American Journal of Epidemiology
Vol. 155, No. 4
Printed in U.S.A.

Endogenous Estrogen Exposure and Cardiovascular Mortality Risk in Postmenopausal Women

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In this study, the authors investigated whether combined information on reproductive factors has additive value to the single reproductive factor age at menopause for assessing endogenous estrogen exposure and cardiovascular mortality risk in postmenopausal women. They conducted a population-based cohort study that included 9,450 postmenopausal women from Nijmegen, the Netherlands, who were aged 35–65 years at enrollment in 1975, with a median follow-up of 20.5 years. A Cox proportional hazards model and Receiver Operating Curves were used to analyze the data. Women aged 52 years or more at menopause had an 18% reduction in cardiovascular mortality (hazard ratio = 0.82, 95% confidence interval (CI): 0.69, 0.98) compared with those aged 44 years or less. Women with more than 18 years of exposure to endogenous estrogen had a statistically significant 20% reduction in cardiovascular mortality (hazard ratio = 0.80, 95 percent CI: 0.67, 0.96) compared with those who had 13 years of exposure or less. The area under the curve of the Receiver Operating Curves for the two models was identical (area under the curve = 0.67, 95 percent CI: 0.66, 0.68). This study shows that age at menopause is related to cardiovascular disease mortality and that a newly developed composite measure of endogenous estrogen exposure does not add to the predictive value of age at menopause for cardiovascular mortality. *Am J Epidemiol* 2002;155:339–45.

cardiovascular diseases; estrogens; menopause; mortality

Premenopausal women are at low risk of cardiovascular disease relative to men of comparable age and to postmenopausal women (1–3). This protection is ascribed to endogenous estrogen production in premenopausal women. Furthermore, a vast amount of observational studies suggest that hormone replacement therapy (HRT) protects postmenopausal women from cardiovascular disease (4), although the only randomized controlled trial published so far of which we are aware, in women with coronary artery disease (Heart and Estrogen/Progestin Replacement Study (5)), did not show an effect of HRT on cardiovascular disease outcome. Several biologically plausible mechanisms were postulated to explain the effect of endogenous and exogenous estrogen on the etiology of cardiovascular diseases, including direct effects on the vascular wall and long-term indirect effects via reduction of cardiovascular risk factors. It is not known to what extent postmenopausal women still benefit from the long-term effects of their premenopausal endogenous estrogen exposure. In several studies, age at menopause was found to be related to risk of cardiovascular disease (6–9). Age at menopause is not the only determinant related to endogenous estrogen exposure. Age at menarche, number and duration of pregnancies, duration of breastfeeding, and oral contraceptive use also determine the duration and level of exposure to endogenous estrogen.

The aims of this study are to describe the association between age at menopause and cardiovascular mortality and to investigate whether duration of premenopausal endogenous estrogen exposure adds to the prediction of cardiovascular mortality risk in postmenopausal women. Since progestogen could attenuate the estrogen effect on cardiovascular disease, we are most interested in the duration of exposure to "unopposed" endogenous estrogen. To quantify the duration of this exposure, we will combine data on the reproductive factors age at menopause, age at menarche, number and duration of pregnancies, duration of breastfeeding, and oral contraceptive use. Furthermore, we want to study whether premenopausal duration of exposure to endogenous estrogen is a better predictor of cardiovascular mortality than age at menopause. For this purpose, we used data from postmenopausal women who participated in a breast cancer screening project in the Netherlands.

Received for publication August 24, 2000, and accepted for publication August 15, 2001.

Abbreviations: CI, confidence interval; HR, hazard ratio; HRT, hormone replacement therapy; ICD-10, *International Classification of Diseases*, Tenth Revision; PAR, population attributable risk; SD, standard deviation.

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MATERIALS AND METHODS

Study population

Between January 1975 and December 1976, 19,702 (86 percent) of the 22,903 invited women aged 35–66 years, living in the city of Nijmegen, the Netherlands, participated in a population-based screening program for breast cancer. Mammographic checkups were repeated biannually from baseline. Questionnaires on general health, cardiovascular risk factors, and reproduction-related events were completed at the first screening visit only. The original screening cohort included both pre- and postmenopausal women. For this study, we included postmenopausal women only. At baseline, 9,528 women reported that their menses had ceased at least 12 months earlier. Seventy-eight women were excluded: Eight women had had their last menstrual period more than a year before because of an episode of pregnancy and/or breastfeeding; age at menopause, menarche, or menstrual cycle length data were missing for 18 women; and follow-up data were missing of 52 women. Thus, 9,450 postmenopausal women were included in this analysis. Women gave oral consent for participation in the study, which was approved by the Institutional Review Board.

