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*Original Article*

# Effects of Gender and Age on the Association of Apolipoprotein E ε4 with Bone Mineral Density, Bone Turnover and the Risk of Fractures in Older People*

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**Abstract.** The aim of this study was to examine whether the presence of apolipoprotein E ε4 (ApoE ε4) is associated with a lower bone mineral density (BMD), lower quantitative ultrasound (QUS) measurements, higher bone turnover and fracture risk, and whether these relations are modified by gender and age. A total of 1406 elderly men and women ($\geqslant$65 years) of the Longitudinal Aging Study Amsterdam (LASA) participated in this study. In all participants, QUS measurements were assessed, as well as serum osteocalcin (OC) and urine deoxypyridinolin (DPD/Cr urine). Follow-up of fractures was done each three months. In a subsample ($n$=604), total body bone mineral content (BMC) and BMD of the hip and lumbar spine were measured. In addition, prevalent vertebral deformities were identified on radiographs. In women, the presence of ApoE ε4 was associated with significantly lower femoral neck BMD (g/cm$^2$; mean ± SEM; ε4+, 0.64 ± 0.01 vs. ε4−, 0.67 ± 0.01; $p$ = 0.04), lower trochanter BMD (g/cm$^2$; mean ± SEM; ε4+, 0.58 ± 0.01 vs. ε4–, 0.61 ± 0.01; $p$ = 0.01) and lower total body BMC (g; mean ± SEM; ε4+, 1787 ± 40.0 vs. ε4–, 1863 ± 23.8; $p$ = 0.04). Women with ApoE ε4 also had a higher risk of severe vertebral deformities (OR=2.78; 95%CI: 1.21–6.34). In men, the associations between ApoE status and both hip BMD and QUS depended on age. Only among the younger men (65–69 years) was the presence of ApoE ε4 associated with lower BMD values. Bone markers and fractures were not associated with ApoE ε4 in either women, or men. In conclusion, this large community-based study confirms the importance of ApoE ε4 as a possible genetic risk factor related to BMD and vertebral deformities and demonstrates that its effect is gender related, and depends on age in men only.

**Keywords:** ApoE; Bone markers; Bone mineral density; Fractures; Ultrasound; Vertebral deformities

## Introduction

Osteoporosis is a complex multifactorial disease, which has an important genetic background [1–3]. Several genes were found to be associated with differences in bone mineral density (BMD) or increased fracture risk, such as polymorphism of the vitamin D receptor (VDR) gene [4], estrogen receptor gene [5] and collagen type 1A1 gene [6]. The identification of genes may be useful for both the prediction of fractures and to elucidate the biological mechanisms underlying osteoporosis.

Recently, apolipoprotein E (ApoE), a well-known genetic risk marker for Alzheimer's disease, has also been shown to be associated with osteoporosis [7–11]. ApoE is a plasma protein, encoded by a gene on chromosome 19 [12], that mediates the uptake of lipoproteins into target tissues, such as the liver and bone. Whether ApoE ε4 is a genetic risk factor for osteoporosis is controversial. Previous studies demon-

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strated that older people with ApoE ε4 compared to people without ApoE ε4 had lower lumbar spine BMD [8–10], higher levels of osteocalcin [8], and a two- to fourfold increase in risk of fractures [7,9,11], but other studies could not confirm these ApoE genotype differences in osteoporosis [13–15].

BMD and the susceptibility to fractures vary between men and women, and change with age. In the third decade of life, peak bone mass is achieved. In women, bone density starts to decline rapidly after the menopause, whereas in men bone loss occurs gradually with age. Few data exist on whether genetic factors are responsible for these gender and age-related differences in the pathogenesis of osteoporosis [16]. Possibly, the association between ApoE ε4 and osteoporosis is also modified by gender or age.

The aim of the present study was to determine whether the presence of ApoE ε4 is associated with a lower BMD, lower quantitative ultrasound (QUS) measurements, higher bone turnover and fracture risk in older men and women, and to explore whether these relations are modified by gender and/or age.

