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The increase in cholesterol with menopause is associated with the apolipoprotein E genotype A population-based longitudinal study

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Abstract

During menopause, a sharp increase in cholesterol concentration occurs with an unexplained wide variation in change. Possibly, this is attributable to genetic variation. The authors prospectively studied the effect of the apolipoprotein E (APOE) genotype on the change in cholesterol level with menopause among 1116 Dutch women. Women with the APOE3E3 genotype were regarded as the reference category and changes were adjusted for age at baseline, years of follow-up, years since menopause, and body mass index. At baseline, the women were on average 50.4 years. After 5.9 years of follow-up, the women were on average 4.3 years (S.D. 1.5 years) postmenopausal. The mean increase in cholesterol with menopause in women with the APOE3E3 genotype was 0.67 mmol/L (95% CI, 0.61–0.72 mmol/L). In women with the APOE2E3 genotype the increase in cholesterol was 0.44 mmol/L (CI, 0.32–0.56 mmol/L). The increase in cholesterol in women with the APOE3E4 genotype did not differ from the increase in women with the APOE3E3 genotype. These results show that the increase in cholesterol level with menopause is 30% lower in women with the APOE2E3 genotype when compared with women with the APOE3E3 genotype, indicating that the APOE genotype contributes to the variation in cholesterol increase with menopause.

Keywords: APOE genotype; Cholesterol; Menopause; Population-based

1. Introduction

The incidence of cardiovascular disease in women rises after middle age. Although still debated [1–4], menopause is thought to be a determinant of this increase [5–7]. Studies consistently show that total and low-density lipoprotein (LDL) cholesterol are the primary cardiovascular risk factors affected by menopause [8–17]. Longitudinal studies show an average increase in total cholesterol with menopause of 0.5 mmol/L, with a wide variation in change [18–21]. It is not known why some women have no or only a slight increase in cholesterol, whereas others exhibit a large increase. Possibly, this difference can be explained by genetic variation.

An important polymorphism associated with cholesterol level is the apolipoprotein E (APOE) genotype [22]. The heterogeneity in APOE genotype is responsible for different isoforms of apolipoprotein E (apoE), which is mainly present on chylomicrons and very-low-density lipoproteins (VLDLs). When associated with these lipoproteins, apoE serves as a ligand for the hepatic lipoprotein receptors. It has been firmly established that the APOE polymorphism affects plasma cholesterol level. Compared with the APOE*3 homozygotes, the most common genotype, the APOE*2 allele is associated with lower levels of cholesterol, whereas the APOE*4 allele has opposite effects [22,23]. In a cross-sectional study, the association between the APOE genotype and cholesterol concentration has been

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found to be weaker in premenopausal compared with postmenopausal women [24], suggesting that estrogen affects the influence of the APOE genotype on cholesterol level.

In a Dutch population-based cohort of women, the Eindhoven Perimenopausal Osteoporosis Studies, we examined prospectively among 1116 women experiencing natural menopause whether the variation in increase in cholesterol with menopause may be explained by the APOE genotype.

2. Subjects and methods

2.1. Study population

The Eindhoven Perimenopausal Osteoporosis Study is a population-based cohort study originally designed to examine determinants of bone mass, with special emphasis on gynecological parameters, in perimenopausal women [25]. The baseline examination was conducted between September 1994 and September 1995. All women living in the city of Eindhoven, The Netherlands, and born between 1941 and 1947 were invited by the Diagnostic Center Eindhoven, a diagnostic center for general practitioners, and the Department of Municipal Public Health Services Eindhoven for screening of their bone mineral density. Of the 8503 eligible women, 6700 (79%) participated and gave informed consent to be invited for future research.

