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Hormone Replacement Therapy and Intima-Media Thickness of the Common Carotid Artery
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Background and Purpose—Observational data suggest that hormone replacement therapy (HRT) reduces morbidity and mortality from cardiovascular disease in healthy postmenopausal women. The mechanisms underlying this protection are not entirely clear but may include inhibition of the atherosclerotic process.

Methods—We studied the association between ever use of HRT and intima-media thickness (IMT) of the common carotid artery in 1103 naturally menopausal women, aged 55 to 80 years, in the Rotterdam Study, a community-based cohort study in a suburban area of Rotterdam, Netherlands. Mean and maximum IMT of the common carotid artery were measured noninvasively with B-mode ultrasound.

Results—Ever use of HRT for ≥1 year was associated with a decreased mean and maximum IMT compared with never users (mean IMT, 0.719 mm [SE 0.01] versus 0.742 mm [SE 0.004], $P=0.03$; maximum IMT, 0.952 mm [SE 0.015] versus 0.983 mm [SE 0.006], $P=0.04$), after adjustment for age, smoking, educational level, systolic blood pressure, and body mass index. No association was found for use <1 year (mean IMT, 0.739 mm [SE 0.013] versus 0.742 mm [SE 0.004], $P=0.69$; maximum IMT, 0.990 mm [SE 0.019] versus 0.983 mm [SE 0.006], $P=0.75$). Additional adjustment for diabetes, frequency of visits to healthcare facilities, or total and HDL cholesterol did not change these results.

Conclusions—The findings of this population-based study show that ever use of HRT is associated with a decreased IMT in the common carotid artery in elderly women. (*Stroke*. 1999;30:2562-2567.)

Key Words: atherosclerosis ■ carotid arteries ■ estrogen ■ hormone replacement therapy ■ intima-media thickness

Observational data suggest that hormone replacement therapy (HRT) reduces morbidity and mortality from cardiovascular disease in postmenopausal women.[1–3] The mechanisms underlying this protection are not entirely clear. Postulated mechanisms for a beneficial effect include changes in lipid levels, hemostatic variables, and blood viscosity; direct effects on the arterial wall; and inhibition of the atherosclerotic process. Recently, however, a randomized trial on the effects of HRT in women with coronary heart disease showed no effect on the overall risk of coronary heart disease after 4 years of treatment (the Heart and Estrogen/Progestin Replacement Study [HERS]).[4] However, an increased risk of coronary heart disease events was found in the HRT group in the first year of the trial, and the risk decreased in subsequent years. This might be explained by an immediate prothrombotic, proarrhythmic, or proischemic effect of treatment, which is gradually outweighed by a beneficial effect on the progression of atherosclerosis. The results of HERS thus do not exclude the possibility that HRT inhibits the development of atherosclerotic disease. It is therefore important to assess whether favorable effects of HRT on atherosclerosis are indeed present. Data on the effects of use of HRT on atherosclerosis are conflicting.[5,6] We investigated the association between ever use of HRT and the presence of atherosclerosis in the carotid artery by measuring common carotid intima-media thickness (IMT) in 1103 naturally postmenopausal women participating in the Rotterdam Study.

Subjects and Methods

The Rotterdam Study

The Rotterdam Study is a prospective population-based follow-up study that aims to assess the occurrence of chronic diseases in an aging population and to clarify their determinants. The study focuses on cardiovascular, neurogeriatric, ophthalmologic, and locomotor diseases and has been described in more detail elsewhere.[7] In brief, all residents aged 55 and over of a defined district in Rotterdam were invited to participate. A total of 7983 men and women (78% of those eligible) entered the study. During the first survey, during 1990–1993, all participants were interviewed at home by a trained research assistant. The participants subsequently visited the study center for a

clinical examination. A second visit to the study center took place in 1993–1994. The study was approved by the Medical Ethical Committee of Erasmus University, and informed consent was given by all participants.

