

Hormone Therapy and the Impact of Estrogen Intake on the Risk of Ovarian Cancer

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Background: The association between menopausal hormone therapy (HT) and risk of ovarian cancer is as yet equivocal, and the effect of estrogen and estrogen-progestogen therapy, specifically the effect of the cumulative hormone intake, is unclear.

Methods: We conducted a nationwide population-based case-control study in Denmark. Cases were women aged 35 to 79 years with incident ovarian cancer diagnosed between January 1, 1995, and May 30, 1999. Controls were frequency age-matched women from the Danish Central Population Register. The analyses included data on 376 cases who have not undergone hysterectomy and 1111 controls.

Results: The risk of ovarian cancer in relation to oral HT increased with the cumulative intake of the estrogen component of HT but not with the duration or the cumulative intake of the progestogen component when

the 3 variables were mutually adjusted. A simple trend was found such that each additional gram of estrogen was associated with the same relative increase. The odds ratio was constant throughout the range of cumulative intake. After adjustment for established risk factors, the estimated odds ratio per each additional gram of cumulative estrogen was 1.056 (95% confidence interval, 1.003-1.112), corresponding to an odds ratio of 1.31 (95% confidence interval, 1.01-1.70) per 5 g of estrogen.

Conclusions: Oral HT is associated with risk of ovarian cancer in women who have not undergone hysterectomy. Our results imply that the risk increases with cumulative oral estrogen intake but not with duration of HT, indicating that the increased ovarian cancer risk associated with oral HT may be diminished substantially by minimizing the daily dose of estrogen from oral HT.

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HORMONE THERAPY (HT) IS increasingly used in developed countries to alleviate menopausal symptoms in healthy women.

Hormone therapy has a preventive effect on some diseases, such as hip fracture and colorectal cancer, but HT is a risk factor for coronary heart disease, stroke, breast cancer, and endometrial cancer.^{1,2} Because cancer of the ovaries shares some risk factors with breast and endometrial cancer, it has been suggested that HT could also increase the risk of ovarian cancer, probably by promoting proliferation and malignant transformation of ovarian epithelial cells.³ Because ovarian cancer is the most lethal of the gynecologic cancers, an increased risk could have important implications.

Several observational studies⁴⁻¹⁵ have examined the association between HT and ovarian cancer, including 2 large prospective cohort studies,^{5,6} a reanalysis of 5 European case-control studies,^{7,8} a reanalysis of 12 US case-control studies,⁹ and 2

meta-analyses.^{10,11} Despite this, results are equivocal, although the more recent studies^{4-6,12} tend to show evidence of an increased ovarian cancer risk. Various preparations are available for perimenopausal and postmenopausal hormonal treatment, but most preparations on the European (Danish) market contain the potent natural unconjugated estrogen estradiol. European women do not commonly use conjugated equine estrogens, which mostly are prescribed in the United States. Furthermore, in Europe, progestogens have been more frequently used than in the United States. Thus, most data thus far concern the use of conjugated estrogens.^{1,5,6,9,12,13,15} Some studies^{4,6,12-15} have included unopposed estrogen therapy and combination therapy with a progestogen, but there is currently no information about the relationship between the cumulative intake of these hormones and risk of ovarian cancer.

We report findings from a large Danish population-based case-control study on

the risk of epithelial ovarian cancer and HT use in women who have not undergone hysterectomy (hereafter referred to as nonhysterectomized women). The study was designed to address especially long-term oral HT intake and different administration modes, making it possible to assess for the first time the impact of cumulative oral HT intake on risk of epithelial ovarian cancer according to different estrogen and progestogen combinations.

METHODS

CASES

The study area consisted of Danish counties with a gynecologic hospital department (ie, the municipalities of Copenhagen and Frederiksberg and the counties of Copenhagen, Frederiksborg, Roskilde, Western Sealand, Storstroem, Funen, and Southern and Northern Jutland). Between January 1, 1995, and May 30, 1999, cases were recruited from the 16 gynecologic departments in the study area. Potential cases were Danish-born women, aged 35 to 79 years, scheduled for an explorative laparotomy because of the suspicion of ovarian malignancy. Potential cases were requested to participate by providing a blood sample and engaging in a personal interview. A first definition of the case group took place on the basis of the preoperative macroscopic or microscopic findings. Women with invasive or borderline tumors were allocated to a personal interview at the hospital. To ensure that all eligible cases in the study area were included, the study database was linked to the Danish Cancer Registry every second month. If a woman was registered in the Danish Cancer Registry with ovarian cancer but had not primarily been included in the study, she was contacted by letter and asked to participate in an interview.