In the year 2000, we selected the population for the current study. To prevent admixture we restricted our population to the 6448 white Dutch women. Of these, we selected the 2892 women who were premenopausal, defined as last menses less than 1 year ago, at the baseline examination (1994–1995). We excluded women using hormone replacement therapy or oral contraceptives ($n = 244$) and women using cholesterol-lowering therapy ($n = 21$) at baseline because these medications influence cholesterol levels. Four women used both types of medication, leaving 2631 women. Of the 2631 women, 2457 had serum samples at baseline, 208 of whom moved outside the area, leaving 2249 subjects to be invited for the follow-up study, which was conducted between November 2000 and May 2001. The study protocol was approved by the medical ethics committee of the Erasmus Medical Center Rotterdam, The Netherlands.

Of the 2249 invited women, 318 did not respond to the invitation, 68 refused to participate, 8 moved outside the area after the selection of women to be invited, 12 were not able to participate because of physical or mental illness, 7 had died, and 7 responded after the ending of the study, resulting in 1829 participating women, which corresponds with a participation rate of 81%.

2.2. Interview and clinical examination

At the baseline examination (1994–1995), women were invited to the Diagnostic Center Eindhoven or the St. Joseph Hospital in Veldhoven, a suburb of Eindhoven, where information on menstruation pattern, menopausal state, and medication use was obtained through an interview by a trained research assistant. Subsequently, weight and height were measured, body mass index (BMI, weight divided by height squared) was computed, and nonfasting blood samples were taken. Serum samples were obtained and stored at −80 °C for future use. After the visit, participants were asked to fill-in a questionnaire on menopausal complaints, smoking habits, and alcohol use, and return this to the Diagnostic Center Eindhoven within 1 week (response 92%).

At the follow-up examination (2000–2001) at the Diagnostic Center Eindhoven, women were interviewed by a trained research assistant. Menopausal state was ascertained by questioning whether the menses had stopped, and if so, at what age and the reason for its cessation (natural or artificial). The type of artificial menopause was subsequently registered. Information on smoking habits and alcohol use was obtained. Participants were asked to bring their current medication to the research center, where preparation names were noted (oral contraceptives, hormone replacement therapy, and cholesterol-lowering medication). Height and weight of the participants were measured, BMI was computed, and nonfasting blood samples were taken by venapuncture.

2.3. Cholesterol

Serum samples of the baseline investigation were retrieved from storage, defrosted at room temperature, and subsequently vortexed. Total cholesterol levels of baseline and follow-up serum samples were assessed in the same batch to prevent interassay variation contributing to differences between baseline and follow-up cholesterol levels, with an automatic enzymatic procedure [26] at the laboratory of the Diagnostic Center Eindhoven. The interassay coefficient of variation was 0.49% and the intraassay coefficient of variation was 0.99% at a level of cholesterol of 7.40 mmol/L.

2.4. DNA isolation and APOE genotyping

EDTA samples obtained at follow-up were frozen at −20 °C until DNA-isolation and genotyping were performed at the genomic laboratory of the department of Internal Medicine, Erasmus MC. Genomic DNA was isolated from peripheral leukocytes using PUREGENE® DNA isolation kit of Gentra Systems (Minneapolis, USA) with slight modifications of the provided protocol. The extracted DNA was amplified using a duplex polymerase chain reaction (PCR) generating a 244 bp PCR fragment of APOE using oligonucleotide primers:

Forward: 5′-TAAGCTTGGCACGGCTGTCCAAGGA-3′.
Reverse: 5′-AGAATTCGCCCCGGCCTGGTACAC-3′.

PCRs were carried out in 10 μl reaction volumes containing 60 ng of genomic DNA, 10*PCR buffer [(Promega) containing 20 mM Tris-HCl (pH 8.0), 100 mM KCl, 0.1 mM EDTA, 1 mM DDT, 50% glycerol, 0.5% Nonidet®-P40,

and 0.5% Tween®20], 1.5 mM $MgCl_2$, 0.2 mM deoxy-NTP, 9 pmol of each ApoE primer, and 1 U of Taq polymerase (Promega). The reactions were performed in 96-well format in a thermocycler (MJ-tetrad). Each reaction mixture was denatured for 5 min at 95 °C and subjected to 35 cycles of amplification by primer annealing (59 °C for 45 s), extension (72 °C for 45 s), and denaturation (94 °C for 45 s). Subsequently, APOE genotyping was performed using the SNaPshot procedure using primers:

Codon 112: 5′-$(T)_{12}$ GGGCGCGGACATGGAGGACG-TG-3′.
Codon 158: 5′-$(T)_{18}$ CGATGCCGATGACCTGCAGAA-G-3′.

