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HORMONE REPLACEMENT THERAPY AND THE RISK OF BREAST CANCER. NESTED CASE-CONTROL STUDY IN A COHORT OF SWEDISH WOMEN ATTENDING MAMMOGRAPHY SCREENING

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There is concern that hormone replacement therapy (HRT) increases the risk of breast cancer. We undertook a case-control study of this risk relationship within a cohort of 40- to 74-year-old women in Uppsala County, Sweden, who participated in mammography screening. Incident cases of breast cancer were ascertained during 5 years of follow-up. In all, 435 cases (87% invasive, 13% *in situ* cancers) were detected, 313 through screening and 122 through clinical diagnosis. As controls, 1,740 women were selected randomly. Information on risk factors and use of HRT was obtained through interviews before the start of follow-up. Multivariate analyses revealed an increased risk among users of any type of HRT for more than 10 years, the odds ratio (OR) being 2.1 (95% confidence interval [CI] 1.1–4.0), as well as when restricting analyses to cases diagnosed through mammography screening. After stratification for compound type, risk estimates were apparently higher among women reporting estradiol–progestin combined treatment *vs.* estradiol or conjugated estrogens alone, with ORs for more than 10 years of intake being 2.4 (95% CI 0.7–8.6) and 1.3 (95% CI 0.5–3.7), respectively. Analyses through a model including both compound type and length of hormone intake confirmed a significant excess risk linked to treatment for more than 10 years, OR = 2.6 (95% CI 1.3–5.1). Our results indicate a moderately increased risk of breast cancer after many years of HRT and, hypothetically, a further enhancement of the risk with added progestins. *Int. J. Cancer* 72:758–761, 1997.
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There is concern that long-term hormone replacement therapy (HRT), used to improve quality of life or to prevent coronary heart disease and osteoporosis, may increase the risk of breast cancer in post-menopausal women (Adami and Persson, 1995). The empirical data are, however, contradictory and equivocal. Epidemiological studies have reported a positive risk relationship with HRT given for many years, with conjugated estrogens (Yang *et al.*, 1992; Colditz *et al.*, 1995) or estradiol compounds (Ewertz, 1988; Bergkvist *et al.*, 1989), whereas others have failed to show any alteration of risk (Stanford *et al.*, 1995; Newcomb *et al.*, 1995). Clinical studies indicate that added progestins may have an additional adverse effect by enhancing proliferation of breast epithelial cells (Key and Pike, 1988; Stanford and Thomas, 1993). However, epidemiological data on estrogen–progestin combined HRT are relatively scarce and inconsistent. Results from long-term follow-up of a cohort of Swedish women are compatible with a greater risk increase in association with progestin-combined HRT as compared with administration of estrogens alone (Bergkvist *et al.*, 1989). A few studies did not show any enhancement of the effect of estrogens by added progestins (Yang *et al.*, 1992; Ewertz, 1988; Hunt *et al.*, 1987), while 2 case-control studies from the United States failed to find any association with either estrogens alone or combined treatments (Stanford *et al.*, 1995; Newcomb *et al.*, 1995).

We used a case-control study nested in a cohort of Swedish women who attended mammography screening to test whether different modern HRT regimens, including progestin-combined treatments, are linked to an excess risk of breast cancer.

SUBJECTS AND METHODS

The cohort

Our source population comprised those 77% of all invited women in the county of Uppsala, Sweden, who attended the second administrative screening round of a population-based mammography screening program during the period February 1990–July 1992. In this county, mammography is offered to all women aged 40–74 years, with average intervals of approx. 21 months at ages 40–54 and 27 months for those older (Thurfjell and Lindgren, 1994).

The cohort at study was made up of the attending women who had not been diagnosed with a breast cancer earlier; altogether, 36,503 women were eligible. A total of 33,319 of these women (91%) had previously undergone mammography, and 30,982 of them (85%) had participated in the first screening round of this county.

Our follow-up of breast-cancer outcome in the cohort started at the second mammography screening round and continued until the end of the present observation period, June 30, 1995. All cases occurring in the cohort were ascertained through mammographic examinations in the county, 89% of the cohort women being re-examined with mammography at least once during the follow-up period, and by searching the roster on all new cases diagnosed at the Department of Pathology, University of Uppsala, serving the entire catchment area. Of all cohort women, 34,736 (95%) were still living in Uppsala County at the end of follow-up.