In the final case definition, hospital files of the included women were scrutinized, and information about the histologic diagnosis and the procedure and findings at surgery was collected. Diagnoses were categorized into 8 main histologic groups (borderline tumors, nonepithelial tumors, serous adenocarcinomas, endometrioid adenocarcinomas, mucinous adenocarcinomas, papillary adenocarcinomas, clear cell adenocarcinomas, and undifferentiated carcinomas).¹⁶ On approximately 30% of the cases, a histopathologic review was performed in a blinded fashion by an experienced ovarian cancer pathologist (L.C.). In case of disagreement, another pathologist reviewed the slides, and a consensus diagnosis was obtained. In terms of invasiveness, agreement between the original hospital diagnosis and the review diagnosis was present in 98% of the cases. For cases in which the histologic slides were not reviewed (approximately 70%), all histologic descriptions were scrutinized by the study pathologist for consistency between the description and the resulting diagnosis. Through this procedure, it was possible to define the histologic subgroup more precisely in 14% of the cases (ie, a diagnosis of undifferentiated carcinoma could be classified as, eg, a papillary carcinoma). This study used the reviewed diagnoses.

By the end of the study, 970 cases of histologically verified malignant ovarian tumors had been identified in the study area. A total of 53 women were considered too ill to participate, and 45 women died before being contacted, leaving 872 cases for the study. In all, 692 women (79.4%) were included in the study; 586 women with an interview were included in the final analyses; 106 with a blood sample only were not included in the final analyses. Women interviewed more than 1 year after the ovarian cancer diagnosis ($n=27$) were excluded from the analyses. More than 75% of the included cases were interviewed earlier than 9 weeks (2.2 months) after the operation, the median time being 1.7 months (range, 0.06-12.0 months).

Because epithelial and nonepithelial ovarian tumors have different embryologic, pathologic, and epidemiologic characteristics,¹⁷ nonepithelial tumors ($n=28$) were excluded, leaving 531 cases for the final analyses (326 serous adenocarcinomas [61.4%], 71 endometrioid adenocarcinomas [13.4%], 47 mucinous adenocarcinomas [8.8%], 36 papillary adenocarcinomas [6.8%], 43 clear cell adenocarcinomas [8.1%], and 8 undifferentiated carcinomas [1.5%]).

CONTROLS

The controls were randomly drawn among Danish-born women in the study area by means of the computerized Danish Central Population Register (includes all inhabitants in Denmark, and each individual has a unique personal identification number). Using the age distribution of the women with ovarian cancer registered in the Danish Cancer Registry between 1987 and 1992, controls were frequency matched in 2- and 3-year age intervals.

Between September 1, 1995, and August 31, 1997, the controls were invited by letter to participate in the study by partaking in a personal interview and providing a blood sample. If a woman did not give notice about her participation, she was contacted by telephone or a second letter, inviting her to take part in the study by engaging in a personal interview and providing a blood sample or by participating in a telephone interview. The telephone interview was less comprehensive than the personal interview, but it covered the most important known risk factors for ovarian cancer. Regarding HT, women who participated in a telephone interview were only asked about ever and never use of HT and duration of HT intake. All women invited to take part in the study as controls were asked whether both their ovaries had been removed previously. If so, these women were excluded as controls.

Of the 3639 women who were invited to take part in the study as controls, contact could not be achieved with 301. Of the remaining 3338 potential controls, 269 were excluded owing to bilateral oophorectomy, 6 had moved out of the study area, and 126 were too ill to participate, leaving 2937 women as eligible controls. We enrolled a total of 1979 controls (1460 women participated via a personal interview and 519 women participated via a telephone interview). Because the telephone interview included less detailed information on HT use than the personal interview, the present analyses are based entirely on women who participated in the personal interview.