The SBE reaction was performed according to details provided by the manufacturer (ABI Prism® SNaPshot™ ddNTP Primer Extension Kit of PE Biosystems) with slight modifications of the provided protocol. Samples were analyzed in a random fashion and the laboratory technician carrying out the genotyping procedures was blinded for the cholesterol levels of the samples concerned.

2.5. Definition of population for analysis

Of the 1829 women participating at follow-up, 133 women still had a normal menstruation pattern, 357 women had an irregular menstruation pattern and 1339 women reported 1 year of amenorrhea. Of these 1339 women, cessation of the menses had occurred in 70 women after surgery of the womb and/or ovaries, in five women after treatment with chemotherapy for breast cancer and in 1264 women spontaneously. We excluded women using hormone replacement therapy ($n = 76$) or anti-estrogens ($n = 4$), and women using cholesterol-lowering medication ($n = 56$) at the time of blood drawing. Two women used two types of medication, leaving 1130 women. Due to logistic reasons, cholesterol levels were missing for six women at baseline and for three women at baseline and at follow-up, leaving 1121 women. DNA isolation was not feasible in blood samples of five women, resulting in a population for analysis of 1116 women.

2.6. Statistical analysis

Initially, we used a paired t-test to compare continuous characteristics measured at baseline and at follow-up, and the McNemar test for paired comparisons of dichotomous variables.

We used a general linear model (univariate analysis of variance) to compute and compare mean values of cholesterol at baseline (premenopausal state) and at follow-up (postmenopausal state) as well as changes in cholesterol during follow-up (change with menopause = follow-up level − baseline level) in strata of the APOE genotype. In these analyses, the APOE3E3 genotype was used as reference category. Differences in cholesterol levels and changes in cholesterol levels relative to the APOE3E3 genotype were tested within the same model with custom hypothesis testing using a K-matrix. In the analyses at the premenopausal and postmenopausal state, we adjusted for age and BMI as measured at premenopausal and postmenopausal state, by putting these variables as continuous variables in the model. In models regarding change in cholesterol with menopause, we adjusted for age at baseline, years of follow-up, years since menopause, BMI at baseline, and change in BMI during follow-up (all continuous variables). No interaction terms were used. For missing data on body mass index the mean value as calculated from the study population was imputed.

By using analysis of variance (ANOVA) we estimated the contribution of the APOE genotype to the phenotypic variation of cholesterol. ANOVA was done on residual values after adjustment for age and BMI at premenopausal and postmenopausal assessments. For the analysis regarding the change of cholesterol, ANOVA was done on residual values after adjustment for age at baseline, years of follow-up, years since menopause, BMI at baseline, and change in BMI during follow-up (all continuous variables). The genotypes of APOE were entered as dummy variables in the analyses.

We considered two-sided probability-values <0.05 to be statistically significant. SPSS 10.0 for Windows (SPSS Inc., Chicago, Illinois) was used for all analyses.

3. Results

Table 1 shows the baseline and follow-up characteristics of the study population. The mean period of follow-up was 5.9 years (standard deviation [S.D.] 0.3 years) and ranged from 5.3 to 6.6 years. The mean age at menopause of the 1116 women was 52.1 years and the women were on average 4.2 years postmenopausal (S.D. 1.5 years) at the follow-up visit. During follow-up, women lost on average 0.8 cm of their height (S.D. 1.3 cm) and gained 4.0 kg (S.D. 5.3 kg). At follow-up, fewer women smoked, whereas the proportion of women drinking alcohol had increased compared with the baseline examination. The mean serum cholesterol level increased with 0.64 mmol/L (95% confidence interval [CI]: 0.60, 0.69 mmol/L) during follow-up. Fig. 1 shows the distribution of the change in cholesterol levels in the 1116 women experiencing natural menopause during follow-up.