Duration of exposure

Women completed questionnaires on age at menarche, regularity, and duration of menstrual cycle and the year and month that menses had stopped. Information was obtained about the number of liveborn children, miscarriages and stillborn children, children breastfed, and years of oral contraceptive use and to estimate a woman's lifetime duration of exposure to endogenous estrogen during premenopausal life.

Before age at menarche, sex hormone levels are very low. During the preovulation part of the menstrual cycle, estrogen levels increase without an increase in progesterone levels. During the postovulation part of the menstrual cycle, progesterone levels are high and estrogen levels are decreasing. Variations in cycle length are almost completely determined by differences in the first part of the menstrual cycle length. Duration of the cycle after the ovulation is 2 weeks, independent of total cycle length. Therefore, only the first part of the menstrual cycle, the duration of which varies among women, contributes to the period of unopposed estrogen exposure. During pregnancy, hormone levels increase substantially, but there is no period in which the increase of estrogen is not accompanied by a progesterone increase. Pregnancy therefore is not an episode with exposure to unopposed endogenous estrogen. During breastfeeding, there is a negligible, very low exposure to estrogen and progesterone. The oral contraceptives that were used before 1975 were "first-generation" pills that contained a very high dose of synthetic estrogen plus progestogen. Therefore, the normal cycle with endogenous estrogen was suppressed. At menopause, estrogen and progesterone levels decrease substantially.

For each woman, age at menarche (in months) was subtracted from age at menopause (in months). For each liveborn child, 9 months (the average duration of pregnancy) were subtracted; for each miscarriage or stillborn child, 3 months (the average duration of pregnancy for a miscarriage/stillborn child) were subtracted, and for each child who was breastfed, 4 months (the average duration of breastfeeding per child) were subtracted. This assumption of the average duration of breastfeeding was based on calculations within a population-based screening program for breast cancer in Utrecht, the Netherlands, in 1975 (10). Furthermore, total duration of oral contraceptive use was subtracted. In this way, an estimation of the lifetime total duration of all menstrual cycles (in months) was obtained. To calculate the duration of unopposed endogenous estrogen exposure, we subtracted from this number the total duration of all postovulatory periods of this cycle (2 weeks multiplied with the total number of menstrual cycles). For example, for a woman with an age at menarche of 14 years and an age at menopause of 52 years with two children born alive, one miscarriage/stillborn child, 1 year of oral contraceptive use, and an average cycle duration of 4 weeks, we estimated a duration of exposure to endogenous estrogen of 17.7 years.

Other determinants

Age at entry was calculated by subtracting date of birth from the date of screening examination. Height and weight were measured at enrollment. Body mass index was calculated as weight (kg)/squared height (m^2). Women reported whether a surgical treatment was the cause of the cessation of menstruation, and if so, what kind of treatment they received. They were asked about the use of medication for menopausal complaints during the 12 months prior to enrollment. Menses were reported as regular or irregular, on average. Women were classified as a prevalent cardiovascular disease case if they had used medication for cardiovascular disease (heart glycosides, antiarrhythmic drugs, nitrates, other antianginal drugs, or anticoagulants) during the year prior to enrollment. They were classified as hypertensive if they had used antihypertensive drugs during the previous year or as diabetic if they reported that they were diabetic and/or that they had used antidiabetic medication in the year prior to enrollment. Socioeconomic class was divided in four categories mainly based on the average educational level in the area in which they were living.

Cardiovascular disease mortality

The municipal registry informed the Department of Epidemiology at the University of Nijmegen weekly about migration and deaths of cohort members before December 31, 1996. We linked the death certificate numbers from the municipal registry in Nijmegen with the cause-of-death registration at the Netherlands Central Bureau of Statistics, and so, we obtained the primary cause of death for women who had died during the 22 years of follow-up since enrollment.