## Methods

### *Study Sample*

The Longitudinal Aging Study Amsterdam (LASA) is an ongoing cohort study on predictors and consequences of changes in autonomy and well being in the aging population in the Netherlands. [17] The sampling and data collection procedures have been described in detail elsewhere [18]. Briefly, a sample of older men and women (aged 55–85 years), stratified by age, sex, urbanization and five years expected mortality, was drawn from the population registers of 11 municipalities in areas in the west (Amsterdam and its vicinity), northeast (Zwolle and vicinity) and south (Oss and vicinity) of the Netherlands. Data collection took place in 1992/1993, in 1995/1996 and in 1998/1999.

Figure 1 shows the recruitment of the subjects. Of the 3805 older persons who were initially approached, 3107 (81.7%) took part in the baseline examination in 1992/1993. Non-response was related to age ($p < 0.001$), the oldest persons being less likely to participate. In 1995/96, 2204 of the 2639 (83.5%) eligible respondents completed the main interview. Loss to follow-up between the first and second cycle was mainly due to mortality [18]. The present study on ApoE ε4 and osteoporosis was performed within a subsample of the LASA sample, which consisted of participants who participated in the medical interview of the second data collection and who were born in 1930 and before (aged 65 years and older as of the first of January 1996). Of the 1720 eligible respondents, 1509 (87.8%) took part in the medical interview. After this interview at home, participants were invited to the VU University Medical Center (VUMC) (respondents living in Amsterdam and vicinity) or a health care center near their homes

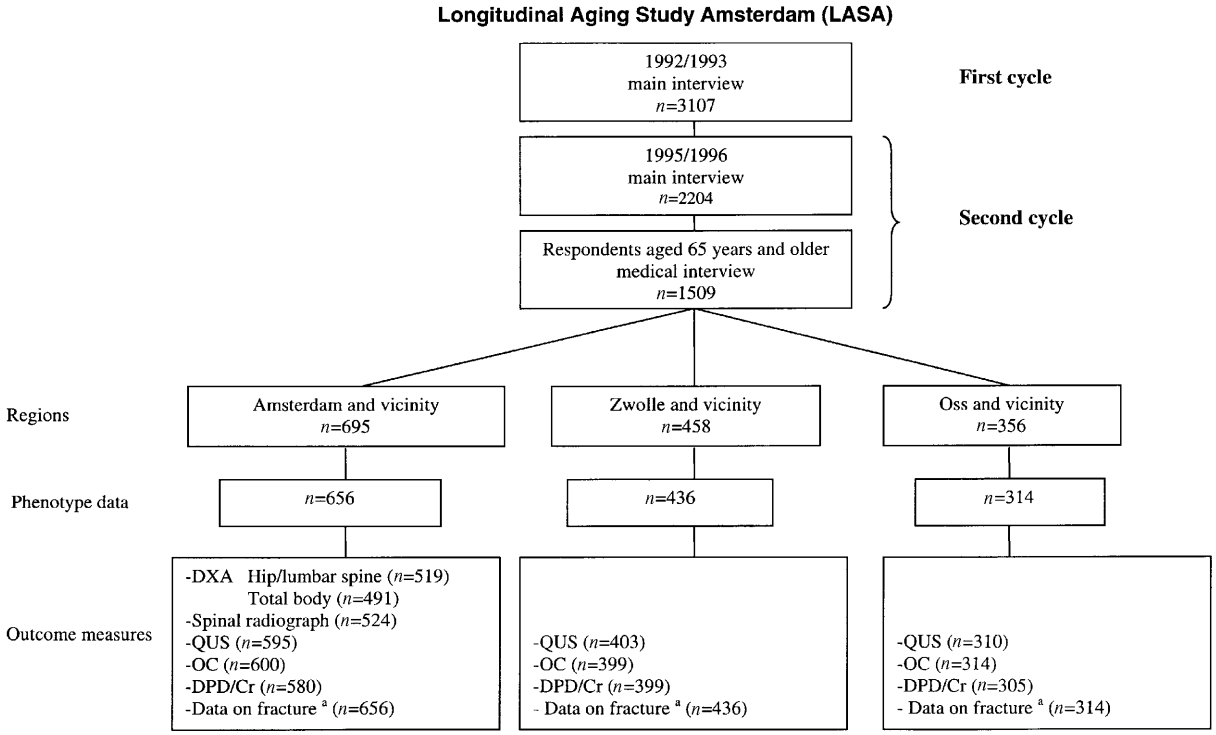


**Fig. 1.** Recruitment of participants. [a]Fractures that occurred between 1992/1993 and 1995/1996 were assessed retrospectively in 1995/1996, whereas fractures that occurred between 1995/1996 and 1998/1999 were assessed prospectively. QUS=quantitative ultrasound measurements; DXA=dual X-ray absorptiometry.

