Menopausal status and risk factors for cardiovascular disease

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Abstract Peters HW, Westendorp ICD, Hak AE, Grobbee DE, Stehouwer CDA, Hofman A, Witteman JCM (Erasmus University Medical School, Rotterdam; Utrecht University, Utrecht; Vrije Universiteit, Amsterdam, The Netherlands). Menopausal status and risk factors for cardiovascular disease. *J Intern Med* 1999; **246**: 521–528.

Objectives. Changes in cardiovascular risk factors with menopausal status are difficult to study, owing 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 oestrogen status between pre- and postmenopausal women of the same age was maximized.

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 40–60 years in the Dutch town of Zoetermeer (n = 12 675; response 54%).

Subjects. Postmenopausal women who were at least 3 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 3 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.

Keywords: apolipoproteins, cardiovascular disease, cholesterol, insulin, menopause, risk factor.

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 the 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 oestrogen status [17, 21].

In the present study, we examined the relationships between natural menopause and several atherogenic factors in a highly selected population in which the contrast in oestrogen status between pre- and postmenopausal women of the same age was maximized.
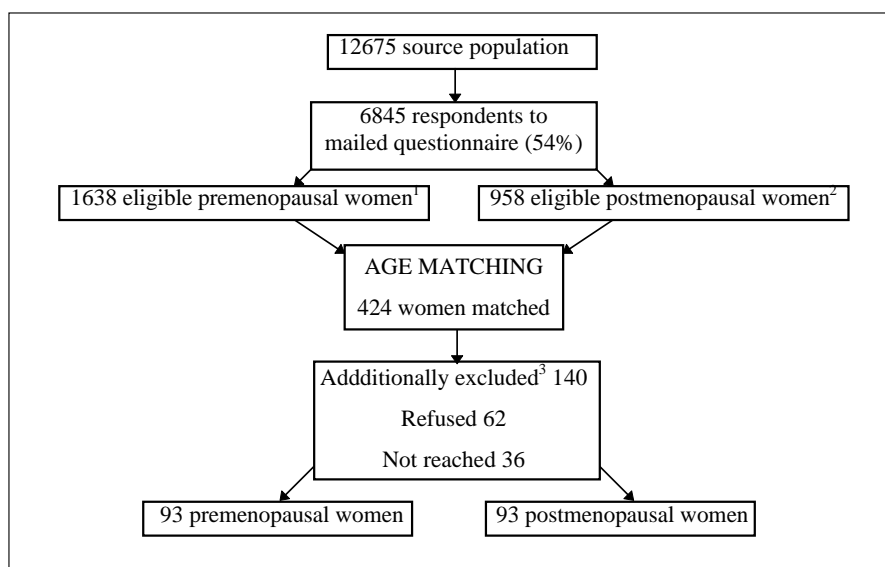
Materials and methods

Study population

Selection of participants in this study was aimed at maximizing the contrast in oestrogen status, in pre- and postmenopausal women of the same age (Fig. 1). A questionnaire, including questions about menopausal status, medical history, medication use, and smoking behaviour, was sent by mail to all women aged 40–60 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 ($n = 1551$). Women were considered pre-

menopausal if they had had one or more bleedings in the past 12 months ($n = 3829$). 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 ($n = 1645$) were excluded. 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 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 ($n = 1242$). Postmenopausal women who reported a history of hormone replacement therapy for over 6 months or use of female hormones within 6 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 vs. 16; 1.7% postmenopausal women), use of antihypertensive medication (31; 1.9% vs. 35;

Fig. 1 Schematic presentation of the selection procedure of the study population. (1) Eligible 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 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 used antihypertensive or cholesterol lowering drugs were excluded. (3) Women who no longer fulfilled the criteria at the moment of examination were excluded.

12675 source population

6845 respondents to mailed questionnaire (54%)

1638 eligible premenopausal women[1]   958 eligible postmenopausal women[2]

AGE MATCHING
424 women matched

Addditionally excluded[3] 140
Refused 62
Not reached 36

93 premenopausal women   93 postmenopausal women

3.7%), use of cholesterol-lowering drugs (3; 0.2% vs. 20; 2.1%) and current smoking of 5 or more cigarettes per day (302; 18.4% vs. 218; 22.8%).

