rather than to provide evidence for HT as CRC prevention.

Conclusions

There is consistent evidence to support an association between EPT and CRC risk reduction. The literature for the association between ET and CRC risk is heterogeneous, but our analysis suggests that current use of ET may be associated with a decreased risk. Further studies that explore the underlying biologic mechanisms for the effect of HT on CRC are needed before a definitive causal relationship between exposure and outcome can be drawn.

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