The distribution of the APOE polymorphism in our study population was in Hardy–Weinberg equilibrium ($\chi^2 = 3.26$; d.f. = 3; $P = 0.35$, Table 2). In Table 3, the mean levels of cholesterol according to menopausal state and changes in levels of cholesterol during follow-up are shown in strata of the APOE genotype. Both at premenopausal and at postmenopausal assessments, age-adjusted cholesterol levels were intermediate in women with the APOE3E3 genotype, lower in women with the APOE2E3 genotype, and higher in women with the APOE3E4 genotype. At the

Table 1
Baseline and follow-up characteristics of 1116 women participating in the Eindhoven Perimenopausal Osteoporosis Studies and experiencing natural menopause during 5.9 years of follow-up, 1994–1995 to 2000

Characteristic	Premenopausal (baseline)	Postmenopausal (follow-up)
Age (years)	50.4 ± 2.2	56.3 ± 2.1[c]
Height (m)	1.65 ± 0.06	1.64 ± 0.06[c]
Weight (kg)	68.5 ± 11.7	72.5 ± 12.8[c]
Body mass index (BMI) (kg/m^2)	25.3 ± 4.3	27.0 ± 4.8[c]
Smoking (%)[a]	30	26[c]
Alcohol use (%)[b]	59	62[d]
Cholesterol (mmol/L)	5.72 ± 0.98	6.36 ± 1.06[c]

Values are unadjusted mean ± S.D. or percentages. At baseline, height was missing in one woman, weight and BMI were missing in four women, and information on smoking was missing in 129 women (12%). At follow-up, weight and BMI were missing in one woman.

[a] More than 1 cigarette per day.

[b] More than 1 glass per week.

[c] $P < 0.001$ compared with baseline measurement.

[d] $P < 0.05$ compared with baseline measurement.

postmenopausal assessment, cholesterol levels in women with the APOE2E2 genotype were no longer different from cholesterol levels in women with the APOE3E3 genotype. By using ANOVA, age and BMI accounted for approximately 3% of the variance of cholesterol at the premenopausal and the postmenopausal assessment. The APOE genotype explained 3.8% of the total phenotypic variation of cholesterol at the premenopausal assessment ($F_{5,1110} = 8.78$, $P < 0.001$), whereas at the postmenopausal assessment it explained 5.8% ($F_{5,1110} = 13.60$, $P < 0.001$), adjusted for age and BMI.

The mean increase in cholesterol level during menopause in women with the APOE3E3 genotype was 0.67 mmol/L (CI: 0.61, 0.72 mmol/L), adjusted for age at baseline, years of follow-up, years since menopause, BMI at baseline, and change in BMI during follow-up. Women with the APOE2E3 genotype showed a 30% smaller increase of 0.44 mmol/L (CI: 0.32, 0.56 mmol/L) with menopause. The increase in cholesterol with menopause in women with the APOE2E2 genotype was 1.45 mmol/L (CI: 0.96, 1.94 mmol/L), although the number of women was low ($n = 9$). The increase in cholesterol with menopause in women with the APOE3E4 or APOE4E4 genotype did not differ from the increase in women with the APOE3E3 genotype. The change in cholesterol level during follow-up according to the most common APOE genotypes is visualized in Fig. 2. By using ANOVA, age at baseline, years of follow-up, years since menopause, BMI at baseline, and change in BMI during follow-up explained 8.2% of the change in cholesterol level with menopause. Adjusted