Pre- and postmenopausal women were matched on age, whilst maximizing the contrast in oestrogen status. 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 pre-menopausal 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 43–55 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 divided by height squared) and waist-to-hip ratio were computed. Alcohol drinking habits and cigarette smoking history were obtained by a standardized questionnaire. Blood pressure was assessed four times at the right upper arm after a 5 min rest in the supine position, with a Dinamap automatic blood pressure recorder (Critikon, Tampa, Florida, USA) and the mean was used in the analyses. Venous blood samples were drawn from each subject after a 12-h fast. The samples were stored at − 80 °C, and subsequently serum parameters were determined using a Kone Specific Analyser (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 colourimetry. 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

Analysis of covariance was used to compare characteristics of pre- and postmenopausal women, with adjustment for age. Since the distribution of insulin was highly skewed, it was natural-log transformed for the analyses. Differences in frequencies of smoking status and alcohol drinking were tested by the Chi-square test. Differences in risk factors between pre- and postmenopausal women 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 analysis 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 ≥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 relationship between the risk factors and time since menopause was estimated using linear regression analysis, with the ordinal variable as the dependent variable.

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 43.3–54.7) than the premenopausal women (mean 50.6, range 44.1–55.3) (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 (Fig. 2).

Table 1 General characteristics of pre- and postmenopausal women

	Premenopausal (n = 93)	Postmenopausal (n = 93)
Mean (SD)		
Age (years)	50.6 (2.4)	51.1 (2.2)
Mean (SD)[a]		
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 per week)	45 (57.0)	45 (57.1)
Percentage (n)		
Current smoking (%)[b]	6 (6)	6 (6)
Past smoking (%)[b]	42 (39)	39 (36)

[a]Adjusted for age. [b]Subjects who smoked five or more cigarettes per day were excluded from study participation.

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 3 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

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

Mean (SE)[a]	Premenopausal (n = 93)	Postmenopausal (n = 93)	Difference (%)	95% confidence interval for the percentage difference
Total cholesterol (mmol L^{-1})	5.89 (0.10)	6.48 (0.10)**	10.0%	(5.1; 14.0)
LDL cholesterol (mmol L^{-1})	3.78 (0.09)	4.32 (0.09)**	14.0%	(6.9; 19.9)
HDL cholesterol (mmol L^{-1})	1.58 (0.04)	1.64 (0.04)	3.7%	(−2.9; 10.3)
Triglycerides (mmol L^{-1})	1.16 (0.06)	1.16 (0.06)	0%	(−13.5; 13.9)
Apolipoprotein A1 (mg dL^{-1})	1.53 (0.03)	1.56 (0.03)	1.9%	(−3.8; 8.0)
Apolipoprotein B (mg dL^{-1})	0.89 (0.03)	1.06 (0.03)*	8.2%	(0.6; 15.5)
Glucose (mmol L^{-1})	5.56 (0.06)	5.55 (0.06)	−0.01%	(−2.9; 2.6)
Insulin (picomol L^{-1})[b]	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)

[a]Adjusted for age. [b]Skewed data, therefore geometric mean is shown. *P < 0.05. **P < 0.001.

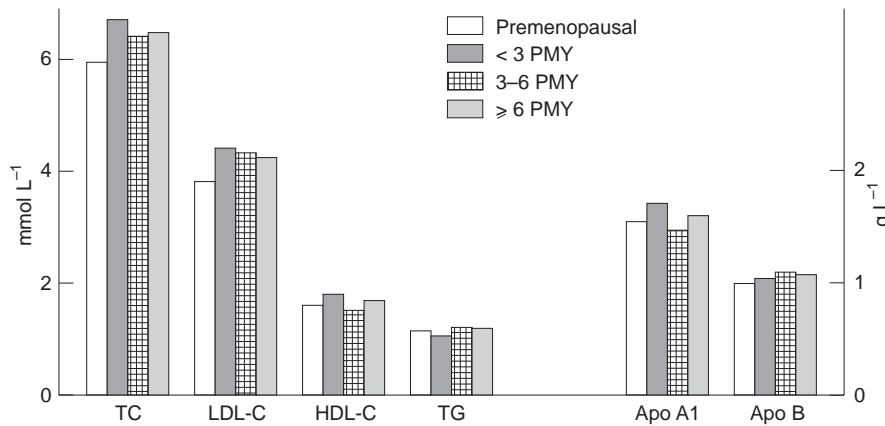Lipid levels by number of postmenopausal years (PMY)

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

important confounding factor. In most cross-sectional studies, the study population includes women in an age-range which 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 women who only recently passed menopause, which decreases the contrast in oestrogen 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 oestrogen 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 from responders to a mailed questionnaire. We assume,

however, that the results from our study are generalizable to the general population even if some selection has taken place, because we have no reason to assume that the relationship between menopause and biological factors will be different in responders and nonresponders.

To ensure that the results are due to true associations between natural menopause and cardiovascular risk factors, bias owing to other factors also has 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 five 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, socioeconomic status, genetic factors or parity, may have been related to early menopause and the difference in lipid levels. This seems unlikely, however, as although socioeconomic 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 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 oestrogen 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 relationship 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 findings that apolipoprotein A1 was not different and that 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 premenopausal 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 relationships 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 maximize the contrast in oestrogen status. The results 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.

Acknowledgements

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