INTERVIEWS

Written informed consent was obtained from each participant before the interview. All interviewers were women and were nurses, medical students, and laboratory technicians. They were all trained by the same physician (E.G.) and were given written guidelines about how to perform the interview. The interviewers were kept unaware of the specific hypotheses tested but could not be kept unaware of the case-control status because interviews of cases took place in hospitals and interviews of controls took place in the women's homes or at the Danish Cancer Society. The questionnaire was designed as a person-to-person interview and was used to ascertain social, reproductive, medical, gynecologic, and dietary history. We assessed well-known risk factors, such as family history of ovarian cancer (whether her mother, sister, or daughter had had ovarian cancer), menopausal status (postmenopausal is defined as no natural menstrual period for the past 12 months), infertility (with medical attention sought), and sterilization. Information about previous hysterectomy was also obtained at the interview, and all women who reported a hysterectomy (75

cases and 137 controls) were excluded, leaving 456 cases and 1323 controls for the analysis.

A life-event calendar especially designed for this study was used to obtain detailed information regarding pregnancies, abortions, births, breastfeeding periods, contraception, fertility drug use, and HT. To obtain as specific and accurate information on the type and the intake of HT as possible, we used color photographs of all hormonal products (tablets, injections, vaginal gels, vagitories, and transdermals) that were on the market in Denmark between January 1, 1955, and December 31, 1994. For HT, we obtained information on the length and calendar year for each period the woman used a specific product, how many days per month she used it, and the reason she used it. If she could not remember the name of the specific brand, photographs of brands marketed in the relevant period were shown to the participant.

The generic name(s), administration mode, intake (and, for sequential oral contraceptives [OCs] and HT, the variation in the dosage) of each product, date (month and year) of registration, date of release for sale, and date of withdrawal from the market were registered for all products. These data were collected from the Danish Board of Health, medical companies, pharmacists, and the Danish Pharmaceutical Register (1964-1995) for 237 different brands. The information was entered into a database and linked to the information from each woman.

CLASSIFICATION OF HT EXPOSURE

In the present study, HT was defined as oral HT administered for at least 1 month. Any HT used by cases after diagnosis was not considered. All women who had ever undergone transdermal HT (5 cases and 35 controls) or preparations for injections (17 cases and 48 controls) were excluded from the analyses because the systemic effect is not fully comparable to that of oral HT. We considered the systemic effect of vaginal HT treatment as negligible. Therefore, we only included oral intake of HT in the calculated cumulative (total lifetime) HT intake, implying that the women who had only used HT vaginally were included in the group of never-users of oral HT and that women who had used oral and vaginal HT contributed only with their oral HT use. Oral HT administration covered continuous unopposed estrogen (estradiol or estriol) regimens, continuous progestogen (cyproterone acetate, medroxyprogesterone acetate, norethisterone acetate, or levonorgestrel) regimens, sequential and cyclical estrogen-progestogen regimens, and continuous combined estrogen-progestogen regimens. We excluded all women for whom we could not calculate the cumulative oral HT intake of estrogen and progestogen because the women could not specify the precise brands used or the exact number of days of use per month (54 cases and 100 controls), and we excluded women who had ever used artificial steroids with combined estrogenic, progestogenic, and androgenic effects (tibolon) (2 cases and 16 controls).

Finally, we excluded 2 cases and 13 controls who had ever used artificial estrogens as HT because this group was too small to allow evaluation of whether artificial estrogen is similar to natural estrogens regarding the association with ovarian cancer.

STATISTICAL ANALYSIS

Associations between different aspects of HT use and the risk of ovarian cancer were analyzed using logistic regression models. All analyses included age categorized according to the grouping used in the sampling of the controls. Duration and the different intake variables were entered as linear variables on the

log-odds scale. The analyses sought to evaluate how ovarian cancer risk is associated with cumulative HT exposure among HT users, so an indicator variable of ever vs never use of oral HT was included to ensure that nonusers did not bias the estimated association for low exposures.

The linearity of the associations with duration and cumulative oral HT intakes of estrogen and progestogen was evaluated in more flexible models using linear splines¹⁸ with 5 boundaries placed close to the sextiles among oral HT users in the analyzed data. No significant deviations from linearity were found for any of the 3 variables ($P > .31$ for all). Tests were based on the likelihood ratio test statistic, and 95% confidence intervals (CIs) were based on the Wald test on the log-odds scale. Statistical analysis software (SAS release 6.12 for UNIX; SAS Institute Inc, Cary, NC) was used.

