Estrogen, atherosclerosis and cardiovascular disease in women

Epidemiological studies on menopause and hormone replacement therapy

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Epidemiologische studies naar menopauze en hormoon substitutie therapie

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Co-promotor	:	Dr J.C.M. Witteman
Overige leden	:	Prof. dr B.C.J.M. Fauser Prof. dr H.A.P. Pols Prof. dr J.R.T.C. Roelandt

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General introduction

Atherosclerosis, the principal cause of ischemic heart disease, stroke and peripheral arterial disease, is the most important cause of morbidity and mortality in Western countries. Atherosclerosis and cardiovascular disease are diseases of the elderly. Demographic data predict that the number of elderly people in our country as well as in most Western countries, will increase in the coming years, especially among women. This means the absolute number of deaths from cardiovascular disease in women is bound to increase, and an increasing awareness of the importance of cardiovascular disease as a major issue in women is warranted.

Premenopausal women seem to be protected from cardiovascular disease compared to postmenopausal women. What part menopause plays in the increased risk of cardiovascular disease after middle age is still debated.[1-3] One might expect that women experiencing an early menopause have more time to develop atherosclerosis and thus carry a high risk of coronary heart disease. But data on the association between menopause and coronary heart disease are conflicting. The inconsistency might be the result of a methodological problem; incidence of cardiovascular disease in women shortly after menopause is quite low, and increases only after age 70. This lag time of 10 to 20 years between menopause and the occurrence of coronary heart disease in women makes the effect of menopause difficult to disentangle from that of age. A better approach to study the role of menopause might be to study its association with atherosclerosis, as the latter is present long before symptomatic coronary heart disease develops. Only few studies have focussed on non-invasively measured atherosclerosis in relation to menopause.

Observational studies consistently show a marked reduction of coronary heart disease associated with the use of hormone replacement therapy. Although results from observational studies are strong, consistent and biologically plausible, potential biases are large and most would be expected to spuriously enhance the observed cardioprotective effect. Nonetheless, because coronary heart disease is the most common and most deadly disease of women, any significant reduction in coronary heart disease risk due to hormone replacement therapy would strongly affect the benefit-risk scale. The mechanisms by which hormone replacement therapy exerts its effect on the cardiovascular system have not yet been fully explained. It is not known, whether the effect is based on influencing atherosclerosis or on other, direct effects, and whether these effects remain present after discontinuation of therapy.

The aim of this thesis is to gain insight into the role of menopause in the increase of cardiovascular disease in women after middle age. Furthermore, in search of possibilities for prevention, it addresses the effect of hormone replacement therapy.

In chapter 2.1, in order to assess the effect of menopause on risk factors for coronary heart disease, we examined cardiovascular risk factors in a meticulously selected population in which the contrast in estrogen status between pre- and postmenopausal women of the same age was maximised. In chapter 2.2, the difference in arterial dis-

tensibility between pre- and postmenopausal women was studied, in the same study population of pre- and postmenopausal women. In chapter 2.3 we investigated the association between age at menopause and the presence of non-invasively measured atherosclerosis in the aorta, the carotid arteries and peripheral arteries, as well as the presence of myocardial infarction in 4853 postmenopausal women, aged 55 and over, participating in the Rotterdam Study.

The results of two observational studies on the effects of hormone replacement therapy are described in chapter 3. In chapter 3.1 the associations between current and past use of hormone replacement therapy and peripheral arterial disease are reported, using the observational data from The Rotterdam Study. The association between hormone replacement therapy and intima-media thickness of the common carotid artery was studied in chapter 3.2. In chapter 4 of this thesis results from a randomised intervention trial (ROMEO I), are reported. Two studies were conducted to examine whether combined hormone replacement therapy would affect arterial stiffness and intima-media thickness of the common carotid artery. In the general discussion the results described in this thesis are briefly summarised. Some methodological problems are discussed. Our findings are addressed in the light of the hypotheses on the effects of menopause and hormone replacement therapy on atherosclerosis and cardiovascular disease in women, and finally, some suggestions for further research are given.

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2

Menopause

2.1 Menopausal status and risk factors for cardiovascular disease

Abstract

Objectives – Changes in cardiovascular risk factors with menopausal status are difficult to study, due to the high correlation of menopausal status with age. Therefore we examined cardiovascular risk factors in a meticulously selected population in which the contrast in estrogen status between pre- and postmenopausal women of the same age was maximised.
Design – Risk factors were compared in 93 premenopausal and 93 postmenopausal women who were matched on age (range 43-55 years).
Setting – The women were selected from respondents to a mailed questionnaire about the menopause, which was sent to all women aged 40 to 60 years in the Dutch town of Zoetermeer (n = 12,675; response 54 %).
Subjects – Postmenopausal women who were at least three years after menopause or whose menses had stopped naturally before age 48 were age-matched with premenopausal women with regular menses and without menopausal complaints.
Results – Compared to premenopausal women, postmenopausal women had significantly increased levels of total cholesterol (10.0%, 95% confidence interval 5.1 - 14.0), low density lipoprotein (LDL) cholesterol (14.0%, 6.9 - 19.9), and apolipoprotein B (8.2%, 0.6 - 15.5). The difference was present within three years after onset of menopause and did not show a trend towards an increase with the number of postmenopausal years. No differences were found in high-density lipoprotein (HDL) cholesterol, triglycerides, apolipoprotein A1, blood glucose, insulin, body mass index, waist-to-hip ratio, and systolic and diastolic blood pressure.
Conclusions – The results of this study add to the evidence that total cholesterol, LDL cholesterol and apolipoprotein B are the primary cardiovascular risk factors affected by menopause.

INTRODUCTION

The incidence of cardiovascular disease in women rises sharply after middle age. Although results of large follow-up studies are inconsistent, menopause is thought to be a major determinant of this increase.[1-3] The mechanism through which menopause exerts its effect on the cardiovascular system is still unknown. Increased levels of serum total cholesterol after cessation of menses have been found in most studies on menopause and risk factors.[4-16] Inconsistent results, however, have been reported with respect to HDL-cholesterol[8-10,12-14,17], apolipoproteins[7,8,12,16-18], blood pressure[4-6,8,9,17,19-22], waist-to-hip ratio [23,24] and insulin.[25-27] A difficulty with studying effects of menopause is the high correlation between menopausal status and age. Studies that included women in a broad age range may not be able to validly remove the confounding effect of age.[13,16,19,24] Studies in a restricted age range around the menopause will include premenopausal women who have irregular menses and postmenopausal women who only recently passed menopause, which reduces the contrast in estrogen status.[17,21]

In the present study, we examined the relations between natural menopause and several atherogenic factors in a highly selected population in which the contrast in estrogen status between pre- and postmenopausal women of the same age was maximised.

MATERIALS AND METHODS

Study population

Selection of participants in this study was aimed at maximising the contrast in estrogen status, in pre- and postmenopausal women of the same age (figure 1). A questionnaire, including questions about menopausal status, medical history, medication use, and smoking behaviour, was sent by mail to all women aged 40 to 60 years and living in the town of Zoetermeer, The Netherlands (n=12,675). The response rate was 54%. Selection of pre- and postmenopausal women was based on the questionnaire. Women with a hysterectomy and/or uni- or bilateral ovariectomy and women with missing information on type or date of menopause (n=233) were excluded from the study population (n=1,551). Women were considered premenopausal if they had one or more bleedings in the past 12 months (n=3,829). Premenopausal women who reported no longer having monthly bleedings (n=938) and women who reported the presence of climacteric symptoms, defined as perspiration and/or hot flushes (n=1,645) were ex-

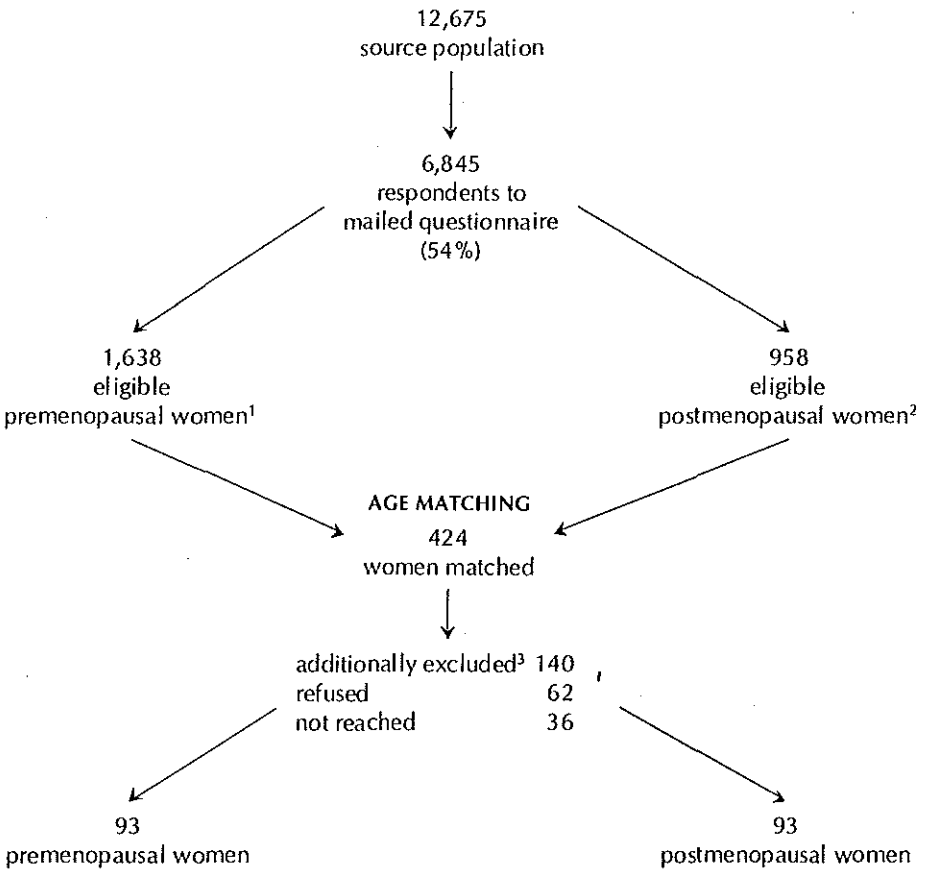

Figure 1 – Schematic presentation of the selection procedure of the study population.
(1) Eligible were women with regular menses and no climacteric symptoms, who did not use hormone replacement therapy or oral contraceptives in the past 6 months. Subsequently women who smoked, who had diabetes mellitus or used antihypertensive or cholesterol lowering drugs were excluded. (2) Eligible were women whose menses had ceased naturally more than 12 months ago and who had not used hormone replacement therapy. Subsequently women who smoked, who had diabetes mellitus, or who used antihypertensive or cholesterol lowering drugs were excluded. (3) Women who no longer fulfilled the criteria at the moment of examination were excluded.

cluded. Furthermore, premenopausal women who reported use of hormone replacement therapy or use of oral contraceptives within 6 months prior to the clinical examination were excluded (n=423).

The total number of premenopausal women excluded for the above mentioned reasons was 2,191, leaving 1,638 eligible premenopausal women.

Women were considered to have had natural menopause if their menses had ceased naturally for at least 12 months (n=1,242). Postmenopausal women who reported a history of hormone replacement therapy for over six months or use of female hormones within six months prior to the clinical examination and women who reported cessation of bleedings immediately upon stopping hormones were excluded (n=241). The total number of postmenopausal women excluded, including those with missing values on hormone use was 284, leaving 958 eligible postmenopausal women. Of these women, we additionally excluded women reporting diabetes mellitus (13 (0.8%) premenopausal versus 16 (1.7%) postmenopausal women), use of antihypertensive medication (31 (1.9%) versus 35 (3.7%)), use of cholesterol-lowering drugs (3 (0.2%) versus 20 (2.1%)) and current smoking of 5 or more cigarettes per day (302 (18.4%) versus 218 (22.8%)).

Pre- and postmenopausal women were matched on age, while maximising the contrast in estrogen status. Postmenopausal women who were at least three years after menopause or whose menses had stopped at least three years before the average age of menopause (51 years) were age-matched with premenopausal women with a regular menses and without menopausal complaints. If it was not possible to find a match within the same year of age, a match was taken from an adjacent year. If one of a matched pair was unwilling to participate a new match was sought. Women were invited for study participation on average 15 months after return of the questionnaire. Out of 424 invited women, 140 were excluded because they no longer fulfilled the inclusion criteria (regular menses, no climacteric symptoms, no hormone replacement therapy or cardiovascular disease) or no proper replacement match could be found. Sixty-two women (15%) were unwilling to participate and 36 could not be reached. This left 93 pre- and 93 postmenopausal women, aged 43 to 55 years, who participated in the study. All women gave written informed consent, and the study was approved by the medical ethical committee of the Erasmus University Medical School.

Measurements

During a visit at the research centre, a medical history was taken by a physician. Height, weight, and waist and hip circumference were measured with indoor clothes without shoes. Body mass index (weight/height^2) and waist-to-hip ratio were computed. Alcohol drinking habits and cigarette smoking history were obtained by a standardised questionnaire. Blood pressure was assessed 4 times at the right upper arm after a 5 minutes' rest in the supine position, with a Dinamap automatic blood pressure recorder (Critikon, Inc, Tampa, Florida, USA) and the mean was used in the analyses. Venous blood samples were drawn from each subject after a 12 hours fast. The samples were stored at -80°C, and subsequently serum parameters were determined using

a Kone Specific Analyzer (Kone Instruments, Espoo, Finland). Total cholesterol was measured with an automated enzymatic method[28], using the CHOD-PAP High Performance reagent kit from Boehringer Mannheim (Germany). HDL-cholesterol was measured by the phosphotungstate method according to Burstein[29] with a minor modification as described by Grove[30]. The overall coefficients of variation for total cholesterol and HDL-cholesterol were 2.9% and 3.7%, respectively. LDL-cholesterol was computed with the Friedewald formula.[31] Serum triglycerides were determined by using a reagent kit from Boehringer Mannheim (Germany) after enzymatic hydrolysis of the triglycerides with subsequent determination of liberated glycerol by colorimetry. No correction was made for serum free glycerol. The overall coefficient of variation of this method did not exceed 3.2%. Apolipoprotein A1 and B were measured by an automated turbidimetric immuno-assay using the reagent kits of Orion Diagnostics (Espoo, Finland). Glucose was enzymatically determined by the Hexokinase method (Instruchemie, Hilversum, The Netherlands). Serum insulin was determined by Metric assay (Biosource Diagnostics, Fleuris, Belgium). This assay has no cross-reactivity with either pro-insulin or C-peptide.

Statistical analysis

The two sample t test was used to compare general characteristics of pre- and postmenopausal women. Differences in frequencies of smoking status and alcohol drinking were tested by the Chi-square test. Analysis of covariance was used to compare risk factors in pre- and postmenopausal women. Since the distribution of insulin was highly skewed, it was natural-log transformed for the analyses. Differences in risk factors were expressed as percentages, and confidence intervals for these percentages were calculated. If a woman could not recall the exact date of onset of menopause, but only the year, the date of menopause was approximated and set on the first of July of that year.

For the risk factors shown to differ significantly between pre- and postmenopausal women, additional analyses were performed. The age-adjusted means of these risk factors were calculated within three groups of postmenopausal women defined according to the number of postmenopausal years: 1.0-2.9 (n=23), 3.0-6.0 (n=39) and $\geq$ 6.0 (n=31). A new ordinal variable was created, comprising the values 1, 2, and 3, corresponding with the three categories of postmenopausal years. The relation between the risk factors and time since menopause was estimated using linear regression analysis, with the ordinal variable as the independent variable. A test of significance for the coefficient of this ordinal variable was considered to be a test for trend.

RESULTS

The number of postmenopausal years was on average 5.4 (SD=3.0), and ranged from 1.3 to 12.8. The postmenopausal women were slightly older (mean 51.1, range 43.3 to 54.7) than the premenopausal women (mean 50.6, range 44.1 to 55.3) (Table 1). The group means of height, weight, body mass index, waist-to-hip ratio, and alcohol consumption showed no significant differences (Table 1). Percentages of current smokers and ex-smokers did not differ significantly between the groups.

Significantly higher levels of serum total cholesterol, LDL-cholesterol and apolipoprotein B were found in postmenopausal women compared with premenopausal women, after adjustment for age (Table 2). Levels of HDL-cholesterol, triglycerides, apolipoprotein A1, blood glucose, insulin, and systolic and diastolic blood pressure were not significantly different between the two groups. Additional adjustment for body mass index, waist-to-hip ratio, cigarette smoking and alcohol consumption influenced the results only slightly. No significant linear trend with number of postmenopausal years was observed for the lipids, apolipoproteins levels and insulin levels, after adjustment for age (figure 2).

Table 1
General characteristics of pre- and postmenopausal women.

	Premenopausal (n = 93)	Postmenopausal (n = 93)
Mean (SD)		
Age (yrs)	50.6 (2.4)	51.1 (2.2)
Mean (SD)		
Height (cm)	166.8 (5.7)	165.6 (7.3)
Weight (kg)	68.8 (11.1)	68.6 (11.5)
Body Mass Index (kg/m^2)	24.7 (3.8)	25.0 (4.1)
Waist-to-hip ratio	0.77 (0.05)	0.77 (0.05)
Alcohol (grams/wk)	45 (57.0)	45 (57.1)
Percentage (n)		
Current smoking (%)†	6 (6)	6 (6)
Past smoking (%)†	42 (39)	39 (36)

† Subjects who smoked 5 or more cigarettes per day were excluded from study participation

Table 2
Risk factors for cardiovascular disease in premenopausal and postmenopausal women.

Mean (SE) *	Pre-menopausal (n = 93)	Post-menopausal (n = 93)	Difference (%)	95 % Confidence interval for the % difference
Total cholesterol (mmol/l)	5.89 (0.10)	6.48 (0.10) ‡	10.0 %	(5.1 ; 14.0)
LDL-cholesterol (mmol/l)	3.78 (0.09)	4.32 (0.09) ‡	14.0 %	(6.9 ; 19.9)
HDL-cholesterol (mmol/l)	1.58 (0.04)	1.64 (0.04)	3.7 %	(-2.9 ; 10.3)
Triglycerides (mmol/l)	1.16 (0.06)	1.16 (0.06)	0 %	(-13.5 ; 13.9)
Apolipoprotein A1 (mg/dl)	1.53 (0.03)	1.56 (0.03)	1.9 %	(-3.8 ; 8.0)
Apolipoprotein B (mg/dl)	0.89 (0.03)	1.06 (0.03) †	8.2 %	(0.6 ; 15.5)
Glucose (mmol/l)	5.56 (0.06)	5.55 (0.06)	-0.01 %	(-2.9 ; 2.6)
Insulin (picomol/l) §	45.7 (1.05)	44.9 (1.05)	-1.8 %	(-11.2 ; 14.8)
Systolic blood pressure (mmHg)	120.8 (1.5)	120.6 (1.5)	- 0.16 %	(-3.4 ; 3.2)
Diastolic blood pressure (mmHg)	67.7 (1.0)	68.6 (1.0)	1.3 %	(-2.8 ; 5.5)

* Adjusted for age
§ Skewed data, therefore geometric mean is shown
† $p < 0.05$
‡ $p < 0.001$

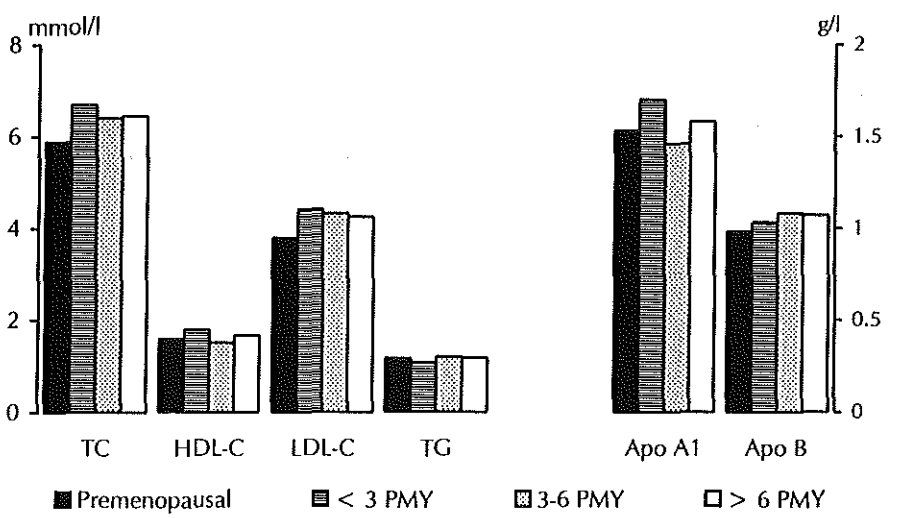

Figure 2 – Mean lipid levels in 93 premenopausal women and 93 postmenopausal women in 3 categories of time since menopause (< 3 yr n=23, 3-6 years n=39, ≥ 6 years n=31).
TC = total cholesterol; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; TG = triglycerides; Apo = apolipoprotein; PMY = postmenopausal years.

DISCUSSION

In the present study we found that mean levels of serum total cholesterol, LDL-cholesterol and apolipoprotein B were significantly higher in postmenopausal women than in premenopausal women of the same age. These higher levels were established within three years after the onset of menopause and did not change over postmenopausal time. Levels of triglycerides, HDL-cholesterol, apolipoprotein A1, blood glucose, insulin, body mass index, waist-to-hip ratio and systolic and diastolic blood pressure, were not significantly associated with natural menopause.

In studying the effect of menopause, age is an important confounding factor. In most cross-sectional studies, the study population includes women in an age-range that encompassed the extreme ages of menopause.[7,8,10,13,14,16,19] Such a study population comprises premenopausal women who have irregular menses and postmenopausal who only recently passed menopause, which decreases the contrast in estrogen status between the two groups. On the other hand, when a large proportion of women is aged in a range with little overlap between pre- and postmenopausal women, it is questionable whether age-adjustment by statistical modelling gives valid results. In some cross-sectional studies, pre- and postmenopausal women were matched in categories of age.[4,9,12] Within age groups, however, the postmenopausal women are still likely to be older than their premenopausal counterparts, resulting in residual confounding. In only one cross-sectional study were women matched on age in one-year categories.[21] In longitudinal studies, women who went through menopause during follow-up were compared with women of the same age who remained premenopausal.[5,6,17,32,33] These studies decrease the within-subject variation but at the expense of contrast in estrogen status: most premenopausal women who go through menopause will have irregular menses at baseline and will only recently have passed menopause at follow-up. By a careful matching procedure in the present study, we composed a population of age-matched pre- and postmenopausal women.

The women in our study were selected form responders to a mailed questionnaire. We assume, however, that the results from our study are generalisable to the general population even if some selection has taken place, because we have no reason to assume that the relation between menopause and biological factors will be different in responders and non-responders.

To ensure that the results are due to true associations between natural menopause and cardiovascular risk factors, bias due to other factors has also to be considered as a possible explanation. We excluded women currently using hormone replacement therapy or oral contraceptives. Moreover, after age-matching and exclusion of women smoking 5 cigarettes per day or more, residual confounding by age, smoking, body

mass index and alcohol drinking habits was dealt with by adjustment in the analyses. Some other determinants of early menopause were not measured in this study. For example, socio-economic status, genetic factors or parity, may have been related to early menopause and the difference in lipid levels. This seems unlikely, however, as although socio-economic status and parity have been shown to be associated with increased lipid levels, the reported effects of these factors are not large enough to explain the difference found in our study. Because of our stringent in- and exclusion criteria, the effect of possible misclassification of menopausal status is likely to be small. Misclassification of age of menopause and number of postmenopausal years might have occurred, as these assessments were based on self-reports.

The observation of an increased total cholesterol level in postmenopausal compared to premenopausal women is in agreement with most other studies, both cross-sectional[4,7-10,12-16,26] and longitudinal.[5,6,11,17,32] We found age-adjusted levels to be increased by 10.0%; in other cross-sectional studies the difference ranged from 8% to 13%.[7,8,10,13,14,16] In accordance with some groups who investigated linear trends in total cholesterol levels with postmenopausal years cross-sectionally[4,8,13] or longitudinally[5], we found that these higher levels were established within the first years after menopause and did not change thereafter.

The results with respect to LDL-cholesterol in our and other studies are consistent with the findings for total cholesterol[7,8,12-15]. In accordance with our observation, HDL-cholesterol was often found not to be associated with menopause[8,10,12,14], but in some cross-sectional[9,13] and longitudinal studies[17] a slightly lower HDL-cholesterol was found in postmenopausal women. The apparent inconsistency may be due to small opposing effects of estrogen deprivation on the HDL subfractions.[13,34]

In contradiction to observations in many cross-sectional studies in which an elevated level of triglycerides after menopause was found[6,8,13,15,16], we found no significant difference in triglycerides between the pre- and postmenopausal women. We have no explanation for this discrepancy. Some other studies, however, including one cross-sectional and one longitudinal study in which subjects were matched on age, found no menopausal effect on triglycerides.[14,17,21]

Few studies examined the relation between menopause and apolipoproteins A1 and B. Findings include a small increase[16], a decrease[7] or no change[8,17,18] in apolipoprotein A1. Apolipoprotein B, which is a strong marker for coronary atherosclerosis in women, was increased in postmenopausal women in some[8,12,16,18], but not all studies.[17] Our finding that apolipoprotein A1 was not different and apolipoprotein B was higher in post- compared with premenopausal women is consistent with our observations of the associated lipoproteins HDL-cholesterol and LDL-cholesterol.

Our finding that blood glucose and insulin levels were not associated with menopause is consistent with results of other studies.[5,8,16,17,21,33] One cross-sectional study did find higher levels of insulin in postmenopausal women compared to premeno-

pausal women of the same age[35] and one longitudinal study found lower levels.[26] Our finding of comparable insulin levels in pre- and postmenopausal women does not exclude the possibility that menopause does have an effect on glucose metabolism. An increased pancreatic insulin secretion in postmenopausal women, together with a compensatory decreased insulin clearance has been suggested.[36] Two studies have suggested an age-independent reduction of insulin sensitivity with time after menopause.[37,38] In our study we did not find an increase in insulin levels with time since menopause.

Although body mass index increases in the perimenopausal period, body mass index does not seem to be affected by menopause after adjustment for age.[5,6,14,16,17,20] Data on changes in fat distribution with menopause are scarce. In a cross-sectional study the proportion of upper body fat was higher in women after menopause, but the results were not adjusted for age.[23] In one small longitudinal study central adiposity increased with menopausal transition, compared to women who remained premenopausal.[32] In the Healthy Women's Study, unadjusted differences in waist-to-hip ratio between pre- and postmenopausal women were present cross-sectionally, but not longitudinally.[24] The latter finding agrees with our observation of no difference between the two groups.

Although conflicting results on the relations between menopause and blood pressure have been found, our observation that blood pressure was not associated with menopause is consistent with most cross-sectional[9,14] and longitudinal studies.[5,6,17] In most cross-sectional studies only the diastolic or only the systolic component was affected by menopause.[4,8,16,19] In a follow-up study, systolic blood pressure was observed to decline from 2 years before until 6 years after menopause, but no control group of premenopausal women was present.[20] One study suggests that menopause affects stress-induced levels of systolic and diastolic blood pressure.[39]

In conclusion, we selected age-matched pre- and postmenopausal women from a large general population in order to maximise the contrast in estrogen status. The results of our study suggest that total cholesterol, LDL-cholesterol and apolipoprotein B are the primary risk factors affected by menopause. Because increased cholesterol levels were established soon after cessation of menses, preventive measures aimed at reduction of heart disease in women should be initiated in early menopause.

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2.2 Menopausal status and distensibility of the common carotid artery

Abstract

Although several studies have shown that exogenous estrogens have beneficial effects on arterial characteristics, the effect of endogenous estrogen on the vascular system is still unknown. In this study distensibility, an indicator of arterial elasticity, of the common carotid artery was compared in pre- and postmenopausal women. The study comprised 93 premenopausal and 93 postmenopausal women of similar age (range 43-55 years). Women were selected from respondents to a mailed questionnaire about the menopause, which was sent to all women aged 40 to 60 years, in the Dutch town of Zoetermeer (n = 12,675).

Postmenopausal women who were at least 3 years past natural menopause or whose menses had stopped naturally before age 48, were age-matched with premenopausal women with regular menses and without menopausal complaints. The selection aimed at maximising the contrast in estrogen status between pre- and postmenopausal women of the same age. Distensibility of the carotid artery was measured non-invasively with B-mode ultrasound and a vessel wall movement detector system. Arterial distensibility is expressed as the change in arterial diameter (distension, ΔD) with the cardiac cycle, adjusted for lumen diameter, pulse pressure and mean arterial blood pressure. Compared to premenopausal women, postmenopausal women had significantly lower arterial distension (ΔD 370.5 μm (SE 9.5) versus 397.3 μm (SE 9.6)). These results suggest that the distensibility of the common carotid artery is negatively affected by natural menopause in presumed healthy women.

INTRODUCTION

The incidence of cardiovascular disease in women rises sharply after middle age, and menopause is thought to be a major determinant of this increase.[1-3] The mechanism through which menopause exerts its effect on the cardiovascular system remains largely unexplained. Unfavourable effects on lipid metabolism have been considered a major intermediary. However, recent studies have increasingly emphasised the direct beneficial effects of estrogens on the arterial wall. In experimental studies in animals, estrogen replacement had direct vasodilatory effects[4-6] and was shown to affect the structure and mechanical properties of large arteries. Improved endothelial function has been shown after hormone replacement therapy in women[7-9] and after estrogen use in transsexual men.[10,11] Use of estrogens in premenopausal women with coronary artery disease had a beneficial effect on exercise induced myocardial ischemia.[12]

Few studies have addressed the effects of endogenous estrogens and natural menopause on the dynamic characteristics of the arterial system. Although changes in distensibility were not found during the menstrual cycle[13], going through menopause has shown to negatively affect the elastic properties of the aortic root in hypertensive women[14], and time since menopause was inversely related to the pulsatility index in the carotid arteries[15] and several parameters of aortic flow.[16] In the current study, we examined the relation between natural menopause and arterial distension in the common carotid artery.

