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Apolipoprotein E gene is related to mortality only in normal weight individuals: The Rotterdam study

M. Carolina Pardo Silva · A. Cecile J. W. Janssens · Albert Hofman · Jacqueline C. M. Witteman · Cornelia M. van Duijn

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Abstract *Objective* To investigate the relationship between the apolipoprotein E (*APOE*) gene and the risk of mortality in normal weight, overweight and obese individuals. *Methods and Results* In a population-based study of 7,983 individuals aged 55 years and older, we compared the risks of all-cause and coronary heart disease (CHD) mortality by *APOE* genotype, both overall and in subgroups defined by body mass index (BMI). We found significant evidence for interaction between APOE and BMI in relation to total cholesterol ($p = 0.04$) and HDL cholesterol ($p < 0.001$). Overall, *APOE**2 carriers showed a decreased risk of all-cause mortality. Analyses within BMI strata showed a beneficial effect of *APOE**2 only in normal weight persons (adjusted hazard ratio (HR) 0.7[95% CI 0.5–0.9]). *APOE**2 was not associated with a lower risk of all-cause mortality in overweight or obese persons. The effect of *APOE**2 in normal weight individuals tended to be due to the risk of CHD mortality (adjusted HR 0.5 [95% CI 0.2–1.2]). *Conclusion* The *APOE**2 allele confers a lower risk of all-cause mortality only to normal weight individuals.

Keywords *APOE* · BMI · Cholesterol · Mortality · CHD mortality

M. C. Pardo Silva · A. Hofman · J. C. M. Witteman · C. M. van Duijn (✉)
Department of Epidemiology & Biostatistics, Erasmus MC University Medical Center Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands
e-mail: c.vanduijn@erasmusmc.nl

A. C. J. W. Janssens
Department of Public Health, Erasmus MC University Medical Center Rotterdam, Rotterdam, The Netherlands

Apolipoprotein E is a plasma protein involved in the metabolism of cholesterol. The apolipoprotein E isoforms ε2, ε3 and ε4, determined by the three alleles of the apolipoprotein E gene (*APOE**2, *APOE**3 and *APOE**4), differ in their binding affinity to serum cholesterol and hence in their ability to clear dietary fat from the blood [1]. Accordingly, total serum cholesterol levels differ between *APOE* genotypes, with *APOE**2 carriers having lower total serum cholesterol levels and *APOE**4 carriers having higher levels than *APOE**3 homozygotes [1–4].

In line with the clear differences in total serum cholesterol levels associated with the *APOE* genotype, one would expect that *APOE**2 carriers have lower risks of cardiovascular morbidity and mortality and *APOE**4 carriers have higher risks. Yet, the evidence for this association is inconsistent. A recent meta-analysis showed that *APOE**4 was associated with a higher risk of coronary heart disease (CHD), but found no association with *APOE**2 [5]. *APOE**2 has been associated with a lower risk of mortality [6] and *APOE**4 with a higher risk [7–11], but also these relationships were not found by others [12–19].

A possible explanation for these inconsistencies may be found in the role of body weight. Several studies have demonstrated that the relationship between the *APOE* gene and lipids differs between normal weight, overweight and obese individuals [20–22]. Total serum cholesterol levels were higher among obese than among non-obese *APOE**4 carriers [20], and obese *APOE**2 carriers had higher serum triglycerides and higher LDL-cholesterol levels than non-obese *APOE**2 carriers [20, 21]. While *APOE**2 carriers generally have the lowest cholesterol levels, those with high BMI were found to have similar total serum cholesterol levels compared to *APOE**3 homozygotes and *APOE**4 carriers [20]. These findings suggest that the adverse effects of high body weight may outweigh the

beneficial effect of *APOE**2 status. If so, a protective effect of *APOE**2 on mortality may be largest among normal weight individuals.