Cases and controls

Overall, 435 women in the cohort were diagnosed with primary breast cancer, 379 (87%) with an invasive cancer and 56 (13%) with an *in situ* cancer. Among all cases, 174 (40%) were ascertained at the second screening round, 139 (32%) at the subsequent rounds and 122 (28%) through clinical work-up.

Altogether, 1,740 women were frequency-matched to cases to serve as controls, with a control:case ratio of 4:1. Frequency matching was based on age (5-year groups) and year of attendance.

Data collection

Women in the cohort were interviewed by a nurse. For those who had attended the first screening round, information was collected on parity, age at first birth, ages at menarche and at menopause (if it had occurred), family history of breast cancer, height and weight. At the second round, the interview focused on previous use of combined oral contraceptives (COCs) and HRT and an update of menstrual history (to ascertain an eventual menopause in previous participants). For those attending for the first time, also items mentioned above were covered. Questions on COC and HRT use

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probed ever-use, age when starting, number of years of intake and name of the compound (if more than one, the one taken for the longest duration). Responses to the questions were noted in a standard protocol. All data were obtained before the mammographic examination of the second round.

The reported exposures were grouped into different compound categories, *i.e.,* estradiol compounds or conjugated estrogens alone, estradiol compounds combined with progestins (mostly norethisterone acetate, cyclically added for 10 days or continuously during the treatment cycle), weak estrogens (oral estriol compounds or vaginally administered low-dose estrogens) and unknown brands (''others''). Estradiol compounds and conjugated estrogens are referred to as ''medium potency'' estrogens, as opposed to weak estrogens (mainly estriol compounds). The former type of estrogens are used mainly for treatment of climacteric symptoms and for prevention of bone loss, the latter chiefly for alleviation of problems associated with urogenital atrophy.

Since information on menstrual bleedings was missing for a large proportion of the subjects and because age at true menopause could not be ascertained in women who had bleedings caused by HRT, we chose to omit this variable from the multivariate analyses.

Statistical methods

Univariate and multivariate analyses were performed using the logistic regression model, estimated by the maximum likelihood method. In addition to univariate models, 2 basic multivariate models were estimated. In the first one, adjustment was made only for the frequency-matching variables age and year of screening attendance, while in the second further adjustment was made for parity (0–4, ≥5 children), age at first birth (<35, ≥35), age at menarche, body mass index (BMI), family history (breast cancer in mother or sister(s)) and use of COCs (never/ever). Adjustment for the matching variables (*i.e.,* age and year of screening) produced results similar to those obtained in the univariate analyses. Stratified analyses for separate compound groups also were performed.

RESULTS

Cases had, as expected, on the average lower parity, higher age at first birth, a more frequent family history of breast cancer and a somewhat higher ever usage of HRT (Table I).

Ever-use (of any HRT type) was not associated with an alteration of risk (Table II). However, in the category of women using any type of HRT for more than 10 years, risk was increased significantly, the adjusted odds ratio (OR) being 2.1 (95% confidence interval [CI] 1.1–4.0).

TABLE I – CHARACTERISTICS OF CASE AND CONTROL SUBJECTS IN THE MAMMOGRAPHY COHORT

Characteristics	Number of subjects (cases/controls)	Cases	Controls
		(mean values)	
Age	435/1,740	56.0	55.8
Age at menarche	425/1,693	13.3	13.3
Number of children	431/1,700	2.0	2.2
Age at first birth	373/1,492	25.2	24.1
Age at menopause[1]	270/1,114	49.2	49.0
BMI[2]	429/1,695	24.8	24.9
		(percentages)	
Family history of breast cancer[3]	435/1,737	13.8	9.0
Ever-use of COCs[4]	434/1,733	41.0	41.1
Ever-use of HRT[5]	430/1,730	21.4	19.8

[1]Menopause defined as having had no menstrual bleeding (natural or treatment-induced) for the last 3–6 months.–[2]Weight/height^2 (kg/m^2). BMI, body mass index.–[3]Breast cancer in grandmother, mother and/or sister.–[4]COCs, combined oral contraceptives.–[5]HRT, hormone replacement therapy.

For all separate compound groups, intake for more than 10 years was associated with larger risk estimates than treatments of shorter duration. None of the separate estimates, crude or adjusted for all of the co-variates, however, was elevated significantly. Intake of progestin-combined regimens for more than 10 years was seemingly associated with higher risk estimates, OR = 2.4 (95% CI 0.7–8.6), as compared with equally long intake of medium-potency estrogens only, OR = 1.3 (95% CI 0.5–3.7), the 2 estimates being not significantly different. Elevated point estimates, however non-significant, were also noted for women reporting long-duration intake of unknown or weak brands.