RESULTS

After all exclusions, the final study population comprised 376 cases (nonhysterectomized women with epithelial ovarian cancer) and 1111 nonhysterectomized controls. The distribution of the different histologic types in this final case group was nearly identical to that in the case group before the different exclusions (data not shown). **Table 1** provides the distribution of selected variables in cases and controls. In general, case-control differences reflected established epidemiologic associations. Controls had more frequently been pregnant, used OCs, and undergone sterilization, whereas more cases than controls had sought medical attention for infertility problems and had a first-degree relative with ovarian cancer.

Cases and controls also had different patterns of HT use (**Table 2**). More cases than controls had ever undergone HT. In cases and controls, HT was most often administered orally, and only a few had received vaginal HT only or a combination of oral and vaginal HT. The detailed registration of HT, including time using each specific brand, made it possible to distinguish between different types of HT use and between duration and cumulative oral HT intake of estrogen and progestogen. The distribution of these variables is given in Table 2. The cumulative oral intake of estrogen among ever-users of oral HT varied between 0.04 and 41.1 g (data not shown), and 19 cases (20% of ever-users among cases) and 29 controls (13% of ever-users among controls) had a cumulative intake of 10 g of estrogen or more. Within the case group and within the control group, the most frequent type of treatment included the combined use of estrogen and progestogen.

In **Table 3**, associations between cumulative oral HT use, duration of HT, and ovarian cancer are given. Higher cumulative oral HT intake of estrogen was associated with a significant increase in ovarian cancer risk ($P = .02$). Less pronounced and nonsignificant increases in risks were seen for cumulative oral HT intake of progestogen ($P = .78$) and duration of oral HT ($P = .09$). When the 3 variables were mutually adjusted, the risk still increased (borderline significantly) with cumulative oral HT estrogen intake ($P = .07$), whereas it decreased nonsignificantly with cumulative oral HT progestogen intake ($P = .75$) and duration of oral HT use

Table 1. Selected Characteristics of Epithelial Ovarian Cancer Cases and Controls Who Have Not Undergone Hysterectomy

Characteristic	Cases, No. (%) (n = 376)	Controls, No. (%) (n = 1111)
Age, y		
35-39	16 (4.3)	74 (6.7)
40-44	20 (5.3)	73 (6.6)
45-49	52 (13.8)	174 (15.7)
50-54	74 (19.7)	170 (15.3)
55-59	57 (15.2)	132 (11.9)
60-64	48 (12.8)	158 (14.2)
65-69	49 (13.0)	173 (15.6)
70-74	34 (9.0)	99 (8.9)
75-79	26 (6.9)	58 (5.2)
Pregnancies, No.		
0	54 (14.4)	73 (6.6)
1	67 (17.8)	109 (9.8)
2	100 (26.6)	350 (31.5)
3	83 (22.1)	287 (25.8)
≥4	72 (19.1)	292 (26.3)
Duration of oral contraceptive use, y		
Never	199 (52.9)	494 (44.5)
<1	45 (12.0)	103 (9.3)
1-5	58 (15.4)	183 (16.5)
6-10	42 (11.2)	148 (13.3)
≥11	31 (8.2)	180 (16.2)
Unknown	1 (0.3)	3 (0.3)
Family history of ovarian cancer		
No	342 (91.0)	1029 (92.6)
Yes	8 (2.1)	17 (1.5)
Unknown	26 (6.9)	65 (5.9)
Menopausal status		
Postmenopausal	258 (68.6)	720 (64.8)
Premenopausal/perimenopausal	104 (27.7)	356 (32.0)
Unknown	14 (3.7)	35 (3.2)
Infertility		
No	304 (80.8)	935 (84.2)
Yes	72 (19.2)	176 (15.8)
Sterilization		
No	347 (92.3)	1000 (90.0)
Yes	29 (7.7)	111 (10.0)