The primary endpoint of the analysis was cardiovascular mortality (*International Classification of Diseases*, Tenth Revision (ICD-10) codes 100–199 (11)). Subgroup analyses were done separately for ischemic heart disease mortality (ICD-10 codes 20–25) and cerebrovascular disease mortal-

ity (ICD-10 codes 60–69). Death from other causes, loss to follow-up due to moving outside Nijmegen, and loss to follow-up due to a changing registration system at the municipal registries in Nijmegen were considered censoring events.

Data analysis

All analyses were performed with the statistical package SPSS (SPSS for Windows, Release 8.0.0., SPSS, Inc., Chicago, Illinois). Correlation statistics were used to quantify the association between the different reproductive factors and the composite measure, duration of exposure to endogenous estrogen, calculated from these factors. Pearson correlation was used for the association between numeric reproductive factors and duration of endogenous estrogen exposure (continuous), and Spearman correlation was utilized for the nominal and ordinal reproductive factors. Cox proportional hazards regression analysis was used to quantify the effect of age at menopause and the duration of exposure to endogenous estrogen on cardiovascular mortality. The time of the event in the Cox analysis was marked by the time since enrollment in 1975 and by an event ((cardiovascular) death, moving, or end of the study (1996)). Since cardiovascular disease mortality increases with age, all analyses were adjusted for age at entry. Age at menopause and duration of exposure to endogenous estrogen were analyzed in quartiles, with the lowest quartiles as reference groups. Hazard ratios are presented with 95 percent confidence intervals. We repeated all analysis with multivariate adjustment for HRT use, hypertension, body mass index, and social economic status.

To study whether duration of exposure to endogenous estrogen is a better predictor of cardiovascular mortality than age at menopause, we compared the Receiver Operating Curves of the two determinants. Using the Cox model, a probability of dying of cardiovascular disease was calculated twice for each woman, first by a probability based on the Cox model with age at menopause (in quartiles) as an independent variable and, second, by a probability based on the Cox model with duration of exposure to endogenous estrogen (in quartiles) as an independent variable. The linear predictor ($\beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_n X_n$) was compared with the outcome, ignoring censoring (12, 13). Receiver Operating Curves were constructed by plotting sensitivity versus one minus specificity at various cutpoints computed for both models. The area under the curve was calculated by using the nonparametric trapezoidal rule, with its standard error according to Hanley and McNeil (14). The standard errors were used to compute 95 percent confidence intervals.

RESULTS

The median age of the participants at entry was 57 years (mean = 56.5, standard deviation (SD) 5.9). Baseline characteristics of the reproductive factors are presented in table 1. The median age at menopause was 48.4 years (mean = 47.1 years, SD 5.9), and the median number of years of endogenous estrogen was 15.7 (mean = 15.3 years, SD 4.0). The correlations between the reproductive factors and the measure calculated from these factors, duration of endogenous estrogen exposure, are presented in table 2. Age at menopause (0.725) and duration of menstrual cycle (0.411) correlate highly with duration of endogenous estrogen exposure. The cardiovascular risk factors and other potential confounders are presented in table 3 in quartiles of duration of exposure to endogenous estrogens. The prevalence of hypertension was more common in the highest quartile of duration of endogenous estrogen. Other potential confounders were equally distributed among quartiles of duration of endogenous estrogen exposure.

Table 4 shows the follow-up results for the 9,450 women in the cohort, yielding 161,742 person-years of observation. At the end of the follow-up period (December 31, 1996), 1,482 women (15.7 percent) had moved outside Nijmegen. The median follow-up for these women was 11.2 years, with a maximum of 21.8 years (median for the entire cohort =

TABLE 1. Reproductive factors of the study population, Nijmegen, the Netherlands, 1975–1976

Reproductive factor	Mean	%	Median	(SD*)
Age at menarche (years)	13.7		14.0	(1.8)
Age at menopause (years)	47.1		48.4	(5.9)
Cause of menopause				
Natural		77.6		
Hysterectomy		11.0		
Ovariectomy		8.1		
Unknown		3.3		
No. of liveborn children	2.8		3.0	(2.4)
No. of miscarriages and stillbirths	0.5		0	(0.9)
No. of children breastfed	2.2		2.0	(2.3)
Ever use of oral contraceptives		8.1		
Duration of use of oral contraceptives (years)	3.6		2.0	(3.3)
Duration of menstrual cycles (weeks)	4.2		4.0	(0.9)
Regular menses		90.1		

* SD, standard deviation.