Despite this evidence for a moderating effect of body weight on the relation between the *APOE* gene and total serum cholesterol, no studies so far have performed a stratified analysis on the relationship between the *APOE* gene and the risk of mortality. The aim of the present study was to investigate the extent to which the relationship between *APOE* genotypes and the risk of mortality differs between normal weight, overweight and obese individuals. We examined this relationship for both all-cause and CHD mortality.

Methods

Study population and procedures

The present analyses were performed in the Rotterdam Study, an ongoing population-based study on the determinants of disease and disability among persons 55 years and older. Details of this study have been described elsewhere [23]. Baseline data were collected between 1990 and 1993. During home visits, a trained investigator obtained information on health status, medical history, medication use and lifestyle. Subsequently, participants were invited to the study center where they underwent an extensive clinical examination. The Medical Ethics Committee of the Erasmus Medical Center approved the study protocol and all participants provided written informed consent. From 10,275 eligible subjects, 7,983 (78%) individuals agreed to participate in the study and were examined at baseline.

Data collection

Clinical and laboratory assessments

The clinical examination included weight, height, systolic and diastolic blood pressure, serum glucose, total serum cholesterol and high-density lipoprotein (HDL) cholesterol levels. BMI was computed as weight (kg) divided by height squared (m^2). Participants were classified as underweight (BMI < 18.5 kg/m^2), normal weight (18.5 kg/$m^2 \leq$ BMI < 25 kg/m^2), overweight (25 kg/$m^2 \leq$ BMI < 30 kg/m^2) or obese (BMI ≥ 30 kg/m^2) [24]. The waist and hip circumferences were measured, and the waist-hip-ratio (WHR) was calculated as indirect assessment of abdominal fat. Systolic and diastolic blood pressures were measured twice in a sitting position after 5 min rest using a random-zero sphygmomanometer. The mean of the two measurements was used for the analyses. Hypertension was defined as systolic blood pressure higher than 160 mmHg, diastolic blood pressure higher than 100 mmHg, or the use of antihypertensive medication indicated to treat high blood pressure (hypertension grades 2 and 3) [25]. Diabetes was diagnosed based on a random or post-load glucose level higher than 11.0 mmol/l and/or the use of anti-diabetic medication [26]. Total serum cholesterol and HDL-cholesterol were measured using an automated enzymatic procedure [27]. Hypercholesterolemia was defined as a total serum cholesterol level above 6.2 mmol/l [28]. The *APOE* genotype was determined on DNA samples using a polymerase chain reaction followed by enzymatic digestion using methods previously described [29]. The frequencies of *APOE* genotypes were *APOE* 2/2 0.8%, *APOE* 2/3 12.8%, *APOE* 2/4 2.7%, *APOE* 3/3 58.4%, *APOE* 3/4 22.9% and *APOE* 4/4 2.4%. The proportions of the *APOE* alleles and genotypes were in Hardy-Weinberg equilibrium ($p = 0.71$).

Mortality data

Information on the vital status of the participants was obtained at regular intervals from the municipal population registry. Causes of death were obtained from the general practitioners by means of a standardized questionnaire relating to the circumstances of death, most likely cause of death and time and place of death. Two independent research physicians coded all events according to the International Classification of Diseases, 10th edition (ICD-10) [30]. CHD mortality was defined as death from diseases coded I20–I25, I46, I50 or R96. Mortality data were available up to December 2003.

Statistical analyses

Of the 7,983 individuals who participated at baseline, 5,817 (73%) had *APOE* genotyped successfully and had complete information on BMI, WHR and cholesterol levels. Because persons with an extremely low BMI may suffer from life-threatening diseases such as cancer, individuals with a BMI below 18.5 kg/m^2 ($n = 51$) were excluded from the analyses. Also, *APOE* 2/4 individuals were excluded from the analyses ($n = 158$) to distinguish the effect of the *APOE* alleles unambiguously. Hence, data from 5,608 participants were available for the analyses.