Measurements

Interview information was obtained by a trained research assistant. Data included medical history, current medication, smoking habits, alcohol intake, highest attained level of education, and age at last menstruation. Menopause was defined as cessation of menses for ≥1 year. Height and weight were measured at the study center. Blood pressure was measured twice with a random zero sphygmomanometer with the subject in a sitting position, and the measurements were averaged. Serum total cholesterol values were assessed by an automated enzymatic procedure in a nonfasting blood sample. Serum HDL cholesterol was measured after precipitation of the non-HDL fraction with phosphotungstate-magnesium. Random and postload serum glucose levels were assessed after an oral glucose tolerance test.

Measurement of IMT

To measure carotid IMT, ultrasonography of the left and right common carotid arteries was performed with a 7.5-MHz linear array transducer (ATL UltraMark IV). On a longitudinal 2-dimensional ultrasound image of the carotid artery, the anterior (near) and posterior (far) walls of the carotid artery are displayed as 2 bright white lines separated by a hypoechogenic space. The distance between the leading edge of the first bright line of the far wall (lumen-intima interface) and the leading edge of the second bright line (media-adventitia interface) indicates the IMT. For the near wall, the distance between the trailing edge of the first bright line and the trailing edge of the second bright line at the near wall provides the best estimate of the near wall IMT.[8,9] After the ultrasound protocol,[10] a careful search was performed for all interfaces of the near and far walls of the distal common carotid artery. When an optimal longitudinal image was obtained, it was frozen on the R wave of the ECG and stored on videotape. This procedure was repeated 3 times for both sides. The actual measurements of IMT were performed offline. From the videotape, the frozen images were digitized and displayed on the screen of a personal computer with the use of additional dedicated software. This procedure has been described in detail previously.[11] With a cursor, the interfaces of the arterial segments were marked over a length of 10 mm. The beginning of the dilatation of the distal common carotid artery served as a reference point for the start of the measurement. The average of the IMT of each of the 3 frozen images was calculated. For each individual, an IMT was determined as the average of near and far wall measurements of the left and right arteries. The readers of the ultrasound images from videotape were unaware of the exposure status of the subject. Reproducibility of IMT measurements was studied among 80 subjects who underwent a second ultrasound scan of both carotid arteries within 3 months of the first scan. Measurements were shown to be highly reproducible.[8] Offline the carotid artery was evaluated from tapes for the presence (yes/no) of atherosclerotic lesions on both the near and the far walls of the arteries. Plaques were defined as a focal widening relative to adjacent segments, with protrusion into the lumen composed of either only calcified deposits or a combination of calcified and noncalcified material. The common and internal carotid arteries and the carotid bifurcation were both evaluated online and offline for the presence or absence of atherosclerotic lesions. In the analyses, carotid plaque was defined as the presence of plaques at the near or far wall at ≥1 site. The size of the lesions was not quantified. A reproducibility study among 166 subjects on the assessment of plaques in the carotid bifurcation revealed a κ of 0.65 for the right carotid artery, indicating moderate agreement.[12]

Assessment of Use of HRT

During the baseline interview (1990–1993), questions on ever use of female hormones for menopausal complaints and duration of use were asked. More information on use of female hormones was collected by a questionnaire during the first follow-up visit to the study center in 1993–1994, on average 2.2 years after baseline. In this questionnaire, information on medication for menopausal complaints, medication after an operation of the womb/ovaries, specific information regarding brand and type of female hormones, duration of use, recent use, and the use of progestins was collected.

Information from both the first and the second visits to the study center was used for classification of subjects. At baseline, 571 women reported the use of female hormones for menopausal complaints. Twenty-six women reported ever use of female hormones in the first follow-up visit to the study center, while they had not reported this in the baseline interview. These women were classified as probable users. Additionally, 95 women reporting the use of medication for menopausal complaints in the questionnaire for the follow-up visit, but who were not sure what type of medication this had been and who had not reported use of female hormones in the baseline interview, were classified as possible users. Women reporting the use of only vaginal creams or ovules were classified as nonusers of female hormones. Thus, 692 women were classified as ever users of female hormones at the follow-up visit. Seventy-four of these women reported to have continued use of female hormones in the period between the baseline and the follow-up visits. Since we cannot be sure whether these women were current users at the time of IMT measurement at baseline, these women were classified as recent users.