TABLE 2. Correlation between reproductive factors in the endogenous estrogen model and duration of exposure to endogenous estrogen, Nijmegen, the Netherlands, 1975–1976

	Duration of exposure to endogenous estrogen (years)
Age at menopause (years)	0.725*
Age at menarche (years)	−0.200*
No. of liveborn children	−0.282*
No. of miscarriages and stillbirths	−0.115*
No. of children breastfed	−0.250*
Duration of use of oral contraceptives (years)	−0.215*
Duration of menstrual cycles (weeks)	0.411*

* Correlation is significant at the 0.001 level (2-tailed).

20.5; maximum = 21.9). The percentage of women who had moved was distributed equally among the quartiles of duration of exposure to endogenous estrogen. Furthermore, 196 women (2.1 percent) were lost to follow-up due to a changing registration system at the municipal registries in Nijmegen. During 22 years of follow-up (161,742 person-years), 2,439 (25.8 percent) women died. A total of 1,063 women died of cardiovascular disease (43 percent), 496 women of ischemic heart disease, and 237 of cerebrovascular disease. A total of 749 (31 percent) women died of cancer, 64 (3 percent) from injury or external causes, including accidents and suicides, and 563 (23 percent) from other causes.

Cardiovascular mortality risk was lower for women with a late menopause (table 5). Postmenopausal women aged 52 years or older at menopause (highest quartile) had a 18 percent reduction in cardiovascular mortality (hazard ratio (HR) = 0.82, 95 percent confidence interval (CI): 0.69, 0.98) compared with women with an age at menopause of 44 years or younger. Adjustment for HRT use, hypertension, body mass index, and socioeconomic class did not change the estimates materially (table 5). A high age at menopause was not associated with mortality from cerebrovascular diseases (HR = 1.02, 95 percent CI: 0.71, 1.47) (table 5).

TABLE 3. Cardiovascular risk factors and other potential confounders* in quartiles of exposure to endogenous estrogen, Nijmegen, the Netherlands, 1975–1976

	Duration of exposure to endogenous estrogen (years)							
	≤ 13		>13 and ≤ 16		>16 and ≤ 18		>18	
	No.	%	No.	%	No.	%	No.	%
No. of women	2,422	25.6	2,664	28.2	2,267	24.0	2,097	22.2
Hormone replacement therapy use	186	7.7	164	6.2	149	6.6	160	7.6
Prevalent cardiovascular disease	134	5.5	143	5.4	133	5.9	115	5.5
Hypertension	229	9.5	254	9.6	254	11.2	280	13.4
Diabetes mellitus	73	3.0	96	3.6	67	3.0	82	3.9
Socioeconomic class								
1 (highest)	530	21.9	589	22.1	555	24.5	590	28.1
2	834	34.4	900	33.8	745	32.9	736	35.1
3	530	21.9	647	24.3	513	22.6	44	21.2
4	528	21.8	528	19.8	454	20.0	327	15.6
	Mean (SD†)		Mean (SD)		Mean (SD)		Mean (SD)	
Age at entry (years)	54.2 (7.3)		56.2 (5.6)		57.6 (4.7)		58.2 (4.3)	
Body mass index (kg/m^2)	26.2 (4.5)		26.6 (4.3)		26.6 (4.3)		26.7 (4.5)	

* Occasionally there are missing data, but this never exceeds 2%.
† SD, standard deviation.

TABLE 4. Follow-up of 9,450 women by quartiles of duration of exposure to endogenous estrogen, Nijmegen, the Netherlands, 1975–1976

	Duration of exposure to endogenous estrogen (years)							
	≤ 13		>13 and ≤ 16		>16 and ≤ 18		>18	
	No.	%	No.	%	No.	%	No.	%
Persons at risk	2,422	25.6	2,664	28.2	2,267	24.0	2,097	22.2
Person-years at risk	41,270		45,937		38,718		35,817	
Moved	378	25.5	426	28.7	325	21.9	353	23.8
Total deaths	585	24.0	672	27.6	631	25.9	551	22.6
Cardiovascular deaths	242	22.8	290	27.3	295	27.8	236	22.2

TABLE 5. Hazard ratios of age at menopause and mortality, Nijmegen, the Netherlands, 1975–1976