We also used a multivariate model which, in addition to the co-variates, included compound groups and duration of intake as variables in the same model (Table III). Duration of intake (of any compound) for more than 10 years was associated with a significant, over 2-fold risk increase OR = 2.6 (95% CI 1.3–5.1). None of the compound groups was associated with an independent risk increment. However, we found that estradiol compounds or conjugated estrogens were linked to a possibly lowered risk (OR = 0.5, 95% CI 0.3–1.0), whereas estradiol–progestin compounds were linked to a non-significant risk increase (relative risk [RR] = 1.4; 95% CI 0.9–2.1).

When analyses were restricted to women diagnosed with a breast cancer through mammographic screening (313 cases, 72% of all cases), analyses in the multivariate model showed an excess risk for intake of HRT for more than 10 years, OR = 2.0 (95% CI 1.0–4.0).

DISCUSSION

In this cohort of Swedish women who participated in a mammography screening program, HRT for more than 10 years was linked to a doubled risk of breast cancer. This risk relationship was present also when the analyses were restricted to cases diagnosed through regular screening. Estrogen–progestin combined regimens seemed to entail a higher risk than treatments with estrogens alone.

One important advantage of our study design is that it should minimize possible bias due to enhanced detection among HRT users. Since 91% of the cohort women had undergone a mammography within a few years, the likelihood would be low that there was a prevalent cancer at the start of follow-up that could be detected clinically in the short-term. All women were examined with mammography directly after the interview, and 89% were re-examined at least once during the follow-up. Further, the vast majority (72%) of the observed breast cancers were detected through mammography. Analyses restricted to participants in the mammography screening program yielded results similar to those for the whole cohort, *i.e.,* an increased risk for long-term use of HRT. Another advantage is the acquisition of exposure data before occurrence of the outcome, which precludes recall bias of HRT exposure.

However, there are methodological limitations that could affect the interpretation of our results. Selection bias could arise if women in the cohort moved out of the county, thereby escaping possible detection of a breast cancer, and if this migration was related to HRT exposure. The impact of such a bias is likely to be negligible as 95% of all cohort women were still residents in the catchment area at the end of the follow-up period. Further, mis-classification of exposure may be a problem. Women were classified to exposure categories on the basis of recall of the compound taken for the longest duration, among several possible compounds. This could entail that some women were exposed to compounds which were not reported, *e.g.,* to medium-potency estrogens when classified to the weak estrogen group or progestin-opposed use when in the estrogen-only group. Also, it may be that exposure to HRT was continued after the interview at the second mammography round, which could entail an under-estimation of the true duration of intake. In spite of such possible non-differential mis-classifications, there were some notable patterns, namely, a clear relationship between many years of intake and an increased risk and a possibly

TABLE II – UNIVARIATE AND MULTIVARIATE ANALYSES OF THE RISK OF BREAST CANCER AFTER HRT, STRATIFIED BY COMPOUNDS AND DURATION OF INTAKE

Type of compounds, duration of intake (years)	Cases	Controls	Univariate		Multivariate[1]	
			OR	(95% CI)	OR	(95% CI)
All types						
Never	317	1,293	Reference		Reference	
Ever	93	345	1.1	(0.9–1.4)	1.1	(0.8–1.4)
Duration						
1–2	40	170	1.0	(0.7–1.4)	0.9	(0.6–1.3)
3–5	22	75	1.2	(0.7–1.9)	1.0	(0.6–1.8)
6–10	14	65	0.9	(0.5–1.6)	0.9	(0.5–1.6)
11+	16	33	2.0	(1.1–3.6)	2.1	(1.1–4.0)
Estradiol-conjugated estrogens[2]						
1–10	12	84	0.6	(0.3–1.1)	0.5	(0.3–1.0)
11+	5	17	1.2	(0.4–3.3)	1.3	(0.5–3.7)
Estradiol + progestins[3]						
1–10	25	71	1.4	(0.9–2.3)	1.4	(0.9–2.2)
11+	4	8	2.0	(0.6–6.8)	2.4	(0.7–8.6)
Weak estrogens[4]						
1–10	26	90	1.2	(0.8–1.9)	1.0	(0.6–1.7)
11+	4	6	2.7	(0.8–9.7)	2.5	(0.7–9.4)
Others[5]						
1–10	13	64	0.8	(0.5–1.5)	0.8	(0.4–1.5)
11+	3	2	6.1	(1.0–36.8)	5.7	(1.0–35.1)