Population for Analysis

In the Rotterdam Study, 4853 postmenopausal women participated. Since data on use of HRT were obtained in both the first and the second follow-up rounds, only women participating in the second follow-up round were analyzed in this study (n=3784). Excluded were women who reported that they had reached menopause by surgery (n=677) or radiation of the womb or ovaries (n=42) and women who were older than 80 years (n=558), since the use of HRT in older women was rare. Furthermore, 55 women had no data on the use of HRT. After the exclusion of women who fulfilled ≥1 of these exclusion criteria, 2401 women remained for analysis. IMT was determined in the first 1103 of these women only. Compared with women with data on IMT, women without data were slightly younger, had a significantly higher systolic and diastolic blood pressure, were significantly less often past smokers. No differences were found in other cardiovascular risk factors.

Statistical Analysis

ANCOVA was used to compare characteristics of HRT users and nonusers, adjusted for present age. Age-adjusted linear regression analysis was used to assess the association between risk factors and IMT. Multivariate ANCOVA was used to assess the association between the use of HRT and IMT. Multivariate analyses included smoking status (current, past, and never), number of pack-years for current and past smokers (the number of years of smoking multiplied by the number of cigarettes smoked daily divided by 20), educational level (in 4 categories: primary education, lower general education/lower vocational education, intermediate vocational education, and higher education/university), systolic blood pressure, and body mass index (BMI). A distinction was made between short-term use (<1 year) and long-term use (≥1 year) because a large group of women had reported short-term use, and no effect on development of atherosclerosis was expected from this. Additional analyses were performed with adjustment for diabetes (defined as a random or postload glucose level of >11.0 mmol/L or current use of antidiabetic drugs), frequency of visits to healthcare facilities in the last month, and levels of total and HDL cholesterol. To assess the effect of past use, the analysis was repeated with the exclusion of recent users. The analysis was repeated after exclusion of probable and possible users. All reported *P* values are 2-sided. Analyses were performed with the use of BMDP software (BMDP Statistical Software, Inc).

TABLE 1. Age-Adjusted Baseline Characteristics of Short-Term, Long-Term, and Never Users of Female Hormones Among Women With Natural Menopause, Aged 55 to 80 Years

Characteristic	Never Users (n=875)	Short-Term Users (<1 y) (n=79)	Long-Term Users (≥1 y) (n=136)
Age, y	67.9 (0.23)	66.5 (0.71)	67.1 (0.56)
Time since menopause, y	18.6 (0.2)	18.1 (0.5)	19.4 (0.4)
Systolic blood pressure, mm Hg	137.1 (0.7)	138.3 (2.2)	136.9 (1.9)
Diastolic blood pressure, mm Hg	71.9 (0.4)	74.2 (1.2)	72.3 (0.9)
BMI, kg/m²	27.0 (0.1)	26.7 (0.5)	26.0 (0.4)*†
Total cholesterol, mmol/L	6.96 (0.04)	6.81 (0.14)	6.98 (0.10)
HDL cholesterol, mmol/L	1.45 (0.01)	1.47 (0.04)	1.48 (0.03)
Diabetes, %	8.0 (0.9)	9.1 (0.3)	8.5 (2.3)
Current smoker, %	29.6 (1.8)	23.3 (5.9)	37.8 (4.6)
Pack-years for smokers, n	28.4 (1.4)	38.9 (5.3)	28.0 (3.0)
Former smoker, %	37.6 (1.8)	35.9 (5.9)	45.3 (4.5)
Pack-years for former smokers, n	17.6 (1.3)	25.8 (4.4)	16.9 (3.0)
Alcohol drinker, %	72 (1.6)	75 (5.2)	75 (3.9)
Visited GP in last month, %	40 (1.7)	53 (5.5)*	45 (4.2)§
Visited specialist in last month, %	19 (1.3)	26 (5.4)	26 (3.4)*†
Higher education, % highest category	20.5 (1.3)	21.3 (4.5)	26.1 (3.4)
Income, Euro per month	1192.24 (18.5)	1279.43 (61.0)	1147.24 (46.9)

Values are means or proportions (SE). Numbers do not add up to totals because of 13 missing values for duration of hormone use.

*P<0.05 vs never users; †P<0.05, ever users vs never users.