Differences in baseline characteristics by *APOE* genotypes were tested using the chi-squared statistic (categorical variables) or ANOVA (continuous variables). P for trend was obtained by testing the linearity of the sum of squares from the ANOVA analyses. Multiple linear regression analysis was used to assess the interaction

Table 1 Baseline characteristics by *APOE* genotype groups

n	*APOE**2 carriers 782	*APOE* 3/3 3,369	*APOE**4 carriers 1,457	*p*
Sex (men)	38	42	42	0.05
Age at entry (years)	68.8 (8.8)	68.9 (8.7)	68.3 (8.5)	0.14
Body mass index (kg/m^2)	26.7 (3.6)	26.4 (3.6)	26.1 (3.4)	<0.01
Normal weight	34	39	40	
Overweight	49	47	47	<0.05
Obese	17	14	13	
Waist-to-hip ratio	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)	0.18
Systolic blood pressure (mmHg)	140 (22)	140 (23)	138 (2)	0.04
Diastolic blood pressure (mmHg)	74 (11)	74 (12)	73 (11)	0.06
Hypertension	36	34	33	0.25
Total serum cholesterol (mmol/l)	6.4 (1.3)	6.6 (1.2)	6.8 (1.2)	<0.001
HDL-cholesterol (mmol/l)	1.4 (0.4)	1.3 (0.3)	1.3 (0.4)	<0.001
Cholesterol lowering medication	3	2	3	0.71
Hypercholesterolemia	49	60	66	<0.001
Smoking status (current)	39	35	33	0.02
Education level				
Lower	43	43	45	
Intermediate	43	42	40	0.21
Higher	14	15	15	
Diabetes mellitus	10	10	9	0.75

Values are means (standard deviations) for continuous variables and percentages for categorical variables. *p* values are obtained by ANOVA for continuous variables and by χ^2 for categorical variables

between *APOE* genotype and BMI on total serum cholesterol and HDL-cholesterol levels. Differences in survival probabilities were examined by comparing Kaplan-Meier survival curves and tested using the log rank test. Kaplan-Meier plots were constructed using age as the time scale to take proper account of the effect of age [31, 32]. Risks of mortality were quantified as hazard ratios (HRs) using Cox proportional hazards analyses with age as the time scale. The most common genotype (*APOE* 3/3) was used as the reference category. The proportionality assumption of all models was verified by testing the Schoenfeld residuals [33]. Because the proportionality assumption was not met, HRs were calculated for early and late mortality using 80 years as the cut-off age. This cut-off age was selected because it led to proportionality of the models below and above the cut-off age. HRs were calculated adjusted for gender, smoking status, education level, total serum cholesterol, HDL-cholesterol, waist to hip ratio hypertension and diabetes mellitus.

Results

The mean age at entry was 68.7 years (SD = 8.7 years) and 41.8% of the participants were men. The mean follow up time was 11.1 years (SD = 3.8 years). Baseline characteristics of *APOE**2 carriers, *APOE**3 homozygotes and *APOE**4 carriers are presented in Table 1. As expected, cholesterol levels differed significantly between the genotype groups. The prevalence of hypercholesterolaemia was lowest among *APOE**2 carriers (49%) and highest among *APOE**4 carriers (66%). Furthermore, there were statistically significant differences in BMI scores between the genotype groups, with *APOE**2 carriers having the highest mean BMI (26.7 ± 3.6 kg/m^2) and *APOE**4 carriers the lowest (26.1 ± 3.4 kg/m^2). There were no statistically significant differences in WHR between the genotype groups.

Figure 1 presents mean total serum cholesterol and HDL-cholesterol levels by *APOE* genotype in normal weight, overweight and obese persons. In all BMI strata, *APOE**2 carriers had the lowest and *APOE**4 carriers the highest total serum cholesterol ($p_{\text{for trend}} < 0.01$). The association between HDL-cholesterol and *APOE* genotype was in the opposite direction ($p_{\text{for trend}} < 0.01$). Note that even though *APOE**2 carriers had the lowest total serum cholesterol levels in each BMI group, still 50% of the overweight and 57% of the obese *APOE**2 carriers had hypercholesterolaemia compared to 41% of the normal weight *APOE**2 carriers ($p = 0.003$). There was no