[1]Adjusted for age, age at menarche, parity, age at first birth, family history of breast cancer, previous ever use of combined oral contraceptives and for body mass index. OR, odds ratio; CI, confidence interval. Where not explicitly indicated, the reference group consists of ''never exposed.''–[2]Estradiol compounds 1 or 2 mg; conjugated estrogens 0.625 or 1.25 mg.–[3]Estradiol 2 mg combined with norethisterone acetate 1 mg, either cyclically or continuously.–[4]Weak estrogens, mainly orally or vaginally administered estriol compounds.–[5]Not possible to classify by compound type.

TABLE III – MULTIVARIATE ANALYSIS OF THE RISK OF BREAST CANCER AFTER HRT, SHOWING INDEPENDENT EFFECTS OF COMPOUNDS AND DURATION OF INTAKE

Variables in model	OR	95% CI
Never exposed	Reference	
Estradiol-conjugated estrogens	0.5	0.3–1.0
Estradiol + progestins	1.4	0.9–2.1
Weak estrogens	1.0	0.6–1.2
Duration 11+ years	2.6	1.3–5.1

Model including co-variates (as specified in Table II), compound type and duration. OR, odds ratio; CI, confidence interval.

greater risk increase for progestin-combined use than for use of estrogen only (when duration of intake was accounted for).

Our findings of an increased risk after long-term use of HRT, also with progestin-combined treatment, are consistent with results from previous Scandinavian studies. In another cohort of Swedish women who received prescriptions for HRT (Bergkvist *et al.*, 1989), as well as in a Danish case-control study (Ewertz, 1988), 6–10 years of estradiol intake was associated with an about 2-fold excess risk, with a positive risk relationship also for progestin-combined HRT. A number of studies from the United States have reported adverse effects on breast-cancer risk with long-term intake of conjugated estrogens (Brinton and Schairer, 1993), generally with RR estimates ranging from 1.3 (Steinberg *et al.*, 1991) to 1.6 (Yang *et al.*, 1992; Colditz *et al.*, 1995). The Nurses' Health Study (Colditz *et al.*, 1995) is the only investigation from the United States that has reported a positive risk relationship between invasive breast cancer and progestin-combined therapy. Notably, 2 large population-based case-control studies in the United States failed to find a link between HRT, including combined regimens, and breast cancer (Stanford *et al.*, 1995; Newcomb *et al.*, 1995).

Possible reasons for discrepant results among studies and between countries need to be considered. As the reported risk relationships between HRT and breast cancer are rather weak, some studies may lack statistical power to show a risk alteration in the relevant sub-groups, *e.g.*, women with long-term intake or progestin-combined treatment (Brinton and Schairer, 1993). Variations in methodological rigor among studies also may play a role (Steinberg *et al.*, 1991). However, differences in the source populations regarding susceptibility factors and in biological effects of the different HRT regimens used by the women in the target populations should also be regarded. For instance, in several studies, estrogen replacement has been shown to increase breast-cancer risk predominantly in lean women (Harris *et al.*, 1996). Sub-group analyses with regard to BMI levels and other possible risk modifiers seem necessary. Further, it may be that specific progestin compounds induce different effects in the breast. Androgen-derived progestins (*e.g.*, levonorgestrel or norethisterone acetate) can reduce sex hormone–binding globulin levels more than progesterone-like compounds (*e.g.*, MPA) (Campagnoli *et al.*, 1996), entailing that the bioavailability of estrogens is greater with the former types. Further, progestins may have direct effects on the breast by augmenting proliferation of epithelial cells (Key and Pike, 1988), presumably depending on the potency and dose. It is noteworthy that the former types of progestin predominate in Sweden, while in the United States the latter compounds have been used almost exclusively. In our study, among the case subjects reporting HRT (21% of all cases), 23% had used a cyclically combined regimen with estradiol + norethisterone acetate (an androgen-derived progestin) for 10 days of each cycle and 16% a continuously combined regimen including these compounds.

In conclusion, we found that very long-term intake (more than 10 years) of HRT was associated with a moderately increased risk of breast cancer, possibly with a stronger adverse effect after addition of progestins. When assessing the overall risks and benefits of HRT, a possible adverse effect on breast-cancer risk needs to be considered. To better characterize risk relationships, future studies should be designed to measure effects in relevant sub-groups of women and for different treatment regimens and compounds.

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