METHODS

Study population

Selection of participants in this study was aimed at maximising the contrast in estrogen status, in pre- and postmenopausal women of the same age (figure 1). A questionnaire was sent by mail to all women aged 40 to 60 years living in the Dutch town of Zoetermeer, The Netherlands (n=12,675). The questionnaire included questions about menopausal status, medical history, medication use, and smoking behaviour. The response rate was 54%. The selection of pre- and postmenopausal women was based on the questionnaire. Women with a hysterectomy and/or uni- or bilateral oophorectomy (n=1,551) and women with missing information on type or date of menopause (n=233) were excluded. Women who had 1 or more bleedings in the past 12 months were considered premenopausal (n=3829). Premenopausal women who reported irregular monthly bleeding (n=938) and women who reported the presence of climacteric symp-

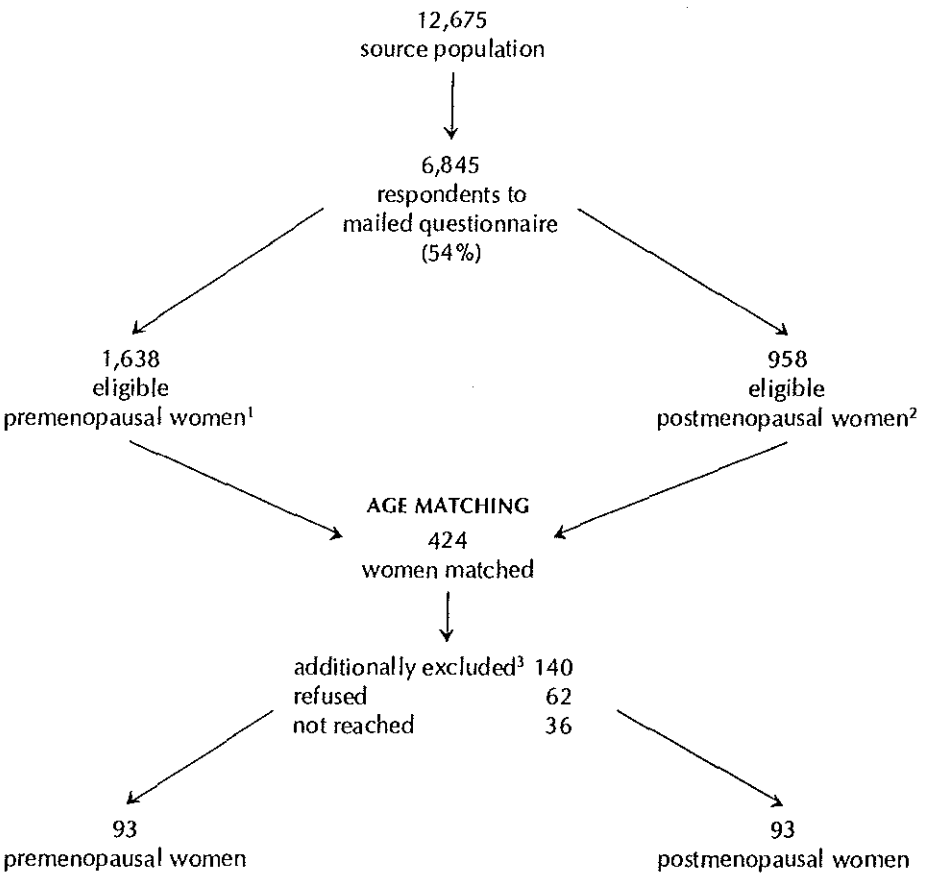

Figure 1 – Schematic presentation of the selection procedure of the study population.
(1) Eligible were women with regular menses and no climacteric symptoms, who did not use hormone replacement therapy or oral contraceptives in the past 6 months. Subsequently women who smoked, who had diabetes mellitus or used antihypertensive or cholesterol lowering drugs were excluded. (2) Eligible were women whose menses had ceased naturally more than 12 months ago and who had not used hormone replacement therapy. Subsequently women who smoked, who had diabetes mellitus, or who used antihypertensive or cholesterol lowering drugs were excluded. (3) Women who no longer fulfilled the criteria at the moment of examination were excluded.

toms, defined as perspiration and/or hot flushes (n=1,645), were excluded for the present study.

Furthermore, premenopausal women who reported use of hormone replacement therapy or use of oral contraceptives within 6 months before the onset of the clinical examination were excluded (n=423). The total number of premenopausal women

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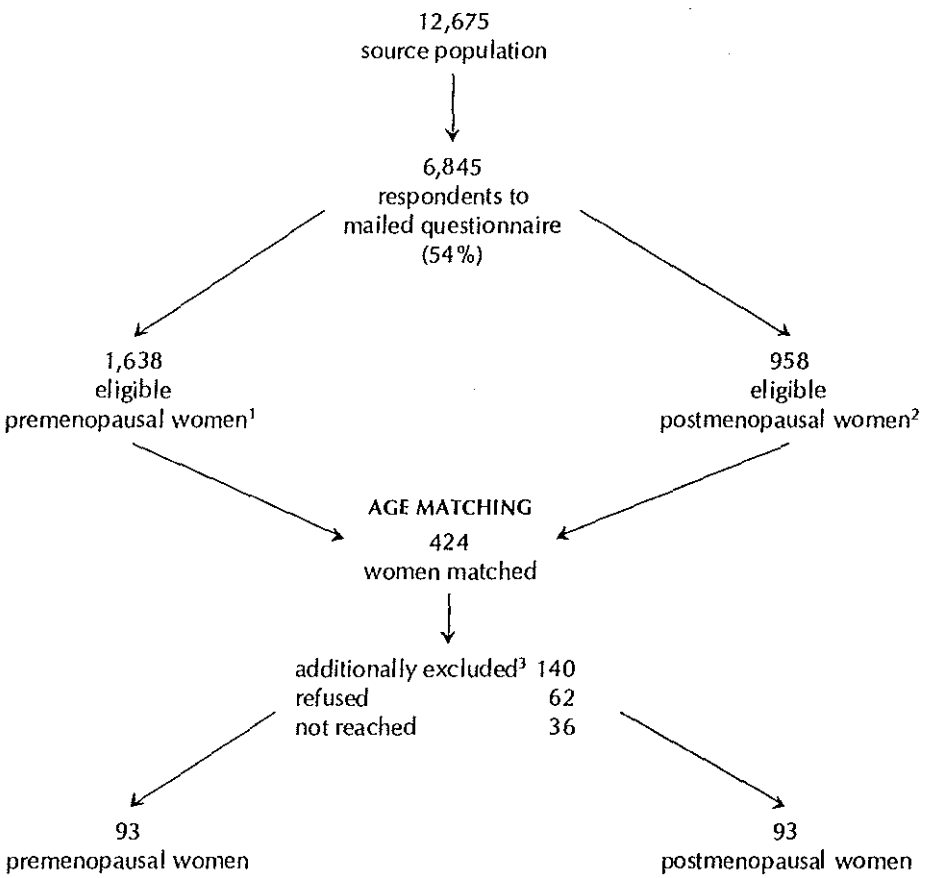

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excluded for the above mentioned reasons was 2191, leaving 1638 eligible premenopausal women.

Women were considered to have had natural menopause if their menses had ceased naturally for at least 12 months (n=1242). Women who reported a history of hormone replacement therapy for over 6 months or use of female hormones within 6 months prior to clinical examination and women who reported cessation of bleeding immediately upon stopping hormones were excluded (n=241). The total number of postmenopausal women excluded, including those with missing values on hormone use was 284, leaving 958 eligible postmenopausal women.

Of the remaining women, we additionally excluded women reporting diabetes mellitus (13 (0.8%) premenopausal and 16 (1.7%) postmenopausal women), use of antihypertensive medication (31 (1.9%) and 35 (3.7%)), use of cholesterol lowering drugs (3 (0.2%) premenopausal and 20 (2.1%) postmenopausal women), and current smoking of 5 or more cigarettes per day (302 (18.4%) premenopausal and 218 (22.8%) postmenopausal women).

To create a sharp contrast in estrogen status we selected women with either an early or a late natural menopause. Postmenopausal women who were at least 3 years after menopause or whose menses had stopped at least 3 years before the average age of menopause (51 years) were age-matched with premenopausal women with regular menses and without menopausal complaints. If it was not possible to find a match within the same year of age, a match was taken from an adjacent year. If one of a matched pair was unwilling to participate a new match was sought. Women were invited for study participation on average 15 months after return of the questionnaire. Out of 422 invited women, 138 were excluded because they no longer fulfilled the inclusion criteria, or no proper replacement match could be found. The primary reasons for no longer fulfilling the inclusion criteria were irregular menses or climacteric symptoms (n=62) and use of female hormones (n=26). Additionally, we excluded women with a history of cardiovascular disease (1 woman with myocardial infarction and 1 with stroke). Sixty-two women (15%) were unwilling to participate and 36 could not be reached. This left 93 pre- and 93 postmenopausal women, aged 43 to 55 years who participated in the study. All women gave written informed consent, and the study was approved by the appropriate local institutional committees on ethical practice.

Measurements

During a visit at the research centre a medical history was taken by a physician. Height, weight, and waist and hip circumference were measured with indoor clothes without shoes. Body mass index (weight/height^2) and waist to hip ratio were computed. Data on alcohol drinking habits and cigarette smoking history were obtained by a question-

naire. Serum total cholesterol was measured with an automated enzymatic method, using the CHOD-PAP High Performance reagent kit from Boehringer Mannheim.

The vessel wall motion of the right common carotid artery was by means of a Duplex scanner (ATL Ultramark IV, operating frequency 7.5 MHz) connected to a vessel wall movement detector system (Wall Track System). The details of this technique have been described elsewhere.[17,18] Briefly, this system enables the transcutaneous assessment of the displacement of the arterial walls during the cardiac cycle and, hence, the time-dependent changes in arterial diameter relative to its diastolic diameter at the start of the cardiac cycle. Subjects were instructed to refrain from smoking and consuming coffee, tea, alcohol or pain-medication on the day of measurement and from taking alcohol on the day before. Subjects were placed in supine position, with the head tilted slightly to the contralateral side for the measurements in the carotid artery. A region at 1.5 cm proximal to the origin of the bulb of the carotid artery was identified using B-mode ultrasonography. Based on the B-mode recording an M-line perpendicular to the artery was selected, and the received radio frequency signals were recorded over 5 cardiac cycles and digitally stored. The displacement of the arterial walls was obtained by processing the radio frequency signals originating from 2 selected sample volumes positioned over the anterior and posterior walls. The successive values of the end-diastolic diameter, the absolute stroke change in diameter during systole (ΔD), and the relative stroke change in diameter ((ΔD)/end-diastolic diameter) were computed from the recording during 5 cardiac cycles. With this system a wall displacement of a few micrometers can be resolved.[17] All measurements were performed by a single observer. A reproducibility study was performed in which 14 participants underwent a second examination within 1 month from the initial examination of the right carotid artery. The coefficient of variation for the absolute diameter change and the lumen diameter was 8.5% and 1.2%, respectively. Measurements were restricted to the right side to save time, as no significant differences in artery wall properties between the right and the left common carotid artery were found in previous studies by the authors.

At the time of the ultrasound examination blood pressure was measured with a Dinamap automatic blood pressure recorder. Blood pressure was read 4 times at the right upper arm during the measurement session, and the mean was taken as the subjects reading. Pulse pressure was defined as systolic blood pressure minus diastolic blood pressure. Mean arterial pressure was calculated as diastolic blood pressure + (1/3 x pulse pressure).

Statistical analysis

Linear regression analysis, with adjustment for age, was used to estimate the differences in characteristics between pre- and postmenopausal women. The difference in

distension of the carotid arteries between pre- and postmenopausal women was also estimated using linear regression analysis, with distension as the dependent variable. Adjustments were made for diastolic lumen diameter and pulse pressure by including these parameters as independent variables in the regression model. This component model allows the inclusion of mean arterial pressure as an additional covariate to account for its effect as well as for pulse pressure.[19-21] Also, additional adjustment could be made for age.

In a separate analysis the relation between distension and time since menopause was estimated using linear regression analysis, adjusting for age, diastolic lumen diameter, pulse pressure and mean arterial pressure. As the independent variable a newly created ordered variable was used which consisted of 3 groups of postmenopausal women: women up to 4 years after menopause, women 5 to 8 years after menopause and women 9 to 12 years after menopause. A test of significance for the coefficient of this ordered variable was considered to be a test for trend. If a woman could recall the year but not the exact date of onset of menopause, the date was approximated and set on the first of July of that year. Statistical significance was considered to be present when $p < 0.05$.

RESULTS

General characteristics of pre- and postmenopausal women are outlined in Table 1. Among postmenopausal women the mean number of years after menopause was 5.4 (SD=3.0), and ranged from 1.3 to 12.8. Age, height, weight, body mass index, waist hip ratio, alcohol consumption, smoking, blood pressure, and pulse pressure and frequency were not significantly different between the 2 groups (Tables 1 and 2). Total cholesterol was significantly higher in postmenopausal women. The end-diastolic lumen diameter was larger in postmenopausal women (6.73 mm) compared to premenopausal women (6.59 mm), but this difference did not reach statistical significance (Table 2).

When comparing the 2 study groups, a significant 7.2% decrease in distension was found in postmenopausal women (ΔD 370.5 μm (SE 9.5)) compared to premenopausal women (ΔD 397.3 μm (SE 9.6)), adjusted for age, diameter during diastole, pulse pressure and mean arterial pressure (Table 2).

When women were categorised in 3 groups by time since menopause, distension in women up to 4 years after menopause was 379.6 μm (SE 15.9), in women 5 to 8 years after menopause distension was 371.0 μm (SE 15.4) and in women 9 to 12 years after menopause distension was 359.6 μm (SE 22.9), adjusted for age, diastolic lumen

Table 1
General characteristics of pre- and postmenopausal women.

	Premenopausal (n = 93)		Postmenopausal (n = 93)	
Mean (SD)				
Age (yrs)	50.6	(2.4)	51.1	(2.2)
Height (cm)	166.8	(5.7)	165.6	(7.3)
Weight (kg)	68.8	(11.1)	68.6	(11.5)
Body Mass Index (kg/m^2)	24.7	(3.8)	25.0	(4.0)
Cholesterol (mmol/l)	5.9	(1.0)	6.5	(0.9)‡
Alcohol (grams/wk)	45	(57.0)	45	(57.1)
Percentage (n)				
Current smoking (%)†	6	(6)	6	(6)
Past smoking (%)‡	42	(39)	39	(36)

† Subjects who smoked 5 or more cigarettes per day were excluded from study participation
‡ $p<0.001$

Table 2
Arterial characteristics in pre- and postmenopausal women.

	Pre-menopausal (n = 93) Mean (SE)	Post-menopausal (n = 93) Mean (SE)	Difference	(95% CI)
ΔD (μm)*	397.3 (9.6)	370.5 (9.5)	-26.8	(-53.5 , -0.19)
ΔD/D (%)†	6.0 (0.14)	5.6 (0.14)	-0.39	(-0.78 , 0.00)
Diastolic lumen diameter (mm)‡	6.6 (0.06)	6.7 (0.06)	0.14	(-0.04 , 0.32)
Systolic blood pressure (mmHg)‡	120.8 (1.5)	120.6 (1.5)	-0.14	(-4.30 , 4.01)
Diastolic blood pressure (mmHg)‡	67.7 (1.0)	68.6 (1.0)	0.90	(-1.97 , 3.77)
Mean arterial pressure (mmHg)‡	85.4 (1.1)	86.0 (1.1)	0.55	(-2.50 , 3.60)
Pulse pressure (mmHg)‡	53.0 (1.1)	52.0 (1.1)	-1.04	(-4.02 , 1.93)
Pulse frequency (beats p/min)‡	67.4 (0.1)	67.0 (0.1)	-0.43	(-2.92 , 2.06)

* Adjusted for age, pulse pressure, mean arterial pressure and diameter
† Adjusted for age, pulse pressure and mean arterial pressure
‡ Adjusted for age
CI = confidence interval; SE = standard error; D = lumen diameter

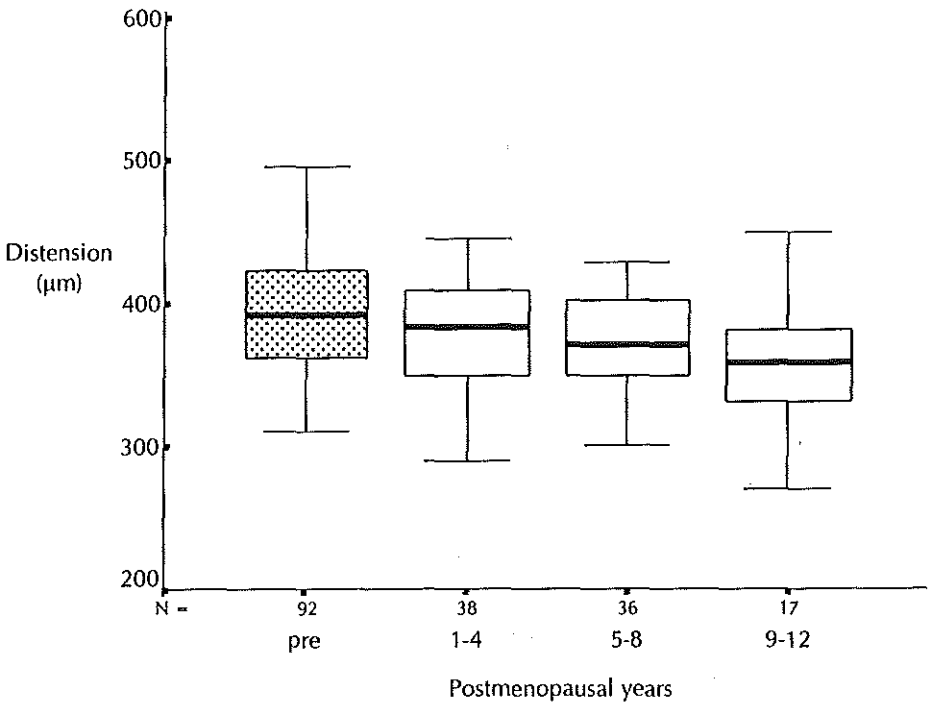

Figure 2 – Distension, adjusted for diastolic lumen diameter, pulse pressure and mean arterial pressure, in premenopausal and three groups of postmenopausal women (test for trend, p = 0.22).

diameter, pulse pressure and mean arterial pressure (figure 2). This shows that distension tended to decrease with time since menopause, but the changes did not reach statistical significance (test for trend p=0.22).

DISCUSSION

The results of the current study show that the distensibility of the common carotid artery is significantly lower in postmenopausal women than in premenopausal women of the same age, indicating increased arterial stiffness after menopause.

In studying the effect of menopause, age is an important confounding factor. By a rigorous selection procedure in the present study, we composed a population of age-matched pre- and postmenopausal women, from a general population. Because of our stringent inclusion and exclusion criteria, the effect of misclassification of menopausal

status is likely to be small. Some misclassification of age of menopause may have occurred, as these assessments were based on self-reports. The slight age difference between the study groups after age matching was dealt with by further adjustment in the analyses. To exclude potential bias due to other factors, such as smoking, lipid lowering medication, antihypertensive medication, current or recent use of hormone replacement therapy or oral contraceptives, or diabetes, we excluded all women with 1 or more of these confounders and furthermore restricted the study to women who had experienced a natural menopause.

We measured distension in the carotid artery and adjusted for pulse pressure measured in the brachial artery. We thereby assume that pulse pressure measured in the brachial artery is representative of pulse pressure in the carotid artery. In dogs, it has been demonstrated that pulse pressure in the brachial artery is linearly related to blood pressure in the carotid artery over a wide range of blood pressures.[22] It is known that the arterial pressure-waves undergo transformation in the arterial tree, and therefore, the pulse pressure is higher in the brachial artery than in more central vessels.[23] With increasing age, however, this difference between central and peripheral pulse pressure decreases. It is not known whether the overestimation of pulse pressure measured at the brachial artery differs between pre- and postmenopausal women. If, in line with the decreasing difference seen with age, the overestimation of pulse pressure is less in postmenopausal women, then the true difference in distensibility between the 2 groups would be even larger than estimated in our study.

Various studies suggest sex-differences in mechanical properties of the large arteries during the reproductive years, but not thereafter[24-26], and a steeper decline in distensibility in women than in men in the age range of 45 to 60 years.[27] This suggests, but does not yet definitively prove the influence of menopause on artery wall properties. Studies aimed directly on the relation between natural menopause and artery wall properties are limited. Gangar et al. found that the pulsatility index, representing impedance to blood flow distal to the point of measurement in the internal carotid artery, decreased with time since menopause.[15] Taquet could not show a relationship between menopausal status and aortic pulse wave velocity in 429 women, but the population consisted of perimenopausal women and therefore the contrast in estrogen levels between pre- and postmenopausal women may have been small.[28] In one study a decrease of elastic properties of the aorta was found in a small group of hypertensive women going through menopause during 3 years of follow-up, compared to age-matched women who remained premenopausal during the same period.[14] In our study decreased distensibility after natural menopause is demonstrated among presumed-healthy women.

The mechanisms through which menopause affects mechanical properties of the arteries are largely unknown. Specific binding of estrogens to receptors in endothelial and vascular smooth muscle cells has been demonstrated in different vascular beds in

animals and in humans.[29,30] Estrogen might change the structure of the arterial wall. In vitro investigations as well as animal studies showed that estrogens decrease collagen production and decrease the elastin/collagen ratio.[31-33] We found a slightly increased lumen diameter in postmenopausal compared to premenopausal women, which may be indicative of remodelling of the vessel wall.[34]

Whether loss of distensibility is an early marker for asymptomatic atherosclerotic changes or whether it reflects other structural changes of the arterial wall is still a matter of debate.[35-38] Decreased distensibility is unfavourably associated with age[27,39] and with several cardiovascular risk factors, like cholesterol[40] and hypertension.[28,41] Loss of distensibility in elastic arteries has been shown to be associated with an increased risk of cardiovascular disease in cross-sectional studies.[42,43] Longitudinal data on the effect of decreased distensibility on cardiovascular morbidity or mortality are, however, still awaited.

In conclusion, our findings suggest that natural menopause adversely affects the distensibility of the common carotid artery. This may indicate one of the mechanisms through which menopause adversely affects cardiovascular disease risk in women after middle age.

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2.3 Age at menopause, atherosclerosis and coronary heart disease

The Rotterdam Study

Abstract

Background – The rise in coronary heart disease in elderly women is thought to be related to the relative estrogen deficiency after menopause. On the relation between menopause and atherosclerosis little is known.

Methods and results – Associations were examined in the Rotterdam Study, a population-based cohort study including 4853 postmenopausal women aged 55 and over. Atherosclerosis was assessed by ultrasonography of the carotid arteries, by radiographic assessment of the aorta, by measurement of the ankle-arm systolic blood pressure index in the peripheral arteries, and by a compound score of these measures. Early menopause was defined as menopause before age 40, except for peripheral arterial disease and for myocardiocardial infarction (< age 45). Adjusted odds ratios for early natural menopause were 2.75 (95% confidence interval 1.13 - 6.67) for carotid atherosclerosis, 1.67 (1.02 – 2.74) for peripheral arterial disease, 3.24 (1.37 – 4.23) for aortic atherosclerosis, 2.41 (1.37 – 4.23) for moderate atherosclerosis (compound score), 5.30 (2.12 – 13.2) for severe atherosclerosis (compound score), compared to women with menopause at or after age 55. Early bilateral oophorectomy, but not early hysterectomy with or without unilateral oophorectomy, was associated with an increased risk of atherosclerosis at all sites. After exclusion of ever-smokers, the increased risk of aortic atherosclerosis with early natural menopause disappeared, but not that with early bilateral oophorectomy. Associations were also found between early menopause and an increased risk of myocardial infarction.

Conclusion – This study shows that women with early menopause, either naturally or after bilateral oophorectomy, remain at increased risk of atherosclerosis at several sites many years after menopause.

INTRODUCTION

Premenopausal women are protected from cardiovascular disease compared to postmenopausal women. Whether the rise in coronary heart disease in older women is related to menopause is still debated.[1-3] Several studies have shown an inverse association between age at natural menopause and risk of cardiovascular disease[4-10], but others did not.[11-15] The inconsistency might be explained by a methodological problem; a lag time of 10 to 20 years between menopause and the occurrence of coronary heart disease in women makes the effect of menopause difficult to disentangle from that of age, and the accompanying change in other risk factors.

The mechanisms through which menopause might exert its effect on the cardiovascular system are not entirely clear. As atherosclerosis is present long before symptomatic coronary heart disease develops, studying the association with atherosclerosis might be a sensible approach to study the role of menopause, as accurate risk assessment will not be hampered by low rates of cardiovascular events. Few studies have focussed on non-invasively measured atherosclerosis in relation to menopause. In these reports no differences were found in the presence of carotid atherosclerosis between pre- and postmenopausal women.[16-18] However, women were relatively young (< 55 years), and the postmenopausal period might have been too short for an effect to become evident.

We investigated the association between age at menopause and the presence of non-invasively measured atherosclerosis in the carotid arteries, the peripheral arteries and the aorta, as well as the presence of myocardial infarction in 4853 postmenopausal women, aged 55 and over, participating in the Rotterdam Study.

METHODS

The Rotterdam Study

The Rotterdam study is a prospective population-based cohort study in 7983 subjects aged 55 and over that aims to assess the occurrence of chronic diseases in an ageing population, and to clarify their determinants.[19] During the first survey, from 1990 to 1993, all participants were interviewed at home by a trained research assistant, and subsequently visited the study centre twice. The study was approved by the Medical Ethical Committee of Erasmus University, and informed consent was given by all participants.

Measurements

Interview information, including medical history, current medication, alcohol intake, smoking habits, use of hormone replacement therapy (HRT), and duration of use, was obtained by a trained research assistant. As an indicator of socio-economic status the highest attained level of education was assessed. At the study centre, height and body weight were measured. Two blood pressure measurements were taken with a random zero sphygmomanometer with the subject in sitting position, and averaged. A twelve lead ECG was recorded and stored digitally. Serum total cholesterol and HDL-cholesterol values were assessed by an automated enzymatic procedure in a non-fasting blood sample.

Ascertainment of age and type of menopause

Data on age and type of menopause were collected during the home-interview. For women reporting natural menopause, age at menopause was defined as the self-reported age of last menstruation. For all women reporting menopause after gynecologic surgery or radiation therapy and for those reporting any other operations before age 50 that might have lead to menopause, information on the exact date and type of operation were verified using general practitioners (GP) records. Of 955 women reporting artificial menopause, 901 following surgery and 54 following radiation therapy, in 97.5 percent of the cases the medical records could be retrieved, and in 84,7 percent information about the date and type of gynecologic operation or radiation therapy was found.

Of all records in which information on the above interventions was found a history of surgical menopause was refuted in 39 women (9.8 percent) and could be confirmed in 862 women (90.2 percent). In 307 women a letter from the gynecologist describing the operation was found. In the other cases information on the type of operation was taken from notes or deduced from other information in the record. In women for whom we found the exact type of menopause from her gynecologist or for whom information from the GP records, sometimes in combination with interview information, was unambiguous, the type of menopause was classified as definite. In all other cases information from the GP records type of menopause was classified as probable. In case no information was found the self-reported type of operation was taken, and type of menopause was classified as possible. In 52 percent of women with hysterectomy, 69 percent of women with hysterectomy plus unilateral oophorectomy and in 45 percent of women with bilateral oophorectomy, the type of operation was classified as definite. Age at operation was taken from the record when the exact date was found

(61.2 percent of women with early surgical menopause); otherwise the self-reported age was used.

Among women for whom a letter from the gynecologist was found 69.6 percent of the women who had reported a bilateral oophorectomy had correctly reported their ovarian status. For women who reported conservation of one, or both ovaries, these percentages were 65.4 percent and 91.2 percent respectively.