($P = .52$). When the analysis was further adjusted for the established risk factors given in Table 1, the association with cumulative oral HT estrogen intake became significant ($P = .02$), whereas no significant association was observed with cumulative oral HT progestogen intake ($P = .96$) or duration ($P = .15$). The estimated odds ratio (OR) per each additional gram of oral HT estrogen intake was 1.136 (95% CI, 1.008-1.282), corresponding to an OR of 1.90 (95% CI, 1.04-3.46) per 5 g of estrogen when adjusted for duration, cumulative intake of progestogen, and the previously mentioned established risk factors (Table 3). If duration and cumulative intake of progestogen were excluded, thus adjusting for the established risk factors only, the estimated OR per each additional gram of estrogen was 1.056 (95% CI, 1.003-1.112), corresponding to an OR of 1.31 (95% CI, 1.01-1.70) per 5 g of estrogen. The **Figure** shows these ORs as a function of the cumulative oral HT estrogen intake. The reference level corresponds to nonusers of oral HT (indicated by a circle), and the association between the

Table 2. Characteristics of Hormone Therapy (HT) Use Among Epithelial Ovarian Cancer Cases and Controls Who Have Not Undergone Hysterectomy

Characteristic	Cases, No. (%) (n = 376)	Controls, No. (%) (n = 1111)
HT use		
Never	265 (70.5)	817 (73.5)
Vaginal only	11 (2.9)	50 (4.5)
Oral only	93 (24.7)	219 (19.7)
Oral and vaginal	7 (1.9)	25 (2.3)
Duration of oral HT use, y		
Never	276 (73.4)	867 (78.0)
<1	18 (4.8)	61 (5.5)
1-5	25 (6.6)	72 (6.5)
6-10	20 (5.3)	45 (4.0)
≥11	37 (9.8)	66 (5.9)
Cumulative oral intake of estrogen, g		
Never	280 (74.5)	887 (79.8)
<0.5	19 (5.1)	50 (4.5)
0.5-<2	13 (3.5)	51 (4.6)
2-<4	19 (5.1)	32 (2.9)
4-<7	16 (4.3)	35 (3.2)
7-<10	10 (2.6)	27 (2.4)
≥10	19 (5.1)	29 (2.6)
Cumulative oral intake of progestogen, g		
Never	289 (76.8)	912 (82.1)
<0.3	19 (5.1)	42 (3.8)
0.3-<1.6	19 (5.1)	62 (5.6)
1.6-<3.7	20 (5.3)	51 (4.6)
≥3.7	29 (7.7)	44 (4.0)
Unopposed estrogen intake, g		
Never	358 (95.2)	1042 (93.8)
<0.5	3 (0.8)	19 (1.7)
0.5-<2	3 (0.8)	20 (1.8)
2-<4	4 (1.1)	12 (1.1)
≥4	8 (2.1)	18 (1.6)
Progestogen only intake, g		
Never	368 (97.9)	1079 (97.1)
<1.6	3 (0.8)	20 (1.8)
≥1.6	5 (1.3)	12 (1.1)
Mixed estrogen and progestogen intake, g		
Never	296 (78.7)	935 (84.2)
Mixed estrogen intake		
<0.5	17 (4.5)	35 (3.2)
0.5-<2	12 (3.2)	37 (3.3)
2-<7	31 (8.3)	66 (5.9)
≥7	20 (5.3)	38 (3.4)
Mixed progestogen intake		
<0.3	17 (4.5)	37 (3.3)
0.3-<3.7	38 (10.1)	101 (9.1)
≥3.7	25 (6.7)	38 (3.4)

OR (on a logarithmic scale) and cumulative oral estrogen intake among users is fitted by a linear spline¹⁸ with boundaries at 0.5, 2, 4, 7, and 10 g. This gives a continuous, piecewise linear curve where the slopes of the line pieces show the estimated dose-response relationships within each category, that is, the change in log (OR) per each additional gram of cumulative oral HT estrogen intake. The curve shows that the OR increased with the cumulative intake in a log linear manner, with no signs of a threshold for safe use of oral HT. The deviations from a straight line were not significant ($P = .80$).

Table 3. Adjusted Odds Ratios (ORs) of Epithelial Ovarian Cancer Among Women Who Have Not Undergone Hysterectomy According to Cumulative Oral HT Estrogen and Progestogen Intake and Duration of HT

Variable	OR* (95% CI) [P Value]	OR† (95% CI) [P Value]	OR‡ (95% CI) [P Value]
Increase per gram of estrogen intake	1.059 (1.010-1.111) [.02]	1.093 (0.986-1.213) [.07]	1.136 (1.008-1.282) [.02]
Increase per gram of progestogen intake	1.006 (0.962-1.053) [.78]	0.992 (0.941-1.045) [.75]	1.001 (0.945-1.061) [.96]
Increase per 1-y use of oral HT	1.031 (0.996-1.067) [.09]	0.976 (0.905-1.053) [.52]	0.941 (0.863-1.026) [.15]

Abbreviations: CI, confidence interval; HT, hormone therapy.