Age at menopause (years)	Cardiovascular mortality (n = 1,063)				Ischemic heart disease mortality (n = 496)				Cerebrovascular disease mortality (n = 237)			
	Adjusted for age at entry		Adjusted for potential confounders*		Adjusted for age at entry		Adjusted for potential confounders*		Adjusted for age at entry		Adjusted for potential confounders*	
	HR†	95% CI†	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
≤ 44‡	1		1		1		1		1		1	
>44 and ≤ 48	0.78	0.65, 0.94	0.77	0.64, 0.93	0.84	0.64, 1.09	0.81	0.62, 1.06	0.74	0.49, 1.12	0.78	0.51, 1.18
>48 and ≤ 51	0.92	0.78, 1.09	0.92	0.78, 1.09	0.95	0.74, 1.20	0.94	0.74, 1.20	0.96	0.67, 1.38	1.00	0.69, 1.45
>51	0.82	0.69, 0.98	0.81	0.68, 0.97	0.79	0.61, 1.02	0.78	0.60, 1.02	1.02	0.71, 1.47	1.03	0.71, 1.50

* Potential confounders: age at entry, hormone replacement therapy use, hypertension, body mass index, and social economic class.
† HR, hazard ratio; CI, confidence interval.
‡ Reference category.

The risk of cardiovascular mortality was lower for women with a long exposure to endogenous estrogen (table 6). Postmenopausal women with an exposure to endogenous estrogen of more than 18 years (highest quartile) had a 20 percent reduction (HR = 0.80, 95 percent CI: 0.67, 0.96) in cardiovascular mortality compared with women with 13 years of exposure or less. Adjustment for HRT use, hypertension, body mass index, and socioeconomic class did not substantially change the estimates (table 6). For the subgroups of ischemic heart disease mortality as well as those of cerebrovascular disease mortality, similar, although not significant, effects were observed for women with the longest exposure to endogenous estrogen; the HRs were 0.87 (95 percent CI: 0.67, 1.14) and 0.79 (95 percent CI: 0.53, 1.17), respectively.

Area under the curve of the association between age at menopause and cardiovascular mortality and between duration of exposure to endogenous estrogen and cardiovascular mortality risk were calculated; they were identical (0.66, 95 percent CI: 0.64, 0.68).

DISCUSSION

Our results show an association between late age at menopause and cardiovascular mortality risk (HR for the highest vs. the lowest quartile = 0.82, 95 percent CI: 0.69, 0.98). Our data also show an association between a long duration of lifetime premenopausal exposure to endogenous estrogen and cardiovascular mortality (HR for the highest vs. the lowest quartile = 0.80, 95 percent CI: 0.67, 0.96). The predictive values for cardiovascular mortality for both parameters (age at menopause and duration of exposure of endogenous estrogen), calculated from the area under the curve, were identical. Therefore, our composite measure of endogenous estrogen exposure did predict cardiovascular mortality risk but did not add to the predictive value for cardiovascular mortality of age at menopause.

Before these results are interpreted, some issues concerning internal validity need to be addressed. Women had to be healthy enough to attend screening and were probably more motivated compared with women who did not attend. Thus, women who attended the screening were probably at lower cardiovascular risk. We do not think that, because of an early menopause and a short duration of exposure to endogenous estrogen, women were less likely to attend screening. Therefore, selection bias due to the use of a screening population is not very likely in this study.

Recall bias did not apply to this study because all history of exposure was collected before the investigated event. If any misclassification has occurred in the reporting of reproductive factors, it will tend to be random. Although, in a rare case, random misclassification has resulted in bias in the estimates away from the null (15), in general, it will lead to a dilution of the estimate of the true association between age and menopause or duration of exposure to endogenous estrogens and cardiovascular mortality (16–19).