(respondents living in Zwolle or Oss and vicinity) where BMD and QUS measurements were performed and blood and urine samples were obtained. ApoE phenotype could be determined in 1406 (686 men and 720 women) of the 1509 respondents (93.2%). Of these, fasting levels of serum osteocalcin (OC) were available in 1313, urine deoxypyridinolin (DPD/Cr urine,) in 1284, and QUS measurements in 1308 respondents. Prospective information on fractures between the first cycle of data collection in 1992/93 until the third data collection in 1998/99 was obtained for all the 1406 respondents. In a subsample ($n$ = 604), which included participants who were living in the west of the Netherlands, BMD of the hip and lumbar spine ($n$ = 519), total BMC ($n$ = 491) and prevalent vertebral deformities ($n$ = 524) were determined at the end of 1995 or in 1996. Except incident fractures, measures were cross-sectionally determined during the second measurement cycle in 1995/96. Of the 1720 respondents who were eligible, the respondents who did not have QUS measurements ($n$ = 412), were more often female, were older, had a lower level of education, were more often cognitively impaired and had lower physical performance scores ($p < 0.05$). The same applies for respondents who did not have BMD measurements or spine X-rays, except that there were no differences in sex.

All interviews were conducted by specially trained and intensively supervised interviewers (main interview) and nurses (medical interview) and were tape-recorded in order to monitor the quality of the data. Informed consent was obtained from all respondents. The study was approved by the Medical Ethics Committee of the VUMC and conducted according to the principles of the Helsinki declaration.

## Measurements

*Apolipoprotein E Phenotyping.* Serum samples were obtained during the examinations in 1992 ($n$ = 909) and in 1995 ($n$ = 497), and centrifuged and stored at –80° C until determination of ApoE phenotype in 1999. ApoE phenotypes were determined by isoelectric focusing of delipidated serum samples, followed by immunoblotting [19]. ApoE status was classified as ε4 carriers for respondents with an ApoE ε4 allele (phenotypes ε2/ε4, ε3/ε4, ε4/ε4) and as no-ε4 carriers for respondents without an ApoE ε4 allele in the phenotype (phenotypes ε2/ε2, ε2/ε3, ε3/ε3).

*Bone Mineral Density.* Total body BMC and BMD of the hip (total hip, femoral neck, trochanter) and lumbar spine (L1–L4) were measured by dual-energy X-ray absorptiometry (DXA, Hologic, QDR 2000, Hologic Inc., Waltham, Massachusetts, USA; software version V5.67A). The coefficients of variation of DXA bone measurements were reported as 1.9% for lumbar spine BMD, 2.3% for femoral neck BMD, 1.6% for total hip BMD, and 0.4% for total body BMC [20]. For hip density, the right hip was scanned. In people with single hip joint replacement, the other hip was scanned. Respondents with both hips replaced were excluded ($n$ = 13). Appendicular muscle mass (AMM) and total body fat mass (FM) were also measured with DXA. AMM was calculated by summing the fat-free, bone-free mass of both legs and both arms. The coefficients of variation of DXA body composition measurements are 2–3% for both total body fat and AMM [21].

*Quantitative Ultrasound Measurements.* QUS data were obtained using the CUBA Clinical instrument (McCue Ultrasonics, Winchester, UK). Broadband ultrasound attenuation (BUA) (dB/MHz) and speed of sound (SOS) (m/s) were measured twice in both the right and left calcaneus. Mean BUA and SOS values were calculated from these four measurements. The short-term precision of SOS and BUA were reported as coefficients of variation (CV) of 1.4% and 3.4%, respectively, whereas long-term precision of SOS and BUA were reported as CV of 1.3% and 4.9%, respectively [22].

*Bone Markers.* Fasting morning serum levels of intact osteocalcin (OC) and urinary excretion of deoxypyridinoline (DPD) were determined at the Endocrinological Laboratory of the VUMC. Serum OC was measured with an immunoradiometric assay (Biosource Diagnostics, Fleuris, Belgium). DPD was determined with a competitive immunoassay on the automated ACS 180 System (Chiron Diagnostics, Emeryville, USA). The values were corrected for creatinine concentration (Cr) in the same urine sample. In our sample, the interassay CVs of OC and DPD were 9% and 8%, respectively.