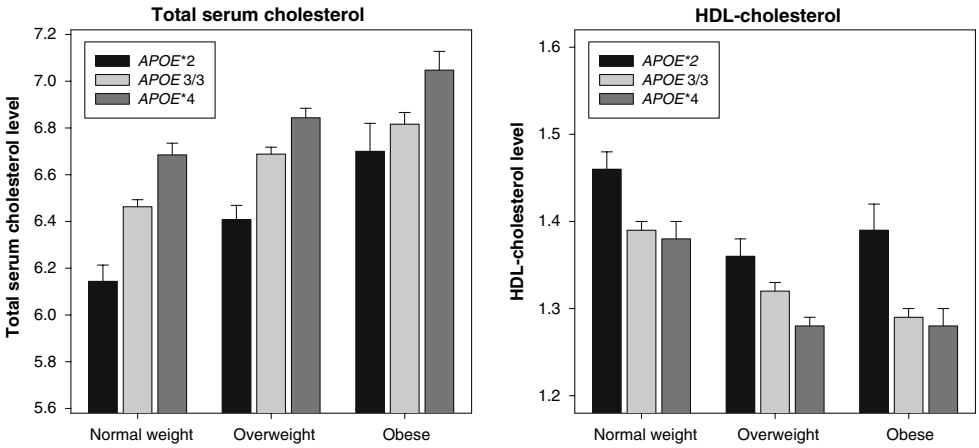

Fig. 1 Mean total serum cholesterol and HDL-cholesterol levels by *APOE* genotypes for normal weight, overweight and obese individuals. HDL: high-density lipoproteins

significant evidence for interaction between *APOE* genotype and BMI in relation to total serum cholesterol ($p = 0.26$) and HDL-cholesterol ($p = 0.25$). However, when BMI was analyzed as a continuous trait, then the p value for interaction was 0.04 for total serum cholesterol and less than 0.001 for HDL-cholesterol.

A total of 1,918 deaths occurred during follow-up. Of those, 257 occurred in 782 *APOE**2 carriers (33%), 1,160 in 3,369 *APOE**3 homozygotes (33%) and 501 in 1,457 *APOE**4 carriers (34%). Differences between the Kaplan-Meier survival curves of the *APOE**2 carriers, *APOE**3 homozygotes and *APOE**4 carriers were small but statistically significant ($p = 0.03$) with *APOE**2 carriers having slightly lower mortality risks (Fig. 2). After adjustment for other cardiovascular risk factors, *APOE* genotype was not associated with all-cause mortality before or after 80 years of age in the overall population (Table 2). The differences between the curves were only statistically significant in normal weight individuals ($p = 0.04$; Fig. 3). Normal weight carriers of the *APOE**2 allele had a significantly lower risk of mortality before age 80 years (HR [95% CI] = 0.7 [0.5–0.9]), but not after age 80 years (crude HR [95% CI] = 1.0 [0.8–1.4]). There were no significant differences in the risk of mortality between genotype groups among overweight or obese persons (Table 2).

To investigate whether the lower risk of mortality observed among normal weight *APOE**2 carriers was due to a lower risk of mortality from CHD, we calculated the HRs separately for CHD mortality. *APOE* genotype was not significantly associated with CHD mortality. Analysis within BMI strata showed that normal weight *APOE**2 carriers tended to have lower risks of CHD mortality (adjusted HR [95% CI] = 0.5 [0.2–1.2]). No differences in the risk of CHD mortality between genotype groups were found in overweight or obese participants (Table 3).