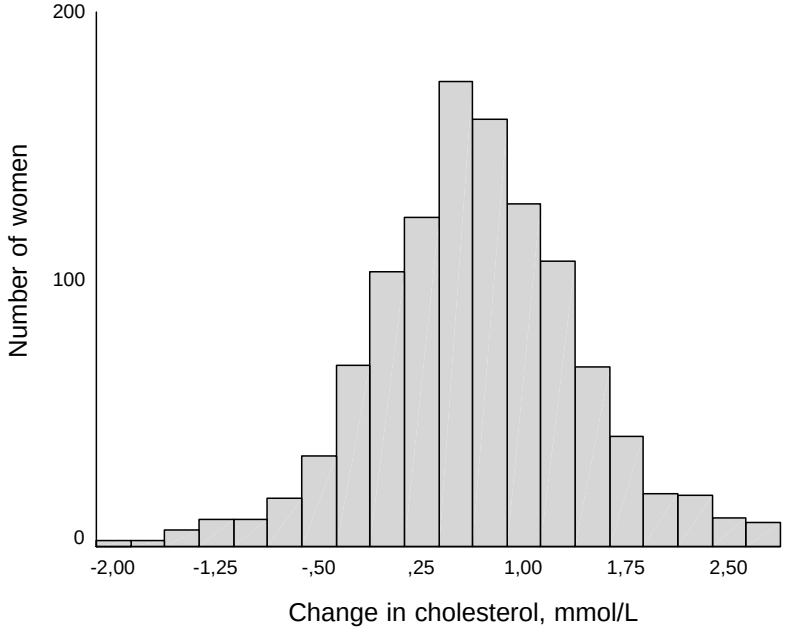

Fig. 1. Change in cholesterol levels (mmol/L) in 1116 women participating in the Eindhoven Perimenopausal Osteoporosis Studies and experiencing natural menopause during 5.9 years of follow-up, 1994–1995 to 2000.

Table 2
Distribution of APOE genotypes and allele frequencies in 1116 women participating in the Eindhoven Perimenopausal Osteoporosis Studies and experiencing natural menopause during 5.9 years of follow-up, 1994-1995 to 2000

APOE genotype[a]	No of women	Relative frequency (%)
E2E2	9	0.8
E2E3	147	13.2
E2E4	25	2.2
E3E3	687	61.6
E3E4	221	19.8
E4E4	27	2.2
Allele	Frequency	
APOE*2	0.085	
APOE*3	0.78	
APOE*4	0.13	

This study includes Dutch white women only.
[a] χ^2 Hardy–Weinberg distribution is 3.26; d.f. = 3; $P = 0.35$.

for these variables, the APOE genotype explained 2.6% of the variation of cholesterol increase with menopause ($F_{5,1110} = 5.99$, $P < 0.001$).

4. Discussion

Our results among 1116 women experiencing natural menopause show that the increase in cholesterol level during menopause is statistically significantly 30% lower in women with the APOE2E3 genotype when compared with women with the APOE3E3 genotype.

In the current large population-based study, we were able to measure intraindividual changes in cholesterol levels in women experiencing natural menopause. The increase in cholesterol was similar to previously described changes in early postmenopausal women [21]. The largest increase in cholesterol with menopause occurs in the perimenopausal years [21]. Because we included women on average 1.7 years before the cessation of their menses we were able to adequately monitor the menopausal increase in cholesterol. The increase in cholesterol level with menopause is most pronounced for LDL cholesterol [20]. Because measures of LDL cholesterol were not available in our study, we used total cholesterol, which is strongly associated with LDL cholesterol. Since any misclassification of cholesterol level is expected to be nondifferential across genotypes, it will not have biased our results. We excluded women using lipid-lowering medication at premenopausal or postmenopausal assessments. APOE*4 carriers are known to have higher cholesterol levels [22,23], therefore, we may preferentially have excluded women carrying this allele.

Whereas all women in our study experienced menopause, the increase in cholesterol level was different among strata of the APOE genotype, indicating that the APOE genotype contributes to the variation in change of cholesterol with menopause. From cross-sectional data among premenopausal and postmenopausal women it was inferred that the increase in cholesterol with menopause would be 9% among women with the APOE2E3 genotype [24], which is similar to our results. Also, the increase in cholesterol with menopause was inferred to be similar in women with the APOE3E3 or APOE3E4 genotype [24]. In the Healthy Women Study, no effect of the APOE genotype on differences in changes in cholesterol level was observed between women who became postmenopausal and age-matched women who stayed premenopausal during 3.5 years of follow-up [27]. However, only 12 and 18 postmenopausal women, respectively, were present in the APOE2E3 and APOE3E4 genotype groups. Furthermore, cholesterol concentration increases from perimenopause onward. Comparing postmenopausal women with age-matched premenopausal women [27], of whom some will be perimenopausal, may therefore lead to an underestimation of the effect of menopause. Also in this study [27], the lower values of cholesterol for women with the APOE2E3 genotype were maintained through menopause despite an increase in cholesterol levels.