Assessment of atherosclerosis and coronary heart disease

Carotid atherosclerosis was assessed by B-mode ultrasonography of the carotid arteries, using a 7.5 MHz linear array transducer (ATL, Ultramark IV). The common and internal carotid artery and the carotid bifurcation were both on-line and off-line evaluated for the presence or absence of atherosclerotic lesions, according to the Rotterdam Study ultrasound protocol.[20] In the analyses, carotid plaque was defined as the presence of plaques at the near or far wall at one or more sites. Intima-media thickness has

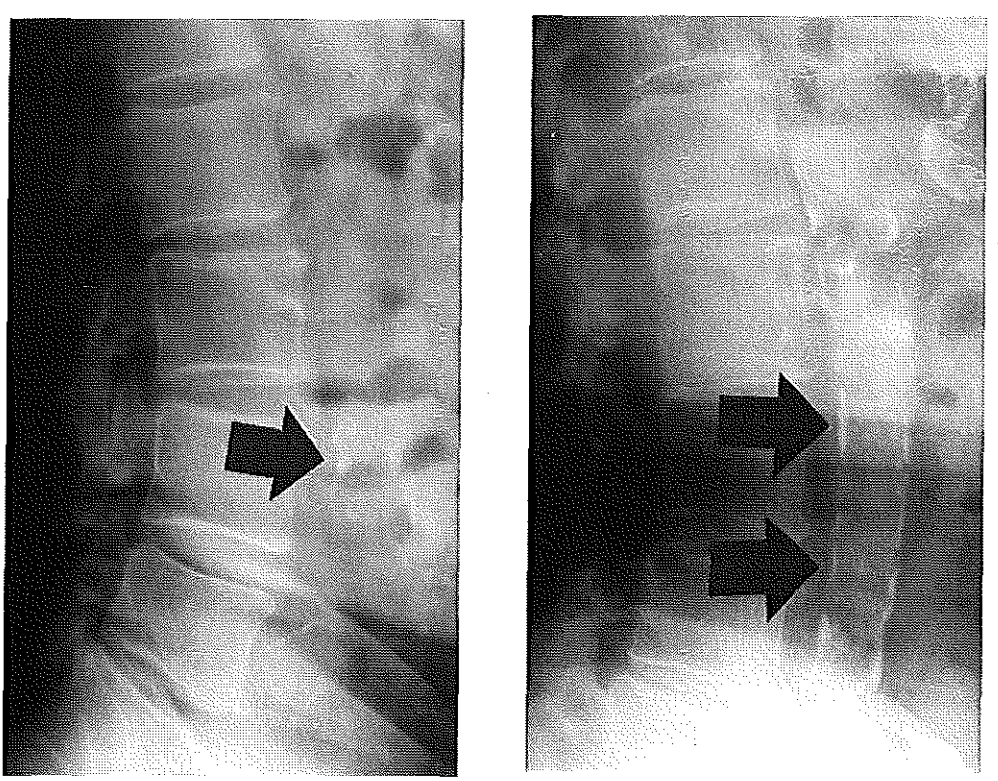

Figure
Detection of aortic atherosclerosis (arrow) on a lateral x-ray of the abdomen. Examples of mild atherosclerosis (left) and severe atherosclerosis (right).

only been measured in digitised images in a subsample of the population and was not analysed. Aortic atherosclerosis was judged present when linear densities were clearly visible in an area parallel and anterior to the lumbar spine (L1-L4) on a lateral x-ray of the abdomen.[21,22] The extent of atherosclerosis was scored in categories according to the length of the involved area: no calcification (0 or ≤ 1 cm), moderate calcification (2-5 cm) and severe calcification (> 5 cm). Radiographic assessment of atherosclerosis has been shown to be highly specific by comparison with assessments made on necropsy material and CT scans.[22,23] Systolic blood pressure was measured at both the left and the right posterior tibial artery using an 8 MHz continuous wave Doppler probe (Huntleigh 500D, Huntleigh Technology, Bedfordshire, UK) and a random zero sphygmomanometer, with the subject in supine position. Peripheral arterial disease (PAD) was considered present when the ankle-arm systolic blood pressure index (AAI) was lower than 0.9 on at least one side, and categorised as moderate (≥ 0.7 AAI < 0.9) or severe PAD (AAI < 0.7). A history of myocardial infarction was based on self-reported information checked with GP or hospital records and/or on ECG evidence. Infarctions detected by the Modular ECG analysis system (MEANS)) without evidence of symptoms (silent myocardial infarctions), were verified by an experienced cardiologist.[24,25] Myocardial infarctions that occurred before menopause (7 cases) were not included in the analysis.

Population for analysis

In The Rotterdam Study 4853 women were postmenopausal at baseline. For this analysis women were excluded who reported a history of HRT for one year or longer (n= 348), and/or cessation of menses after stopping HRT or oral contraceptives, as in these cases the exact age at menopause can not be defined (n =188). Furthermore, women who could not recall age at natural menopause (n=446), and who did not visit the research centre (n= 601) were excluded. After excluding women who fulfilled one or more of the exclusion criteria 3384 women remained for analyses. For each analysis, subjects with missing data on the relevant outcome variables were excluded. Missing data were primarily due to logistic reasons.

Data analysis

The association between age at menopause in 5 categories and the presence of atherosclerosis was assessed using logistic regression analysis. Multivariate analyses were adjusted for current age, smoking status (current, former and never) and number of pack-years (number of years of smoking times number of cigarettes smoked daily divided by twenty, for current and former smokers), body mass index (weight/height^2, in

three categories), education level (in four categories: primary education, lower general education/lower vocational education, intermediate vocational education, and higher education/university) and alcohol intake (in four categories). Missing value indicators were made for women lacking data on confounders.[26] A composite measure of atherosclerosis was constructed, which resulted in a score ranging from zero to six. Points were given to the atherosclerosis score according to the following algorithm: one point for moderate (1-3 carotid plaques) and two points for severe (4-6 carotid plaques), one point for moderate and two points for severe PAD and one point for moderate and two points for severe aortic calcifications. We defined atherosclerosis on the basis of this score into moderate (1-2 points) or severe (3-6 points). Subjects with missing values for one or more measures of atherosclerosis were coded missing on the atherosclerosis score.

To rigorously exclude confounding by smoking, we re-examined the associations among never-smokers only (n = 1880 for natural menopause, 55%, n = 1078 for artificial menopause, 56%). The presence of a log-linear trend in odds ratios (OR) across exposure categories was evaluated using the categories as an ordinal variable, with values from 1 (≥55 yrs) to 5 (<40 yrs). All odds ratios are presented with 95 percent confidence intervals (CI), and all P values are two-sided.

RESULTS

Baseline Characteristics

Menopause had been reached naturally by 2713 women (80.2 percent), surgically by 628 women (18.6 percent) and by radiation therapy by 43 women (1.3 percent). Reported age at natural menopause varied from 25 to 60 years (mean 49.6, median 50.0). Women who reported to have passed menopause before age 45 were statistically significantly older than women in the reference group consisting of women with menopause at or after age 55, and were statistically significantly more often current and former smokers (table 1).

Women in the earliest category of age at menopause had a slightly higher blood pressure than the women in the reference group, but these differences did not reach statistical significance. Women with hysterectomy with or without unilateral oophorectomy were statistically significantly younger than women in the reference group (table 2). Women with artificial menopause were significantly more often current smokers, while women with bilateral oophorectomy were more often former smokers than the women in the reference group.

Table 1
Age-adjusted baseline characteristics of 2713 naturally postmenopausal women, by categories of age at menopause.

Age at menopause	< 40	40–44	45–49	50–54	≥ 55 (reference)
	n=52	n=253	n=717	n=1439	n=252
Mean (SE)					
Age (yr)	72.9 (10.5)¶	72.6 (8.9)¶	71.2 (9.6)	70.3 (9.7)	70.3 (8.6)
*Mean (SD)**					
Syst. blood pressure (mmHg)	147.8 (3.0)	141.8 (1.4)	140.4 (0.8)	140.6 (0.6)	142.3 (1.4)
Diast. blood pressure (mmHg)	77.6 (1.6)	73.4 (0 .7)	72.9 (0.4)	72.9 (0.3)	74.1 (0.7)
Body Mass Index	27.6 (0.6)	26.4 (0.3)	26.8 (0.2)	26.8 (0.1)	27.2 (0.3)
Total cholesterol (mmol/l)	6.85 (0.17)	6.88 (0.08)	6.79 (0.05)	6.80 (0.05)	6.78 (0.08)
HDL cholesterol (mmol/l)	1.46 (0.05)	1.38 (0.02)	1.43 (0.01)	1.44 (0.01)	1.42 (0.02)
Glucose (mmol/l)	6.60 (0.37)	6.85 (0.17)	6.80 (0.10)	6.93 (0.07)	6.70 (0.17)
Diabetes (%)	8.7 (3.8)	10.9 (1.8)	10.0 (1.1)	10.6 (0.8)	9.7 (1.8)
Current smokers (%)	41.6 (6.2)¶	28.9 (2.8)¶	27.1 (1.7)¶	21.5 (1.2)	18.8 (2.7)
Pack-years (n)†	33.3 (3.5)	28.5 (2.5)	28.2 (1.5)	25.4 (1.1)	24.5 (2.8)
Former smokers (%)	45.9 (6.7)¶	36.2 (3.0)	31.0 (1.8)	30.9 (1.3)	27.9 (2.9)
Pack-years (n)‡	16.4 (4.2)	14.1 (2.1)	18.1 (1.3)	16.0 (0.9)	13.8 (2.2)
Alcohol drinkers (%)	79 (7.3)	71 (3.3)	71 (1.9)	74 (1.3)	73 (3.0)
Alcohol (g/wk)§	6.3 (2.0)	9.0 (0.9)	8.3 (0.5)	8.3 (0.4)	7.8 (0.9)
Parous (%)	76 (5.4)	71 (2.5)	73 (1.6)	75 (1.1)	78 (2.5)
Education (% higher ed./university)	15.9 (4.8)	18.7 (2.2)	20.1 (1.4)	18.1 (1.0)	18.6 (2.2)

Values are means or percentages with standard errors. Syst. = systolic. Diast. = diastolic.
* adjusted for age
† among smokers
‡ among former smokers
§ among drinkers
¶ compared to reference group, $p < 0.05$

Natural Menopause

Table 3 shows the odds ratios for atherosclerosis for women with early menopause, compared to women in the reference group. Plaques in the carotid artery were present in 60.5 percent of women. Women with menopause before age 40 compared to women in the reference group had a more than two and a half times increased risk of carotid atherosclerosis, after adjustment for age, smoking, number of pack-years smoked, body mass index, alcohol intake, hormone use and education. PAD was present in 20.2 percent of women.

Table 2
Age-adjusted baseline characteristics of women after artificial menopause at or before age 45, and a reference group of women with natural menopause at or after age 50, by type of menopause.

	Hysterectomy only	Hysterectomy + unilateral oophorectomy	Bilateral oophorectomy ± Hysterectomy	Reference group
	n = 146	n = 72	n = 60	n = 1691
Mean (SD)				
Age (yr)	65.2 (7.0)	65.4 (7.4)	69.6 (8.1)	70.9 (9.4)
Age at menopause (yr)	41.2 (3.2)	41.1 (3.5)	40.2 (4.3)	53.3 (2.0)
*Mean (SD)**				
Systolic blood pressure (mmHg)	137.9 (1.8)	133.9 (2.5)	137.6 (2.8)	139.2 (0.6)
Diastolic blood pressure (mmHg)	72.7 (1.0)	70.5 (1.4)	72.0 (1.5)	72.9 (0.3)
Body Mass Index	27.0 (0.3)	26.9 (0.5)	27.0 (0.5)	26.9 (0.1)
Total cholesterol (mmol/l)	7.0 (0.09)	7.0 (0.13)	6.9 (0.14)	6.8 (0.04)
HDL-cholesterol (mmol/l)	1.40 (0.03)	1.42 (0.04)	1.41 (0.04)	1.43 (0.01)
Glucose (mmol/l)	6.8 (0.21)	7.4 (0.31)	7.1 (0.3)	6.8 (0.08)
Diabetes (%)	9.8 (2.4)	13.1 (3.5)	16.9 (3.8)	10.0 (0.8)
Current smokers (%)	30 (4.0)¶	28 (5.5)	37 (6.2)¶	22 (1.3)
Pack-years (n)†	31.5 (2.8)	37.1 (3.9)¶	25.3 (4.2)	24.4 (1.2)
Former smokers (%)	38 (4.3)	32 (6.1)	44 (6.8)¶	32 (1.4)
Pack-years (n)‡	15.9 (2.9)	10.6 (4.3)	16.1 (4.5)	16.2 (1.1)
Alcohol drinkers (%)	75 (3.9)	68 (5.5)	76 (6.3)	75 (1.4)
Alcohol (g/wk)§	7.8 (1.1)	8.0 (1.6)	9.5 (1.8)	8.5 (0.4)
Parous (%)	81 (3.3)	75 (4.7)	77 (5.2)	78 (1.1)
Education (% higher ed./university)	21 (3.2)	26 (4.6)	14 (4.6)	21 (1.1)

Values are means or percentages with standard errors. ± = with or without.
* adjusted for age
† among smokers
‡ among former smokers
§ among drinkers
¶ compared to reference group, $p < 0.05$

To maintain adequate numbers of events in all categories, the two categories with earliest menopause were combined for the analyses on PAD and myocardial infarction. The adjusted odds ratio for PAD increased gradually with earlier menopause to 1.67 (1.02 – 2.74) in women with menopause before age 45. The magnitude of the association increased with increasing severity of PAD with odds ratios 1.28 (0.67 –

Table 3
Odds ratios for atherosclerosis according to categories of age at natural menopause.

Age at menopause (yr)	No. of cases/ subjects	OR*	(95% CI)	OR†	(95% CI)
Carotid atherosclerosis					
≥ 55	86/166	1	reference	1	reference
50 – 54	567/928	1.56	(1.11 ; 2.19)	1.55	(1.10 ; 2.19)
45 – 49	250/436	1.23	(0.85 ; 1.77)	1.14	(0.79 ; 1.66)
40 – 44	116/165	2.13	(1.35 ; 3.38)	2.00	(1.25 ; 3.21)
< 40	28/36	3.32	(1.39 ; 7.93)	2.75	(1.13 ; 6.67)
P for trend		p=0.01		p=0.04	
Peripheral arterial disease					
≥ 55	32/228	1	reference	1	reference
50 – 54	239/1290	1.37	(0.91 ; 2.08)	1.32	(0.86 ; 2.01)
45 – 49	144/645	1.62	(1.05 ; 2.51)	1.46	(0.94 ; 2.28)
< 45	74/269	1.93	(1.19 ; 3.13)	1.67	(1.02 ; 2.74)
P for trend		p<0.01		p =0.02	
Aortic calcifications					
≥ 55	60/204	1	reference	1	reference
50 – 54	438/1143	1.58	(1.13 ; 2.21)	1.50	(1.06 ; 2.12)
45 – 49	228/559	1.62	(1.13 ; 2.33)	1.46	(1.01 ; 2.11)
40 – 44	88/196	1.76	(1.14 ; 2.71)	1.56	(1.00 ; 2.43)
< 40	22/36	3.95	(1.82 ; 8.58)	3.24	(1.47; 7.13)
P for trend		p=0.03		p=0.18	
Atherosclerosis score – moderate					
≥ 55	45/117	1	reference	1	reference
50 – 54	288/591	1.66	(1.10 ; 2.52)	1.60	(1.05 ; 2.45)
45 – 49	134/260	1.83	(1.16 ; 2.89)	1.71	(1.07 ; 2.73)
< 45	67/109	2.58	(1.49 ; 4.47)	2.41	(1.37 ; 4.23)
P for trend		p < 0.002		p < 0.01	
Atherosclerosis score – severe					
≥ 55	15/87	1	reference	1	reference
50 – 54	119/422	2.33	(1.18 ; 4.63)	2.29	(1.08 ; 4.85)
45 – 49	61/187	2.94	(1.40 ; 6.14)	2.93	(1.31 ; 6.59)
< 45	41/83	5.21	(2.27 ; 11.9)	5.30	(2.12 ; 13.2)
P for trend		p < 0.0001		p < 0.0002	
Myocardial infarction					
≥ 55	14/240	1	reference	1	reference
50 – 54	117/1335	1.55	(0.87 ; 2.76)	1.60	(0.89 ; 2.86)
45 – 49	75/671	1.91	(1.05 ; 3.48)	1.91	(1.05 ; 3.49)
< 45	39/275	2.40	(1.26 ; 4.57)	2.49	(1.30 ; 4.76)
P for trend		p=0.003		p=0.01	

OR denotes odds ratio. CI denotes confidence interval. * Values are adjusted for age. † Values are adjusted for age, smoking (never/past/current [pack-years of smoking for past and current smokers]), body mass index (3 categories), alcohol intake (4 categories), short term hormone replacement therapy (yes/no) and education (4 categories). Numbers do not always add up to totals due to missing values.

Table 4
Odds ratios for atherosclerosis in women with artificial menopause at or before age 45 compared to women with natural menopause at or after age 50.

	no. of events	no.of subjects	OR*	95 % CI	OR†	95 % CI
Carotid atherosclerosis						
Spontaneous ≥ 50	655	1096	1	reference	1	reference
hyst. ≤ 45	51	104	0.84	(0.56 ; 1.28)	0.81	(0.53 ; 1.24)
hyst. + unilat. ooph. ≤ 45	22	50	0.69	(0.38 ; 1.25)	0.66	(0.36 ; 1.23)
hyst. + bilat. ooph. ≤ 45	28	42	1.47	(0.75 ; 2.89)	1.38	(0.69 ; 2.76)
Peripheral arterial disease						
Spontaneous ≥ 50	276	1522	1	reference	1	reference
hyst. ≤ 45	25	134	1.67	(1.04 ; 2.70)	1.61	(0.99 ; 2.62)
hyst. + unilat. ooph. ≤ 45	12	65	1.58	(0.81 ; 3.10)	1.38	(0.68 ; 2.79)
hyst. + bilat. ooph. ≤ 45	14	56	1.82	(0.97 ; 3.44)	1.97	(1.02 ; 3.80)
Aortic calcifications						
Spontaneous ≥ 50	495	1341	1	reference	1	reference
hyst. ≤ 45	37	125	0.94	(0.62 ; 1.43)	0.87	(0.57 ; 1.34)
hyst. + unilat. ooph. ≤ 45	22	66	1.13	(0.65 ; 1.94)	1.04	(0.59 ; 1.84)
hyst. + bilat. ooph. ≤ 45	29	51	3.37	(1.64 ; 6.94)	2.14	(1.17 ; 3.92)
Atherosclerosis score – moderate						
Spontaneous ≥ 50	333	707	1	reference	1	reference
hyst. ≤ 45	31	75	0.87	(0.53 ; 1.42)	0.85	(0.52 ; 1.42)
hyst. + unilat. ooph. ≤ 45	15	35	0.97	(0.48 ; 1.95)	0.87	(0.42 ; 1.79)
hyst. + bilat. ooph. ≤ 45	15	29	1.24	(0.59 ; 2.64)	1.26	(0.58 ; 2.75)
Atherosclerosis score – severe						
Spontaneous ≥ 50	135	509	1	reference	1	reference
hyst. ≤ 45	11	55	1.11	(0.52 ; 2.35)	1.12	(0.94 ; 2.56)
hyst. + unilat. ooph. ≤ 45	5	25	0.96	(0.31 ; 2.97)	1.04	(0.31 ; 3.46)
hyst. + bilat. ooph. ≤ 45	9	23	2.38	(0.91 ; 6.21)	2.28	(0.72 ; 7.28)

OR denotes odds ratio. CI denotes confidence interval. *Values are adjusted for age. † Values are adjusted for age, smoking (never/past/current [pack-years of smoking for past and current smokers]), body mass index (3 categories), alcohol intake (4 categories), short term hormone replacement therapy (yes/no) and education (4 categories). Numbers do not always add up to totals due to missing values.

2.44) for moderate and 2.11 (1.05 – 4.22) for severe PAD, after adjustment for confounders. Aortic atherosclerosis was present in 39.0 percent of women. Women with menopause before age 40 had a more than threefold increased risk of aortic atherosclerosis after adjustment for age and confounders.

The atherosclerosis scores showed that early menopause (< age 40) was associated with a nearly two and a half times increased risk for moderate and a more than 5 times increased risk of severe atherosclerosis. Menopause before age 45 was associated with a two and a half times increased risk of myocardial infarction.

Analyses were repeated in women over 70 years of age. In these analyses, the magnitude of the odds ratios associated with early menopause remained elevated, although somewhat decreased, and most estimates lost significance as a result of small numbers. The odds ratios in women over 70 years of age were 1.56 (0.45 – 5.35) for carotid plaques, 1.76 (0.95 – 3.25) for PAD, 1.68 (0.87 – 3.22) for aortic atherosclerosis, 1.81 (0.30 – 11.0) for the moderate and 7.91 (2.48 – 25.3) for the severe atherosclerosis score. The risk of myocardial infarction could not be examined in age strata because of small numbers.

Artificial Menopause

Table 4 shows odds ratios of atherosclerosis in women with early artificial menopause, at or before age 45, compared to women with a natural menopause at or after age 50 (reference group). Early bilateral oophorectomy was associated with an increased risk of atherosclerosis in the peripheral arteries, in the aorta and the atherosclerosis score while early hysterectomy and unilateral oophorectomy were not. The risk of PAD was also increased in women with early hysterectomy alone, but not in women with early hysterectomy with unilateral oophorectomy. Risk of myocardial infarction was increased for women with early bilateral oophorectomy compared to the reference group, while this was not seen for women with early hysterectomy with or without unilateral oophorectomy. The estimate for bilateral oophorectomy, based on 7 cases, did not reach statistical significance (odds ratio 1.62 (0.69 – 3.75)). Additional adjustment for diabetes or systolic blood pressure did not change the results.

Repeating analyses with only women whose gynecologic operations were classified as definite left the point estimates for the odds ratios for aortic atherosclerosis (odds ratio 2.26 (0.90 – 5.73)), PAD (odds ratio 1.47 (0.50 – 4.29), the moderate atherosclerosis score (odds ratio 0.84 (0.26 – 2.73)), the severe atherosclerosis score 3.68 (0.81 – 16.7) and myocardial infarction (odds ratio 1.83 (0.51 – 6.57)) with bilateral oophorectomy virtually unchanged. The odds ratio for carotid atherosclerosis remained non-significant (0.54 (0.20 – 1.46)). Also, odds ratios for hysterectomy with and without unilateral oophorectomy remained unchanged.

Analysis in never smokers

In never-smokers only (55% of women), the association between early natural menopause and the risk of carotid atherosclerosis was unchanged. The association between PAD and natural menopause decreased, losing statistical significance, while the association between risk of aortic atherosclerosis and natural menopause disappeared (table 5). The results for the atherosclerosis scores and myocardial infarction were

Table 5
Odds ratios for atherosclerosis and myocardial infarction according to categories of age at natural menopause, in all women and in never smokers, adjusted for age and confounders.

Age at menopause (yr)	No. of cases/ subjects	OR* All women	(95% CI)	No. of cases/ subjects	OR* Never smokers	(95% CI)
Carotid atherosclerosis						
≥ 55	86/166	1	reference	49/99	1	reference
50 – 54	567/928	1.55	(1.10 ; 2.19)	320/529	1.67	(1.05 ; 2.66)
45 – 49	250/436	1.14	(0.79 ; 1.66)	125/233	1.01	(0.60 ; 1.66)
< 45	144/201	2.11	(1.34 ; 3.30)	77/101	2.81	(1.48 ; 5.36)
P for trend		p=0.46			p=0.77	
Peripheral arterial disease						
≥ 55	32/228	1	reference	22/139	1	reference
50 – 54	239/1290	1.32	(0.86 ; 2.01)	130/714	1.14	(0.68 ; 1.93)
45 – 49	144/645	1.46	(0.94 ; 2.28)	78/345	1.25	(0.72 ; 2.18)
< 45	74/269	1.67	(1.02 ; 2.74)	35/132	1.48	(0.79 ; 2.79)
P for trend		p=0.02			p=0.50	
Aortic calcifications						
≥ 55	60/204	1	reference	38/123	1	reference
50 – 54	438/1143	1.50	(1.06 ; 2.12)	226/617	1.04	(0.68 ; 1.51)
45 – 49	228/559	1.45	(1.00 ; 2.10)	113/283	1.07	(0.68 ; 1.91)
< 45	110/232	1.74	(1.14 ; 2.67)	52/107	1.10	(0.61 ; 1.96)
P for trend		p=0.09			p=0.61	
Atherosclerosis score – moderate						
≥ 55	45/117	1	reference	27/72	1	reference
50 – 54	288/591	1.60	(1.05 ; 2.45)	166/341	1.87	(1.08 ; 3.24)
45 – 49	134/260	1.71	(1.07 ; 2.73)	64/135	1.59	(0.86 ; 2.93)
< 45	67/109	2.41	(1.37 ; 4.23)	31/53	2.34	(1.09 ; 5.04)
P for trend		p < 0.01			p= 0.13	
Atherosclerosis score – severe						
≥ 55	15/87	1	reference	7/52	1	reference
50 – 54	119/422	2.29	(1.08 ; 4.85)	56/231	2.19	(0.81 ; 5.91)
45 – 49	61/187	2.93	(1.31 ; 6.59)	30/101	2.81	(0.96 ; 8.19)
< 45	41/83	5.30	(2.12 ; 13.2)	26/46	6.63	(2.08 ; 21.1)
P for trend		p < 0.0002			p<0.0001	
Myocardial infarction						
≥ 55	14/240	1	reference	10/148	1	reference
50 – 54	117/1335	1.60	(0.89 ; 2.86)	62/743	1.26	(0.62 ; 2.54)
45 – 49	75/671	1.91	(1.05 ; 3.49)	46/362	1.80	(0.87 ; 3.72)
< 45	39/275	2.49	(1.30 ; 4.76)	20/130	2.22	(0.98 ; 5.01)
P for trend		p=0.01			p=0.01	

OR denotes odds ratio. CI denotes confidence interval. * Values are adjusted for age, smoking (never/past/current [pack-years of smoking for past and current smokers]), body mass index (3 categories), alcohol intake (4 categories), short term hormone replacement therapy (yes/no) and education (4 categories). Numbers do not always add up to totals due to missing values.

essentially the same as those in the whole population. The risk estimates for the associations with artificial menopause in never-smokers only estimates remained comparable to those in the whole population, although most estimates lost significance, due to the smaller number of subjects in these analyses. Odds ratios associated with bilateral oophorectomy in never smokers were 1.82 (0.58 - 5.67) for carotid plaques, 2.57 (0.94 – 7.04) for PAD, 3.03 (1.16 – 7.89) for aortic atherosclerosis, 0.88 (0.23 – 3.35) and 3.59 (0.74 – 17.4) for the moderate and severe atherosclerosis score respectively, and 2.06 (0.57 – 7.43) for myocardial infarction. Estimates for never smokers with hysterectomy with or without bilateral oophorectomy were unchanged.

DISCUSSION

Our study showed that women who experience an early natural menopause have increased risks of atherosclerosis at several sites. Also a history of early bilateral oophorectomy, but not early hysterectomy with or without unilateral oophorectomy, was associated with an increased risk of atherosclerosis. When restricting the analysis to never smokers only, the association of early natural menopause with the increased risk of aortic atherosclerosis disappeared, but not that of bilateral oophorectomy. The association with peripheral arterial disease weakened, but also this association remained elevated with bilateral oophorectomy. The associations of early natural and artificial menopause with the atherosclerosis scores remained essentially the same in never smokers as in the whole population. Associations were also found between early menopause and myocardial infarction. The increased risks persisted into older age.

Women experiencing early menopause may differ in several characteristics from women experiencing a later menopause. Smoking, socio-economic status, alcohol intake and BMI may possibly confound the association between menopause and atherosclerosis. In this study, age at menopause and confounders were assessed in women who were already postmenopausal. This will not have affected the measurement of socio-economic status (highest attained level of education), which remains relatively stable over time. BMI, alcohol intake and smoking may have changed over time, and it could be that some residual confounding has remained after adjustment for these factors. For alcohol and BMI we do not expect this to have a large effect on our results, because these factors have relatively weak associations with atherosclerosis and cardiovascular disease.

In particular, cigarette smoking is likely to be a serious confounder. Smoking is strongly related to early menopause and increases the risk of atherosclerosis. Apart from adjustment for smoking, associations were re-examined in never smokers only, in order to rigorously exclude residual confounding by smoking. The risk of aortic ath-

erosclerosis with early natural menopause virtually disappeared, and the association for atherosclerosis in the peripheral arteries decreased, loosing statistical significance. This could indicate residual confounding by smoking, but as smoking has been found to reduce endogenous estrogen levels, we cannot exclude the possibility of effect modification. The associations of early natural menopause with carotid atherosclerosis, with the overall atherosclerosis scores and with myocardial infarction remained virtually unchanged among never smokers. Furthermore, associations between artificial menopause and all measures of atherosclerosis remained unchanged in never smokers, although as a result of small numbers in these analyses, statistical significance was lost in all analyses, except for aortic atherosclerosis. In a recently published report form the Nurses' Health Study, the association between younger age at menopause and higher risk of coronary heart disease was found to be present among current and past smokers, but not among never smokers, which suggests residual confounding by smoking.[27] However, this observation was based on only nine cases of coronary heart disease in never smokers with menopause before age 45. Confidence intervals of the risk estimates were wide, and thus this finding does not exclude the possibility of an association. The largest study to date studying the association between early menopause and cardiovascular disease mortality did not make a distinction between never smokers and past smokers, and thus residual confounding in this study cannot be excluded.[9] In our study, the association of early natural menopause with myocardial infarction remained borderline statistically significant.

Age at natural menopause was self-reported. Misclassification is likely to be independent of atherosclerosis and coronary heart disease, and therefore, if present, caused underestimation of the effect. The distribution of age at menopause in our study was in accordance with two leading studies on age at menopause in the Netherlands.[28,29] In these studies 7 percent of women reached menopause before age 45 and 10 percent after age 55. The mean age at menopause in our study population was slightly lower than the mean age at menopause in The Netherlands, which is usually seen when assessing age at menopause retrospectively. Early or late menopause, being special events, are recalled better than 'average' menopause.[30]

Misclassification of bilateral oophorectomy can obscure the true associations. In this study, self-reported gynecological operations were verified with patient records, minimising errors in classification. Repeating analyses with only women whose gynecologic operations were classified as definite did not change the results.

In our study atherosclerosis was measured at three sites. Ultrasonographical assessment of carotid plaques, measurement of the AAI, and radiographical assessment of aortic atherosclerosis have all been shown to be accurate non-invasive methods of measuring atherosclerosis. A series of studies has demonstrated their associations with cardiovascular risk factors, atherosclerosis at other sites of the vessel bed, and cardiovascular morbidity and mortality.[20,23,31-38] Myocardial infarction was self-reported or

from ECG evidence and checked with GP records.[24] Only seven of the reported myocardial infarctions had occurred before menopause. Although we can not know at what age silent infarctions have occurred, only a very small percentage is expected to have occurred before menopause.