Results

At baseline, no differences were seen between ever users and never user of HRT in age, educational level, and income (Table 1). Long-term users had a significantly lower BMI and were more often current smokers, although this comparison did not reach statistical significance (P=0.11). Short-term users had a slightly higher diastolic blood pressure (P=0.07) and more pack-years of smoking (P=0.06 for current and P=0.08 for past smokers) than never users. In comparison to never users, short-term users reported significantly more visits to the general practitioner (GP), while long-term users reported more visits to medical specialists in the last month.

Of the 1103 women in this study, 228 reported a history of use of HRT (20.7%). Of these, 13 did not report duration of hormone use. Duration of use ranged from 1 month to >15 years. Seventy-nine women (36.7%) reported use for <1 year, 68 (31.6%) for 1 to 4 years, and 68 for (31.6%) >5 years. Twenty-one women (9% of all users) reported that they had used HRT between the first and second visits. Twenty women (9% of all users) reported a history of use of progestins in addition to the use of estrogens.

Mean IMT in our study group was 0.740 mm (SD 0.131) and ranged from 0.410 to 1.518 mm. Maximum IMT was on average 0.980 mm (SD 0.187) and ranged from 0.550 to 2.295 mm.

Table 2 shows age-adjusted associations between cardiovascular risk indicators and IMT. Age, systolic blood pressure, total and HDL cholesterol levels, diabetes, and current smoking were all independently and significantly associated with IMT. The association of BMI with IMT did not reach

statistical significance (95% CI, −0.0002 to 0.0031; P=0.08).

ANCOVA showed that users of HRT had a mean age-adjusted IMT of 0.730 mm compared with a mean IMT of 0.743 mm in nonusers (P=0.13; Table 3). Stratification for duration of use, however, showed that while use for <1 year was not associated with a reduction of IMT, use for ≥1 year was associated with a statistically significant reduction of

TABLE 2. Age-Adjusted Regression Coefficients for Association Between Cardiovascular Risk Factors and IMT

Characteristic	β*	95% CI
Age (10 y)	0.087	0.077, 0.079
Systolic blood pressure (10 mm Hg)	0.012	0.008, 0.015
Diastolic blood pressure (10 mm Hg)	−0.005	−0.012, 0.001
BMI (kg/m²)	0.0015	−0.0002, 0.0031
Total cholesterol (10 mmol/L)	0.081	0.071, 0.092
HDL cholesterol (10 mmol/L)	−0.241	−0.433, −0.049
Diabetes (yes/no)	0.041	0.0154, 0.0657
Current smoker (yes/no)	0.0297	0.0110, 0.0485
Former smoker (yes/no)	0.0085	−0.0073, 0.0242
Alcohol drinker (yes/no)	−0.0043	−1.0020, 0.0034
Visited GP in last month (yes/no)	0.0193	0.0051, 0.0335
Visited specialist in last month (yes/no)	−0.0004	−0.0177, 0.0169
Higher education (highest category)	−0.001	−0.010, 0.008
Income (per 1000 Euro)	−0.005	−0.019, 0.009

*Increase of IMT in millimeters per unit increase in the cardiovascular risk factor.

TABLE 3.   Age-Adjusted IMT in Never Users, Short-Term Users, and Long-Term Users of HRT

Characteristic	Never Users (n=984)	Short-Term Users (<1 y) (n=79)	P*	Long-Term Users (≥1 y) (n=136)	P*	Ever Users (All) (n=228)	P*
CCA IMT, mean, mm†	0.743 (0.004)	0.739 (0.013)	0.72	0.722 (0.010)	0.05	0.730 (0.008)	0.13
Adjusted full‡	0.742 (0.004)	0.739 (0.013)	0.69	0.719 (0.010)	0.03	0.728 (0.008)	0.10
CCA IMT, maximum, mm†	0.983 (0.006)	0.990 (0.019)	0.76	0.956 (0.015)	0.09	0.970 (0.011)	0.32
Adjusted full‡	0.983 (0.006)	0.990 (0.019)	0.75	0.952 (0.015)	0.04	0.968 (0.011)	0.23

Values in parentheses are SE. CCA indicates common carotid artery. Numbers do not add up to totals because of missing values for duration of hormone use.

*Compared with never users.