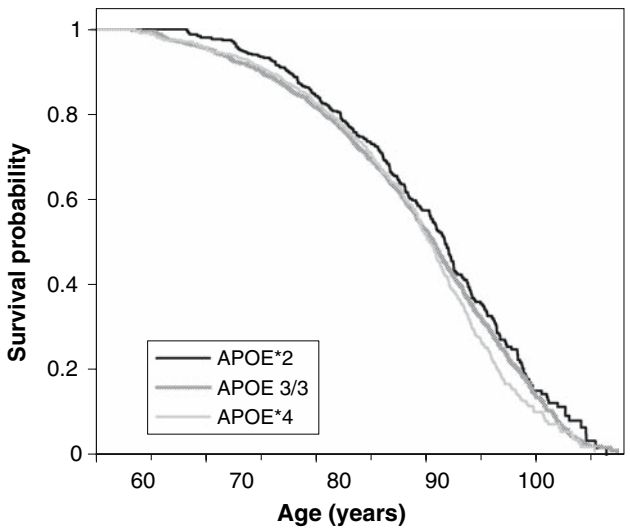

Fig. 2 Kaplan Meier survival curves for all-cause mortality by *APOE* genotypes

Discussion

Our study shows that the *APOE**2 allele has a protective effect on overall mortality. This protective effect seems to be limited to mortality from CHD. Analyses within BMI strata demonstrated that this genetic advantage of *APOE**2 was observed only in normal weight individuals, but not in overweight and obese *APOE**2 carriers.

The results of the present analyses may seem to contradict our earlier work in this population. We previously reported that the *APOE* gene was not significantly related to mortality [12]. While our findings come from the same population, the mean follow-up time of the participants has increased from 5.4 years to 11.1 years and the number of deaths increased by 53% from 18% to 34%. This extended follow-up has improved the statistical power of our study to show a modest effect of *APOE* genotype on overall mortality. Research on the relationship between *APOE* and mortality has merely provided conflicting results [6–19, 34].

Table 2 Incidence rates and hazard ratios for all-cause mortality by *APOE* genotypes and BMI categories

	Follow-up Deaths	(py)	Incidence rate Deaths/1,000 py (95% CI)	Hazard ratio (95% CI)	Follow-up Deaths	(py)	Incidence rate Deaths/1,000 py (95% CI)	Hazard ratio (95% CI)
	Before 80 years of age				After 80 years of age			
All								
APOE*2 carriers	101	6,782	14.9 (12.3–18.1)	0.9 (0.7–1.1)	156	21,764	7.2 (6.1–8.4)	0.9 (0.8–1.1)
APOE 3/3	481	28,703	16.8 (15.3–18.3)		679	95,808	7.1 (6.6–7.6)	
APOE*4 carriers	198	12,693	15.6 (13.6–17.9)	1.0 (0.8–1.2)	303	41,637	7.3 (6.5–8.1)	1.2 (1.0–1.4)
Total	780	48,178	16.2 (15.1–17.4)		1,138	159,209	7.1 (6.7–7.6)	
Normal weight								
APOE*2 carriers	28	2,370	11.8 (8.2–17.1)	0.7 (0.5–0.9)	57	7,289	7.8 (6.0–10.1)	1.0 (0.8–1.4)
APOE 3/3	206	11,314	18.2 (15.9–20.9)		256	38,045	6.7 (6.0–7.6)	
APOE*4 carriers	78	5,080	15.4 (12.3–19.2)	1.0 (0.7–1.3)	127	17,305	7.3 (6.2–8.7)	1.3 (0.9–1.7)
Total	312	18,764	16.6 (14.9–18.6)		440	62,639	7.0 (6.4–7.7)	
Overweight								
APOE*2 carriers	59	3,305	17.9 (13.8–23.0)	1.1 (0.9–1.5)	65	11,064	5.9 (4.6–7.5)	0.9 (0.7–1.1)
APOE 3/3	217	13,573	16.0 (14.0–18.3)		310	45,366	6.8 (6.1–7.6)	
APOE*4 carriers	90	6,047	14.9 (12.1–18.3)	0.9 (0.7–1.2)	137	19,398	7.1 (6.0–8.4)	1.2 (1.0–1.5)
Total	366	22,924	16.0 (14.4–17.7)		512	75,827	6.8 (6.2–7.4)	
Obese								
APOE*2 carriers	14	1,107	12.6 (7.5–21.4)	1.0 (0.5–1.8)	34	3,411	10.0 (7.1–14.0)	1.0 (0.6–1.5)
APOE 3/3	58	3,816	15.2 (11.7–19.7)		113	12,398	9.1 (7.6–11.0)	
APOE*4 carriers	30	1,566	19.2 (13.4–27.4)	1.3 (0.9–2.0)	39	4,934	7.9 (5.8–10.8)	1.1 (0.8–1.6)
Total	102	6,489	15.7 (12.9–19.1)		186	20,742	9.0 (7.8–10.4)	