Most information on menopause and atherosclerosis has been obtained from autopsy studies. In two out of three autopsy studies, women who had a bilateral oophorectomy had an excess of coronary atherosclerosis, which approached that of men, compared to women with intact ovaries.[39-41] Parrish et al. found that excessive coronary atherosclerosis became apparent only 14.4 years after oophorectomy.[40] In female cynomolgus monkeys atherosclerosis was increased in oophorectomised compared to intact females after a period of 30 months on an atherogenic diet.[42] More recently, studies have been performed on the association of menopause with non-invasively measured atherosclerosis. In one study, postmenopausal women had four to five times the odds of carotid plaque compared to premenopausal women, but no adjustment was made for age.[43] Other studies showed no differences in carotid plaques and intima-media thickness between pre- and postmenopausal women after adjustment for age.[17,18] In the Atherosclerosis Risk in Communities Study no overall effect of menopause on carotid intima-media thickness was found[16], however, women in the ARIC study were relatively young (< 55 years of age). An increased risk of radiographically assessed aortic atherosclerosis was shown in one study comparing postmenopausal to premenopausal women.[44]

Evidence on the association between menopause and coronary heart disease is conflicting. One reason for this could be that different studies have adjusted for age and smoking with varying efficacy, which could have lead to biased estimates.[27] Another problem is that a change in cardiovascular disease due to menopause will not be seen until 10 or 20 years later, by which time the effects of menopause are difficult to distinguish from the effects of ageing. Furthermore, studies on associations with early natural menopause have mostly been performed in women who developed heart disease at young age (< 50 and < 55 years of age), which indicates that the postmenopausal period in these studies was short.[8,12,45,46] Our study population consisted of women between 55 and 106 years old, which allowed assessment of the associations in women long after menopause. Although the effect of artificial menopause on atherosclerosis could not be studied in age strata due to small numbers, we found that the effect of natural menopause on atherosclerosis remained present in women over age 70.

Several studies have reported the risk of cardiovascular disease to be increased in women after hysterectomy independently of oophorectomy. It has been hypothesised that hysterectomy increases risk of cardiovascular disease by compromising blood flow to the ovaries affecting their function, or through a hormonal or other secretory function of the uterus.[47-52] Our study showed that the increased risk of atherosclerosis was

restricted to women with early bilateral oophorectomy, implying ovarian function is the key factor in the association. The finding of an increased risk of PAD after hysterectomy alone could be a true association but also may be a chance finding.

If menopause mediates the risk of cardiovascular disease through increased atherogenesis starting after cessation of ovarian function, this may have implications for the practice of treatment with HRT. Because the presently available drugs for HRT are not suitable for long-term use due to an increased risk of breast- and endometrial cancer, it has been suggested that treatment with HRT could be delayed until the age at which cardiovascular disease and osteoporotic fractures frequently occur. Our study, however, suggests that early estrogen deprivation has a long-term effect and that prevention might be indicated in an early phase. The extent to which HRT in the early postmenopausal phase may delay the onset or progression of atherosclerosis needs to be determined, in clinical trials adequately designed to study atherosclerosis as well as clinical endpoints.

The results of this population-based study demonstrate that early menopause, both natural and by bilateral oophorectomy, is associated with increased levels of atherosclerosis in several vessel beds, and with myocardial infarction. Women with early menopause remain at increased risk of atherosclerosis many years after menopause.

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3

Hormone replacement therapy – Observational studies

3.1 Hormone replacement therapy and peripheral arterial disease

Abstract

Objective – To evaluate the effect of ever use of hormone replacement therapy on the presence of peripheral arterial disease in women 55 to 80 years of age.
Design – Cross-sectional analysis in population based cohort study.
Setting – A community based cohort study in a suburban area of Rotterdam, The Netherlands (the Rotterdam Study).
Subjects – 2196 naturally menopausal women aged 55 to 80 years.
Outcome measures – Peripheral arterial disease defined as an ankle/arm systolic blood pressure index < 0.9.
Results – Ever use of hormone replacement therapy for one year or longer was associated with a 52% decreased risk of peripheral arterial disease (odds ratio (OR) 0.48 (95% confidence interval (CI) 0.24 - 0.85)), while no association was found for use during shorter than 1 year (OR 0.97 (95 % CI 0.58 - 1.63), after adjustment for age, smoking and socio-economic status. Additional adjustment for body mass index, age at menopause, total cholesterol and high-density lipoprotein (HDL) cholesterol, alcohol intake or frequency of visits to health care facilities did not change these results.
Conclusions – The findings of this population-based study suggest that ever use of hormone replacement therapy is associated with a decreased risk of peripheral arterial disease in postmenopausal women.

INTRODUCTION

Peripheral arterial disease is a growing clinical and public health issue in elderly women. Prevalence rates have been estimated to range from 6 to 25% in women over 55 years of age, and increase sharply with age. With the relatively large expected increase in the number of elderly women compared to elderly men, women will represent the majority of patients with peripheral arterial disease in the next century. Several studies have demonstrated that patients with peripheral arterial disease, with or without complaints of intermittent claudication, are at an increased risk of cardiovascular morbidity and mortality compared to people without peripheral arterial disease.[1]

Evidence from observational studies suggests that hormone replacement therapy (HRT) reduces morbidity and mortality from cardiovascular disease in postmenopausal women.[2,3] Recently, a randomised trial in women with coronary heart disease showed no effect of hormone replacement therapy on the incidence 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, proarrythmic or proischemic effect of treatment, which is gradually outweighed by a beneficial effect on the progression of atherosclerosis. Thus, a protective effect of this treatment may be present on the development of atherosclerotic disease, but data are scarce.

We investigated the association between ever use of HRT and the presence of atherosclerosis in the peripheral arteries by measuring the ankle/arm systolic blood pressure index (AAI), in 2196 naturally postmenopausal women, aged 55 to 80 years of age, participating in the Rotterdam Study.

METHODS

The Rotterdam study is a prospective population-based follow-up study in 7983 subjects aged 55 and over that aims to assess the occurrence of chronic diseases in an ageing population, and to clarify their determinants.[5] During the first survey, from 1990 to 1993, all participants were interviewed at home by a trained research assistant, and visited the study centre for clinical examination. A second visit to the study centre has taken place in 1993-1994. The study was approved by the Medical Ethical Committee of Erasmus University, and informed consent was given by all participants.

Interview information included medical history, current medication, smoking habits, alcohol intake, highest attained level of education and age at last menstruation. At

the study centre height and weight were measured. Serum total cholesterol and HDL-cholesterol values were assessed by an automated enzymatic procedure. Random and postload serum glucose levels were assessed after an oral glucose tolerance test.

The presence of peripheral arterial disease was assessed at baseline. Systolic blood pressure was calculated as the mean of two consecutive measurements at the right brachial artery while the patient was in a sitting position. A single systolic blood pressure reading was taken both at the left and the right posterior tibial artery as described previously.[6] The ratio of the systolic blood pressure at the ankle to the systolic blood pressure at the arm (AAI) was calculated for each leg. Peripheral arterial disease was considered present when the AAI was lower than 0.9 on at least one side.

In 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 in the first follow-up visit to the study centre 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 and specific information on type and brand of medication, duration of use, recent use and the use of progestins was collected. Information from both the first and the second visit to the study centre 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 centre, 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 follow-up visit's questionnaire, 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 non-users. Thus, 692 (18 %) of women were classified as ever users of female hormones. Seventy-four of these women reporting to have continued use of female hormones in the period between the baseline and the follow-up visit were classified as recent users.

As data on use of HRT were obtained both in the first and the second follow-up round, for this study only women participating in the second follow-up round 3784 (78%) were considered. Women were excluded who reported to have reached menopause by surgery or radiation of the womb/ovaries (n=719) or who were older than 80 (n=496). Thus remained 2569 women. The AAI was not determined in 349 of these women (13.6 %). Eight women with an AAI > 1.5 were excluded as this AAI usually reflects arterial rigidity preventing arterial compression, which leads to spuriously high ankle blood pressure values. Information on use of HRT was missing in 8 women. Thus remained 2196 women for analyses.

Statistical analysis

Analysis of covariance adjusted for age was used to compare continuous variables between groups. Smokers were categorised as current, past or never smokers and additional adjustment was made for the number of pack-years (the number of years of smoking multiplied by the number of cigarettes smoked daily, divided by twenty). Analyses were stratified for short-term (< 1 year) and long-term hormone use (≥ 1 year). For further stratification numbers were inadequate. Logistic regression analysis was used to calculate relative risks of peripheral arterial disease for ever-users compared to never-users. All OR's are presented with 95% confidence intervals (CI). The odds ratios derived from logistic regression analyses were used as an approximation of relative risk. Reported P-values are two-sided. Analyses were performed using BMDP software (BMDP Statistical Software, Inc).

RESULTS

Of the 2196 women in the study population 351 reported ever use of HRT (16.2%). Duration of use ranged from 1 month to more than 15 years. Thirty-two women (9% of users) reported use of progestins, additional to the use of estrogens. Thirty-one women (9% of users) were recent users.

Age was comparable in the three study groups (Table 1). Ever users of female hormones had a lower systolic blood pressure, were more often past smokers, and had a higher frequency of visits to health care facilities in the last month than never users. Both short-term and long-term users were more often past smokers.

Mean AAI was 1.08 (SD 0.19), and 284 women had an AAI < 0.9 (12.9%, 95%CI 11.5-14.3). Age, systolic blood pressure, total-cholesterol, glucose levels, the percentage of current smokers, and among smokers the number of pack-years of smoking, were all significantly higher in women with peripheral arterial disease (Table 2).

Of the 351 ever users 34 had peripheral arterial disease, compared to 247 out of 1837 of the never users. Logistic regression analysis, with adjustment for age showed that, overall, ever users had a 30 % lower risk of peripheral arterial disease (Table 3). While use for a period shorter than one year was not associated with a protective effect, use during 1 year or more was associated with a statistically significant 47% reduction of risk. Adjustment for smoking, number of pack-years smoked and level of education reduced the risk to 52%. Additional adjustment for body mass index, alcohol intake, age at menopause, levels of total and HDL-cholesterol, or the number of visits to health care facilities in the last month did not change the risk estimates. When repeating the analyses after exclusion of women reporting recent use of female hor-

mones, to assess the effect in past users only, a similar association was found (OR 0.39 (95%CI 0.20 – 0.80). Also, excluding women classified as probable or possible users, did not change our results (OR 0.42 (95%CI 0.20 – 0.88)). In 31 recent users 3 women had peripheral arterial disease (9%), in the 32 users of progestins additional to estrogens, 3 women had peripheral arterial disease (9%).

Table 1
Age-adjusted baseline characteristics of short-term, long-term and never users of hormone replacement therapy among women with natural menopause, aged 55 - 80. Values are means or proportions with standard errors.

Characteristics	Never use (n = 1845)	Short-term use Use < 1 year (n = 157)	Long-term use Use ≥ 1 year (n = 169)
Age (years)	66.9 (0.15)	66.5 (0.51)	67.3 (0.50)
Time since menopause (years)	17.0 (0.1)	16.6 (0.3)	17.8 (0.3)‡
Systolic blood pressure (mmHg)	138.5 (0.5)	135.7 (1.6)	135.5 (1.6)
Diastolic blood pressure (mmHg)	73.3 (0.3)	73.1 (0.9)	72.9 (0.9)
Antihypertensive medication (%)	29.1 (1.0)	27.6 (2.4)	26.3 (3.4)
Body mass index (kg/m^2)	26.7 (0.1)	26.5 (0.3)	26.2 (0.3)
Total Cholesterol (mmol/l)	6.91 (0.02)	6.83 (0.09)	6.95 (0.09)
HDL-Cholesterol (mmol/l)	1.45 (0.01)	1.46 (0.03)	1.52 (0.03)*
Glucose (mmol/l)	6.69 (0.06)	6.49 (0.19)	6.61 (0.19)
Diabetes Mellitus§ (%)	8.5 (0.6)	6.2 (2.1)	7.4 (2.1)
Current smoker (%)	29.4 (1.2)	24.2 (4.2)	32.0 (4.3)
Pack-years for current smokers (n)	27.0 (0.01)	32.3 (3.4)	28.0 (3.0)
Past smoker (%)	35.8 (1.2)	37.6 (4.1)*	46.0 (4.1)*
Pack-years for past smokers (n)	17.4 (0.9)	20.8 (2.7)*	18.7 (2.5)
Alcohol drinker (%)	74 (1.0)	75 (3.6)	74 (3.5)
Visited General Practitioner in last month (%)	38 (1.1)	45 (3.8)	43 (3.7)
Visited specialist in last month (%)	19 (0.9)	24 (3.0)	28 (3.0)†
Higher education¶ (% highest category)	21.9 (0.9)	25.9 (3.2)	27.6 (3.2)
Income (Euro/month)	1276 (13.6)	1303 (45.4)	1221 (40.8)

* compared to never users, $p < 0.05$
† compared to never users, $p < 0.01$
‡ long term users compared to short term users, $p < 0.05$
§ Diabetes was defined as a random or postload glucose levels of > 11.0 mmol/l or current use of antidiabetic drugs.
¶ Educational level was divided into four categories.
Numbers do not add up to totals due to 25 missing values on duration of use.

Table 2
Age-adjusted cardiovascular risk indicators in subjects with an AAI < 0.9 or an AAI ≥ 0.9, among women with natural menopause, aged 55 - 80. Values are means or proportions with standard errors (SE).

Cardiovascular risk indicators	AAI ≥ 0.9 1912	AAI < 0.9 284
Age (years)	66.3 (0.15)	69.1 (0.40)‡
Time since menopause (years)	16.7 (0.1)	17.5 (0.3)†
Systolic blood pressure (mmHg)	136.7 (0.5)	144.2 (1.2)‡
Diastolic blood pressure (mmHg)	73.2 (0.3)	74.2 (0.7)
Body mass index (kg/m^2)	26.7 (0.1)	26.4 (0.2)
Total Cholesterol (mmol/l)	6.88 (0.02)	7.14 (0.07)†
HDL-Cholesterol (mmol/l)	1.47 (0.01)	1.43 (0.02)
Glucose (mmol/l)	6.57 (0.06)	7.03 (0.15)†
Diabetes Mellitus¶ (%)	7.6 (0.6)	10.8 (1.6)
Current smoker (%)	26.3 (1.2)	44.3 (3.1)§
Pack-years for current smokers (n)	27.1 (1.0)	30.3 (2.0)
Past smoker (%)	35.9 (1.2)	41.2 (3.4)
Pack-years for past smokers (n)	16.7 (0.8)	23.6 (2.3)†
Alcohol drinker (%)	76.0 (1.0)	68.3 (0.03)*
Higher education# (% high)	23.8 (1.0)	21.0 (2.5)
Income (Euro/month)	1276 (13.6)	1235 (31.7)
Intermittent claudication (% present)	0.32 (0.2)	4.6 (0.6)‡
History of myocardial infarction (% present)	5.9 (0.6)	9.6 (1.5)*
History of angina pectoris (% present)	5.6 (0.5)	7.4 (1.4)
History of stroke (% present)	1.9 (0.4)	7.3 (1.0)‡
Carotid artery intima-media thickness (μm)	73.3 (0.4)	78.4 (1.0)‡
Carotid artery plaques (% present)	52.7 (1.3)	63.4 (3.4)†

* $p < 0.05$
† $p < 0.01$
‡ $p < 0.001$
§ $p < 0.0001$
¶ Diabetes was defined as a random or postload glucose levels of > 11.0 mmol/l or current use of antidiabetic drugs.
# Educational level was divided into four categories.
Numbers do not add up to totals due to 25 missing values on duration of use.

Table 3
Relative risk of peripheral arterial disease associated with use of hormone replacement therapy among postmenopausal women with natural menopause, aged 55 - 80.

HRT	No. of cases	No. of subjects	Age-adjusted Relative Risk* (95% Confidence Interval)	Multivariate-adjusted Relative Risk† (95% Confidence Interval)
Never users	247	1837	1.00 reference	1.00 reference
Short term users (< 1 year)	20	157	0.97 (0.60 ; 1.60)	0.97 (0.58 ; 1.63)
Long term users (≥ 1 year)	13	169	0.53 (0.30 ; 0.93)	0.48 (0.24 ; 0.85)
All users	34	351	0.70 (0.48 ; 1.02)	0.65 (0.44 ; 0.98)

* adjusted for age using the logistic regression model.
† adjusted for age, smoking and education using the logistic regression model.
Numbers do not add up to totals due to 25 missing values on duration of use.

DISCUSSION

Our results show a lower risk of atherosclerosis in the peripheral arteries in ever users of HRT compared to never users.

Before interpreting these results several issues need to be addressed. In a study of elderly subjects there is the possibility of selection bias. Women had to survive until at least age 55 to be in our study. If women who never used female hormones more frequently died of atherosclerotic complications before the start of our study, this may have led to an underestimation of the effect. The AAI gives a good indication of atherosclerosis in the lower limbs, assessing the adequacy of blood flow through the peripheral arteries.[7,8] It has been shown to be associated with preclinical carotid atherosclerosis and predicts cardiovascular mortality in elderly women and men.[1,9] The mean value of the AAI in this population was 1.08. The prevalence of peripheral arterial disease was 12.9 %. This is comparable to values reported in other studies.[6,7,10-14]

Several studies[15,16] have demonstrated that estrogen users are healthier than never users, even prior to use of replacement therapy. This supports the hypothesis that part of the apparent benefit associated with HRT is due to pre-existing characteristics of the users. We cannot exclude the possibility that part (or the whole) of our findings is based on this selection bias. In our study we dealt with the issue of confounding in the following ways. We stratified for duration of hormone use, and found

that in women who had used female hormones for a period shorter than a year, although they were alike long-term users with respect to the presence of several socio-economic and risk factors, no association was found with the presence of peripheral arterial disease. This reduces, but not fully excludes, 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. Socio-economic status (level of education), is a major confounder, but remains relatively stable over time. The frequency of visits to health care facilities (possibly representing health conscious behaviour 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 the latter to have a large effect on our results, however, because of the relatively weak associations of these factors with peripheral arterial disease, especially in women.[17,18]

Assessment of use of hormone replacement therapy by interview might have led to misclassification. Goodman et al. showed a 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 up to 74 years of age.[19] The reported frequency and duration of use was similar to the those in studies in perimenopausal women in The Netherlands, where 12% of women between 45 and 65 used HRT and 50 % of women discontinued use within one year.[20-22] The most frequently prescribed hormone therapy in the studied period was unopposed estrogen therapy in a dose of 0.625 mg daily.[23] Progestins were added in 0.6% of prescriptions in 1970, gradually increaing to 11% of prescriptions in 1986. Our observation of 9% agrees with this.

The finding that HRT inhibits development of atherosclerosis in the coronary arteries and aorta has been reported in several animal studies.[24,25] In women, two out of three angiography studies showed a lower degree of coronary atherosclerosis in HRT users compared to never users.[26-28] Another study, using ultrasonographic examination, found lower degrees of atherosclerosis of the carotid arteries, aorta, and iliac arteries in 40 users of combined replacement therapy compared to never users.[29] Most studies, however, focussed on comparing current users with never users. The effect of past use of female hormones was studied in the Cardiovascular Health Study (CHS), which showed no significant differences in IMT between past users and never users.[30] No tests were presented for differences in AAI between past and never users. The CHS-finding that current users had a significantly lower measure of IMT than never users could not be confirmed in the Atherosclerosis Risk In Communities (ARIC) study.[31] In our study the number of recent users was small, but exclusion of recent users from the

analysis showed that prevalence of peripheral arterial disease was lower in past users compared to never users. Thus, our results suggest that the advantage in levels of atherosclerosis for women who have used HRT remains present after discontinuation of therapy.

Only one large randomised placebo-controlled trial on the effects of HRT has been conducted. This trial in women with diagnosed cardiovascular disease showed no favourable effect of HRT on the prevention of incident coronary heart disease after 4 years of follow-up.[4] This might indicate that bias in observational studies is 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 in the first year of the trial, while risk decreased in subsequent years. This time-trend can be explained by an immediate prothrombotic, proarrythmic 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. Furthermore, in HERS, the effect of opposed estrogen was studied, while in our study mainly unopposed estrogen was used. Progestins have been shown to have unfavourable effects on several determinants of cardiovascular disease, which may have contributed to the absence of a positive trial result.[32]

As the number of elderly women increases in western society, peripheral arterial disease is likely to become an increasing problem. The results of this study suggest that the use of HRT after menopause might protect against the development of peripheral arterial disease later in life. Confirmation of these findings should be provided by randomised trials including peripheral arterial disease as an outcome measure.

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3.2 Hormone replacement therapy and intima-media thickness

Abstract

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 hormone replacement therapy 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, The Netherlands. Mean and maximum IMT of the common carotid artery were measured non-invasively with B-mode ultrasound.

Results – Ever use of hormone replacement therapy for one year or more was associated with a decreased mean and maximum IMT compared to never users ((mean IMT 71.9 μm (standard error (SE) 1.0) versus 74.2 μm (SE 0. 4), p=0.03), maximum IMT 95.2 μm (SE 1.5) versus 98.3 μm (SE 0.6), p= 0.04)), after adjustment for age, smoking, educational level, systolic blood pressure and body mass index. No association was found for use less than one year ((mean IMT 73.9 μm (SE 1.3) versus 74.2 μm (SE 0.4), p=0.69), maximum IMT 99.0 μm (SE 1.9) versus 98.3 μm (SE 0.6), p = 0.75)). Additional adjustment for diabetes, frequency of visits to health care facilities or total cholesterol and high density lipoprotein (HDL) cholesterol did not change these results.

Conclusions – The findings of this population based study show that ever use of hormone replacement therapy is associated with a decreased intima-media thickness in the common carotid artery in elderly women.

INTRODUCTION

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, in haemostatic variables, in blood viscosity, direct effects on the arterial wall and inhibition of the atherosclerotic process. Recently, however, a randomised trial on the effects of hormone replacement therapy 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, proarrythmic or proischemic effect of treatment, which is gradually outweighed by a beneficial effect on the progression of atherosclerosis. The results of HERS are thus supportive of the possibility that HRT inhibits the development of atherosclerotic disease. It is therefore important to assess whether favourable effects of hormone replacement therapy on atherosclerosis are indeed present. Data on the effects of use of hormone replacement therapy 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 in 1103 naturally postmenopausal women, participating in the Rotterdam Study.

METHODS

The Rotterdam Study

The Rotterdam Study is a prospective population-based follow-up study that aims at assessing the occurrence of chronic diseases in an ageing 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, from 1990 to 1993, all participants were interviewed at home by a trained research assistant. The participants subsequently visited the study centre for a clinical examination. A second visit to the study centre 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 or more. At the study centre height and weight were measured. Blood pressure was measured twice with a random zero sphygmomanometer with the subject in sitting position, and averaged. Serum total cholesterol values were assessed by an automated enzymatic procedure in a non-fasting blood sample. Serum HDL-cholesterol was measured after precipitation of the non-HDL fraction with phosphotungstate-magnesium. Random and post-load serum glucose levels were assessed after an oral glucose tolerance test.

Measurement of intima-media thickness

To measure carotid intima-media thickness, ultrasonography of the left and right common carotid artery was performed with a 7.5 MHz linear array transducer (ATL Ultra-Mark IV). On a longitudinal 2-dimensional ultrasound image of the carotid artery, the anterior (near) and posterior (far) wall of the carotid artery are displayed as two bright white lines separated by a hypo-echogenic space. The distance of 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 intima-media thickness. For the near wall, the distance between the trailing edge of the first bright line to the trailing edge of the second bright line at the near wall provides the best estimate of the near wall intima-media thickness.[8,9] Following the ultrasound protocol[10], a careful search was performed for all interfaces of the near and far wall of the distal common carotid artery. When an optimal longitudinal image was obtained, it was frozen on the R wave of the electrocardiogram and stored on videotape. This procedure was repeated three times for both sides. The actual measurements of intima-media thickness were performed off-line. From the videotape, the frozen images were digitised and displayed on the screen of a personal computer using 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 intima-media thickness of each of the three frozen images was calculated. For each individual an intima-media thickness was deter-

mined as the average of near and far wall measurements of the left and right artery. The readers of the ultrasound images from videotape were unaware of the exposure status of the subject. Reproducibility of intima-media thickness measurements was studied among eighty subjects who underwent a second ultrasound scan of both carotid arteries within three months of the first scan. Measurements were shown to be highly reproducible.[8] Off-line the carotid artery was evaluated from tapes for the presence (yes/no) of atherosclerotic lesions on both the near and the far wall of the arteries. Plaques were defined as a focal widening relative to adjacent segments, with protrusion into the lumen either composed of only calcified deposits or a combination of calcification and non-calcified material. The common and internal carotid artery and the carotid bifurcation were both on-line and off-line evaluated 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 one or more sites. 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 kappa of 0.65 for the right carotid artery, indicating moderate agreement.[12]

Assessment of use of hormone replacement therapy

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 in the first follow-up visit to the study centre 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 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 visit to the study centre 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 centre, 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 follow-up visit's questionnaire, 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 non-users 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 visit. As we cannot be sure whether these women were cur-

rent 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. As data on use of HRT were obtained both in the first and the second follow-up round, only women participating in the second follow-up round were analysed in this study (n=3784). Women were excluded who reported to have reached menopause by surgery (n=677) or radiation of the womb or ovaries (n=42) and women who were older than 80 (n=558), as the use of hormone replacement therapy in older women was rare. Furthermore, 55 women had no data on the use of HRT. After excluding women who fulfilled one or more of these exclusion criteria, 2401 women remained for analysis. Intima-media thickness was determined in the first 1103 of these women only. Compared to 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, and were less often former smokers. No differences were found in other cardiovascular risk factors.

Statistical analysis

Analysis of covariance was used to compare characteristics of HRT-users and non-users, adjusted for present age. Age-adjusted linear regression analysis was used to assess the association between risk factors and IMT. Multivariate analysis of covariance 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), educational level (in four 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 (up to one year) and long-term use (one year or more), 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 adjusting for diabetes (defined as a random or post-load glucose level of > 11.0 mmol/l or current use of anti-diabetic drugs), frequency of visits to health care facilities in the last month and levels of total cholesterol and HDL-cholesterol. To assess the effect of past use, the analysis was repeated excluding recent users. The analysis was repeated after exclusion of probable and possible users. All reported P-

values are two-sided. Analyses were performed using BMDP software (BMDP Statistical Software, Inc).

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

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. Values are means or proportions with standard errors.

Characteristics	Never use (n = 875)	Short term use Use < 1 year (n = 79)	Long-term use Use ≥ 1 year (n = 136)
Age (years)	67.9 (0.23)	66.5 (0.71)	67.1 (0.56)
Time since menopause (years)	18.6 (0.2)	18.1 (0.5)	19.4 (0.4)
Systolic blood pressure (mmHg)	137.1 (0.6)	138.3 (2.2)	136.9 (1.9)
Diastolic blood pressure (mmHg)	71.9 (0.4)	74.2 (1.2)	72.3 (0.9)
Body mass index (kg/m^2)	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)
Packyears 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)
Packyears for past 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/month)	1192 (18.5)	1279 (61.0)	1147 (46.9)

* compared to never users, $p < 0.05$, † compared to never users, $p < 0.01$, ‡ long term users compared to short term users, $p < 0.05$, § ever users compared to never users, $p < 0.05$. Numbers do not add up to totals due to 13 missing values on duration of use.

(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 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 more than 15 years. Seventy-nine women (36.7%) reported use for a period up to one year, 68 (31.6%) 1 to 4 years and 68 (31.6%) more than 5 years. Twenty-one women (9% of all users) reported to have used HRT in the period between the first and second visit. Twenty women (9% of all users) reported a history of use of progestins, additional to the use of estrogens.

Mean IMT in our study group was 72.6 μm (standard deviation 13.5) and ranged from 43.0 μm to 145.8 μm. 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 –0.02 ; 0.31, p=0.08).

Table 2
Age-adjusted regression coefficients for association between cardiovascular risk factors and intima-media thickness. β is the increase of IMT in μm per unit increase in the cardiovascular risk factor.

Characteristic	β	95 % confidence interval
Age (years)	0.87	(0.77 ; 0.79)
Systolic blood pressure (mmHg)	0.12	(0.08 ; 0.15)
Diastolic blood pressure (mmHg)	-0.05	(-0.12 ; 0.01)
Boby mass index (kg/m^2)	0.15	(-0.02 ; 0.31)
Total Cholesterol (mmol/l)	0.81	(0.71 ; 0.92)
HDL-Cholesterol (mmol/l)	-2.41	(-4.33 ; -0.49)
Diabetes (yes/no)	4.05	(1.54 ; 6.57)
Current smoker (yes/no)	2.97	(1.10 ; 4.85)
Former smoker (yes/no)	0.85	(-0.73 ; 2.42)
Alcohol drinker (yes/no)	-0.43	(-1.20 ; 0.34)
Visited GP in last month (yes/no)	1.93	(0.51 ; 3.35)
Visited specialist in last month (yes/no)	-0.04	(-1.77 ; 1.69)
Higher education (highest category)	-0.13	(-1.03 ; 0.78)
Income (per 100 euro)	-0.046	(-0.185 ; 0.093)

HDL = high density lipoprotein. GP = general practitioner.

Analysis of covariance showed that users of HRT had a mean age-adjusted IMT of 73.0 μm compared to a mean IMT of 74.3 μm in non-users (p=0.13, table 3). Stratification for duration of use, however, showed that while use shorter than one year was not associated with a reduction of IMT, use during 1 year or more was associated with a statistically significant reduction of IMT. Analysis adjusted for smoking, for number of packyears 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 last month or total cholesterol and high density lipoprotein cholesterol did not change the risk estimates. No associations were found with duration of use in women who had used one year or more.

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 (69.6 μm (SE 2.5) versus 74.1 μm (SE 0.4), p = 0.08).

When repeating the analyses excluding women reporting recent use of female hormones, in order to assess the effect in past users only, similar association were found for past users versus never users for mean IMT (71.9 μm (SE 1.3) versus 74.3 μm (SE 0.4), p = 0.07) and for maximum IMT (94.0 μm (SE 1.9) versus 98.4 μm (SE 0.6), p = 0.03). Also when repeating 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.

Table 3
Intima-media thickness in women with and without a history of hormone use. Values are means or proportions with standard errors.