In the Framingham Offspring Study, the association between the APOE genotype and cholesterol concentration was absent in premenopausal women, whereas it was present in postmenopausal women [24], suggesting that the decrease in estrogen level at the time of menopause fully

Table 3
Mean levels of cholesterol (95% CI) (mmol/L) according to APOE genotype in 1116 women participating in the Eindhoven Perimenopausal Osteoporosis Studies and experiencing natural menopause during 5.9 years of follow-up, 1994–1995 to 2000

APOE genotype	*n*	Premenopausal[a] (baseline)	Postmenopausal[a] (follow-up)	Menopausal increase[b] (absolute)	Menopausal increase[b] (relative) (%)
All women	1116	5.72 (5.66; 5.77)	6.36 (6.30; 6.42)	0.64 (0.60; 0.69)	12.3 (11.4; 13.1)
E2E2	9	4.97 (4.33; 5.60)[c]	6.31 (5.62; 6.99)	1.45 (0.96; 1.94)[c]	34.0 (24.9; 43.1)[c]
E2E3	147	5.37 (5.21; 5.52)[c]	5.81 (5.64; 5.97)[c]	0.44 (0.32; 0.56)[c]	9.2 (6.9; 11.4)[c]
E2E4	25	5.37 (4.99; 5.74)	5.92 (5.51; 6.33)[c]	0.55 (0.26; 0.84)	11.8 (6.5; 17.2)
E3E3 (reference)	687	5.73 (5.66; 5.80)	6.39 (6.32; 6.47)	0.67 (0.61; 0.72)	12.6 (11.6; 13.7)
E3E4	221	5.91 (5.79; 6.04)[c]	6.62 (6.49; 6.76)[c]	0.71 (0.61; 0.80)	12.6 (10.8; 14.4)
E4E4	27	6.18 (5.81; 6.54)[c]	6.87 (6.48; 7.26)[c]	0.71 (0.43; 0.99)	13.1 (7.8; 18.3)

[a] Adjusted for age and body mass index.
[b] Adjusted for age at baseline, years of follow-up, years since menopause, body mass index at baseline, and change in body mass index during follow-up.
[c] Statistically significantly different from APOE3E3 ($P < 0.05$).

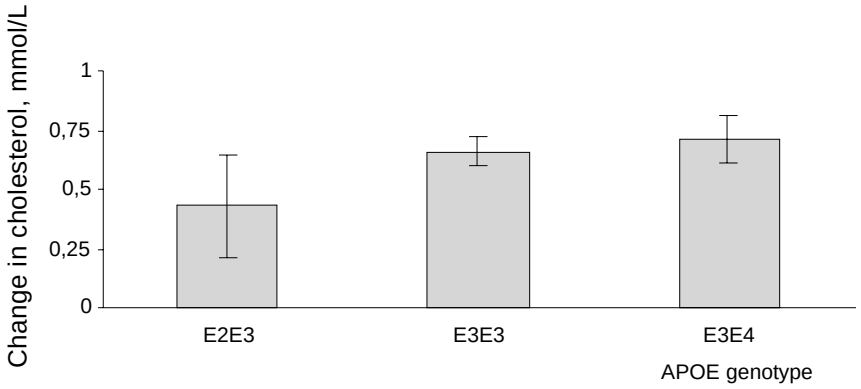

Fig. 2. Change in cholesterol levels (mmol/L)* according to APOE genotype in 1055 women participating in the Eindhoven Perimenopausal Osteoporosis Studies and experiencing natural menopause during 5.9 years of follow-up, 1994–1995 to 2000. Symbol (*) denotes the values adjusted for age at baseline, years of follow-up, and years since menopause and (†) women with the E2E2 ($n = 9$), E2E4 ($n = 25$), or E4E4 ($n = 27$) genotype are excluded.