*Prevalent Vertebral Deformities.* Lateral radiographs of the thoracic and lumbar spine (T4–L5) were made at the end of 1995 or in 1996 in each participant according to the protocol of the European Vertebral Osteoporosis Study [23]. The thoracic film was centred at T7 and the lumbar film at L2. The X-ray tube-to-film distance was 115 cm. The presence and degree of prevalent vertebral deformity were assessed by a semiquantitative method [24] (mild deformity: 20–25% reduction in anterior, central or posterior vertebral height; moderate deformity: 25–30% reduction in vertebral height; severe deformity: $>$30% reduction in vertebral height), as described elsewhere [25]. In a random sample of 50 radiographs, the intra-observer agreement of this semiquantitative method was tested with weighted kappa-scores. Weighted kappa-scores for presence of deformity (y/n), severity and number of deformities were 0.80, 0.75 and 0.63, respectively [25].

## Ascertainment of Fractures

Fractures that occurred between the first examination in 1992/1993 and the second LASA examination in 1995/1996, were retrospectively assessed in 1995/1996. Data on fractures that occurred between the second and the third examination in 1998/1999 were prospectively

collected with a calendar. Eighty-two percent of all reported fractures were verified by a physician or by radiographs. Duration of follow-up was calculated as the time from the first examination to the first occurrence of a fracture, either a hip fracture or any other fracture. Fractures caused by an (motor vehicle) accident were excluded (9%) as well as fractures of the head, fingers and toes (15%).

### *Potential Confounders*

All baseline information on age and sex were derived from the municipal registries. During the first and second data examination body weight, body height, cognitive function, current smoking (y/n), alcohol use (number of drinks per week), physical activity, mobility, postmenopausal hormone replacement therapy (HRT) (y/n) (women only), and time since menopause (years) were assessed in a face-to-face interview. Body weight was measured without clothes and without shoes using a calibrated bathroom scale. Height was measured using a stadiometer. Body mass index (BMI) was calculated from height and weight (weight (kg)/height ($m^2$)). Overall cognitive function was assessed with the Mini-Mental State Examination (MMSE) (score range: 0–30) [26]. Physical activity was assessed with a questionnaire for the elderly, covering housekeeping activities, sports and leisure activities during the previous two weeks [27,28]. Walking outside, bicycling, sport activities, doing light and heavy housekeeping activities were summed up to a physical activity score (range 0–5). Level of mobility was assessed with three physical performance tests [29] which included: time needed to walk three meters back and forth along a rope (walking test), time needed to stand up and sit down five times with arms folded (chair stands), and the time needed to put on a cardigan and take it off (cardigan test). For each test a score of 1 to 4 points was assigned corresponding to the quartile of the time needed. The more time was needed, the lower the score. Participants who were not able to perform a test obtained a score of zero points. The scores of the three tests were summed up to a physical performance score (range 0–12). Sex hormone binding globulin (SHBG) (only determined in 1995/1996) was measured by means of an immunoradiometric assay (Orion Diagnostica, Espoo, Finland, and Incstar Corporation, Stillwater, USA). Serum total cholesterol (TC) was measured with an automated analyser.

### *Data Analyses*

The T-test was used to examine differences in continuous variables between ApoE ε4 carriers and non-ε4 carriers. The chi-square test was used to test for differences in categorical variables. Analysis of covariance was performed to adjust the associations between ApoE ε4 and the continuous outcome measures BMD, BUA, SOS, OC and DPD/$Cr_{urine}$ for potential confounders. The distributions of OC and DPD/$Cr_{urine}$ were normalized by transformation to their natural logarithm to improve the plots of the residual analyses. To examine the association between ApoE ε4 and vertebral deformities, odds ratios (OR) and 95% confidence intervals (95% CIs) were calculated by multivariate logistic regression analysis. The relative risk (RR) (95% confidence intervals) of a hip fracture or any fracture was assessed with Cox regression analysis. All multivariate models included adjustments for potential confounders.