The potential for information bias due to a limited reliability and validity of self-reported information about cardiovascular and diabetes medication is recognized. On the other hand, it is not very likely that there is an association between

TABLE 6. Hazard ratios of duration of exposure to endogenous estrogen and cardiovascular mortality, Nijmegen, the Netherlands, 1975–1976

Duration of endogenous estrogen exposure (years)	Cardiovascular mortality (n = 1,063)				Ischemic heart disease mortality (n = 496)				Cerebrovascular disease mortality (n = 237)			
	Adjusted for age at entry		Adjusted for potential confounders*		Adjusted for age at entry		Adjusted for potential confounders*		Adjusted for age at entry		Adjusted for potential confounders*	
	HR†	95% CI†	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
≤ 13	1		1		1		1		1		1	
>13 and ≤ 16	0.92	0.77, 1.09	0.91	0.77, 1.09	0.97	0.75, 1.24	0.95	0.74, 1.22	1.01	0.71, 1.45	1.05	0.73, 1.51
>16 and ≤ 18	1.00	0.84, 1.19	1.00	0.84, 1.19	1.07	0.83, 1.38	1.06	0.83, 1.37	0.98	0.68, 1.42	1.02	0.70, 1.48
>18	0.80	0.67, 0.96	0.81	0.67, 0.97	0.84	0.64, 1.10	0.87	0.67, 1.14	0.77	0.52, 1.14	0.79	0.53, 1.17

* Potential confounders: age at entry, hormone replacement therapy use, hypertension, body mass index, and social economic class.
† HR, hazard ratio; CI, confidence interval.

the validity of self-reported medication and age at menopause or duration of endogenous estrogen exposure. Misclassification due to the limited validity of self-reported information will therefore be nondifferential.

In our model, the assumption is made of an average duration of pregnancy and breastfeeding. With this model, we do not have the possibility of measuring the differences in duration of exposure to endogenous estrogen more precisely. This will decrease our ability to find an association between our exposure measure and cardiovascular disease mortality.

It is very unlikely that our results are due to confounding. Age at entry is strongly related to the risk of cardiovascular mortality, and in our data, it is also related to the duration of exposure to endogenous estrogen (table 2). This is probably an artifact of the data, due to the inclusion of only postmenopausal women and, therefore, including relatively more young women with an early menopause compared with older women with a late menopause. A cohort effect as an explanation for this relation between age at entry and duration of exposure to endogenous estrogen is less likely. If any cohort effect had been present, it would have been in the opposite direction, with the youngest birth cohort having a longer duration of exposure due to an earlier age at menarche, a later age at menopause, and fewer pregnancies. The entire postmenopausal age range is represented in the cohort, so selection bias is unlikely. To adjust for confounding bias, we corrected for age at entry in all analyses.

Only 7 percent of the women used HRT during the year before the start of the study. Exclusion of these women from the analysis did not substantially alter the estimates of the hazard ratios. Exclusion of women with a surgical menopause (hysterectomy, ovariectomy, or unknown type of surgical menopause) or of women with an irregular menses did not alter the results either. Adjustment for all potential confounders was achieved by adding them simultaneously into the Cox model. These confounders did not materially influence the estimation of the hazard ratio for age at menopause or that of duration of exposure to endogenous estrogen. Residual confounding by other factors might be possible. Information on a lipoprotein profile was not available. A favorable change in this profile is one of the mechanisms through which estrogens influence the cardiovascular risk. Therefore, lipoprotein profile is part of the causal chain, and it would be inappropriate to adjust for this factor (20). Diabetes and prevalent cardiovascular disease can be considered as mediators in the pathway between estrogen exposure and cardiovascular death as well, so we did not control for these variables. Information on smoking was not available either. However, in comparable studies of age at menopause and (cardiovascular) mortality, smoking was not a strong confounder (6, 8, 9, 21). Hu et al. (9) also studied age at menopause and cardiovascular risk in strata of smoking and suggested that age at natural menopause was related only to cardiovascular disease in (past) smokers, although the null association among nonsmokers could well be caused by chance because of the low number of cases in this stratum.

In the Netherlands, causes of death are registered with the Netherlands Central Bureau of Statistics. This registration was used to collect the information on primary causes of death of the women in the cohort. Although misclassification may have occurred, we do not believe this was related to the determinants of interest, therefore causing most likely an underestimation of the true effect.

We studied cardiovascular mortality instead of cardiovascular disease. Studies on age at menopause and cardiovascular mortality (6) and on age at menopause and cardiovascular disease (9) showed very comparable results.

The Cox regression model is based on the assumption that women who were withdrawn from the study did not differ in their outcome risk compared with women in the cohort. Censoring affected comparable percentages of the duration of exposure categories (approximately 16 percent in each quartile), and mean duration of endogenous estrogen exposure for women who were withdrawn was similar compared with the mean of the whole cohort.