†Adjusted for age.

‡Adjusted for age, smoking, education, systolic blood pressure, and BMI.

IMT. Analysis adjusted for smoking, number of pack-years smoked, level of education, systolic blood pressure, and BMI did not change these results. Additional adjustments for diabetes, frequency of visits to a GP or to a medical specialist in the last month, or total and HDL cholesterol did not change the risk estimates. No associations were found with duration of use in women who had used HRT for ≥1 year.

The numbers of recent users and users of combined estrogen-progestin therapy were small. Comparison of mean IMT in the 21 recent users with never users after adjustment for age and confounders showed a decrease in IMT (0.696 mm [SE 0.025] versus 0.741 mm [SE 0.004]; P=0.08).

When we repeated the analyses excluding women reporting recent use of female hormones to assess the effect in past users only, similar associations were found for past users versus never users for mean IMT (0.719 mm [SE 0.013] versus 0.743 mm [SE 0.004]; P=0.07) and for maximum IMT (0.940 mm [SE 0.019] versus 0.984 mm [SE 0.006]; P=0.03). In addition, when we repeated the analysis after exclusion of women who were classified as probable and possible users (121 women), similar associations were found for both mean and maximum IMT.

Carotid plaques were measured in 1887 of the 2401 women eligible for this study (79%). Plaques were found present in 48% of ever users and 53% of never users after adjustment for age and confounders (P=0.15).

Discussion

We found a lower level of IMT in the common carotid artery in women who had used HRT for ≥1 year compared with never users. Use of HRT for <1 year was not associated with increased IMT.

Before these results are interpreted, several issues need to be addressed. There is the possibility of selection bias in this study of elderly women. Women had to survive until at least age 55 years to be in our study. If a protective effect of HRT was present, and women who had never used female hormones had died of or had not responded because of atherosclerotic complications before the start of our study, this may have led to an underestimation of the effect.

Several studies demonstrated that estrogen users are healthier than never users, even before use of replacement therapy, which supports the hypothesis that part of the apparent benefit associated with HRT is due to preexisting characteristics of the users.[13,14] Women who take hormones are a self-selected group and may have healthier lifestyles with fewer risk factors than women who do not. Additionally, compliant women who stay on estrogen represent a minority of all women who are ever prescribed estrogen, and these women may differ from the less compliant women. We cannot exclude the possibility that part (or the whole) of our findings is based on this selection bias. In this study we have dealt with the issue of confounding in the following ways. We stratified for duration of hormone use and found that short-term users were similar to long-term users with respect to the presence of several socioeconomic and risk factors, such as income, frequency of visits to a GP or medical specialist in the last month, alcohol consumption, and total and HDL cholesterol levels. Among women who had used female hormones for <1 year, no association was present with IMT. This diminishes but does not fully exclude the probability of selection bias. Furthermore, we adjusted for known risk factors. We measured the current status of risk factors, while the exposure to hormone use had largely taken place in the past. Socioeconomic status (level of education) may be a major confounder but remains relatively stable over time. The frequency of visits to healthcare facilities (possibly representing health-conscious behavior now and in the past) differed between ever and never users of HRT, but adjustment did not change our results. Smoking habits might have changed over time, but misclassification of smoking habits would have given an underestimation of the effect, because users were more frequently smokers. BMI and alcohol intake may undergo changes with age, and it could be that some residual confounding has remained after adjustment. We do not expect this to have a large effect on our results, however, because of the relatively weak associations of these factors with IMT.

Use of HRT was assessed by interview. This might have led, to a certain extent, to misclassification. Greendale et al[15] demonstrated that a single self-report question is adequate to ascertain ever use of postmenopausal estrogen use in women aged <64 years. Another study showed moderate to substantial agreement between users and physicians on ever/never use of estrogens and no differential misclassification with disease status of the subject in women aged <74 years.[16] The reported frequency and duration of use of HRT seemed to be similar to those in studies in perimenopausal women in the Netherlands, where 12% of women aged 45 and 65 years used HRT and 50% of women discontinued use within 1 year.[17–19]