Because BMD, BUA, SOS, OC or DPD/$Cr_{urine}$, and vertebral deformity were determined during the second data examination, the models that used these measures as outcome variables were also adjusted for the covariates measured during the second data examination, whereas the data on fractures were adjusted for covariates determined in 1992/93. For each association, we evaluated which of the potential confounders changed the size of the effect measure. Those which changed the strength of the association substantially ($>10\%$) were included as confounders [30]. Because age is a very well known risk factor for BMD, markers of bone turnover and fracture risk, all analyses were adjusted for age. In addition body height was included in all models with BMD as dependent variable because it has been shown to influence DXA measurements [31,32]. To test whether there was significant effect modification ($p<0.05$), interaction terms defined as ApoE ε4 multiplied by sex, and ApoE ε4 multiplied by age, were included in all multivariate models. If interaction terms were significant, analyses were stratified by gender and/or age. For these analyses, age was divided into five-year age groups (65–69, 70–74, 75–79, 80 +).

## Results

### *Respondent Characteristics*

Table 1 shows the distribution of ApoE ε4 alleles of the total sample and the subsample.

Analysis of covariance revealed significant interactions for gender multiplied by ApoE status for BMD (femoral neck, trochanter, total body BMC ($p<0.05$);

**Table 1.** Distribution of ApoE ε4 alleles of the total sample and the subsample

| ApoE allele | Total sample ($n$ = 1406) | Subsample ($n$ = 604) |
|---|---|---|
| | % | % |
| ε2/ε2 | 0.7 | 1.2 |
| ε2/ε3 | 11.5 | 11.8 |
| ε3/ε3 | 61.4 | 61.9 |
| ε2/ε4 | 2.6 | 2.6 |
| ε3/ε4 | 20.6 | 19.9 |
| ε4/ε4 | 3.1 | 2.6 |

**Table 2.** Characteristics of respondents in 1995/1996 (total sample and subsample) by ApoE ε4 allele, stratified by sex

| | Women ($n$ = 720) | | | Men ($n$ = 686) | | |
|---|---|---|---|---|---|---|
| Characteristics of total sample ($n$ = 1406) | ApoE ε4 + ($n$ = 184)[a] | ApoE ε4 – ($n$ = 536)[a] | $p$ value | ApoE ε4 + ($n$ = 187)[a] | ApoE ε4 – ($n$ = 499)[a] | $p$-value |
| Age (years) | 75.2 ± 6.5 | 75.6 ± 6.6 | 0.44 | 75.5 ± 6.6 | 75.8 ± 6.6 | 0.58 |
| Height (cm) | 160.3 ± 6.5 | 160.1 ± 6.3 | 0.79 | 173.2 ± 7 | 172.9 ± 6.6 | 0.51 |
| Body weight (kg) | 71.0 ± 12.7 | 70.9 ± 13.1 | 0.86 | 77.6 ± 11.9 | 78.2 ± 11.8 | 0.57 |
| BMI (kg/m$^2$) | 27.6 ± 4.6 | 27.6 ± 5.0 | 0.99 | 25.8 ± 3.3 | 26.1 ± 3.4 | 0.28 |
| Total cholesterol (mmol/l) | 6.4 ± 1.2 | 6.0 ± 1.1 | 0.00 | 5.8 ± 1.1 | 5.6 ± 1.1 | 0.11 |
| MMSE (total score) | 28 [25–29][c] | 28 [26–29][c] | 0.46[d] | 27 [26–29][c] | 28 [26–29][c] | 0.29[d] |
| Current smoker (%) | 14.7 | 11.0 | 0.12[b] | 17.1 | 27.8 | 0.02[b] |
| Alcohol use (drinks/week) | 0 [0–6][c] | 1 [0–6][c] | 0.61[d] | 6 [1–21][c] | 7 [2–21][c] | 0.59[d] |
| Physical activity (total score 1–5) | 3 [3–4][c] | 3 [2–4][c] | 0.24[d] | 3 [3–4][c] | 3 [3–4][c] | 0.21[d] |
| Physical performance | 6.8 ± 2.6 | 6.6 ± 2.9 | 0.38 | 7.1 ± 2.7 | 6.8 ± 2.8 | 0.21 |
| HRT use after menopause (%) | 10.3 | 13.6 | 0.15 | NA | NA | NA |
| Time since menopause (years) | 26.5 ± 8.4 | 27.0 ± 8.3 | 0.45 | NA | NA | NA |
| SHBG | 49.6 [35.6–65.8][c] | 48.1 [34.1–65.7][c] | 0.21[d] | 42.8 [28.4–61.7][c] | 42.0 [31.7–54.1][c] | 0.59[d] |
| Characteristics of the subsample ($n$ = 604)[e] | ApoE ε4 + ($n$ = 83)[a] | ApoE ε4 – ($n$ = 228)[a] | $p$ value | ApoE ε4 + ($n$ = 69)[a] | ApoE ε4 – ($n$ = 224)[a] | $p$-value |
| Fat mass (kg) | 33.8 ± 11.3 | 30.4 ± 10.1 | 0.02 | 22.4 ± 8.2 | 22.3 ± 7.2 | 0.87 |
| Appendicular muscle mass | 15.1 ± 2.4 | 14.6 ± 2.2 | 0.13 | 21.1 ± 2.8 | 21.5 ±2.7 | 0.37 |
| HRT use after menopause (%) | 6.0 | 17.6 | 0.01[b] | NA | NA | NA |