We analyzed in quartiles the variables age at menopause and duration of exposure to endogenous estrogen. The results seem to indicate a quadratic or threshold protective effect for cardiovascular mortality that was particularly pronounced in the highest quartile of age at menopause and duration of exposure to endogenous estrogen . This indicates that an increasing exposure does not reduce the risk of cardiovascular mortality until a older age at menopause or a high level of exposure to endogenous estrogen.

Our model of duration of exposure to endogenous estrogen allows us to combine all data on reproductive endogenous estrogen-related events in one measure that we can use to address the association between endogenous estrogen and estrogen-related diseases. To our knowledge, no other studies have been performed using this or comparable endogenous estrogen exposure algorithms. Our model has not been validated in other studies yet. Theoretically, it is still possible that duration of endogenous estrogen exposure is a better predictor of cardiovascular disease risk compared with age at menopause. This will be the case when the model is not valid or when the model can be improved by a more precise measurement of duration of endogenous estrogen exposure. It would be interesting to validate the algorithm in observational studies with other estrogen-related diseases such as breast or endometrial carcinoma or osteoporosis.

Indirect evidence of a role of endogenous hormones on cardiovascular disease risk comes from studies relating cardiovascular risk to the timing or frequency of events associated with endogenous sex hormone levels. These reproductive events have been studied in relation to known cardiovascular disease risk factors as well as cardiovascular disease events. There was much variation in the study design, domain, and endpoints of the studies considering these reproductive factors in association with cardiovascular risk (22). Age at menopause was the only reproductive factor that was clearly associated with cardiovascular disease risk in these studies. Therefore, we compared the predictive value of duration of exposure to endogenous estrogen with that of the single reproductive factor age at menopause by the use of Receiver Operating Curves. It is possible that the duration of endogenous estrogen exposure does not affect cardiovascular disease risk but that lack of exposure to estrogen does. In that case,

age at menopause is the only reproductive factor associated with cardiovascular disease risk because this age demarcates the transition from high to low exposure to endogenous estrogen. The duration of exposure to endogenous estrogen is, by definition, strongly related to age at menopause (Pearson correlation coefficient = 0.73, $p < 0.001$), which could explain the equality of predictive values of cardiovascular mortality.

Research on the protective cardiovascular effects of estrogen has focused mostly on the effects of HRT. A recent meta-analysis estimated a 50 percent reduction in coronary heart disease risk (4). Most studies in this meta-analysis addressed the use of unopposed, conjugated equine estrogens. The only randomized controlled trial published so far of which we are aware, assessing secondary prevention by HRT (Heart and Estrogen/Progestin Replacement Study (5)), did not show an effect of combined therapy on cardiovascular disease outcome after 4 years of treatment. A possible explanation for these results was the adverse effect of conjugated estrogens combined with medroxyprogesterone on arterial thrombosis, annulling the positive effects on lipoproteins. The favorable association in observational studies between hormonal replacement therapy and cardiovascular disease risk could also be explained by selection bias, introduced by the "healthy estrogen user effect" (23). Ongoing primary prevention trials should provide us with more definitive answers on the beneficial effects of HRT.

The population attributable risk (PAR) represents the proportion of women who died of a cardiovascular event that was attributable to a specific risk factor. A 20 percent reduction in cardiovascular mortality risk in the highest quartile of endogenous estrogen exposure and age at menopause means a PAR of –5 percent. The PAR of a body mass index above 29 in this population is 14 percent, and the PAR for hypertension is 11 percent. Cardiovascular deaths account for an important proportion of all deaths among postmenopausal women (43.6 percent of total deaths in this population). A PAR of –5 percent for cardiovascular mortality in the highest quartile of endogenous estrogen exposure and age at menopause can therefore be clinically relevant.

In conclusion, this prospective study shows that age at menopause is related to cardiovascular disease mortality. However, a newly developed composite measure of endogenous estrogen exposure did not add to the predictive value for cardiovascular mortality of age at menopause. Therefore, age at menopause will provide the clinician sufficient information on history of endogenous estrogen exposure and its associated cardiovascular disease risk.

ACKNOWLEDGMENTS

Supported by research grant 96.034 from the Netherlands Heart Foundation.

The authors thank E. Brummelkamp and H. Otten for collecting data. The authors are grateful to the Netherlands Central Bureau of Statistics, who allowed them to work in their office with Central Bureau of Statistics data on causes of death.

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