*Adjusted for age (categorical) and ever/never use of oral HT. Includes 376 cases and 1111 controls.

†Mutually adjusted and adjusted for age (categorical) and ever/never use of oral HT. Includes 376 cases and 1111 controls.

‡Mutually adjusted and adjusted for age (categorical), ever/never use of oral HT, ever/never pregnant, number of pregnancies (linear), ever/never oral contraceptive use, length of oral contraceptive use (linear), family history of ovarian cancer (yes/no), menopausal status (postmenopausal/premenopausal), infertility (yes/no), and sterilization (yes/no). Includes 338 cases and 1011 controls.

The association with the cumulative intake of progestogen did not depend of the type of progestogen (cypoterone acetate, medroxyprogesterone [acetate], norethisterone acetate, or levonorgestrel) ($P = .15$), and the association with the cumulative intake of estrogen did not depend on the type of estrogen (estradiol or estriol) ($P = .98$), but the associations with the cumulative intakes of progestogen and estrogen differed borderline significantly from each other ($P = .05$) (data not shown).

We also evaluated whether the effects of cumulative oral HT intakes of estrogen and progestogen on the risk of ovarian cancer depended on whether the hormones were administered synchronously (**Table 4**). In these analyses, we calculated for each woman 4 oral HT intake variables: the cumulative intake of estrogen from products containing only estrogen ("unopposed estrogen"), the cumulative intake of estrogen from products also containing progestogen ("mixed estrogen"), the cumulative intake of progestogen from products containing only progestogen ("progestogen only"), and the cumulative intake of progestogen from products also containing estrogen ("mixed progestogen"). These 4 intake variables were included simultaneously in the model. The change in ovarian cancer risk with increasing cumulative estrogen intake was higher for combined HT regimens but was not significantly different from the association with unopposed estrogen intake ($P = .55$). Likewise, the association with progestogen did not depend significantly on whether it was administered together with estrogen ($P = .28$).

Finally, we evaluated whether the data showed any indications of latency of the effect of cumulative oral HT intake on ovarian cancer, but we found no systematic effect of time since first use of oral HT. Neither did the recency of the oral HT intake have any significant impact on the risk of ovarian cancer, since the estimated OR for current users was between the estimated ORs for 6 to 10 years since last use and more than 10 years since last use (data not shown).

COMMENT

We found strong evidence of an increased risk of epithelial ovarian cancer in nonhysterectomized women who had used oral HT. The risk increased evenly with the cumulative oral HT intake of estrogen. The risk was not associated with the cumulative intake of the progestogenic component of the HT regimens.

The risk increased with cumulative oral HT estrogen intake when adjusted for duration, whereas the risk decreased nonsignificantly with duration when adjusted for estrogen intake, indicating that cumulative estrogen intake is the more important factor and that the increase in risk with duration in our unadjusted analyses, and in other studies,⁴⁻⁶ is caused by confounding with cumulative oral HT estrogen intake. We found the increased risk of ovarian cancer with increased cumulative oral HT intake of estrogen to be present over the entire range of intake, with a linear relation between the log-transformed OR and the cumulative estrogen intake. Thus, each extra gram of estrogen led to the same proportional increase in the odds of low and high cumulative intakes. The decrease with duration, although not statistically significant, indicates that perhaps the adverse effect of cumulative HT estrogen intake is diminished if estrogen is administered over a longer period by lowering the daily dose.

This study has several strengths. We eliminated the selection biases that can arise in hospital-based designs by drawing the controls randomly from the source population, and we reduced the likelihood of recall bias by interviewing the incident cases shortly after their operation. The participation rates among cases and controls were reasonably high, although 18% of eligible controls participated via a telephone interview only. Cumulative oral HT intake could not be calculated for these controls because of less detailed registration on HT use in the telephone interview, but they did not differ from the remaining controls with respect to duration of HT use, number of pregnancies, length of OC use, menopausal status, and frequency of hysterectomy (data not shown).