[a]Values indicate means ± standard deviations (SD), unless otherwise indicated.
[b]Differences in frequencies were examined with $\chi^2$ test.
[c]Median and interquartile range.
[d]Differences in skewed parameters were examined with Mann-Whitney test.
[e]Only variables that were not determined in the total sample are presented. Means ± SD of the other variables are comparable to the total sample.
NA = not applicable; MMSE = Mini-Mental State Examination.

lumbar spine, total hip ($p < 0.10$)) and both QUS measures ($p < 0.05$)).

Table 2 shows the respondent characteristics of the second examination in 1995/1996 for the total sample and the subsample according to ApoE ε4 status. Differences in characteristics of the total population at baseline (data not shown) were similar to the second examination.

## *Association Between ApoE Phenotype and BMD*

Analysis of covariance revealed that, female ApoE ε4 carriers had lower values than women without ApoE ε4 for BMD of the femoral neck ($p$ = 0.04), trochanter ($p$ = 0.01) and total body BMC ($p$ = 0.04), after adjustment for age, body height, HRT use and fat mass (Table 3). Differences in total hip BMD ($p$ = 0.06) and lumbar spine BMD ($p$ = 0.09) were borderline significant.

In men, but not in women, significant interaction between ApoE status and age was found for BMD of the total hip ($p$ = 0.01), femoral neck ($p$ = 0.004) and trochanter ($p$ = 0.03). Figure 2(a) shows the age-related differences of total hip BMD by ApoE status in men. The figures for BMD of the femoral neck and the trochanter were very similar (data not shown).

**Table 3.** Means (SEM) of hip, lumbar spine BMD and total body BMC by ApoE ε4 allele in women

| BMD and total body BMC | ApoE ε4 + ($n$ = 68) | ApoE ε4 – ($n$ = 194) | $p$-value |
|---|---|---|---|
| Total hip (g/cm$^2$) | 0.76 ± 0.02 | 0.79 ± 0.01 | 0.06 |
| Femoral neck (g/cm$^2$) | 0.64 ± 0.01 | 0.67 ± 0.01 | 0.04 |
| Hip trochanter (g/cm$^2$) | 0.58 ± 0.01 | 0.61 ± 0.01 | 0.01 |
| Lumbar spine (g/cm$^2$) | 0.90 ± 0.02 | 0.94 ± 0.01 | 0.09 |
| Total body BMC (g) | 1787 ± 40.0 | 1863 ± 23.8 | 0.04 |

All values are presented as means ± SEM; values were adjusted for age, height, fat mass and HRT use.

## *Association Between ApoE Phenotype and QUS*

In women, no significant differences in BUA and SOS with ApoE ε4 status were found (BUA: mean (dB/MHz) ± SEM; ε4+, 60.9 ± 1.2 vs. ε4–, 61.1 ± 0.7; $p$ = 0.84; SOS: mean (m/s) ± SEM; ε4+, 1603 ± 3.5 vs. ε4–, 1609 ± 2.0; $p$ = 0.09). In men, the associations between ApoE ε4 and QUS values were modified by age (BUA: $p$ = 0.01 for the interaction term age × ApoE ε4; SOS: $p$ = 0.08 for the interaction term age × ApoE ε4). Figure 2(b,c) shows the age-related differences in BUA and SOS by ApoE status in men.