Characteristic	Never users (n = 984)	Short-term users < 1yr (n = 79)	p‡	Long-term users ≥ 1 year (n = 136)	p‡	Ever users All (n = 228)	p‡
CCA IMT mean (μm)*	74.3 (0.4)	73.9 (1.3)	0.72	72.2 (1.0)	0.05	73.0 (0.78)	0.13
adjusted full†	74.2 (0.4)	73.9 (1.3)	0.69	71.9 (1.0)	0.03	72.8 (0.78)	0.10
CCA IMT maximum (μm)*	98.3 (0.6)	99.0 (1.9)	0.76	95.6 (1.5)	0.09	97.0 (1.14)	0.32
adjusted full†	98.3 (0.6)	99.0 (1.9)	0.75	95.2 (1.5)	0.04	96.8 (1.14)	0.23

CCA = common carotid artery. IMT = intima-media thickness. * Adjusted for age, † Adjusted for age, smoking, education, sbp and BMI. ‡ p-value for comparison to never users. Numbers do not sum up to totals due to missing values on duration of hormone use.

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 one year or longer, compared to never users. Use of HRT for a period shorter than one year was not associated with a decreased IMT.

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

Several studies demonstrated that estrogen users are healthier than never users, even prior to use of replacement therapy, which supports the hypothesis that part of the apparent benefit associated with HRT is due to pre-existing characteristics of the users.[13,14] Women who take hormones are a self-selected group and may have healthier life-styles with fewer risk factors than women who do not. Also, 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 socio-economic and risk factors, like income, frequency of visits to GP or medical specialist in last month, alcohol consumption and total and HDL-cholesterol levels. Among women who had used female hormones for a period shorter than a year no association was present with IMT. This diminishes, but not fully excludes, 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. Socio-economic status (level of education) may be a major confounder, but remains relatively stable over time. The frequency of visits to health care 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 hormone replacement therapy was assessed by interview. This might have led, to a certain extent, to misclassification. Greendale et al. demonstrated that a single self-report question is adequate to ascertain ever-use of postmenopausal estrogen use in women up to 64 years of age.[15] 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 up to 74 years of age.[16] The reported frequency and duration of use of HRT seemed to be similar to the those in studies in perimenopausal women in The Netherlands, where 12% of women between 45 and 65 used HRT and 50 % of women discontinued use within one 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. Besides conjugated equine estrogens, also oestradiol preparations were prescribed. Progestins were added in 0.6% of prescriptions in 1970, gradually increasing to 11% of prescriptions in 1986. Our observation of 9% agrees with this 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], with atherosclerosis in other locations [9,12] and with cardiovascular disease.[23,24] Thus, IMT can be used as an indicator for generalised 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 endpoints showed a lower degree of coronary atherosclerosis in HRT users compared to 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 non-users. 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 artery compared to never users.[35] Another large population-based study in women under age 55 (the Atherosclerosis Risk In Communities study, ARIC), however, did not find an association between HRT and IMT.[5] In two 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 to nonusers.

Results from the Asymptomatic Carotid Atherosclerosis Progression Study (ACAPS) among 186 postmenopausal women suggested that hormone replacement therapy may halt progression of atherosclerosis, as measured by carotid intima-media thickness.[38]

Most studies focussed 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 excluding 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, as 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 [41,42,43], several large population based studies [6 39 40], 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, and a diminishing of the protective effect after cessation of therapy, suggest that other mechanisms than the inhibition of atherosclerosis are also active. Our results suggest use of HRT for one year or more decreases the development of atherosclerosis, but no effect of duration of use was found.

Only one randomized trial on the effects of HRT on cardiovascular disease has been conducted (Heart Estrogen/Progestin Replacement Study (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 to the placebo group in the first year of the trial, but decreased in subsequent years. This time-trend should be interpreted with caution, but might be explained as being attributable to an immediate prothrombotic, proarrythmic or pro-ischemic 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 immediate effects of hormone replacement therapy mentioned above 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 to 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 hormone replacement therapy is associated with a favorable atherogenic status. Further understanding of the effects of hormone replacement therapy on atherogenesis can be obtained only in randomized trials, ade-

quately designed to take the distinct effects of hormone replacement therapy on the short- and the longer term into account.

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4

Hormone replacement therapy – Experimental studies

4.1 The effect of hormone replacement therapy on changes in arterial distensibility and compliance

Abstract

A single centre randomised placebo-controlled trial was performed to assess the two-year effects of hormone replacement therapy compared to placebo on mechanical arterial properties in 99 perimenopausal women recruited from the general population. The trial was double-blind with respect to a sequential combined regimen of oral 17β-oestradiol and desogestrel (17βE_2-D) and the placebo group and open with respect to combination of conjugated equine oestrogens and norgestrel (CEE-N). At baseline, distensibility and compliance of the common carotid artery was measured non-invasively with B-mode ultrasound and a vessel wall movement detector system, and the distensibility coefficient (DC) and compliance coefficient (CC) were calculated. Measurements were repeated after 6 and 24 months. Changes in DC and CC in treatment groups were compared to placebo. After 24 months, changes for 17βE_2-D compared to placebo were -1.4 x 10^{-3} / kPa (95% CI -4.4 ; 1.7, p=0.39) for DC and 0.26 mm^2 / kPa (95% CI -0.01 ; 0.53, p=0.07) for CC. Changes for CEE-N compared to placebo were 0.4 x 10^{-3} / kPa (95% CI (-1.0 ; 1.9, p=0.79) and 0.11 mm^2 / kPa (95% CI .-0.14 ; 0.37, p= 0.40). For systolic blood pressure, diastolic blood pressure and arterial lumen diameter no changes were found. In this study no significant differences in changes in distensibility and compliance were found between perimenopausal women using 17βE_2-D or CEE-N and women using placebo after 6 and 24 months.

INTRODUCTION

Epidemiological studies have shown a lower risk of coronary heart disease among postmenopausal users of oestrogen supplements compared to non-users.[1-3] Postulated mechanisms for a beneficial effect include changes in lipid and haemostatic variables and direct effects on the arterial wall. In experimental studies in animals oestrogens had direct vasodilatory effects[4-7] and were shown to affect the structure and mechanical properties of large arteries.[8-10] Improved endothelial function has been shown after hormone replacement therapy (HRT) in animal studies[11], in postmenopausal women[12-14] and after oestrogen use in transsexual men.[15,16] Also, HRT has been suggested to decrease the pulsatility index, a measure reflecting decreased peripheral vascular resistance.[17-20] Data on effects of HRT on distensibility and compliance of large arteries in humans are scarce. In a recent study oestrogen users had a higher arterial distensibility than non-users while oestrogen-progestin users had a lower arterial distensibility, and systemic arterial compliance was higher in both oestrogen and oestrogen-progestin users compared to non-users.[21] In another study HRT-users had a more favorable augmentation index, a measure of pulsatile vascular afterload, than non-users, while there was no difference in Pulse Wave Velocity (PWV), a measure of arterial stiffness.[22] Both studies, however, were observational. Rajkumar et al. showed women using HRT did not only have better systemic arterial compliance than non-users, but withdrawal of therapy, in a small group of women, resulted in a significant decrease of compliance.[23] To our knowledge no randomised studies have been performed to date on effects of HRT on distensibility and compliance of large arteries.

The present study was conducted to assess the long-term effect of Liseta®, a 24-day active, 28-day sequential combined regimen of oral 17β-oestradiol and desogestrel, and Prempak®, a combination of conjugated oestrogens and norgestrel on several cardiovascular risk factors (Bak, 1998, unpublished results) and structural and dynamic arterial characteristics of the carotid artery. Here we present the results of the effects of HRT on six months and two-year change in arterial distensibility and compliance in comparison to placebo in perimenopausal women.

METHODS

Study population and methods

The design of the study was randomised, double-blind with respect to $17\beta E_2$-D and placebo groups and open with respect to CEE-N. The study was conducted in one

centre and included 121 perimenopausal women. Participants were recruited from the general population in the town of Zoetermeer (The Netherlands). They completed a questionnaire on menopause and gynaecological issues that was sent to all women between 40 and 60 years of age. Women who were eligible based on the questionnaire and interested in participating in a hormone replacement study were invited for the screening procedure. The study was performed from October 1992 to July 1995. The study was approved by the Medical Ethics Committee of Erasmus University, and written consent was obtained from all participants.

Subject selection was based on the following criteria: age between 40 and 60 years; not hysterectomised; climacteric symptoms (hot flushes and/or outbreaks of sweating), body weight between 80% and 130% of the ideal body weight (Metropolitan Life Insurance Company Tables for Women, 1983). The main exclusion criteria were: absence of spontaneous vaginal bleeding for more than 5 years; use of sex-steroids currently or within the last two months or ethinyl-oestradiol or injectable sex steroids within the last six months or hormone implants at any time previously; history or presence of any malignant disorder; history or presence of cardiovascular or cerebrovascular disease or thromboembolism / thrombosis; history or presence of hepatic or renal disease, uncontrolled hypertension (systolic blood pressure > 170 mmHg and/or diastolic blood pressure > 105 mmHg); significant hyperlipidaemia (fasting total cholesterol > 9.5 mmol/l and/or fasting triglycerides > 2.5 mmol/l). A cervical smear and a mammography were done unless results were available dated less than one and two years previously, respectively.

17βE_2-D and placebo tablets were supplied in identical looking push-through-strips. CEE-N was supplied in the original, commercially available, strips. The composition of the three study drugs was as follows. Each strip of 17βE_2-D contained 12 tablets with 1.5 mg 17β-oestradiol (micronised), 12 tablets with 1.5 mg 17β-oestradiol (micronised) + 0.15 mg desogestrel (Liseta®; NV Organon), and 4 placebo tablets. Placebo was 17βE_2-D matched and contained 28 placebo tablets. Each strip of CEE-N contained 28 tablets with 0.625 mg conjugated oestrogens and 12 tablets with 0.15 mg norgestrel (Prempak®; Novo Nordisk). Subjects in the 17βE_2-D or 17βE_2-D-matched placebo groups took one tablet per day, on a continuous basis. Subjects treated with CEE-N took one tablet per day from day 1 to 16 and two tablets per day from day 17 to 28, for each cycle. Tablets were taken after breakfast. Sex steroids other than the study medication, hydantoins, barbiturates, primidone, carbamazepine, rifampicin, griseofulvin, and lipid lowering agents were not allowed during the study.

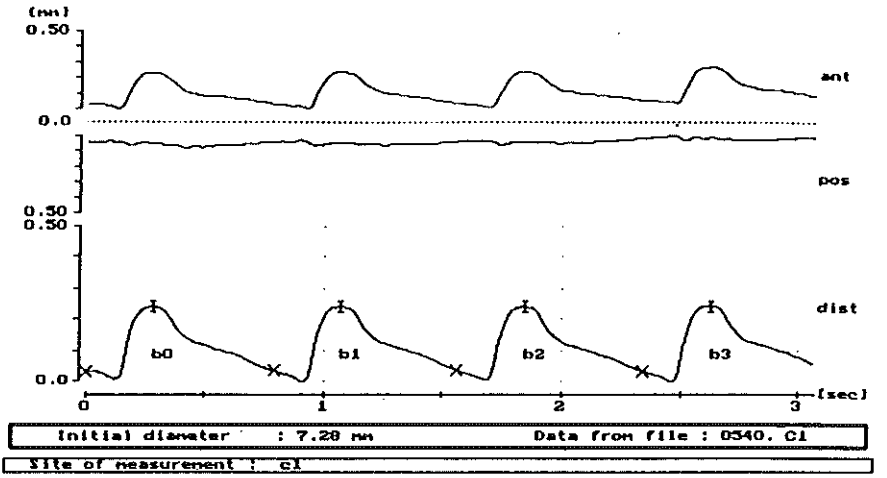
Randomisation to treatment with 17βE_2-D, CEE-N, or placebo was performed in a ratio of 3:2:2 using a computerised allocation algorithm. The treatment allocation of 3:2:2 was chosen to assure the gain of sufficient information on the relatively new combination 17βE_2-D. The design was double-blind with respect to 17βE_2-D and placebo groups, and open with respect to CEE-N. The first part of the study comprised six

consecutive cycles of 28 days, after which the blind was broken and the trial was continued as an open trial for another 18 months for the $17\beta E_2$-D and CEE-N groups. As in studies of HRT blinding is difficult to maintain throughout the study, because of the clear effects on menstrual cycle (participants were asked to complete a bleeding diary), the ultrasound technician was blinded for the intervention groups. All participants, including those in the placebo group, were invited for a final ultrasound examination 18 months after the blind was broken. Arterial characteristics were measured non-invasively at day of randomisation, and at cycle 6 and 24. Measurements were performed on day 21 ± 2 of the cycle for the $17\beta E_2$-D group and on 25 ± 2 for the CEE-N group.

At baseline, information was obtained about smoking. Body weight and body height were measured and body mass index was calculated. Fasting blood samples were obtained between 8.00 and 10.00 a.m. Cholesterol and triglycerides were assayed enzymatically with a Hitachi 747 automated analyser with kits from Boeringher Mannheim (Germany). HDL-cholesterol was measured after precipitation with phosphowolfram/phosphotungstic acid and 2 mmol of manganese chloride per litre. The LDL-cholesterol concentration was calculated with the Friedewald formula.[24]

Measurement of arterial distensibility and compliance

The vessel wall motion of the right common carotid artery was measured by means of a Duplex scanner (ATL Ultramark IV, operating frequency 7.5 MHz) connected to a vessel wall movement detector system (WTS).[25] The details of this technique have been described elsewhere.[26-28] Briefly, this system enables the transcutaneous assessment of the displacement of the arterial walls during the cardiac cycle and, hence, the time-dependent changes in arterial diameter relative to its diastolic diameter at the start of the cardiac cycle. Subjects were instructed to refrain from smoking and from taking coffee, tea, alcohol or pain-medication on the day of measurement, and from taking alcohol on the day before. Subjects were placed in supine position, with the head tilted slightly to the contra-lateral side for the measurements in the carotid artery. A region at 1.5 cm proximal to the origin of the bulb of the carotid artery was identified using B-mode ultrasonography. Based on the B-mode recording an M-line perpendicular to the artery was selected, and the received radio frequency signals were recorded over five cardiac cycles and digitally stored. The displacement of the arterial walls was obtained by processing the radio frequency signals originating from two selected sample volumes positioned over the anterior and posterior walls. The successive values of the end-diastolic diameter (Dd), the absolute stroke change in diameter during systole (ΔD), and the relative stroke change in diameter ($(\Delta D)/Dd$) were computed from the recording during five cardiac cycles (Figure 1). Blood pressure was measured with a Dinamap automatic blood pressure recorder (Critikon, Inc, Tampa, Florida, USA), and



beat	dist (μm)	diam (mm)	dist (%)
0	234	7.25	3.23
1	239	7.24	3.30
2	235	7.25	3.24
3	243	7.24	3.36
mean	238	7.25	3.28
stdev	4	0.00	0.06

Figure 1
Example of distensibility measurement. The upper figure shows movements of the anterior (ant) and posterior wall (pos), the lower line the change in lumen diameter resulting from these movements (distensibility). Below the figure the calculated change in lumen diamater (dist in μ), end-diastolic lumen diameter (diam in mm) and relative distension (dist in %) are shown.

read 4 times at the right upper arm during the measurement session. The mean was taken as the subjects reading. Pulse pressure (PP) was defined as systolic blood pressure (SBP) minus diastolic blood pressure (DBP). The arterial wall distensibility coefficient (DC) and compliance coefficient (CC) were calculated according to the following equations[29]:

$$DC = (2\Delta D/D) / \Delta P$$
$$CC = (\pi D \times \Delta D) / 2\Delta P)$$

With this system a wall displacement of a few micrometers can be resolved[26], and D, ΔD, ΔD/D, DC and CC can be assessed reliably.[28] The arterial wall properties, as determined in this way, are defined as the relative and the absolute change in arterial

cross-sectional diameter (distensibility) and arterial volume compliance for a change in pressure. They reflect a combination of passive elastic properties and active components induced by smooth muscle cells. All measurements were done by a single performer, who was blinded for the treatment group. A reproducibility study was performed in which 15 participants underwent a second examination within one month from the initial examination of the right carotid artery. The coefficients of variation for distension and lumen diameter were 8.5% and 1.2%, respectively.

Data analysis

In total 121 perimenopausal women were randomised. Baseline distensibility measurements were not performed in 22 subjects for logistic reasons, who were distributed randomly over the treatment groups. The following results are based on the 99 subjects with baseline assessment. Forty-four subjects received 17βE_2-D, 29 CEE-N and 26 placebo (randomisation code 3:2:2). Data are presented by treatment group. The individual changes in common carotid artery DC and CC from baseline to cycle 6 and to cycle 24 were calculated. Analyses for the changes in the first 6 months were performed using those subjects of whom information on distensibility at baseline and at 6 cycles (n=61) were present. Analyses for changes over 24 months were performed using those subjects for whom data at baseline and at 24 cycles (n=48) were present. For this analysis, two dummy variables were constructed: the first with placebo (value 0), 17βE_2-D (value 0) and CEE-N (value 1) and the second with placebo (value 0), 17βE_2-D (value 1) and CEE-N (value 0). A linear regression analyses with change in DC and CC as dependent variables and the two dummy variables as independent variables were used to study whether the change in DC and CC from baseline in the treatment groups differed among treatment groups. Results are presented as mean differences in change in DC and CC relative to the placebo group with 95% confidence intervals. Analyses were performed with and without adjustment for values of baseline measurements, and analyses with adjustment for baseline are presented. In a similar way analyses were performed to study whether change in lumen diameter, systolic and diastolic blood pressure and pulse pressure from baseline differed across the treatment groups. All analyses were performed using SAS software (SAS Statistical Software, Inc).

The sample size calculations for the present trial showed that with 44 subjects in the 17βE_2-D group a standard deviation of the distensibility coefficient of 7.1, a two-sided α of 0.05 and a power of 0.80, a difference in change of distensibility coefficient of 4.9 x 10^{-3}/kPa between the treated and placebo group could be detected after 24 months. For the compliance coefficient, with a standard deviation of 0.37, a difference in change of 0.27 mm^2 / kPa between the treated and the placebo groups could be detected after 24 months.

Table 3
Change in blood pressure, distensibility and compliance after 6 months and 24 months in 3 treatment groups.

	6 months n = 61			24 months n = 48		
	difference	95% CI for the difference	p	difference	95% CI for the difference	p
17β-oestradiol + Desogestrel	(n = 24 versus placebo (n = 20)			n = 13 versus n = 18		
Systolic blood pressure (mmHg)	3.33	(-0.3 – 7.0)	0.43	1.13	(-5.5 – 7.7)	0.74
Diastolic blood pressure (mmHg)	-2.73	(-6.4 – 1.0)	0.31	-1.16	(-6.7 – 4.4)	0.68
Distensibility Coefficient (10^{-3} / kPa)	-0.08	(-3.0 – 2.8)	0.56	-1.37	(-4.4 – 1.7)	0.39
Compliance Coefficient (mm^2 / kPa)	-0.003	(-0.12 - 0.11)	0.97	0.26	(-0.01 – 0.53)	0.07
Conjugated equine estrogens + Norgestrel	(n = 17 versus placebo (n = 20)			n = 17 versus n = 18		
Systolic blood pressure (mmHg)	1.59	(-2.3 – 5.5)	0.08	-2.25	(-8.3 – 3.8)	0.48
Diastolic blood pressure (mmHg)	-2.15	(-6.2 – 1.9)	0.15	0.05	(-1.3 – 1.4)	0.98
Distensibility Coefficient (10^{-3} / kPa)	0.96	(-2.2 – 4.2)	0.96	0.41	(-1.0 – 1.9)	0.79
Compliance Coefficient (mm^2 / kPa)	-0.003	(-0.14 – 0.13)	0.96	0.11	(-0.14 – 0.37)	0.40

ADVERSE EVENTS

The total number of subjects that discontinued drug treatment after 6 months was 23; 11 (21%) in the 17βE_2-D group, 8 (23.5%) in the CEE-N group, 4 (11.4%) in the placebo group. After cycle 6, 5 women decided not to enter the second part of the study: 4 (10%) in the 17βE_2-D group, and 1 (4%) in the CEE-N group. Between cycle 6 and cycle 24, 20 (45%) women in the 17βE_2-D group and 19 (56%) in the CEE-N group completed the study. Of the women that had been randomised to the placebo group, 22 out of 35 who completed the 6 months were willing to visit the research centre for the cycle 24 visit. During the study, the occurrence of unacceptable adverse events was the main reason for withdrawal. The number of evaluable subjects at baseline, 6 and 24 months was 44, 24 and 13 for the 17βE_2-D group, 29, 17 and 17 for the CEE-N group and 26, 20 and 18 for the placebo group. Ninety-six women had at least one adverse experience in this period: 42 in the 17βE_2-D group (80.8%), 29 in the CEE-N group (85.5%) and 25 in the placebo group (71.4%). The type and severity of the adverse experiences recorded were consistent with those sometimes seen with this type of therapy, such as headache, depressive feelings, abdominal pain and nausea. During treatment, four of the adverse experiences recorded were classified as serious but probably not drug-related (one myocardial infarction in the placebo group, and cases of epileptic seizure, syncope and unspecified uterine disorder in the 17βE_2-D group).

DISCUSSION

In the present study in perimenopausal women, no significant differences in change in arterial distensibility of the common carotid artery could be demonstrated between women using 17βE_2-D or CEE-N and women using placebo after 6 and 24 months. Also changes in systolic and diastolic blood pressure and lumen diameter did not differ significantly between intervention and placebo groups. In our study no significant differences were found between the effects of 17βE_2-D and CEE-N.

Before interpreting these data, some issues need to be addressed. Participants were healthy subjects from the general population. Compliance to study medication, as assessed by tablet count and diary-checks, was satisfactory in all three groups with a mean compliance rate of more than 97%. The double-blind design with respect to 17βE_2-D and placebo intervention was difficult to maintain throughout the study, because the clear effects on menstrual cycle (participants were asked to complete a bleeding diary) and climacteric symptoms could not be ignored. However, the ultrasound technician was blinded for the intervention group. The percentage of women

who had withdrawn from the study or had missing data on distensibility (52 %) was comparable in the 17βE_2-D and the CEE-N groups. Theoretically, the drop-out rate may have influenced the effectiveness of the randomisation process, i.e. resulting in incomparability in prognosis of the three groups. However, women who completed the study had similar levels of baseline cardiovascular risk factors and distensibility and compliance compared to women who had withdrawn. A high drop-out rate does have an unfavourable effect on the precision of the estimates of change in distensibility and compliance, which decreases the ability to observe differences across groups. After 6 months of study the blind was broken and the trial continued for 18 months as an open trial. The fact that, in contrast to the placebo group, the treatment groups were subject to intensive follow-up from cycle 6 to 24, cannot explain our results, as it would, if anything, have resulted in a more health-conscious behaviour of the treatment group, and thus can be expected only to have increased a possible difference between the treated and placebo groups.

We measured distension in the carotid artery and adjusted for pulse pressure measured in the brachial artery. We assume that pulse pressure measured in the brachial artery is representative for pulse pressure in the carotid artery. In dogs, it has been demonstrated that pulse pressure in the brachial artery is linearly related to blood pressure in the carotid artery over a wide range of blood pressures.[27] It is known that the arterial pressure-waves undergo transformation in the arterial tree and therefore the pulse pressure is higher in the brachial artery than in the more central vessels.[30] With increasing age and arterial stiffness, however, this difference between central and peripheral pulse pressure decreases. If, in line with the decreasing difference seen with age and arterial stiffness, the overestimation of pulse pressure is less in untreated women, the DC would be overestimated in this group, and a true difference in distensibility between the groups would only be larger than the difference observed in our study. Although it has been demonstrated that DC and CC do not differ in the different phases of the menstrual cycle, in spite of distinct changes in endogenous oestrogen levels[31], all measurements were performed on a defined day in the second phase of the cycle, to exclude incomparability due to differences in treatment phase.

A body of evidence on favourable effects of oestrogen on the vascular system is available from animal and observational studies, as well as in a limited number of randomised studies. Our randomised study, however, did not show an effect of HRT on carotid artery distensibility and compliance in a group of 99 perimenopausal women. Favourable effects of oestrogen on vasodilatory responses have been shown in animal experiments, but these findings are based mainly on oestrogen administration and not on oestrogen-progestin combinations.[4-7,11] The effects of progestins on vascular properties are not well understood.[32,33] In a recent study Register et al. demonstrated that oestrogen alone, but not in combination with medroxyprogesterone acetate inhibit potentially detrimental aortic connective tissue alterations in ovariectomised monkeys

on lipid lowering therapy.[34] In a randomised cross-over trial Luckas et al. showed that the addition of progestagen to oestrogen replacement therapy partly antagonises the favourable effect of oestrogen on pulsatility index in the peripheral arteries in postmenopausal women. Other studies have found similar antagonistic effects of progestins in uterine arteries in oophorectomised ewes and women[35-37], and in the carotid arteries in women.[38] Possibly, addition of progestagens to oestrogen replacement therapy in our study might have attenuated oestrogen-mediated changes in vascular properties. Furthermore, the difference between our findings and the positive findings in observational studies could be explained by the better vascular condition of HRT users in these studies might be, in part, due to the selection of healthy women for use of HRT.[3,39] Another explanation for the apparent difference between our findings and that of other studies could be the different endpoints used. Studies have used pulsatility index (peripheral vascular flow), flow-mediated dilatation (endothelial function), systemic arterial compliance (compliance over the total arterial system), and augmentation index (reflecting both arterial stiffness and peripheral pressure wave reflections), which probably reflect essentially different arterial properties. The two studies examining local carotid artery distensibility as in our study, found no differences in DC between HRT-users and non-users[40] and no change of DC with the menstrual cycle.[31] It is not established whether changes in arterial distensibility are the result of short term-effects or of structural changes in the arterial wall. Short-term effects on arterial distensibility have been shown as a response to treatment with calcium antagonists.[41] Also structural changes may underly a decrease in arterial distensibility. In vitro investigations as well as animal studies showed that oestrogens decrease collagen production and decrease the elastin/collagen ratio.[8-10] Possibly, these structural changes need more time to develop than the two years of this intervention trial. In a recent study, a difference in arterial distensibility was shown between premenopausal women and postmenopausal women who were on average 5.4 years after menopause.[25]

The lack of change in blood pressure with HRT in our study is in agreement with a large body of literature showing that HRT is not associated with changes in blood pressure. The finding of an increased systolic blood pressure in the $17\beta E_2$-D group after 6 but not after 24 months, which was not statistically significant after adjustment for baseline values, could be a true transient effect of the treatment, specific for $17\beta E_2$-D, but considering the literature, probably should be seen as a chance finding.

In the present study all women had climacteric complaints and were predominantly perimenopausal. Although in women with symptoms oestradiol levels are decreased compared to premenopausal women[42], the endogenous oestradiol production in these women will probably influence the effects of HRT. Because symptomatic women are the target population for HRT, the vast majority of HRT in the Netherlands is prescribed for the indication menopausal complaints[43], it is of interest and of practical importance to know the effects of HRT on cardiovascular risk factors in this popu-

lation. However, it is not perimenopausal women, but postmenopausal women in whom prevention of coronary heart disease is of most importance. We have to be careful to extrapolate our findings from this relatively young population to the use in older, postmenopausal women. Possibly, when studying postmenopausal women, with low endogenous estrogen levels, the contrast between the placebo and the intervention groups would have been larger, and an effect might have been present. The well-known beneficial effects of HRT on total-cholesterol, LDL-cholesterol, HDL-cholesterol and the HDL-c/LDL-c ratio in our study (unpublished results) were all present and in the same direction as those in studies in postmenopausal women[44], although the magnitude of the effect might have been larger in the postmenopausal women.

Up to date, no information is available as to what increase in arterial distensibility or compliance is clinically relevant. Sample size calculations showed the trial could have detected changes of 4.9 10^{-3}/kPa in DC and of 0.27 mm^2/kPa in CC. We detected a mean change in DC of 0.4 10^{-3}/kPa in the CEE-N group relative to the mean change in the placebo group, and of 0.11 mm^2/kPa in CC. In the 17βE_2-D we detected a mean change of -1.4 10^{-3}/kPa in DC and 0.26 mm^2/kPa in CC. This suggests there are no effects of treatment on distensibility, but we cannot exclude the possibility that in a larger study or in a study of longer duration, the effects of 17βE_2-D would have reached statistical significance. This trial is the first randomised study of HRT on local carotid artery distensibility and compliance.

Whether loss of distensibility or compliance are early markers for asymptomatic atherosclerotic changes is still a matter of debate.[45-49] Decreased distensibility is unfavourably associated with age[50,51] and with several cardiovascular risk factors, like cholesterol[52] and hypertension.[53,54] Loss of distensibility in elastic arteries has been shown to be associated with an increased risk of cardiovascular disease in cross-sectional studies.[55,56] Longitudinal data on the effect of decreased distensibility on cardiovascular morbidity or mortality are, however, still awaited.

To conclude, in the present study in healthy perimenopausal women from a general population, no significant changes in arterial distensibility and compliance could be demonstrated for women using 17β-oestradiol plus desogestrel or conjugated equine oestrogens plus norgestrel during two years of observation.

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4.2 Hormone replacement therapy in perimenopausal women and two year change of carotid intima-media thickness

Abstract

Objectives – To assess the two-year effects of a combined regimen of oral 17ß-estradiol and desogestrel (17βE-D), and a sequential combination of conjugated equine estrogens and norgestrel (CEE-N) on common carotid intima-media thickness and end-diastolic lumen diameter in comparison to placebo in perimenopausal women.

Methods – The study was a single center, randomized, group-comparative, double-blind study with respect to the 17βE-D and placebo groups and open with respect to CEE-N. After cycle 6, the blind was broken and the trial was continued as an open trial for another 18 months for the active study arms. The study included 121 perimenopausal women recruited from the general population. Common carotid intima-media thickness and end-diastolic lumen diameter were measured at baseline and cycle 24 with B-mode ultrasonography.