unmasks sensitivity to the effects of the APOE genotype. In our study, we found the APOE genotype to be associated with cholesterol level at both the premenopausal and the postmenopausal assessment. Therefore, our results do not entirely support the hypothesis that menopause unmasks genetic susceptibility to the effects of the APOE genotype. However, also in our study the contribution of the APOE genotype to the total phenotypic variation of cholesterol tended to be higher in postmenopausal women than in premenopausal women, indicating that the effect of the APOE genotype on cholesterol level is amplified by menopause.

Although the number of women with the APOE2E2 genotype in our study was small ($n = 9$), women with this genotype displayed a very large increase in cholesterol level with menopause. Homozygosity for the APOE*2 allele is a very common, albeit not sufficient, cause for type III hyperlipoproteinemia (type III HLP), which is characterized by both hypercholesterolemia and hypertriglyceridemia [28]. Even though the frequency of the APOE2E2 genotype is about 1 in 100 in the general population, as in our study population, the disorder occurs only about 1 in 5000 [28]. Additional metabolic factors are usually required for full clinical expression [29]. Menopause is considered to be a factor contributing to the expression of this disorder [30,31], which gives support for the hypothesis that estrogen modifies the effect of the APOE genotype on cholesterol level.

The beneficial response of cholesterol to hormone replacement therapy in early postmenopausal women has also been found to be related to the APOE genotype [32,33]. In Finnish [32] and Japanese [33] postmenopausal women, the cholesterol-lowering effect of hormone replacement therapy, as studied in a randomized controlled trial design, was absent in women carrying the APOE*4 allele [32,33]. In the Japanese study [33], results were presented separately for women with the APOE2E3 or the APOE3E3 genotype. The cholesterol-lowering effect of hormone replacement therapy was most pronounced in women with the APOE2E3 genotype [33]. Also in our study, women with the APOE2E3 genotype showed statistically significantly different changes in cholesterol during follow-up when compared with women with the APOE3E3 genotype. Together with our results, these results suggest that estrogen modifies the effects of the APOE genotype on cholesterol level.

The mechanism relating menopause to the increase in cholesterol level is primarily thought to be due to a reduction in LDL receptor number or activity in response to the decline in blood estrogen level [34]. Although our data indicate that the APOE genotype contributes to the variation in increase in cholesterol with menopause, the variation is far from completely explained by the APOE genotype. Other factors, such as expression of estrogen receptors, which mediate the activation of the LDL receptor in the liver [35], may be involved in the increase of cholesterol with menopause.

Studies on the association between the APOE genotype and either atherosclerosis or cardiovascular disease have shown inconsistent results [36–39]. However, few population-based investigations including women have been performed on this topic. In a Dutch population-based study, the APOE2E3 genotype was inversely related to carotid artery atherosclerosis in elderly men and women [40]. This result agrees with the results of our study, which showed the increase of cholesterol level during follow-up to be lowest in women with the APOE2E3 genotype. Although cholesterol level was not an intermediate in the association between APOE genotype and carotid atherosclerosis [40], it seems reasonable to speculate that the amount of change of cholesterol with menopause would have an impact on the development or progression of atherosclerosis and cardiovascular disease. In the Healthy Women Study, the amounts of coronary and aortic atherosclerosis measured shortly after menopause were not found to be related to changes in levels of LDL cholesterol with menopause [41]. However, a longer follow-up time may be necessary for effects of higher cholesterol levels on atherogenesis to become detectable.

In conclusion, our results in 1116 women experiencing natural menopause show that the increase in cholesterol level with menopause is 30% lower in women with the APOE2E3

genotype when compared with women with the APOE3E3 genotype, indicating that the APOE genotype contributes to the variation in the increase in cholesterol with menopause.

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