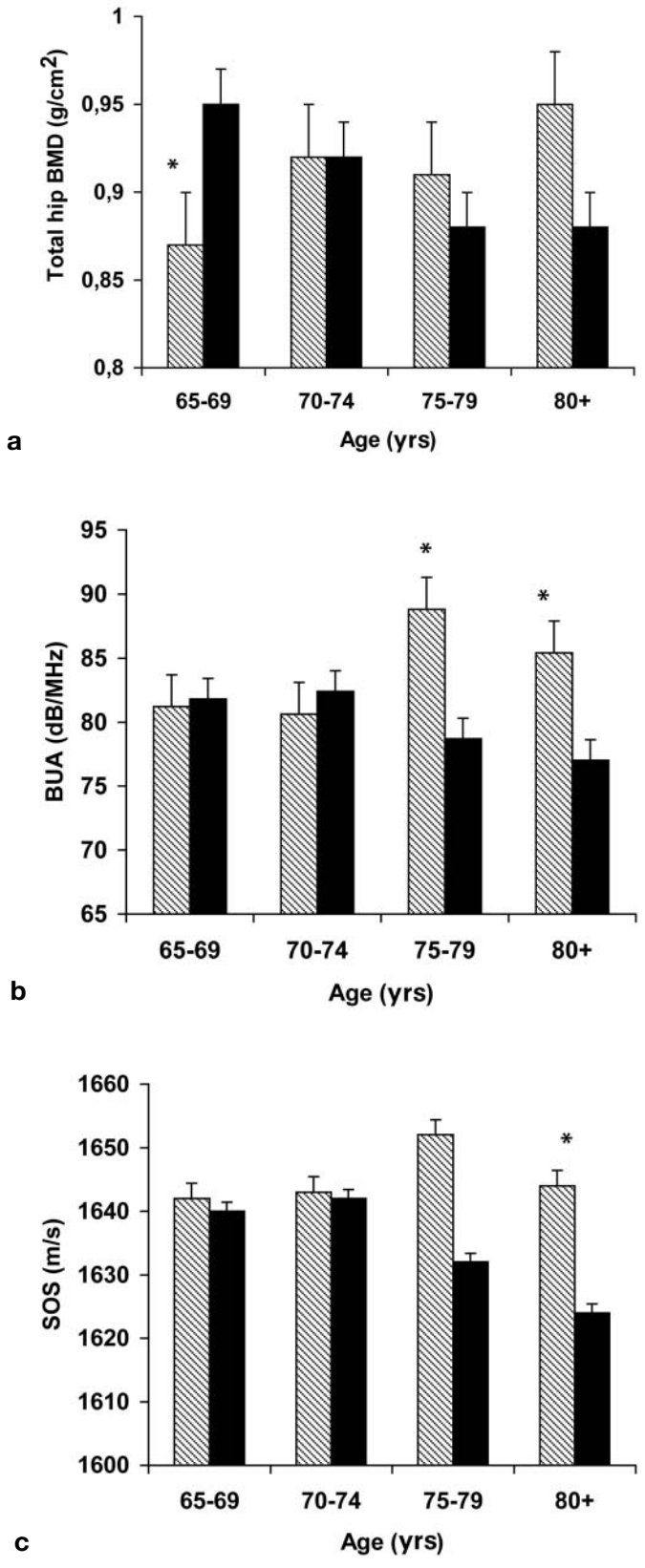


**Fig. 2.** Mean (± SEM) age-related differences in BMD (**a**), BUA (**b**) and SOS (**c**) according to ApoE ε4 status in men. *$p < 0.05$ within the same age group. ▨ ApoE ε4+, ■ ApoE ε4 –.

### *Association Between ApoE Phenotype and Bone Markers*

In men and women, urine levels of DPD/$Cr_{urine}$, and serum OC concentrations were not significantly different among the two phenotype groups. No significant interactions were found (data not shown).

### *Association Between ApoE Phenotype and Fracture Risk*

Table 4 shows the distribution of incident non-vertebral fractures that occurred between 1992 and 1999, and prevalent vertebral deformities (in 1996), according to ApoE ε4 status and stratified by sex.