Results – At cycle 24 small changes in intima-media thickness and lumen diameter were observed. Relative to placebo changes in intima-media thickness were -0.009 mm [95% CI -0.045; 0.027] for 17βE-D and -0.016 mm [95% CI -0.055; 0.024] for CEE-N. For end-diastolic lumen diameter the changes were -0.091 mm [95% CI -0.236; 0.055] and -0.125 mm [95% CI -0.820; 0.032] for 17βE-D and CEE-N, respectively.

Conclusions – In this study among perimenopausal women a significant effect of 17βE-D and CEE-N on common carotid intima-media thickness and lumen diameter could not be demonstrated. Although the sample size of the present trial is too limited to provide definite conclusions, the direction of the effect is in agreement with evidence from earlier studies on the effects of hormone replacement therapy in postmenopausal women.

INTRODUCTION

It is widely believed that ovarian estrogen production is responsible for the lesser degree of atherosclerosis and cardiovascular risk in premenopausal women compared to men and postmenopausal women. In a recent Dutch study, each year of delayed natural menopause represented a 2% decrease in the annual hazard of cardiovascular death.[1] If ovarian estrogen production is responsible for protecting the arteries in premenopausal women, then peri- and postmenopausal women could possibly benefit from estrogen replacement therapy. In support of this view, results from observational studies demonstrated that estrogen replacement therapy is associated with a 40-50% reduction in coronary heart disease risk in postmenopausal women.[2,3] Postulated mechanisms for a beneficial effect include favorable changes in cardiovascular risk factors[2,3,4], favorable effects on endothelial function[5,6], and favorable effects on atherosclerosis.[7,8,9]

In the Netherlands, hormone replacement therapy is most commonly used by women with climacteric complaints like hot flushes, vaginal dryness and disturbances in menstruation pattern. Women with climacteric complaints have lower endogenous estrogen levels compared to women without these complaints. In a Dutch study on determinants of first prescription of hormone replacement therapy perimenopausal women aged 45 to 50 were most likely to start using this therapy and they did not use it for a very long period of time.[10] In populations at large, atherosclerosis can be studied non-invasively by measurement of intima-media thickness of superficial arteries with B-mode ultrasound.[11,12,13,14] Increased carotid intima-media thickness has been associated with unfavorable levels of cardiovascular risk factors, prevalent cardiovascular disease, atherosclerosis elsewhere in the arterial system[11,12,13,15,16,17] and with risk of future myocardial infarction and stroke.[18,19] There is a growing belief that carotid intima-media thickness can be regarded as an indicator of generalized atherosclerosis, and may be used as an intermediate endpoint or proxy endpoint in observational studies and trials as a suitable alternative for cardiovascular morbidity and mortality.[20] Data on non-invasively assessed intima-media thickness and hormone replacement therapy are limited. Unopposed estrogen replacement therapy (without progestins) was in one large cross-sectional study in elderly postmenopausal women inversely related to carotid intima-media thickness[9], but this finding could not be confirmed in another large cross-sectional study among middle-aged postmenopausal women.[21] In a recent study among postmenopausal women indices of arterial function in long-term hormone replacement therapy users were compared with age matched controls.[22] Intima media thickness was significantly lower in the hormone replacement therapy users. In a lipid intervention study, estrogen replacement therapy was shown to be re-

lated with a reduction of progression of carotid intima-media thickness among postmenopausal women who did not use lipid lowering agents.[8] Data from intervention studies on the effect of hormone replacement therapy on progression of atherosclerosis are currently lacking, as well as data from studies in perimenopausal women.

The present study was primarily conducted to assess the effect of hormone replacement therapy in perimenopausal women on several cardiovascular risk indicators (Bak AAA, Witteman JCM, Planellas J, VanderBom JG, Coelingh Bennink HJT, Grobbee DE. Effects of hormone replacement therapy on fibrinolytic activity and coagulation inhibitors in perimenopausal women. 1998. Submitted), whereas structural and dynamic arterial characteristics of the carotid artery were also measured. Here we present the results of the effects of 17βE-D, a 24 day active tablet, 28-day sequential combined regimen of oral 17ß-estradiol and desogestrel (Org32818; Liseta^{R}) and CEE-N, a sequential combination of conjugated equine estrogens and norgestrel (Prempak-C^{R}), on two-year change in common carotid intima-media thickness and end-diastolic lumen-diameter in comparison to placebo in perimenopausal women.

MATERIALS AND METHODS

Population

The design of the study was randomized, group-comparative, double-blind the first six months with respect to 17βE-D and placebo groups and open with respect to CEE-N . The study was conducted in one center and included 121 perimenopausal women. Participants were recruited from the general population in the town of Zoetermeer (The Netherlands). They completed a questionnaire on menopause and gynecological issues that was sent to all women between 40 and 60 years of age. Women who were eligible based on the questionnaire and interested in participating in a hormone replacement study were invited for the screening procedure. Subject selection was based on the following criteria: age between 40 and 60 years; not hysterectomized; climacteric symptoms (hot flushes and/or outbreaks of sweating); and body weight between 80% and 130% of the ideal body weight (Metropolitan Life Insurance Company Tables for Women, 1983). The main exclusion criteria were: absence of spontaneous vaginal bleeding for more than 5 years; use of sex-steroids currently or within the last two months or ethinylestradiol or injectables within the last six months or hormone implants at any time previously; history or presence of any malignant disorder; history or presence of cardiovascular or cerebrovascular disease or thrombo-embolism / thrombosis; history or presence of hepatic or renal disease, uncontrolled hypertension (systolic blood pressure > 170 mmHg and/or diastolic blood pressure > 105 mmHg) and

significant hyperlipidaemia (fasting total cholesterol > 9.5 mmol/l and/or fasting triglycerides > 2.5 mmol/l). A cervical smear and a mammography were done to exclude any cervical or breast-pathology unless results were available dated less than one and two years previously, respectively.

The study was performed from October 1992 to July 1995. The study was approved by the Medical Ethics Committee of the Medical School, Erasmus University Rotterdam, and written consent was obtained from all participants.

Design

17βE-D and placebo tablets were supplied in identical looking push-through-strips. CEE-N was supplied in the original, commercially available, strips. The composition of the three study drugs was as follows. Each strip of 17βE-D contained 12 tablets with 1.5 mg 17β-estradiol (micronized), 12 tablets with 1.5 mg 17β-estradiol (micronized)+ 0.15 mg desogestrel, and 4 placebo tablets. Placebo was 17βE-D matched and contained 28 placebo tablets. Each strip of CEE-N contained 28 tablets with 0.625 mg conjugated estrogens and 12 tablets with 0.15 mg norgestrel. Subjects in the 17βE-D or 17βE-D - matched placebo groups took one tablet per day, on a continuous basis. Subjects treated with CEE-N took one tablet per day from day 1 to 16 and two tablets per day from day 17 to 28, for each cycle. Tablets were taken after breakfast. Sex steroids other than the study medication, hydantoins, barbiturates, primidone, carbamazepine, rifampicin, griseofulvin, and lipid lowering agents were not allowed during the study.

Randomization to treatment with 17βE-D, CEE-N, or placebo was performed in a ratio of 3:2:2 using a computerized allocation algorithm. The first part of the study comprised six consecutive cycles of 28 days, after which the blind was broken and the trial was continued as an open trial for another 18 months for the 17βE-D and CEE-N groups. All participants, including those in the placebo group, were invited for a final ultrasound examination at 24 months (cycle 24). Arterial characteristics were measured non-invasively at day of randomization and after 24 months (cycle 24).

At baseline, information was obtained about smoking. Body weight and body height were measured and body mass index was calculated. Blood pressure was measured twice, separated by a pulse count, at the right upper arm in sitting position using a Hawksley random-zero sphygmomanometer. Fasting blood samples were obtained between 8.00 and 10.00 a.m. Cholesterol and triglycerides were assayed enzymatically with a Hitachi 747 automated analyzer with kits from Boeringher Mannheim. HDL-cholesterol was measured after precipitation with phosphowolfram/phosphotungstic acid and 2 mmol of manganese chloride per liter. The LDL-cholesterol concentration was calculated with the Friedewald formula.[23]

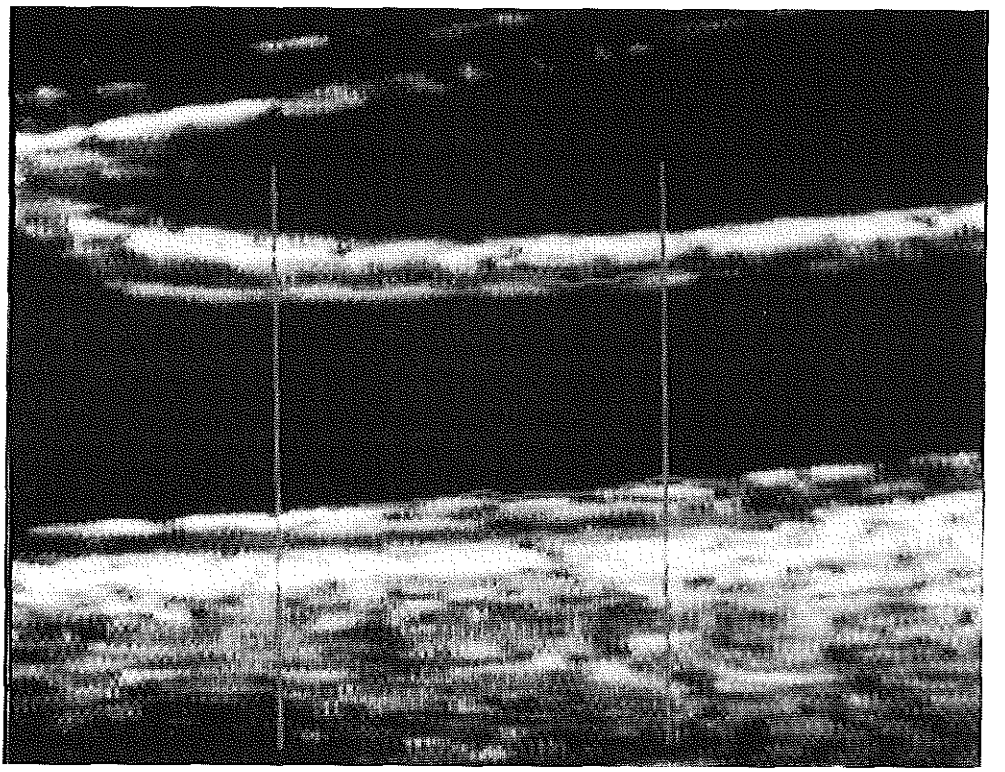

Figure 1
A characteristic longitudinal ultrasound image of the distal common carotid artery, on which the interfaces are marked over a length of 10 mm.

Measurement of arterial characteristics: Intima-media thickness

To measure carotid intima-media thickness, ultrasonography of the right common carotid artery and carotid bifurcation 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) wall of the carotid artery are displayed as two bright white lines separated by a hypo-echogenic space. The distance of 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 intima-media thickness. For the near wall, the distance between the trailing edge of the first bright line to the trailing edge of the second bright line at the near wall provides the best estimate of the near wall intima-media thickness. The distance between the lead-

ing edge of the second bright line (intima-lumen interface) on the near wall and the lumen-intima interface at the far wall reflects the lumen diameter (figure 1).

Following the ultrasound protocol[14,16,18], a careful search was performed for all interfaces of the near and far wall of the distal common carotid artery. When an optimal longitudinal image was obtained, it was frozen on the R wave of the electrocardiogram and stored on video tape. This procedure was repeated three times. The actual measurements of intima-media thickness were performed off-line. From the videotape, the frozen images were digitized and displayed on the screen of a personal computer using additional dedicated software. This procedure has been described in detail previously.[24] In short, with a cursor the interfaces of the distal common carotid artery 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 intima-media thickness and lumen diameter of each of the three frozen images was calculated. For each individual a common carotid intima-media thickness was determined as the average of near and far wall measurements of the right artery. The reader of the ultrasound images from videotape was unaware of the intervention status of the subject. Results from a reproducibility study of intima-media thickness measurements have been published elsewhere.[25]

Data analysis

Data are presented by treatment group. Analyses were performed using those subjects of which information on intima-media thickness at baseline and 24 cycles was present. In this analysis the individual change in common carotid intima-media thickness at cycle 24 from baseline was calculated. Two dummy variables were constructed: the first dummy with placebo (value 0), 17βE-D (value 1) and CEE-N (value 0); and the second dummy with placebo (value 0) 17βE-D (value 0) and CEE-N (value 1). A linear regression analysis with change in intima-media thickness as dependent variable and the two dummy variables as independent variable was used to study whether the change in intima-media thickness from baseline differed across the treatment groups. Results are presented as mean differences in change in intima-media thickness with corresponding 95% confidence interval. Analyses were performed with and without adjustment for values of baseline intima-media thickness. Since the findings in both analyses were similar, only results of the first approach is presented. In a similar way analyses were performed to study whether change in lumen diameter from baseline differed across the treatment groups.

The a priori sample size calculation for the present trial showed that with 52 subjects in the 17βE-D group, a standard deviation of measurement of progression of intima-media thickness of 0.07 mm, a two-sided alpha of 0.05 and a power of 80%, a

two-year difference in progression rate between 17βE-D and placebo of 0.047 mm could be detected.

RESULTS

One hundred and twenty one perimenopausal subjects were randomized. Fifty two subjects received 17βE-D, 34 CEE-N and 35 placebo. The general characteristics of the study groups are presented in table 1. Most cardiovascular risk factors were well balanced between the three groups and at baseline there were no major differences in common carotid intima media thickness and end-diastolic lumen diameter between the three study groups. Current smoking was more common in the CEE-N group compared to the 17βE-D and the placebo group (table 1). Mean time since last menstruation was 3 months (range 1 –11) in the perimenopausal women, and was similar in the three study groups. Thirteen of the 121 women were postmenopausal, defined as cessation of menses for more than one year.

The baseline characteristics of women who completed 24 cycles and of whom ultrasound data were available were compared to women who withdraw or had missing ultrasound data (table 2). No statistically significant differences in cardiovascular risk factors were found between these two groups of women.

Table 1
General characteristics of the study population.

	17βE-D (n=52)	CEE-N (n=34)	Placebo (n=35)
Age (years)	46.9 (4.0)	47.5 (3.9)	47.2 (4.1)
Body Mass Index (kg/m^2)	23.4 (2.8)	23.9 (2.9)	23.7 (2.9)
Systolic blood pressure (mmHg)	111.8 (12.4)	112.5 (14.8)	115.9 (15.6)
Diastolic blood pressure (mmHg)	73.3 (8.1)	73.4 (9.7)	74.6 (10.8)
Total Cholesterol (mmol/l)	5.7 (1.0)	5.8 (0.9)	5.9 (0.9)
LDL Cholesterol (mmol/l)	3.7 (0.9)	3.8 (0.8)	3.9 (0.8)
HDL Cholesterol (mmol/l)	1.5 (0.3)	1.4 (0.3)	1.5 (0.3)
Current Smoking	13.5%	32.4%	20.0%
Intima media thickness (mm)	0.62 (0.077)	0.62 (0.077)	0.62 (0.077)
Lumen diameter (mm)	5.62 (0.50)	5.65 (0.44)	5.78 (0.64)

Values are proportions or means with standard deviation in parentheses.

After 6 cycles, 5 women decided not to enter the second part of the study: 4 (10%) in the 17βE-D group and 1 (4%) in the CEE-N group. Between cycle 6 and cycle 24, 8 women (22%) in the 17βE-D group and 4 (16%) in the CEE-N group discontinued medication. Twenty-nine (56%) women in the 17βE-D group and 21 (62%) in the CEE-N group completed the study. Of the women that had been randomized to the placebo group, 22 out of 31 who completed the six months were willing to visit the research center for the cycle 24 visit. From the subjects who completed the study ultrasound data were available for 24 women in the 17βE-D group; 17 women in the CEE-N group and 21 subjects in the placebo group.

Change in intima-media thickness

The mean change of intima media thickness was an increase of 0.0088 mm (SD 0.058) in the placebo-group; a decrease of 0.0071 mm (SD 0.062) in the CEE-N group and a decrease of 0.0014 mm (SD 0.064) in the 17βE-D group. Results of comparison of the effects of 17βE-D, CEE-N and placebo on change in intima-media thickness are given in table 3. The results are adjusted for baseline value of intima-media thickness. Relative to placebo changes in intima-media thickness were –0.009 [95% CI –0.045; 0.027] for 17βE-D and -0.016 [95% CI –0.055; 0.024] for CEE-N .

Table 2
Baseline characteristics of women who completed the study and of whom ultrasound-data are available and those who had withdrawn or had missing ultrasound data.

	withdrawn or missing data			completed study and ultrasound measurements		
	17βE-D	CEE-N	Placebo	17βE-D	CEE-N	Placebo
Number	28	17	14	24	17	21
Age (years)	47.7	47.1	48.8	46.0	47.9	46.1
Body mass index (kg/m^2)	23.0	24.2	23.3	23.9	23.7	24.0
Systolic pressure (mmHg)	114	113	117	109	112	115
Diastolic pressure (mmHg)	75	75	75	71	72	74
Total cholesterol (mmol/l)	5.6	5.8	6.1	5.7	5.8	5.8
LDL (mmol/l)	3.64	3.71	4.05	3.66	3.85	3.83
HDL (mmol/l)	1.51	1.42	1.46	1.55	1.32	1.45
Current smoking	7%	35%	7%	21%	29%	29%
Intima media thickness (mm)	0.632	0.635	0.630	0.602	0.610	0.612
Lumen diameter (mm)	5.70	5.74	5.65	5.59	5.57	5.86

Table 3
Difference in change in intima-media thickness (IMT, in mm) and end diastolic lumen diameter (in mm) between active treatment groups and placebo at cycle 24, adjusted for baseline-value of intima media thickness or lumen diameter, respectively.

	17βE-D vs placebo	CEE-N vs placebo	17βE-D + CEE-N vs placebo
Change in IMT	-0.009 (-0.045; 0.027)	-0.016 (-0.055; 0.024)	-0.012 (-0.044; 0.020)
Change in lumen diameter	-0.091 (-0.236; 0.055)	-0.125 (-0.282; 0.032)	-0.105 (-0.235; 0.025)

Values are linear regression coefficients with 95% confidence intervals in parentheses.

Change in end-diastolic lumen diameter

The mean change of end-diastolic lumen diameter was an increase of 0.0897 mm (SD 0.229) in the placebo-group; a decrease of 0.0063 mm (SD 0.261) in the CEE-N group and an increase of 0.0259 mm (SD 0.243) in the 17βE-D group. Results of the comparison of the effects of 17βE-D, CEE-N and placebo on change in end-diastolic lumen diameter are given in table 3. The results are adjusted for baseline value of lumen diameter. The changes were -0.091mm [95% CI –0.236; 0.055] in the 17βE-D group and –0.125 mm [95%CI –0.282; 0.032] in the CEE-N group compared to placebo.

Adverse events

During the first 6 cycles the number of subjects that discontinued drug treatment was 23; 11 (21.2%) in the 17βE-D group, 8 (23.5%) in the CEE-N group and 4 (11.4%) in the placebo group. Ninety-six women had at least one adverse experience during this period: 42 subjects in the 17βE-D group (80.8%), 29 in the CEE-N group (85.3%) and 25 in the placebo group (71.4%).

During the study, the occurrence of unacceptable adverse experiences was the main reason for withdrawal from the study. The type and severity of the adverse experiences observed were consistent with those sometimes seen with this type of therapy, such as headache, depressive feelings, abdominal pain and nausea.

DISCUSSION

In the present study among perimenopausal women a significant effect on change in intima-media thickness and end-diastolic lumen diameter of 17βE-D and CEE-N compared to placebo could not be demonstrated. Although the estimates of the effects were in a favorable direction, i.e. reduced progression of intima-media thickness and smaller end-diastolic lumen diameter, the 95% confidence limits were wide.

Before interpreting the data, some issues need to be addressed. The percentage of women that withdrew at cycle 6 (19%) is not unusual for this type of study. However, at the end of the study it was possible to evaluate only 52% of the randomized women, because of subsequent withdrawal in the hormone-treated groups, loss to follow up in the placebo-group and missing ultrasound data in all three groups. Compliance, as assessed by tablet count and diary checks, was satisfactory in all three groups with a mean compliance rate of more than 97% in the first 6 cycles. The dropout rate may have affected the randomization process, i.e., resulting in incomparability in prognosis of the three groups. Women who completed the study had on average lower, although not statistical significant, levels of baseline cardiovascular risk factors and intima-media thickness than women who had withdrawn. This may have resulted in a lower progression rate of intima-media thickness in all three treatment groups. Also, the mean levels of some cardiovascular risk factors in women who had withdrawn in the placebo group were slightly higher than those in women who had withdrawn in one of the two active treatment groups. Furthermore, a high withdrawal rate has an unfavorable effect on the precision of the estimates on change in intima-media thickness and lumen diameter and consequently on the ability to observe differences across groups. Secondly, in the study the blind was broken after 6 cycles and the trial continued for 18 cycles as an open trial. In contrast to the treatment group, the placebo-group was not subjected to follow-up between cycle 6 and cycle 24. Therefore possible differences in healthy behavior could lead to an overestimation of the effects of hormone replacement therapy on intima media thickness.

Observational studies have demonstrated that estrogen replacement therapy is associated with a 40-50% reduction in cardiovascular disease risk in postmenopausal women.[2,3] Postulated mechanisms for a beneficial effect include favorable changes in LDL and HDL levels, in carbohydrate metabolism[27], in endothelial function[5,6] and in haemostatic parameters.[28] Unopposed estrogen is not suitable for long-term use due to the higher incidence of endometrial hyperplasia and endometrial cancer.[29] Therefore, treatment with combined estrogen and progestin is usually given. It has been suggested that the beneficial effect of estrogen is reduced by the addition of progestins. Recently the results were presented of the first trial on the effects of combined hor-

mone replacement therapy on cardiovascular endpoints (HERS).[30] After four years of treatment the relative risk of cardiovascular disease in hormone replacement therapy users was one compared to non users; although there was a trend in the last two years of the study of a decreasing risk in the active treatment group. This trial was conducted in postmenopausal women with coronary heart disease and therefore the results could not automatically be generalized to healthy women.

Several observational studies examined the relation between hormone replacement therapy and intima media thickness. In a cross-sectional analysis the vessel wall characteristics of elderly women using estrogen and progestin were similar to those of women using estrogens alone. Both groups had a reduced intima-media thickness of the internal and common carotid artery compared to never users.[9] Results from the Asymptomatic Carotid Atherosclerosis Progression Study (ACAPS) among 186 postmenopausal women suggested that hormone replacement therapy might decrease progression of atherosclerosis, as measured by carotid intima-media thickness.[8] The investigators showed a reduction in the annual progression of carotid intima-media thickness among hormone replacement therapy users compared to non-users. Until now, the studies evaluating the effects of hormone replacement therapy on intima media thickness all have an observational design in which the effect of "the healthy estrogen user" may have led to biased estimates. Most of these studies focus on postmenopausal women using hormone replacement therapy. In the Netherlands most hormone replacement therapy users are perimenopausal. Therefore it is of interest and of practical importance to evaluate the effects of hormone replacement therapy on intima media thickness in perimenopausal women in a controlled randomized trial. Although not statistically significant, the estimates of the hormonal effect in the present trial are in agreement with the findings from most observational studies in postmenopausal women, despite of possible variable endogenous estrogen levels in perimenopausal women.

Our finding that treatment may have a favorable effect on end-diastolic lumen diameter, i.e., a diameter reduction, is intriguing. In the present study, B-mode images are frozen on the R-wave of the ECG, thereby generating images of the carotid artery at end-diastole. As such, the size of the lumen diameter may be regarded as an indicator of arterial stiffness: the stiffer the artery, the larger the lumen diameter at end-diastole. In an earlier study we showed that a larger end-diastolic lumen diameter was associated with presence of isolated systolic hypertension[17], a condition predominantly characterized by stiffness of the large arteries. The results of the present study are in agreement with the studies suggesting a beneficial role of combined hormone replacement therapy on arterial stiffness.[32,33]

In conclusion, in the present experimental study among perimenopausal women a significant effect of 17βE-D and CEE-N on common carotid intima-media thickness and lumen diameter could not be demonstrated. Although the sample size of the pres-

ent trial is too limited to provide definite conclusions, the direction of the observed effects is in agreement with evidence from earlier studies on the effects of hormone replacement therapy in postmenopausal women. Therefore perimenopausal women possibly benefit from the effects of hormone replacement therapy on the intima media thickness in the carotid artery which could lead to a decrease in their cardiovascular disease risk.

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5

General discussion

This thesis describes the effects of two specific determinants of cardiovascular disease in women in the general population. On the one hand it describes the associations of menopause and the decrease in estrogen levels that accompanies it, with cardiovascular disease. On the other hand it examines the effects of replacement of estrogen. In this chapter the main findings of our and other studies will be summarised. The shortcomings and merits of the individual studies presented in this thesis have been discussed in the previous chapters, but several specific methodological aspects of studies on this topic will be discussed. Possible clinical implications will be discussed. Finally, recommendations for future research will be made.

BACKGROUND

Cardiovascular diseases are the major cause of death in men and women alike. Based solely on the demographic evolution, it is predicted that the proportion of elderly people in our country as well as in most Western countries, will increase in the coming years. As the ageing of the population is more pronounced in women than in men, the absolute number of deaths from cardiovascular disease in women is bound to increase. Cardiovascular disease is generally considered a disease of men. One reason for this is the low incidence of the disease in women at young age. At older age, however, cardiovascular disease also becomes the most important cause of mortality for women. In the 1950's it was suggested that estrogen is an important mediator of a woman's risk of cardiovascular disease. It was noticed that coronary heart disease is rare in young women and increases only after middle age, and that this might be ascribed to the decreasing estrogen levels after menopause. An increased risk of coronary heart disease was found in young women who had both ovaries removed. Although the assumption that the lower incidence of cardiovascular disease among older women is due to menopause is long and widely held, it is still debated.[1-3] Data on the association between menopause and cardiovascular disease are conflicting. Several studies have shown an inverse association between age at natural menopause and risk of cardiovascular disease [4-10], but others did not.[11-15] This inconsistency might be the result of a methodological problem; a lag time of at least 10 years between menopause and the occurrence of coronary heart disease in women makes the effect of menopause difficult to disentangle from that of age.

Hormone replacement therapy might offer possibilities for prevention. On the other hand, it may increase the risk of endometrial hyperplasia and cancer[16,17], breast cancer[18], venous thromboembolic events[19,20] and gallbladder disease.[16,20] As coronary heart disease is the most common and most deadly disease of women, any significant

reduction in coronary heart disease risk due to hormone replacement therapy would strongly affect the benefit-risk scale of this therapy.

The first randomised trial of estrogen and heart disease was performed in the 1960s, in men. Men with heart disease were prescribed 2.5 mg or 5 mg of conjugated estrogen daily, which is much higher than the dose commonly used in women today. The estrogen arm of the trial was stopped early as treated men were observed to have an increased rate of thromboembolic events, myocardial infarction and cancer (as well as gynecomastia and impotence). It is now thought that the high doses of estrogen may have been responsible for this effect, or that men and women might respond differently to estrogen therapy. Observational evidence from the last 20 years suggests that hormone replacement therapy reduces morbidity and mortality from cardiovascular disease in healthy postmenopausal women. It has been demonstrated that estrogen replacement therapy raises HDL and lowers LDL cholesterol levels in men and women, and that estrogen therapy decreases diet-induced atherosclerosis in primates. Although, the mechanisms underlying the protective effect of estrogen are not fully known, these findings suggested that the correction of the low levels of estrogen after menopause might prevent disease. Only recently the next randomised trial with clinical outcomes was conducted, this time in women (the Heart and Estrogen/progestin Replacement Study, HERS[20]).

Insight in the associations between estrogens and cardiovascular disease in women might shed light on the aetiology of cardiovascular disease, and on possibilities for prevention and treatment.

MENOPAUSE

Main Findings

Menopause and risk factors for cardiovascular disease

The mechanisms through which menopause might exert its effect on the cardiovascular system remain largely unknown. Increased levels of serum total cholesterol after cessation of menses have been found in most studies on menopause and risk factors.[21-33] Inconsistent results, however, have been reported with respect to HDL-cholesterol [25-27,29-31,34], apolipoproteins [24,25,29,33-35], blood pressure [21-23,25,26,34,36-39], waist-to-hip ratio [40,41] and insulin. [42-44] When comparing cardiovascular risk factors between pre- and postmenopausal women (see chapter 2.1) we found that total cholesterol, LDL cholesterol and apolipoprotein B are the primary cardiovascular risk factors affected by menopause. No differences between pre- and postmenopausal women were found in

high density lipoprotein (HDL) cholesterol, triglycerides, apolipoprotein A1, blood glucose, insulin, body mass index, waist-to-hip ratio, and systolic and diastolic blood pressure. Because increased cholesterol levels were established soon after cessation of menses, preventive measures aimed at reduction of cholesterol in women might best be initiated in early menopause. Many other risk factors are thought to relate to menopausal status. Haemostatic factors, homocysteine metabolism, endothelial function, glucose metabolism and inflammatory markers all seem to play a role in atherosclerosis and cardiovascular disease. Data on relations to menopause are sparse, however, and their specific role in atherogenesis after menopause needs to be investigated.