Hazard ratios adjusted for sex, waist-hip-ratio, smoking, education level, total serum cholesterol, HDL cholesterol hypertension and diabetes; py: person-years; CI: Confidence interval

Three out of eleven studies showed a protective effect of *APOE**2 [6, 14, 18]. and three a deleterious effect of *APOE**4 [8–10]. The latter effect may partly mediated by the increased risk of Alzheimer disease in association with *APOE**4 and mortality [10, 11].

Note that our analysis showed small significant difference in the Kaplan-Meier survival curves, demonstrating a protective effect of *APOE**2 before age 80. This may be due to the effect of age that becomes apparent when performing survival analysis with age as time scale, as we did in the present paper. Such finding is compatible with previous reports suggesting that genes affecting human lifespan might be age-specific [35]. Genetic and environmental interactions at older ages vary from those at early ages and therefore explain the differences in the association between *APOE* and mortality before and after 80 years of age. A previous report showed that among nonagenarians, *APOE* genotype has no effect on cognitive function, cognitive decline or survival [36].

In line with previous studies [20–22], we found that the total serum cholesterol levels associated with *APOE* genotype differed between normal weight, overweight and obese individuals. As expected, mean levels of total serum cholesterol increased with BMI and *APOE**2 carriers had the lowest mean total serum cholesterol in each BMI group. *APOE**4 carriers had higher cholesterol levels and lower HDL-cholesterol concentrations than *APOE**2

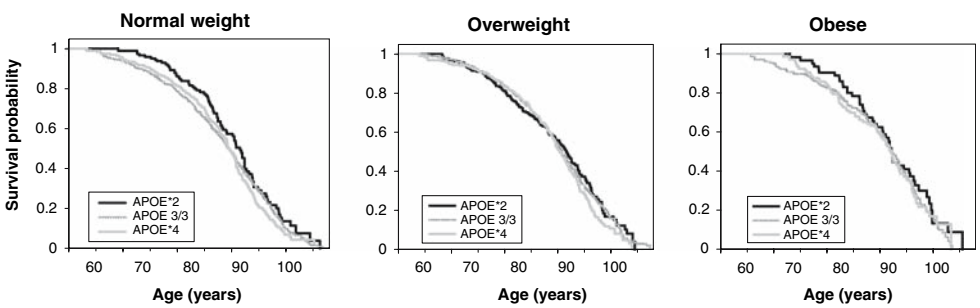

Fig. 3 Kaplan Meier survival curves for all-cause mortality by *APOE* genotypes for normal weight, overweight and obese individuals

Table 3 Incidence rates and hazard ratios for CHD mortality by *APOE* genotypes and BMI categories