The reliability of self-reported use of HT has been found to be high in a population similar to ours,¹⁹ and it was further optimized in the present study by the use of picture books and life-event calendars, resulting in a high percentage of women who remembered their lifetime intake of oral HT (75.7% of controls and 72.3% of cases). Thus, this large population-based case-control study had sufficient statistical power to explore specific associations between ovarian cancer risk and modern regimens of HT.

Confounding by known risk factors is unlikely to account for our results, since the estimates were adjusted for most of the established risk factors. Of particular importance is that the results are adjusted for ever-never

use and duration of OC use, since OC use reduces the risk of ovarian cancer and women who have used OC are more likely than other women to use HT.²⁰ We excluded women who had undergone hysterectomy from the present analyses because of the possible misclassification regarding oophorectomy among these women, a misclassification that by nature can only be differential in any study of ovarian cancer. This means that our results cannot be confounded by hysterectomy. As pointed out in a comprehensive review,² results on the association of ovarian cancer and HT may differ according to whether a potential confounding effect of hysterectomy is taken into account. Our results are in accordance with an increased risk of ovarian cancer with ever-use of HT vs never-use, as found in studies^{4-7,12} adjusting for hysterectomy. Regarding duration of HT, our results are also similar to those of studies⁴⁻⁶ that have examined prolonged use while simultaneously accounting for the effect of hysterectomy.

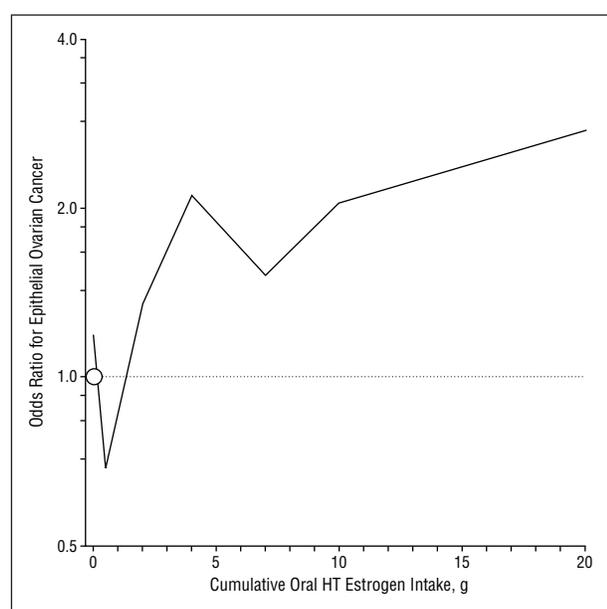


Figure. The relationship between the risk factor-adjusted odds ratio (logarithmic axis) for epithelial ovarian cancer and the cumulative oral hormone therapy (HT) intake of estrogen among ever-users of oral HT. The relationship is fitted by a linear dose-response relationship within each category delimited by boundaries at 0.5, 2, 4, 7, and 10 g (linear spline). The reference level is the odds among never-users of oral HT, indicated by a circle.

A recently published cohort study⁵ claimed that there was a threshold effect of the increased risk of ovarian cancer by 10 years of HT use. However, this study only considered a categorical variable with a pre-specified cutoff point at 10 years of use, thereby creating a threshold at this point by the chosen modeling of duration such that the longer durations below the cutoff point are assigned the same low risk as the shorter durations. We used linear splines in the present study to evaluate the adequacy of the modeling of the association between the log-odds and the quantitative variables measuring oral HT intake. This allows examination of nonlinear associations without assuming constant risk within categories. We found no evidence of a threshold for safe use of oral HT. Rather, a simple trend with the cumulative oral HT intake of estrogen was found, and the association between ovarian cancer risk and duration could be explained by the association between duration and cumulative estrogen intake. Thus, we confirm that menopausal estrogen intake increases the risk of ovarian cancer, but the allegation that estrogen use for less than 10 years is not associated with increased risk is questionable.