Besides effects on risk factors, recent studies have increasingly emphasised the direct beneficial effects of estrogens on the arterial wall. Although several studies have shown that estrogen replacement has beneficial effects on arterial characteristics, the effect of endogenous estrogen on the vascular system is still unknown. Few studies have addressed the effects of endogenous estrogens and natural menopause on the dynamic characteristics of the arterial system. Although no changes in distensibility were found during the menstrual cycle[45], going through menopause has been shown to negatively affect the elastic properties of the aortic root in hypertensive women.[46] We studied the relation between natural menopause and arterial distensibility in the common carotid artery, comparing pre- and postmenopausal women. The results of our study suggest that the distensibility of the common carotid artery is negatively affected by natural menopause in healthy women (chapter 2.2). Whether loss of distensibility is an early marker for asymptomatic atherosclerotic changes or whether it reflects other structural changes of the arterial wall is still a matter of debate.[47-50] Loss of distensibility in elastic arteries has been shown to be associated with an increased risk of cardiovascular disease in cross-sectional studies.[51,52] If abnormal arterial characteristics occur before clinical manifestation of disease are apparent, this could be important for identifying patients at risk. Longitudinal data on the effect of decreased distensibility on cardiovascular morbidity or mortality are, however, still awaited.

Menopause and atherosclerosis

A change in incidence in coronary heart disease will not be seen immediately after menopause but probably only 10 to 20 years later, by which time the effects of menopause are difficult to distinguish from the effects of ageing. As one of the main mechanisms through which menopause is thought to exert its effect on the cardiovascular system is the inhibition of the atherosclerotic process, and atherosclerosis is present long before symptomatic coronary heart disease develops, studying the association with atherosclerosis might be a sensible approach to study the role of menopause. In chapter 2.3 we studied the effect of age at menopause on development of non-invasively measured atherosclerosis. Our study population consisted of women between 55

and 106 years old, which allowed assessment of the associations in women long after menopause. Age at natural menopause was significantly and inversely related to the risk of atherosclerosis in different vessel beds and with myocardial infarction. Analyses were repeated after stratification for smoking habits. Although risk of early natural menopause with aortic atherosclerosis disappeared in women who had never smoked, and risk of peripheral arterial disease decreased, loosing statistical significance, the associations of early artificial menopause with these two measures of atherosclerosis remained. Also the association between early natural menopause and the other measures of atherosclerosis remained among never smokers. These results suggest an association is present between a decrease in estrogen levels and atherosclerosis, independent of smoking. Early bilateral oophorectomy, but not hysterectomy alone or hysterectomy with unilateral oophorectomy, was associated with an increased risk of atherosclerosis at different vessel beds and with myocardial infarction. Several studies have found the risk of cardiovascular disease to be increased in women after hysterectomy independently of oophorectomy. It has been hypothesised that hysterectomy increases risk of cardiovascular disease by compromising blood flow to the ovaries affecting their function, or through a hormonal or other secretory function of the uterus.[53-58] The results from our study, where self-reported gynecological operations were verified with patient records, thus minimising errors in classification, imply ovarian function is the key factor. The results support the hypothesis that endogenous estrogens protect women from developing atherosclerosis, and that increased atherogenesis might be one mechanism mediating the observed epidemiological link between menopause and cardiovascular disease. The results further suggest that the moment of cessation of ovarian estrogen production may have consequences for the cardiovascular system well into high age. The extent to which hormone replacement therapy in the early postmenopausal phase may postpone or delay the development of atherosclerosis needs to be determined.

Menopause and cardiovascular disease

Mean age at menopause seems to have remained constant over the last 100 years. If no cohort effect is present for age at menopause, we should be able to use vital statistics to see whether an increase in coronary heart disease incidence exists after menopause. A semi-logarithmic plot of coronary heart disease mortality rates by age does not show an increased acceleration in women after the age of 50, that is, there is no inflection at the age of menopause (figure 1). This in contrast to the pattern in breast cancer, which is a strongly estrogen dependent disease, and which clearly decreases after menopause. This argument is often used to argue that menopause does not affect the risk of coronary heart disease, and that the increase in risk of coronary heart disease in

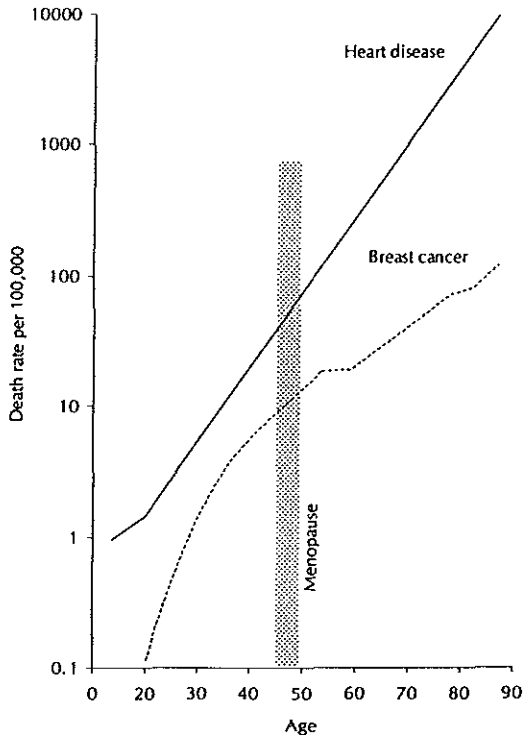

Figure 1
Semi-logarithmic plot of age-specific death rates versus age, in women in 1962, USA (after R.E. Tracy, J Chron Dis 1966)

women after middle age is merely an effect of ageing. But perhaps, this approach is not justified for examining the effect of menopause.

Firstly, an increased acceleration of coronary heart disease mortality rates at or after the age of menopause implies that the relative risk associated with menopause continuously increases with age, which is not seen for any other coronary heart disease risk factor, including hypertension and smoking. Secondly, since the acceleration of coronary heart disease mortality rates declines with age in men, it is not likely to find an increased acceleration in women, even when menopause elevates the risk. To illustrate the effect of menopause under more realistic assumptions, we conducted a simulation study. We used mortality rates of UK men [2], the Framingham risk function [59] and estimates of relative risks at young ages from the MRFIT Study [60], to construct a graph of coronary heart disease mortality rates in men in the first decile of the Framingham risk score to represent women premenopausal until the end of life. The curve was gently smoothed (figure 2). Based on estimates of the effect of age at menopause[9], we assumed menopause to enhance coronary heart disease mortality with 20, 60 and 100% at ages 50-54, 55-59 and 60-69 years, respectively, taking into account variation in menopausal age and a lag-time. We assumed the menopause-

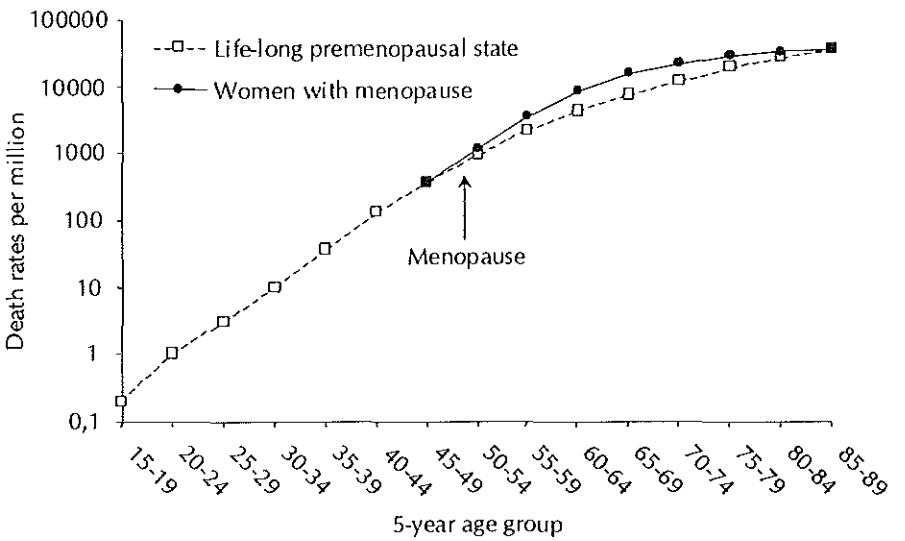

Figure 2
Coronary heart disease mortality rates by age on a logarithmic scale.
The lower curve gives mortality rates in women staying premenopausal until the end of life. The upper curve gives mortality rates when an effect of menopause is imposed on the curve reflecting the life-long premenopausal state.

related risk to decrease gradually thereafter until no excess risk at age 85-89. The upper curve in the figure represents the curve when an effect of menopause is imposed upon the curve reflecting the lifelong premenopausal state. The thus constructed curve does not show an inflection around the age of menopause and is comparable in shape to that of UK women.[2] Although we will probably never know the true effect of menopause, and cannot be certain that our assumptions are correct, our data suggest that the observed coronary heart disease mortality pattern in women in the population is not incompatible with an effect of menopause.

Methodological considerations for studies on menopause

Design

In a cross-sectional study, the exposure and outcome variables are measured at the same point in time. This generally complicates interpretation of study results in terms of a cause-effect relation. Our study in chapter 2.3, however, although exposure (menopause) and outcome (atherosclerosis and coronary heart disease) were measured at the same moment, is conceptually a follow-up study. Since we studied only postmenopausal women, it is clear that menopause preceded the measurement of ath-

erosclerosis. A history of coronary heart disease was verified using medical record from general practitioners and ECG diagnoses, and it was shown that 97% of detected infarctions had occurred after menopause.[61] Although we cannot know when silent myocardial infarctions have occurred, only a very small percentage is thus expected to have occurred before menopause. Whereas a very long follow-up time would have been necessary to asses effects of menopause in a prospective follow-up study, this design, in which menopause was assessed in retrospect and data were verified with the general practitioners records, enabled us to study women long after menopause. A selective survival which might have occurred if women with early menopause would have died more often of atherosclerotic complications before the start of our study, will, if anything, have given an underestimation of the effect.

In studies on effects of menopause on cardiovascular disease several other methods can be used. One method is to follow women from premenopausal state, through menopause, into the postmenopausal phase. In this design it is necessary to have a control group of premenopausal women of the same age, that does not become postmenopausal during the study period. Such studies in a restricted age range around the menopause will include mostly perimenopausal women, women with irregular menses and recent postmenopausal women, which reduces the contrast in estrogen status. With this design either a long follow-up time or a large group of women is necessary. Another method could be to compare pre and postmenopausal women. In this design inevitably an age-difference between the groups will endanger comparability. Studies that include women in a broad age range may not be able to validly remove the confounding effect of age. In our study, we examined the effects of natural menopause in a selected population in which the contrast in estrogen status between pre- and postmenopausal women of the same age was maximised, which enabled us to efficiently study differences associated with menopausal status (chapter 2.1 and 2.2).

Exposure assessment – Menopause

The onset of menopause is defined as the age at which a woman's last menstruation takes place. In fact, however, menopause is not an acute event, but a process of about 10 years in which the ovaries slowly decrease their function (a process called "shutting down"). The exact moment when menstruation ceases is the moment when estrogen levels decrease below the level necessary to stimulate the endometrium, and in epidemiological studies it is this moment which is used as a proxy for onset of menopause. A difficulty in the assessment of age at menopause is that a menstruation can be taken as the last menstruation only in retrospect. Recalling this age can thus pose a problem. However, early or late menopause, being special events, are recalled better than 'average' menopause[62] which reduces the risk of misclassification of extremes.

Misclassification of exposure can obscure a true association. Non-differential error can lead to a false negative finding (a type II error). Differential error may arise when women with more atherosclerosis would have reported bilateral oophorectomy more often than women with less atherosclerosis, which we do not assume to be likely. If this type of misclassification has occurred, this would have given an underestimation of the true effect. In our study (chapter 2.3), self-reported gynecological operations were verified with patient records, in order to minimise errors in classification.

Outcome assessment – subclinical atherosclerosis

Traditionally, epidemiologists have studied cardiovascular disease by examination of the relation between potential risk factors and the presence or occurrence of cardiovascular events. Yet, the use of the discrete event as a study outcome has two disadvantages. The first is that cardiovascular events result from a combination of processes, which makes understanding of the aetiology difficult. The second is that the events reflect a near end-stage of the disease, which prohibits the study of risk factor effects at earlier stages of the disease. The study of determinants of underlying processes will enhance understanding of the aetiology. As atherosclerosis is present long before symptomatic coronary heart disease develops, it is an interesting approach to study the role of menopause by studying its association with atherosclerosis.

To study atherosclerosis non-invasively in asymptomatic, non-hospitalised subjects it is necessary to rely on vessel beds other than the coronary and cerebral arteries. Atherosclerosis in these vessel beds needs to represent a generalised process. In this thesis aortic calcification, carotid atherosclerosis and atherosclerosis in the arteries of the lower extremities were used as measures of atherosclerosis, and assumed to be a proxy for generalised atherosclerosis. Radiographically assessed aortic atherosclerosis, ultrasonographically assessed carotid plaques and the ankle-arm systolic blood pressure index have all been shown to be accurate non-invasive methods of measuring atherosclerosis. A series of studies have demonstrated their associations with cardiovascular risk factors, atherosclerosis at other sites of the vessel bed, and cardiovascular morbidity and mortality.[63-72]

Outcome assessment – Mechanical arterial properties

Pathofysiologic changes in the blood vessels are associated with a wide variety of cardiovascular events, but our ability to assess vascular function is limited. Imaging techniques provide information regarding intimal pathology, but they provide little information about the functional properties of the arterial wall. Recently several new methods have been developed to non-invasively assess mechanical properties of arteries in humans. Various parameters are in use to characterise these properties. Parameters commonly used are distensibility and compliance, defined as the relative (Δ-diameter /

end-diastolic diameter) and absolute (ΔV) change in arterial volume for a change in pressure (ΔP). Although with the use of these parameters valuable information can be obtained about changes in artery wall properties, it should be realised that the methods presently in use still have their limitations. For calculation of arterial distensibility, not only local distension of the artery, but also pulse pressure at the site of measurement is needed. This is approximated by pulse pressure measured in the brachial artery, as discussed in chapter 2.2.

Confounding and mediating factors

It has been suggested that early menopause can be seen as a marker for premature ageing. If this were the case, ovarian failure and the development of cardiovascular disease could both be the result of the premature ageing process in these women, and not necessarily cause and effect. We do not believe this is the case as the results of our studies in chapter 2.1 show that lipid levels differ between pre- and postmenopausal women, but do not increase with time since menopause (figure 2, chapter 2.1, page 14). The hypothesis would also involve that other risk factors known to increase with ageing, like blood pressure and glucose levels, should have differed between pre- and postmenopausal women, which was not observed in our study.

Theoretically, other factors like socio-economic status, parity or genetic factors related to early menopause can confound or modify the studied associations. Although some of the mentioned confounders are known to increase lipid levels, the effects on cardiovascular disease of these factors reported in the literature do not seem to be large enough to explain the difference found in our study. Knowledge on genetic factors in coronary heart disease is limited. Evidence that genetic factors are related to age at menopause has been observed previously in family and twin studies.[73-75], but the genes involved remain ill defined. Until now, only a limited number of genes have been studied in the association with the onset of menopause.[76,77] In a recent study, it was shown that a common allelic variation in the estrogen-receptor gene is associated with age at menopause as well as hysterectomy.[78] This receptor also mediates the activation of the LDL-receptor in the liver. Possibly, the increase of cholesterol with menopause is modified by polymorphisms in the estrogen receptor, or by other polymorphisms like the apolipoprotein-E gene.

Cigarette smoking is likely to be a serious confounder. Smoking is strongly related to early menopause and increases the risk of atherosclerosis. As some misclassification in smoking habits is inevitable when using interview data, adjustment for smoking might not suffice and residual confounding can only be excluded by examining associations in never smokers only. In a recently published report form the Nurses' Health Study, the association between younger age at menopause and higher risk of coronary heart disease was found to be present among current and past smokers, but not among

never smokers, which suggests residual confounding by smoking.[79] However, this observation was based on only a small number of cases. In our study in chapter 2.3 the risk of aortic atherosclerosis with early natural menopause virtually disappeared among never smokers only, and the association for atherosclerosis in the peripheral arteries decreased, loosing statistical significance. This could indeed indicate residual confounding by smoking, but as smoking has been found to reduce endogenous estrogen levels, one cannot exclude the possibility of effect modification. We found the associations of early natural menopause with carotid atherosclerosis and with two compound scores of atherosclerosis remained virtually unchanged among never smokers. Furthermore, point estimates for the associations between artificial menopause and all measures of atherosclerosis remained unchanged in never smokers. Thus, our results do suggest the presence of an association between age at menopause and the risk of atherosclerosis, independent of smoking.

Many studies did not examine the associations by smoking status. Stratified analysis in these and future studies would increase their validity.

HORMONE REPLACEMENT THERAPY

Main Findings

Hormone replacement therapy and atherosclerosis

Observational evidence suggests that hormone replacement therapy reduces morbidity and mortality from cardiovascular disease in healthy postmenopausal women.[16,80,81] The mechanisms underlying this protection are not entirely clear, but may include inhibition of the atherosclerotic process. We investigated the association between ever use of hormone replacement therapy and the presence of atherosclerosis in the arteries of the lower extremities and in the carotid arteries in women from the Rotterdam Study. Although randomised trials are indispensable to determine to what extent the findings from observational studies are biased by confounding, our observational study in women aged 55 to 80, showed that the level of atherosclerosis was lower in past users compared to never users. This suggests that the advantage of women who have used hormone replacement therapy remains present after discontinuation of therapy.

Observational studies have found hormone replacement therapy reduces cardiovascular morbidity and mortality by as much as 40 %. Many studies have tried reducing selection bias by adjusting for confounding variables in the analyses. Many large studies, however, did not have information on important factors, like smoking, socio-economic status or use of other medication. Other studies assumed to have reduced selec-

tion bias by restricting the study population, for example to a single socio-economic class (the Nurses Health Study). Still, in most studies selection bias can not be excluded. Only one large randomised trial has been conducted on the effect of hormone replacement therapy on cardiovascular disease in women (HERS). This was a secondary prevention trial among 2763 postmenopausal women with known heart disease (and an intact uterus) who were randomly assigned to daily conjugated equine estrogen plus medroxyprogesteron acetate therapy or placebo. The trial showed no favourable effect of hormone replacement therapy on the prevention of further cardiovascular disease after 4.1 years of follow-up, and an increased risk of gall-bladder disease and venous thromboembolic disease.[20] These results might indicate that the bias in observational studies is larger than thought until now. On the other hand, in the trial an increased risk for coronary heart disease events was found in the first year of the trial, while risk decreased in subsequent years. This time-trend should be interpreted with caution, but can be explained as being attributable to an immediate prothrombotic, proarrythmic or proischemic effect of treatment that is gradually outweighed by a beneficial effect on the underlying progression of atherosclerosis. Thus, the results of the HERS-trial support the hypothesis that hormone replacement therapy can affect the development of atherosclerosis.

In the ROMEO trial (chapter 4.2) among perimenopausal women a significant effect on change in intima-media thickness and end-diastolic lumen diameter of 17β-estradiol plus desogestrel and conjugated equine estrogens plus norgestrel compared to placebo could not be demonstrated. Although the direction of the observed effects was favourable, i.e. reduced progression of intima-media thickness and smaller end-diastolic lumen diameter, the sample size of this trial was insufficient to provide definite conclusions. While it was expected that the measured anti-atherogenic effects of hormone replacement therapy would be smaller in perimenopausal women than in postmenopausal women, as endogenous estrogen production will reduce the contrast in estrogen levels between the treatment and the placebo group, the majority of hormone replacement therapy in The Netherlands is prescribed to perimenopausal women, and it is of importance to assess the effect of hormone replacement therapy in this group of women. Larger randomised trials, with measures of atherosclerosis as outcome measure are needed to provide definitive evidence.

Hormone replacement therapy and mechanical arterial properties

A difficulty in comparing our results with respect to mechanical arterial properties with the results from other studies in the relatively new field of non-invasively measured mechanical arterial properties is the variety of different endpoints that is being used to describe arterial properties. Studies have shown favourable effects of estrogen therapy on pulsatility index (peripheral vascular flow), flow-mediated dilatation (endothelial

function), systemic arterial compliance (compliance over the total arterial system), and on augmentation index (reflecting both arterial stiffness and peripheral pressure wave reflections), which probably all reflect essentially different arterial properties. Two studies examined the associations between estrogens and arterial distensibility by the same method as we used. One cross-sectional observational study did not find a difference in arterial distensibility between users and non-users of combined hormone replacement therapy, but a higher distensibility in users of estrogen replacement only[82] Another study showed no changes in arterial distensibility over the menstrual cycle. Our study is the first randomised trial of hormone replacement therapy on local distensibility and compliance of the carotid artery. No significant changes in arterial distensibility and compliance could be demonstrated for women using 17β-estradiol plus desogestrel or conjugated equine estrogens plus norgestrel during two years of observation. This suggests there are no effects of treatment on distensibility, but as compliance was lower in the treated group than in the placebo group, we cannot exclude the possibility that in a larger study or in a study of longer duration, the effects would have reached statistical significance. To date, no information is available as to what increase in arterial distensibility or compliance is clinically relevant, by what mechanisms estrogen can affect distensibility, and how long the exposure to estrogen is needed to achieve an effect. Longitudinal studies of the consequences of decreased arterial distensibility on coronary heart disease are awaited, and special attention is indicated to examine differences between men and women.

Methodological considerations for studies on hormone replacement therapy

Exposure assessment

In our study use of hormone replacement therapy was assessed by interview. This might have led, to a certain extent, to misclassification. Greendale et al. demonstrated that a single self-report question is adequate to ascertain ever-use of postmenopausal estrogen use in women up to 64 years of age.[83] 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 up to 74 years of age.[84] In the Rotterdam Study, we compared reported use of hormone replacement therapy with the general practitioners records in a random sample of women. These records did not provide a gold standard to which reported hormone use could be verified. When prescriptions for hormone replacement therapy are found in the medical record the use of hormone replacement therapy can be assumed. However, when no information is found, this could have been due to an inadequate registration system. The reported frequency and duration of use in our study was compara-

ble to the those in studies in perimenopausal women in The Netherlands, where 12% of women between 45 and 65 use hormone replacement therapy and 50 % of women discontinue use within one year.[85-87] If differential misclassification were present, this could have biased the estimates. In the study of subclinical atherosclerosis, however, we do not assume this to be likely.

Confounding

HRT seems to improve women's risk factor profile and reduce risk of cardiovascular disease and mortality. Results from observational studies are strong, consistent and biologically plausible. Still, potential biases are large and most would be expected to spuriously enhance the observed cardioprotective effect. Several studies demonstrated estrogen users are healthier than never users, even prior to use of replacement therapy [88,89], which supports the hypothesis that part of the apparent benefit associated with HRT is due to pre-existing characteristics of the users. Women who take hormones are a self-selected group and may have healthier life-styles with fewer risk factors than women who do not. Not only self-selection, but also selection of low risk women by prescribing physicians may play a role in this respect. Thirdly, compliant women who stay on estrogen represent a minority of all women who are ever prescribed estrogen, and these women may differ in several ways from less compliant women. We cannot exclude the possibility that part (or the whole) of the findings is based on this self-selection, doctors-selection or compliance bias.

In order to evaluate possible bias in observational epidemiological studies, ideally, important characteristics are measured and adjusted for in analyses. Not all confounders may be known, however, or measurable. In our study we dealt with the issue of confounding in the following ways. Firstly, in the Rotterdam Study a large number of risk factors were measured, for which we adjusted in the analysis. As a result of our retrospective follow-up design, we used the current status of risk factors, while the exposure to hormones had largely taken place in the past (chapter 2.3). Secondly, we stratified in the analyses for duration of use of HRT, and found that in women who had used female hormones for a period shorter than a year, although they were alike long-term users with respect to the presence of several socio-economic and risk factors, no association was found with atherosclerosis. This increases the probability of a biological mechanism and reduces the probability of selection bias, although it can not fully exclude it. Randomised trials are needed to provide definitive evidence.

MECHANISMS

The mechanisms through which estrogens exert their effects on cardiovascular disease are diverse. Most attention has been directed to the effects of estrogen on lipid metabolism. In the first place, estrogen causes induction (up-regulation) of LDL receptors in the liver, which increases catabolism of LDL-cholesterol, and lower LDL-cholesterol levels. Also, lipoprotein(a) levels can be lowered by estrogens, possibly by the same mechanism, as lipoprotein(a) has a similar structure to LDL-cholesterol. Estrogen increases catabolism of apo-lipoprotein B, and this results in a net reduction in circulating apolipoprotein B levels. Furthermore, estrogen increases HDL-cholesterol, partly by enhancement of HDL constituents – apolipoprotein A-I – and partly by inhibition of the enzyme hepatic lipase, which degrades HDL-cholesterol. Oral administration of estrogens enhances triglyceride synthesis in the liver, possibly through induction of fatty-acid binding protein in the liver cell membrane by estrogens. Furthermore oral estrogen increases production and immediate catabolisation of VLDL-cholesterol, which also results in increased triglyceride levels, as well as increased VLDL-levels.[90]

Estrogens probably have other ways of inhibiting atherosclerosis. Effects, such as inhibition of platelet aggregation and inflammatory cell attachment to the vessel wall, enhanced fibrinolytic activity, release of growth factors that stimulate smooth muscle cell migration, inhibition of oxidative modification of LDL and HDL-cholesterol, reduction of blood viscosity, and calcium channel blocking properties may be beneficial.[91]

Estrogen has been shown to have chronic and immediate vascular effects. The mechanisms of these vascular effects are not fully elucidated, but as discussed in chapter 2.2, estrogens might change the structure of the arterial wall by decreasing collagen production and the elastin/collagen ratio.[91-93] Specific binding of estrogens to receptors in endothelial and vascular smooth muscle cells has been demonstrated in different vascular beds in animals and in humans.[94,95] Furthermore, estrogen may be related to the augmentation of nitric oxide release, which leads to a relaxation of the vessel wall.[96]

CLINICAL IMPLICATIONS

Although evidence on the effects of menopause on coronary heart disease is conflicting, the results of our studies suggested that menopause affects risk factors for cardiovascular disease, mechanical arterial properties, levels of atherosclerosis and the risk of myocardial infarction. The major risk factor affected by menopause is cholesterol (chapter 2.1). Because higher cholesterol levels are reached in the first years after

menopause, preventive measures aimed at reducing cholesterol levels might best be initiated immediately after menopause. Our study further suggests that early estrogen deprivation may have a long-term effect; increased risks of atherosclerosis remained detectable in women after age 70. To what extent hormone replacement therapy can be used to delay the onset and progression of atherosclerosis and cardiovascular disease needs to be determined.

The results from our studies showed that HRT users have lower levels of atherosclerosis and this advantage remains after discontinuation of therapy. However, this study remains observational. As mentioned, the HERS trial, a secondary prevention trial, showed that HRT did not diminish the risk of recurrent cardiovascular events. Furthermore, in a recent observational study it was shown that women with a history of coronary artery disease starting HRT after a myocardial infarction had an increased risk of hospitalisation for unstable angina compared to women who had never used HRT.[97] Thrombogenic properties of estrogens are likely to play a part in this. Until findings from large randomised trials confirm the benefit of estrogen therapy for prevention of cardiovascular disease, no definitive conclusions can be drawn on the use of HRT in primary prevention.

FUTURE RESEARCH

Studies in the past have focussed on men for practical reasons, including the accessibility of the working population, and their higher frequency of cardiovascular disease. However, coronary heart disease is also the leading cause of death in women. Data for women are needed, not only to obtain direct evidence in women for their own benefit, but also because a better understanding of why men are at so much greater risk of coronary heart disease may lead to better methods of prevention and treatment for both sexes. In general, studies in women will benefit from the opportunity of non-invasive measurement of atherosclerosis because accurate risk assessment will not be hampered by low rates of cardiovascular events. The non-invasive measurement of mechanical arterial properties will provide valuable methods to study the association of not only structural but also functional mechanical arterial properties with a number of disease processes.

Knowledge on cardiovascular risk factors in women lags behind that in men. It is often assumed that the magnitude of effects of classical risk factors or their interactions are the same in men and women, but physiological differences between the sexes may increase risk factor levels or modify the effect of risk factors. Estrogens are associated with a diversity of factors influencing atherogenesis, like haemostatic and fibrinolytic factors, homocysteine metabolism, endothelial function and inflammatory factors,

which provide interesting fields of research. The roles of these factors and their relationship to other life-style factors, such as anti-oxidants, intake of specific fatty acids, or physical activity, their interactions with classical risk factors, such as blood pressure and lipids, and sex-hormone profiles need to be documented in women and in men separately. Insulin resistance has been put forward as a potentially important risk factor. Diabetes mellitus is the only risk factors for which the relative effect is stronger in women than in men, and elucidating the reasons behind this may provide information about the causes of the female protection for cardiovascular disease. Insulin resistance has been found to cluster with elevated blood pressure and lipid abnormalities.[98] Abdominal obesity and increased levels of serum androgens in women have been suggested to be associated with several of these risk factors, but the biological mechanisms underlying these associations need further investigation.

In contrast to the extensive research on exogenous hormones, little is known on the relation between endogenous hormones with lipid levels and coronary heart disease. For LDL-cholesterol, premenopausal women have lower levels than men of the same age, but after age 50 the increase in LDL levels in women is greater. These sex-differences might be due to the decrease of circulating hormone levels with menopause, and may explain the sex-differences in coronary heart disease. Whether the levels of estrone and estradiol that establish after menopause are associated to the development of atherosclerosis is not yet known. Since both exogenous and endogenous estrogens may be important determinants of coronary heart disease risk, information about the serum sex hormone levels to lipid levels, other risk factors, atherosclerosis and coronary heart disease would enhance our understanding of the pathogenesis of coronary heart disease in women.