	Follow-up Deaths	(py)	Incidence rate Deaths/1,000 py (95% CI)	Hazard ratio (95% CI)	Follow-up Deaths	(py)	Incidence rate Deaths/1,000 py (95% CI)	Hazard ratio (95% CI)
	Before 80 years of age				After 80 years of age			
All								
APOE*2 carriers	26	6,782	3.8 (2.6–5.6)	1.0 (0.6–1.5)	41	2,103	19.5 (14.4–26.5)	1.1 (0.8–1.5)
APOE 3/3	126	28,703	4.4 (3.7–5.2)		171	8,744	19.6 (16.8–22.7)	
APOE*4 carriers	59	12,693	4.6 (3.6–6.0)	1.1 (0.8–1.4)	66	3,472	19.0 (14.9–24.2)	1.1 (0.8–1.4)
Total	211	48,178	4.4 (3.8–5.0)		278	14,319	19.4 (17.3–21.8)	
Normal weight								
APOE*2 carriers	5	2,370	2.1 (0.9–5.1)	0.5 (0.2–1.2)	15	663	22.6 (13.6–37.5)	1.6 (0.9–2.9)
APOE 3/3	56	11,314	4.9 (3.8–6.4)		48	3,075	15.6 (11.8–20.7)	
APOE*4 carriers	26	5,080	5.1 (3.5–7.5)	1.2 (0.7–1.9)	21	1,286	16.3 (10.6–25.1)	1.1 (0.6–1.8)
Total	87	18,764	4.6 (3.8–5.7)		84	5,024	16.7 (13.5–20.7)	
Overweight								
APOE*2 carriers	16	3,305	4.8 (3.0–7.9)	1.4 (0.8–2.4)	15	989	15.2 (9.1–25.2)	0.7 (0.4–1.3)
APOE 3/3	51	13,573	3.8 (2.9–4.9)		92	4,035	22.8 (18.6–28.0)	
APOE*4 carriers	21	6,047	3.5 (2.3–5.3)	0.8 (0.5–1.4)	35	1,619	21.6 (15.5–30.1)	1.0 (0.7–1.5)
Total	88	22,924	3.8 (3.1–4.7)		142	6,643	21.4 (18.1–25.2)	
Obese								
APOE*2 carriers	5	1,107	4.5 (1.9–10.8)	1.1 (0.4–3.4)	11	450	24.4 (13.5–44.1)	1.4 (0.7–3.1)
APOE 3/3	19	3,816	5.0 (3.2–7.8)		31	1,634	19.0 (13.3–27.0)	
APOE*4 carriers	12	1,566	7.7 (4.4–13.5)	1.6 (0.8–3.5)	10	568	17.6 (9.5–32.7)	1.1 (0.5–2.2)
Total	36	6,489	5.5 (4.0–7.7)		52	2,651	19.6 (14.9–25.7)	

Hazard ratios adjusted for sex, waist-hip-ratio, smoking, education level, total serum cholesterol, HDL cholesterol hypertension and diabetes; py: person-years; CI: Confidence interval

carriers despite the presence of a lower BMI and equal WHR. Previous reports showed that *APOE* genotype, BMI and WHR determine together the lipid levels [20–22, 37]. However, in our study abdominal fat, as measured by WHR, did not have any effect on the lipid levels by *APOE* genotype, while BMI was an important determinant. The significant interaction between *APOE* genotype and BMI in relation to total serum cholesterol found previously [20], was also supported by this study. Although the mechanism of such interaction has not been yet elucidated, evidence suggests that obesity and abdominal fat increase low density lipoprotein cholesterol and therefore total cholesterol at a higher degree among *APOE**4 carriers [20–22, 37]. This is in line with our observation that the deleterious effect of increased BMI prevails over the beneficial effects of the *APOE**2 allele. Furthermore, previous research in the Rotterdam Study and the Dutch population indicated that among *APOE**2 homozygous carriers, the expression of Hyperlipoproteinemia type III is determined to a great extent by hyperinsulinemia and the presence of insulin resistance syndrome [38], conferring and increased risk of CHD through accelerated atherosclerosis [39].

Although the association between high BMI and high cholesterol levels is well known and the *APOE* gene is associated with cholesterol levels as previously described, this is the first study that examined the effect of *APOE* genotype on mortality in BMI strata. We found that presence of the *APOE**2 allele was associated with a decreased risk of mortality only in normal weight individuals and that this lower risk was partly explained by lower risk of CHD mortality. These results were in line with the lower total cholesterol and higher HDL levels among normal weight *APOE**2 carriers. Our finding that the protective effect of *APOE**2 was only found in normal weight individuals suggests that the negative influences of increasing BMI outweigh the positive effects of genetic predisposition. When these results are confirmed by other population-based studies, a challenging question remains whether weight loss in overweight and obese *APOE**2 carriers restores the genetic advantage of their *APOE* status.

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