According to data from the Institute of Medical Statistics (an institution reporting yearly updates on prescriptions per indication), the most frequently prescribed hormone therapy in the studied period was unopposed estrogen therapy in a dose of 0.625 mg daily. In addition to conjugated equine estrogens, estradiol preparations were also prescribed. Progestins were added in 0.6% of prescriptions in 1970 and in 11% of prescriptions in 1986. Our observation of 9% agrees with this statistic and with that of others.[18]

Increased IMT of the common carotid artery has been shown to be associated with risk factors for atherosclerosis,[20–22] atherosclerosis in other locations,[9,12] and cardiovascular disease.[23,24] Thus, IMT can be used as an indicator for generalized atherosclerosis. Ultrasonographic measurements of IMT have been shown to be highly reproducible.[8,25]

The finding that HRT inhibits development of atherosclerosis in the coronary arteries and aorta has been reported in several animal studies.[26–31] Studies with angiographic end points showed a lower degree of coronary atherosclerosis in HRT users than in nonusers.[32–34] Detection bias could have been introduced, however, if women on estrogen were selected for an angiogram on the basis of less severe symptoms in comparison with nonusers. Results from population-based studies have been conflicting. In the Cardiovascular Health Study, carotid IMT and stenosis in elderly women using estrogen and progestin were similar to those of women using estrogens alone, and both groups had a lower IMT of the internal and common carotid arteries compared with never users.[35] Another large population-based study in women aged <55 years (the Atherosclerosis Risk in Communities Study [ARIC]), however, did not find an association between HRT and IMT.[5] In 2 small cross-sectional studies, a lower IMT was found on ultrasonographic examination of the carotid arteries[36] and in the aorta and iliac arteries[37] in users of combined replacement therapy compared with nonusers. Results from the Asymptomatic Carotid Atherosclerosis Progression Study (ACAPS) among 186 postmenopausal women suggested that HRT may halt progression of atherosclerosis, as measured by carotid IMT.[38]

Most studies focused on current users. The effect of past use of female hormones was studied in an earlier report from the Cardiovascular Health Study, which showed that differences in mean carotid wall thickness were greater between current and past users than between past and never users.[6] Maximum wall thickness did not differ between past and never users. In our study the number of recent users was small, but the exclusion of recent users from the analysis clearly demonstrated the association in past users. Possibly, this difference in findings can be explained by the longer period since hormone use among women in the Cardiovascular Health Study, since these women were older than the women in our study population.

Although some studies have found an effect of duration of use of female hormones on atherosclerosis,[39–41] several large population-based studies,[6,42,43] did not find this association. This may be explained as a reflection of unreliability of data on duration. On the other hand, the observations of a stronger protective effect of HRT in current than in past users, as well as a diminishing of the protective effect after cessation of therapy, suggest that mechanisms other than the inhibition of atherosclerosis are also active. Our results suggest that use of HRT for ≥1 year decreases the development of atherosclerosis, but no effect of duration of use was found.

Only 1 randomized trial on the effects of HRT on cardiovascular disease has been conducted (HERS). This trial in women with diagnosed cardiovascular disease showed no favorable effect of HRT on incident coronary heart disease after 4.1 years of follow-up.[4] This result indicates that bias in observational studies may be larger than thought until now. On the other hand, in the trial an increased risk for coronary heart disease events was found in the HRT group compared with the placebo group in the first year of the trial, but a decreased risk was found in subsequent years. This time trend should be interpreted with caution but might be explained as attributable to an immediate prothrombotic, proarrhythmic, or proischemic effect of treatment that is gradually outweighed by a beneficial effect on the progression of atherosclerosis. Thus, the results of the HERS trial do not exclude the possibility that HRT might inhibit the development of atherosclerosis. The aforementioned immediate effects of HRT might be expected to be of more importance in women with previous cardiovascular disease. Furthermore, in the HERS trial the effects of opposed estrogen were compared with placebo, while in our study mainly unopposed estrogen was used. The effects of different kinds of progestins on development of atherosclerosis remain unclear.[44–46]

Our results suggest that past use of HRT is associated with a favorable atherogenic status. Further understanding of the effects of HRT on atherogenesis can be obtained only in randomized trials that are adequately designed to take the distinct effects of HRT on the short and the longer term into account.

Acknowledgments

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