HRT has several limitations for disease prevention, like the resumption of menstrual bleeding with estrogen therapy, breast tenderness, fluid retention and mood fluctuations with progestins, and a potentially increased risk of breast- and endometrial cancer, venous thromboembolic events and gallbladder disease. Optimal disease prevention therapy for postmenopausal women might be better realised by estrogen-like compounds that mimic the actions of estrogens on the bones and cardiovascular system (including serum lipids), while at the same time minimising estrogenic effects on the breast and endometrial tissues. Selective estrogen receptor modulators (SERMs) comprise a group of structurally diverse compounds which are distinguished from estrogens by their ability to interact with the estrogen receptor but to act either as a receptor agonist or antagonist depending on the target tissue and hormonal milieu. Raloxifene, the most investigated SERM to date, has been shown to have estrogenic effects on bone and lipids, but estrogen antagonistic effects on breast and uterus.[99] Although Raloxifene was shown to decrease LDL-cholesterol, lipoprotein (a) and increase HDL_2-cholesterol, a disadvantage of this medication is that it does not decrease, and possibly even increases, the incidence of hot flushes.[100] Large-scale clinical testing

currently in progress for raloxifene, and soon for other SERMs, will have to evaluate the potential of this new group of drugs as a potential substitute for long-term female hormone replacement therapy.

The effects of progestins on vascular properties and on development of atherosclerosis are not yet well understood. The many different types of progestins in use, with their different androgenic qualities, have prevented clear conclusions on their effects. As estrogen therapy should always be combined with progestins to reduce the risk of endometrial carcinoma this field needs further investigation.

Incidence of menopausal complaints is much lower in Asian countries than in the western world, as is the incidence of chronic and degenerative diseases. Dietary factors have been thought to be responsible for this. This dietary hypothesis became more specific with the detection and identification of two groups of compounds of plant origin (lignans and isoflavonoids) with molecular weight and structure similar to those of steroids, the precursors of which are found especially in soy and unrefined grain products which are highly prevalent in the Asian diet. It has been proposed that these dietary phyto-estrogens (plant estrogens) might be modulators of the human hormonal system, and thus may affect the incidence of hormone-sensitive diseases, among which cardiovascular disease, without having the unfavourable side-effects known of estrogen therapy.[101] Studies have shown that phyto-estrogens bind to estrogen receptors, and show significant estrogenic effects in animals and humans.[102] The lowering effect of soy intake on plasma-lipids has been well known, and it might be the high phyto-estrogen concentration in soy that, at least partially, is responsible for the favourable effects.[103] However, also the plant proteins in themselves may affect lipid proteins. Most information on effects of phyto-estrogens in vivo is based on consumption of phyto-estrogen-rich diets and the causal relationship and the mechanisms of phyto-estrogen action in humans still remain to be demonstrated. The potential of phyto-estrogens, especially soy-intake, in prevention of cardiovascular disease provides interesting fields for further research.

As mentioned before, genetic factors affecting age at menopause might play a role in modifying the risk of cardiovascular disease. Evidence that genetic factors are related to age at menopause has been observed previously in family and twin studies.[73-75] Yet, the genes involved remain ill defined. A promising line of study is the estrogen receptor gene. In a recent study, the different polymorphisms of this gene were found to be associated with age at menopause as well as the risk of hysterectomy.[78] This receptor also mediates the activation of the LDL-receptor in the liver, and it would be of interest to relate the polymorphism with cholesterol levels in women.

Due to the problems of selection bias, inevitably the definitive proof on effects of HRT will have to come from large randomised trials. After the results of the PEPI-trial, which showed favourable effects of HRT compared to placebo on several cardiovascular risk factors, the HERS trial was the first randomised study on cardiovascular

events, performed in women with existing coronary heart disease. In primary prevention, a large randomised trial is underway in the United States. The Women's Health Initiative (WHI) is a primary prevention trial among 27.500 postmenopausal women. In this trial, women with a uterus are being randomised to conjugated equine estrogens plus medroxyprogesteron acetate or placebo and women without a uterus are being randomised to conjugated equine estrogens or placebo. Women will be followed for 10 years for cardiovascular events, osteoporotic fractures and cancer. Similarly, in the UK, the Medical Research Council has started the recruitment for the Women's International Study on long Duration Oestrogen after Menopause (WISDOM), an international trial of 30,000 women. Results of these studies are not expected until 2006. Until then a careful weighing of individual factors should provide the basis for counselling of peri- and postmenopausal women considering long-term hormone replacement therapy, but general considerations for treatment of all asymptomatic women is not justified at present.

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Summary

In this thesis, the association between estrogen, atherosclerosis and coronary heart disease in women is described. The first part of the thesis deals with the associations of menopause with risk factors for coronary heart disease, mechanical properties of the large arteries, atherosclerosis and myocardial infarction. In the second part, in search of possibilities for prevention, the effects of hormone replacement therapy are addressed.

The incidence of cardiovascular disease in women rises sharply after middle age. Although results of large follow-up studies are inconsistent, menopause is thought to be a major determinant of this increase. The mechanism through which menopause might exert its effect on the cardiovascular system is still unknown. Increased levels of serum total cholesterol after cessation of menses have been found in most studies on menopause and risk factors. Inconsistent results, however, have been reported for HDL-cholesterol, apolipoproteins, blood pressure, waist-to-hip ratio and insulin. A difficulty when studying differences between pre- and postmenopausal women is the high correlation between menopausal status and age. Studies that included women in a broad age range may not be able to validly remove the confounding effect of age. Studies in a restricted age range around the menopause will include premenopausal women who have irregular menses and postmenopausal women who only recently passed menopause, which reduces the contrast in estrogen status.

Chapter 2.1 describes the relations between natural menopause and several atherogenic factors in a highly selected population in which the contrast in estrogen status between pre- and postmenopausal women of the same age was maximised. Risk factors were compared in 93 premenopausal and 93 postmenopausal women who were matched on age (range 43-55 years). The women were selected from respondents to a mailed questionnaire about the menopause, which was sent to all women aged 40 to 60 years, living in the Dutch town of Zoetermeer (n=12,675; response 54 %). Postmenopausal women who were at least three years after menopause or whose menses had stopped naturally before age 48 were age-matched with premenopausal women with regular menses and without menopausal complaints. Compared to premenopausal women, postmenopausal women had significantly increased levels of total cholesterol (10.0%, 95% confidence interval 5.1 ; 14.0), low density lipoprotein (LDL) cholesterol (14.0%, 6.9 ; 19.9), and apolipoprotein B (8.2%, 0.6 ; 15.5). The difference was present within three years after onset of menopause and did not show a trend towards an increase with the number of postmenopausal years. No differences were found in HDL-cholesterol, triglycerides, apolipoprotein A1, blood glucose, insulin, body mass index, waist-to-hip ratio, and systolic and diastolic blood pressure. The results of this study add to the evidence that total cholesterol, LDL cholesterol and apolipoprotein B are the primary cardiovascular risk factors affected by menopause.

In **chapter 2.2** distensibility of the common carotid artery, an indicator of arterial elasticity, was compared in pre- and postmenopausal women in the same study. Although the beneficial effects that exogenous estrogens have on arterial characteristics have been shown in several studies, the effect of endogenous estrogen on the vascular system is not known. Distensibility of the carotid artery was measured non-invasively with B-mode ultrasound and a vessel wall movement detector system. Arterial distensibility is expressed as the change in arterial diameter (distension, ΔD) with the cardiac cycle, adjusted for lumen diameter, pulse pressure and mean arterial blood pressure. Compared to premenopausal women, postmenopausal women had significantly lower arterial distension (ΔD 370.5 μm (SE 9.5) versus 397.3 μm (SE 9.6)). These results suggest that the distensibility of the common carotid artery is unfavourably affected by natural menopause in presumed healthy women.

In **Chapter 2.3** associations of early natural and artificial menopause with atherosclerosis and myocardial infarction were examined in 4,853 postmenopausal women aged 55 and over in the Rotterdam Study. Atherosclerosis was assessed in the aorta by detection of calcified plaques on lateral X-ray's of the abdomen, in the carotid arteries by ultrasonography, and in the peripheral arteries by measurement of the ankle-arm systolic blood pressure index. A history of myocardial infarction was based on self-reported information checked with GP or hospital records and/or on ECG evidence. We found that age at natural menopause was significantly and inversely related to the risk of atherosclerosis and that of myocardial infarction. After adjustment for confounders, odds ratios for women with menopause before age 40 compared to women with menopause after age 55 were 3.24 (95 percent confidence interval 1.47 – 7.13) for aortic atherosclerosis, 2.75 (1.13 – 6.67) for carotid atherosclerosis, and for women with menopause before age 45 odds ratios were 1.67 (1.02 ; 2.74) for peripheral arterial disease, and 2.49 (1.30 – 5.74) for myocardial infarction. The associations were present both in women above and below age 70. Early bilateral oophorectomy, but not hysterectomy with or without unilateral oophorectomy, was associated with an increased risk of atherosclerosis at all sites and with myocardial infarction.

If endogenous estrogens protect women from developing atherosclerosis, substitution of exogenous estrogens might provide opportunities for prevention. Thus, in **chapter 3** of this thesis the extent to which hormone replacement therapy in the early postmenopausal phase may postpone or delay the development of atherosclerosis was examined. The association of ever use of hormone replacement therapy with the presence of peripheral arterial disease, a major representative of atherosclerotic disease, was evaluated in **chapter 3.1**. A total of 2,196 naturally postmenopausal women aged 55 to 80 years were studied in the Rotterdam Study. Peripheral arterial disease was defined as an ankle/arm systolic blood pressure index < 0.9. Ever use of hormone replacement therapy for one year or more was associated with a 52% decreased risk of

peripheral arterial disease (Odds Ratio (OR) 0.48 (95% confidence interval (CI) 0.24 ; 0.85)), while no association was found for use during 1 year or shorter (OR 0.97 (95 % CI 0.58 ; 1.63), after adjustment for age, smoking and socio-economic status. Additional adjustment for body mass index, age at menopause, total cholesterol and HDL-cholesterol, alcohol intake or frequency of visits to health care facilities did not change these results. The results of this study suggest that the use of hormone replacement therapy after menopause might protect against the development of peripheral arterial disease later in life.

In **chapter 3.2**, the association of hormone replacement therapy with intima-media thickness of the common carotid artery was examined. Mean and maximal intima-media thickness of the common carotid artery were measured in a total of 1,103 naturally menopausal women aged 55 to 80 years in the Rotterdam Study. A history of ever use of hormone replacement therapy longer than one year was associated with a lower intima-media thickness (71.9 μm in hormone users versus 74.2 μm in nonusers, p=0.03)), while no association was found for use during 1 year or shorter (73.9 μm in hormone users versus 74.2 μm in nonusers (p=0.40)), after adjustment for age, smoking and socio-economic status. Additional adjustment for body mass index, age at menopause, alcohol intake or frequency of visits to health care facilities did not change these results.

In **chapter 4** of this thesis results from ROMEO, a randomised intervention trial, are reported. The ROMEO trial is a single centre randomised placebo-controlled trial, conducted to assess the two-year effects of a sequential combined regimen of oral 17β-estradiol plus desogestrel (17βE_2-D) and a combination of conjugated equine estrogens plus norgestrel (CEE-N), compared to placebo on several cardiovascular risk factors in perimenopausal women. Also, dynamic and structural arterial characteristics of the common carotid artery were measured. In **chapter 4.1**, the results are presented from an analysis on two dynamic arterial characteristics, distensibility and compliance. The ROMEO study included 121 perimenopausal women recruited from the general population. The trial was double-blind with respect to the 17β-estradiol plus desogestrel and placebo groups and open with respect to conjugated equine estrogen plus norgestrel. After six cycles, the blind was broken and the trial was continued as an open trial for another 18 months for the active study arms. At baseline distensibility and compliance of the common carotid artery was measured non-invasively with B-mode ultrasound and a vessel wall movement detector system, and the distensibility coefficient (DC) and compliance coefficient (CC) were calculated. Measurements were repeated after 6 and 24 months. Changes in DC and CC in treatment groups were compared to placebo. After 24 months changes for 17β-estradiol plus desogestrel compared to placebo were -1.4 x 10^{-3} / kPa (95% CI -4.4 ; 1.7, p=0.39) for DC and 0.26 mm^2 / kPa (95% CI -0.01 ; 0.53, p=0.07) for CC. Changes for conjugated equine estrogens plus

norgestrel compared to placebo were 0.4 x 10^{-3} / kPa (95% CI (-1.0 ; 1.9, p=0.79) and 0.11 mm^2 / kPa (95% CI -0.14 ; 0.37, p= 0.40). Also for systolic blood pressure, diastolic blood pressure and arterial lumen diameter no changes were found.

In **chapter 4.2** the effect of both treatments on intima-media thickness was described. Common carotid intima-media thickness and end-diastolic lumen diameter were measured at baseline and cycle 24 with B-mode ultrasonography. At cycle 24 small changes in intima-media thickness and lumen diameter were observed. Relative to placebo changes in intima-media thickness were -0.016 mm (95% CI -0.055, 0.024) for 17β-estradiol plus desogestrel and -0.009 mm (95% CI -0.045, 0.027) for conjugated equine estrogens plus norgestrel. For end-diastolic lumen diameter the changes were -0.125 mm (95% CI -0.820, 0.032) and -0.091 mm (95% CI -0.236, 0.055) for 17β-estradiol plus desogestrel and conjugated equine estrogens plus norgestrel, respectively.

We concluded that in this study among perimenopausal women a significant effect of 17β-estradiol plus desogestrel and conjugated equine estrogens plus norgestrel on common carotid intima-media thickness and lumen diameter could not be demonstrated. Although the duration of the present trial is too limited to provide definite conclusions, the direction of the effect is in agreement with evidence from earlier studies on the favourable effects of hormone replacement therapy in postmenopausal women.

In **chapter 5**, the general discussion, the results described in this thesis and several methodological issues are discussed. We conclude that menopause has an unfavourable association with several cardiovascular risk factors, structural characteristics of the large arteries, on atherosclerosis and on coronary heart disease. Hormone replacement therapy, which has been shown in earlier observational studies to be associated with a lower risk of coronary heart disease, was shown to protect women from development of atherosclerosis in the lower extremities and in the common carotid artery. This suggests that the mechanisms underlying this protection include inhibition of the atherosclerotic process. Our analyses of a randomised, placebo-controlled study could not confirm the effect on hormone replacement therapy on atherosclerosis of the common carotid artery, but the duration of this study was small, and the favourable direction of the effect was in agreement with expectations. Until findings from large randomised trials confirm the benefit of estrogen therapy for prevention of cardiovascular disease no definite conclusions can be drawn, and hormone replacement therapy should not be routinely recommended for this purpose in clinical practice.

Samenvatting

Dit proefschrift beschrijft de relatie tussen oestrogenen, atherosclerose en hart- en vaatziekten bij vrouwen. In het eerste deel worden de gevolgen beschreven van de menopauze voor risicofactoren voor hart- en vaatziekten, voor de arteriële elasticiteit, voor de kans op atherosclerose en voor het krijgen van een hartinfarct. Het tweede gedeelte van het proefschrift beschrijft de effecten van hormoon substitutie therapie op arteriële elasticiteit en op atherosclerose.

De incidentie van hart- en vaatziekten neemt snel toe bij vrouwen na de middelbare leeftijd. Hoewel de resultaten van verschillende grote follow-up onderzoeken hierover niet consistent zijn, lijkt de menopauze een determinant te zijn van deze toename. De mechanismen waardoor de menopauze het risico op hart- en vaatziekten beïnvloedt zijn nog niet geheel opgehelderd. De meeste studies vinden een toename van totaal-cholesterolspiegels na de menopauze, maar wisselende resultaten worden gevonden voor HDL-cholesterolspiegels, apolipoproteïnen, bloeddruk, middel-heupratio en insulinespiegels. Studies naar de effecten van menopauze worden bemoeilijkt door de sterke correlatie tussen menopauze en leeftijd. Studies die vrouwen binnen een brede leeftijdsgroep onderzoeken, kunnen niet goed corrigeren voor het effect van leeftijd op de te bestuderen variabelen. In studies onder vrouwen rond de menopauze zullen weer veel vrouwen deelnemen die reeds een onregelmatige cyclus hebben en postmenopauzale vrouwen die pas de menopauze gepasseerd zijn. Dit verzwakt het contrast in oestrogeenstatus tussen pre- en postmenopauzale vrouwen.

Hoofdstuk 2.1 beschrijft de relaties tussen natuurlijke menopauze en verschillende atherogene factoren. In dit onderzoek is getracht het contrast in menopausale status te maximaliseren tussen pre- en postmenopauzale vrouwen van dezelfde leeftijd. Risicofactoren werden vergeleken in 93 premenopauzale en 93 postmenopauzale vrouwen die op leeftijd gematcht waren (leeftijd 43 - 55 jaar). De vrouwen werden geselecteerd middels een vragenlijst over de menopauze die aan alle vrouwen tussen de 40 en 60 jaar in Zoetermeer werd verzonden (n=12675, respons 54%). Postmenopauzale vrouwen die ten minste voor hun 48e spontaan in de menopauze waren gekomen en vrouwen die al ten minste 3 jaar niet meer menstrueerden, werden gematcht met vrouwen van dezelfde leeftijd die nog regelmatig menstrueerden en nog geen overgangsklachten hadden. Vergeleken met premenopauzale vrouwen hadden postmenopauzale vrouwen significant verhoogde spiegels van totaal cholesterol (10.0%, 95% betrouwbaarheidsinterval (BI) 5.1 ; 14.0), LDL-cholesterol (14.0%, 6.9 ; 19.9) en apolipoproteïne B (8.2%, 0.6 ; 15.5). De hogere waarden in de postmenopauzale groep waren al aanwezig binnen 3 jaar na de laatste menstruatie en namen niet toe met het aantal jaren na de menopauze. Er werden geen verschillen gevonden in HDL-cholesterol, triglyceriden, apolipoproteïne A1, glucosespiegels, insulinespiegels, middel-heupratio, body mass index en systolische en diastolische bloeddruk. De resultaten van deze studie

laten zien dat vooral totaal cholesterol, LDL cholesterol en apolipoproteïne B beïnvloed worden door de menopauze.

In **hoofdstuk 2.2** werd de distensibiliteit van de arteria carotis communis, een maat voor vaatwandelasticiteit, vergeleken tussen pre- en postmenopauzale vrouwen in dezelfde studie. Hoewel eerder is aangetoond dat toegediende oestrogenen een gunstig effect hebben op verschillende vaatwandeigenschappen, is niet bekend wat het effect is van het wegvallen van de eigen oestrogenen ten gevolge van de menopauze. De distensibiliteit van de vaatwand werd gemeten door middel van echografie en een vaatwandbewegingsdetectiesysteem. Distensibiliteit werd uitgedrukt als de verandering in de diameter van het vat (distensie (D)) met de hartslag, gecorrigeerd voor de originele diameter van het vat, de polsdruk en de gemiddelde arteriële bloeddruk. Vergeleken met premenopauzale vrouwen hadden postmenopauzale vrouwen een significant lagere distensie ((D 370.5 μm (SE 9.5) versus 397.3 μm (SE 9.6)). Deze resultaten geven aan dat de elasticiteit van de arteria carotis communis in gezonde vrouwen ongunstig wordt beïnvloed door de menopauze.

In **hoofdstuk 2.3** worden de relaties onderzocht tussen de leeftijd waarop de menopauze is ingetreden en het vóórkomen van atherosclerose en myocardinfarct in 4853 postmenopauzale vrouwen van 55 jaar en ouder in het ERGO onderzoek. Atherosclerose werd gemeten op drie plaatsen. In de aorta, door middel van detectie van gecalcificeerde plaques op dwarse röntgenfoto's van de buik, in de arteria carotis door middel van echografie en in de arteriën van de benen door meting van de enkelarmindex. Een voorgeschiedenis van myocardinfarct werd vastgesteld op basis van zelf gerapporteerde gegevens en op ECG-informatie, geverifieerd met behulp van gegevens uit de huisartsenstatus. We vonden dat een vroege menopauze was geassocieerd met een verhoogd risico op atherosclerose en myocardinfarct. Na correctie voor verstorende factoren hadden vrouwen met menopauze voor hun 40ste een 3.2 maal verhoogde kans op atherosclerose in de aorta (95% BI 1.47 ; 7.13), een 2.8 maal verhoogde kans op atherosclerose in de carotiden (95% BI 1.13 ; 6.67), en voor vrouwen met een menopauze voor hun 45ste een 1.7 maal verhoogde kans op perifeer vaatlijden (95% CI 1.02 ; 2.74) en een 2.49 maal verhoogde kans op een myocardinfarct (95% BI 1.30 ; 5.74). Deze associaties werden gevonden in vrouwen onder en vrouwen boven de 70 jaar. Een vroege bilaterale ovariëctomie gaf een verhoging van de kans op atherosclerose in de drie onderzochte locaties en op een myocardinfarct, terwijl een vroege hysterectomie, met of zonder unilaterale ovariëctomie geen risicoverhoging gaf.

Als het wegvallen van endogene oestrogenen het risico op atherosclerose verhoogt, zou substitutie van oestrogenen (hormoon substitutie therapie) deze toename mogelijk kunnen uitstellen of vertragen. In **hoofdstuk 3** van dit proefschrift worden de resultaten beschreven van twee observationele studies naar het verband tussen hormoon substitutie therapie en atherosclerose.

Het effect van hormoon substitutie therapie in het verleden op de aanwezigheid van perifeer vaatlijden werd onderzocht in **hoofdstuk 3.1**. Binnen het ERGO onderzoek werden 2196 vrouwen met een natuurlijke menopauze tussen de 55 en 80 jaar bestudeerd. Perifeer vaatlijden werd gedefinieerd als een enkel-armindex lager dan 0.9. Hormoon substitutie therapie in het verleden gedurende meer dan 1 jaar was geassocieerd met een 50% verlaagde kans op perifeer vaatlijden (odds ratio 0.48, 95% BI 0.24 ; 0.85), terwijl geen verband werd gevonden bij gebruik van hormoon substitutie therapie gedurende een periode korter dan een jaar (odds ratio 0.97, 95% BI 0.58 ; 1.63). Deze associaties waren onafhankelijk van leeftijd, roken of sociaal-economische status. Aanvullende correcties voor body mass index, leeftijd van menopauze, totaal cholesterol en HDL cholesterol, alcoholgebruik of het aantal bezoeken aan voorzieningen in de gezondheidszorg (een maat voor medische consumptie) veranderden de resultaten niet. De resultaten van deze studie suggereren dat het gebruik van hormoon substitutie therapie na de menopauze mogelijk bescherming biedt tegen perifeer vaatlijden op latere leeftijd.

In **hoofdstuk 3.2** wordt de associatie van hormoon substitutie therapie op de vaatwanddikte (de intima-media dikte) van de arteria carotis onderzocht. De gemiddelde en de maximale wanddikte van de arteria carotis communis werden echografisch gemeten in 1103 natuurlijk postmenopauzale vrouwen tussen de 55 en 80 jaar in het ERGO onderzoek. Gebruik van hormoon substitutie therapie in het verleden gedurende langer dan een jaar was geassocieerd met een dunnere vaatwand (71.9 μm in gebruiksters versus 74.2 μm in vrouwen die nooit hormoon substitutie therapie hadden gebruikt, p = 0.03). Gebruik gedurende een periode korter dan een jaar was niet geassocieerd met een verandering in wanddikte (73.9 μm in gebruiksters versus 74.2 μm in vrouwen die nooit gebruikt hadden (p=0.40)), na correctie voor leeftijd, roken, systolische bloeddruk, body mass index en sociaal-economische status. Additionele correcties voor diabetes, het aantal bezoeken aan voorzieningen in de gezondheidszorg, totaal cholesterol en HDL-cholesterol veranderden deze resultaten niet.

In **hoofdstuk 4** van dit proefschrift worden de resultaten van de ROMEO studie, een gerandomiseerde placebo-gecontroleerde interventie-trial, gepresenteerd. De ROMEO trial werd uitgevoerd met het doel de effecten van een twee jaar durend sequentieel gecombineerd regime van oraal 17β-oestradiol plus desogestrel en een combinatie van geconjugeerde oestrogenen plus norgestrel te vergelijken met placebo, ten aanzien van verschillende cardiovasculaire risicofactoren in perimenopauzale vrouwen. Binnen het kader van deze studie onderzochten wij de dynamische en structurele eigenschappen van de arteria carotis communis. In **hoofdstuk 4.1** worden de resultaten beschreven van het onderzoek naar twee veelgebruikte dynamische arteriële karakteristieken: distensibiliteit en compliantie. In de ROMEO studie namen 121 vrouwen deel, die waren gerecruteerd uit de algemene bevolking. De trial was dubbelblind ten aanzien van de

17β-oestradiol plus desogestrel groep en de placebogroep, en open ten aanzien van de geconjugeerde oestrogenen plus norgestrel groep. Na de 6e cyclus werd de blindering losgelaten en werd de trial voortgezet als een open trial gedurende 18 cycli. Bij aanvang van de studie werden distensibiliteit en compliantie niet-invasief gemeten met behulp van echografie en een vaatwandbewegingsdetectiesysteem en werden de distensibiliteitscoëfficiënt (DC) en de compliantiecoëfficiënt (CC) berekend. De metingen werden herhaald na 6 en na 24 cycli. Veranderingen in DC en CC in de behandelde groepen werden vergeleken met die in de placebogroep. Na 24 maanden waren de relatieve veranderingen ten opzichte van de placebogroep klein. In de 17β-oestradiol groep waren de relatieve veranderingen -1.4 x 10^{-3} / kPa (95% BI -4.4 ; 1.7) in DC en 0.26 mm^2 / kPa (95% BI -0.01 ; 0.53) in CC. Relatieve veranderingen voor de gebruiksters van geconjugeerde oestrogenen bedroegen 0.4 x 10^{-3} / kPa (95% BI -1.0 ; 1.9) in DC en 0.11 mm^2 / kPa (95% BI -0.14 ; 0.37) in CC. Ook voor veranderingen in systolische en diastolische bloeddruk werden tussen de groepen geen statistisch significante verschillen gevonden.

In **hoofdstuk 4.2** wordt het onderzoek naar de effecten van de twee behandelingen op de structurele eigenschappen van de vaatwand beschreven. De vaatwanddikte en de lumendiameter van de arteria carotis communis werden gemeten met behulp van echografie. Bij cyclus 24 werden kleine veranderingen in vaatwanddikte en lumendiameter gevonden. Ten opzichte van de veranderingen in de placebogroep werden een verandering van -0.016 mm (95% BI -0.055 ; 0.024) voor 17β-oestradiol en van -0.009 mm (95% BI -0.045 ; 0.027) voor gebruiksters van geconjugeerde oestrogenen gevonden. Voor de einddiastolische lumendiameter werden relatieve veranderingen van -0.125 mm (95% BI -0.820 ; 0.032) en -0.091 mm (95% BI -0.236 ; 0.055) gevonden voor respectievelijk 17β-oestradiol en geconjugeerde oestrogenen vergeleken met placebo. Concluderend kan worden gezegd dat, hoewel de resultaten laten zien dat – overeenkomstig de bestaande hypothesen – het effect van hormoon substitutie therapie gunstig lijkt, in deze 2-jarige studie geen significant effect van hormoon substitutie therapie op de vaatwanddikte en lumendiameter kon worden aangetoond. Mogelijk is de studie te kort om een effect aan te tonen, of mogelijk is het effect van hormoon substitutie therapie op atherosclerose in de arteria carotis communis in perimenopauzale vrouwen inderdaad klein.

In **hoofdstuk 5**, de algemene discussie, worden de resultaten van de in dit proefschrift beschreven studies samengevat en worden verschillende methodologische aspecten besproken. Wij concluderen dat de menopauze ongunstige gevolgen heeft voor verschillende cardiovasculaire risicofactoren, voor de arteriële elasticiteit, voor de kans op atherosclerose en voor de kans op het krijgen van een hartinfarct. Hormoon substitutie therapie, die in eerdere studies vrouwen lijkt te beschermen tegen hart- en vaatziekten, lijkt in onze observationele studies bescherming te bieden tegen atherosclerose in de

beenvaten en de arteria carotis communis. Bovendien lijkt het gunstiger atherosclerotische profiel bij vrouwen die in het verleden hormoon substitutie therapie hebben gebruikt te blijven bestaan na het stoppen met de therapie. Mogelijk is dus de remming van het proces van atherosclerose een van de mechanismen waardoor hormoon substitutie therapie vrouwen beschermt tegen hart- en vaatziekten. De resultaten van de gerandomiseerde ROMEO studie konden de effecten van hormoon substitutie therapie op de remming van atherosclerose in de arteria carotis niet bevestigen. De resultaten lieten wel zien, overeenkomstig de bestaande hypothesen, dat het effect van hormoon substitutie therapie, hoewel niet statistisch significant, gunstig leek. Mogelijk was deze studie te kort om een effect aan te tonen in perimenopauzale vrouwen. Totdat grote gerandomiseerde onderzoeken het gunstige effect van hormoon substitutie therapie op het optreden van hart- en vaatziekten bevestigen, kunnen hieromtrent geen definitieve conclusies worden getrokken en kan hormoon substitutie therapie niet worden aangeraden voor dit doel.

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CURRICULUM VITAE

The author was born on October 10th, 1967 in Durham (North-Carolina), in the United States of America. She attended the Haags Montessori Lyceum in The Hague, after which she started her medical study at the University of Amsterdam in 1986. During her studies she was involved in a research project on drug utilisation in Georgetown, Malaysia, and did clinical electives in Hospital Durand, in Buenos Aires, Argentina. She graduated in 1994 and worked in De Kruispost, a medical and social health post for those without medical insurance in Amsterdam. In 1995 she started the studies described in this thesis at the Department of Epidemiology & Biostatistics at the Erasmus University in Rotterdam (head: Prof. Dr. A. Hofman). In 1997 she obtained a Master of Science in Clinical Epidemiology at the Netherlands Institute for Health Sciences in Rotterdam. During this period she was member of the executive committee of Apollo, the Society for PhD-students of the University of Amsterdam. Currently she is working as a resident in cardiology in the Onze Lieve Vrouwe Gasthuis in Amsterdam, where she will start her training as a cardiologist on January 1st 2000.