There is increasing concern that combined HT regimens may have an additional adverse effect on the breast epithelium so that the addition of progestogen entails a further increase in the breast cancer risk than the use of preparations containing estrogen alone.²¹ Because breast cancer and ovarian cancer share many risk factors, the concern may also be applicable to the risk of ovarian cancer. However, few studies have compared the effects of different types of HT on ovarian cancer risk.^{4,6,12-15} Three of these studies^{6,12,13} had a limited number of women who had used combination therapy; 2 of the studies found no overall association between ovarian cancer risk and HT but an increased risk¹⁴ and a decreased risk¹⁵ among non-hysterectomized women using unopposed estrogen therapy. Finally, a Swedish case-control study⁴ found an elevated risk of ovarian cancer in women who had used unopposed HT and in women who had used estrogen combined with sequential progestogens. In our study, we could not reject the hypothesis of similar effects of the cumulative intake of estrogens from estrogen-only preparations and the cumulative intake of estrogen from combination preparations, but the hypothesis of an additional adverse effect of the combined HT regimen could not be rejected either.

Table 4. Adjusted Odds Ratios (ORs) of Epithelial Ovarian Cancer Related to Cumulative Oral HT Intakes Among Women According to Mode of Oral Administration Who Have Not Undergone Hysterectomy

Administration Mode	OR* (95% CI)	P Value†	OR‡ (95% CI)	P Value†
Cumulative intake of unopposed estrogen	1.047 (0.976-1.124)	.51	1.048 (0.966-1.138)	.55
Cumulative intake of mixed estrogen	1.079 (1.010-1.152)		1.081 (1.005-1.162)	
Cumulative intake of progestogen only	0.923 (0.763-1.117)	.46	0.859 (0.639-1.155)	.28
Cumulative intake of mixed progestogen	0.987 (0.929-1.048)		0.992 (0.928-1.060)	

Abbreviations: CI, confidence interval; HT, hormone therapy.

*Mutually adjusted and adjusted for age (categorical) and ever/never HT use. Includes 376 cases.

†P value for comparison of the effects of a mixed hormone intake and intake of 1 hormone component only.

‡Mutually adjusted and adjusted for age (categorical), ever/never HT use, ever/never pregnant, number of pregnancies (linear), ever/never oral contraceptive use, length of oral contraceptive use (linear), family history of ovarian cancer (yes/no), menopausal status (postmenopausal/premenopausal), infertility (yes/no), and sterilization (yes/no). Includes 338 cases and 1011 controls.

Hormone therapy and OCs are the 2 therapy forms of exogenous hormones that most frequently have been stated to affect cell proliferation and therefore risk of hormone-dependent cancers. In relation to the etiology of ovarian cancer, although OC use reduces the risk of ovarian cancer in a duration-dependent but dose-independent manner,^{22,23} the risk of ovarian cancer in relation to HT increases with the cumulative intake of estrogen. Thus, the mechanisms are likely to be different. Estrogens and progestogens are not primarily mutagenic,²⁴ but it has been suggested that estrogens may increase the risk of ovarian cancer through a process in which estrogens would act as promoting agents, increasing epithelial cell proliferation and thus the opportunity for the accumulation of random genetic errors.^{3,25} A similar effect may exist for OCs, but with that type of hormone use the association may be dominated by the effect of the reduced cell proliferation, that is, the induced anovulation. Thus, the difference in the resulting effect of estrogen use in HT and OC use may be due to the different timing of the use of the estrogens, since menopausal use of hormones does not induce anovulation. A less likely explanation of the difference in the effect of HT and OC use may be that the increase in risk of ovarian cancer occurs only for natural estrogens, as used in HT, and not for synthetic estrogens, as used in OCs.

Various preparations are available for perimenopausal and postmenopausal HT, but most preparations on the European market contain the potent estrogen estradiol. European women do not commonly use conjugated equine estrogens, which are prescribed especially in the United States. We found identical effects of the 2 natural estrogens that were used in the present study (estradiol and estriol). This may indicate that our results could be relevant even for other types of natural estrogens, such as conjugated equine estrogens.

Our findings have important clinical implications for the widespread use of HT. The oral intake of HT estrogen should be diminished whenever possible. If the indication for HT is simply irregular bleeding during perimenopause, treatment with progestogen only may be preferable, and if an estrogen component is required for alleviation of menopausal symptoms, then the lowest possible daily dose should be used. Because ovarian cancer is a highly fatal disease, our result can be helpful in the individual counseling of women receiving HT, balancing the severity of menopausal symptoms against the risks and benefits of HT.

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