Cox regression analysis, adjusted for age, body weight, MMSE and BUA, did not reveal any significant differences in risk of hip or any fracture in relation to ApoE phenotype in men and women. Because the number of fractures was too small, we could not examine effect modification by age. The risk of severe vertebral deformities was increased in ApoE ε4+ women as compared to ApoE ε4– women, but no significant differences were found in men. When women with prevalent vertebral deformities of at least 25% vertebral height loss (moderate deformity) were contrasted with those with vertebral deformities less than 25% vertebral height loss, the adjusted OR was slightly weaker, but also significantly increased in ApoE ε4 carriers compared to those without the ApoE ε4 allele (OR=2.03; 95%CI: 1.00–4.15).

## Discussion

In this study, we showed that female ApoE ε4 carriers had lower total body BMC and BMD values of the femoral neck and trochanter compared to women without an ApoE ε4 allele. Women with ApoE ε4 also had a higher risk of severe vertebral deformities. In men, the effect of ApoE ε4 on BMD and QUS depended on age. The presence of ApoE ε4 was associated with a lower hip BMD only in the younger men aged 65–69 years and with higher QUS measurements only in the oldest men. Finally, ApoE ε4 did not appear to be associated with increased levels of bone turnover markers or increased risk of hip or any fracture, in either in women or men.

Our finding that women with ApoE ε4 had 4–5% lower total body BMC and hip BMD values than women without ApoE ε4 confirms the results of two previous studies [8,10]. Shiraki et al. [8] found a gene-dose effect between ApoE phenotype and lumbar spine BMD in Japanese postmenopausal women with ApoE ε4 having the lowest BMD. Salamone et al. [10] demonstrated that loss of lumbar spine BMD was greater in peri-and postmenopausal women with ApoE ε4 than in women without the ApoE ε4 phenotype. In contrast, some other

**Table 4.** Relative risks (RR) of incident non-vertebral fractures (1992-1999) and prevalent vertebral deformities (1996) by ApoE ε4 allele in men and women

| Type of fracture | ApoE ε4 + | | ApoE ε4 – | | RR (95% CI)/ OR (95% CI)[a,b] |
|---|---|---|---|---|---|
| | *n* | % | *n* | % | |
| Women | | | | | |
| Any fracture (1992–1999) | 22 | 12.2 | 57 | 10.9 | 1.11 (0.66-1.87) |
| Hip fracture (1992–1999) | 4 | 2.2 | 13 | 2.4 | 0.87 (0.28–2.68) |
| Moderate vertebral deformity (in 1996) | 17 | 25.0 | 34 | 17.3 | 2.03 (1.00–4.15)* |
| Severe vertebral deformity (in 1996) | 13 | 19.1 | 20 | 10.2 | 2.78 (1.21–6.34)* |
| Men | | | | | |
| Any fracture (1992–1999) | 11 | 6.0 | 31 | 6.4 | 0.92 (0.41–2.05) |
| Hip fracture (1992–1999) | 4 | 2.1 | 11 | 2.2 | 1.14 (0.36–3.63) |
| Moderate vertebral deformity (in 1996) | 7 | 12.1 | 36 | 17.9 | 0.61 (0.25–1.46) |
| Severe vertebral deformity (in 1996) | 4 | 6.9 | 16 | 8.0 | 0.82 (0.25–2.60) |

[a]The association between ApoE ε4 and incident non-vertebral fracture (any fractures or hip fracture) was expressed as relative risk (RR), whereas the association between ApoE ε4 and prevalent vertebral deformity was expressed as odds Ratio (OR).
[b]For fractures, RRs were adjusted for age, body weight, MMSE and BUA. In men, prevalent vertebral deformities were adjusted for age, fat mass, and lumbar spine BMD. In women, prevalent vertebral deformities were adjusted for age, fat mass, HRT use and lumbar spine BMD.
*$p < 0.05$.

studies did not find differences in baseline BMD [13,15] or rate of BMD loss [9,14,15].

In our study, the impact of ApoE ε4 on hip BMD and QUS appeared to depend on age in men. Among men of the younger age group (65–69 years), the presence of ApoE ε4 was associated with a lower BMD, whereas among the oldest men (75+) ApoE ε4 was associated with higher BMD and QUS values. There are two possible explanations for this age-related association in men. First, these findings may be explained by selection bias; the ApoE ε4 carriers who survive into old age, are a stronger and healthier group. The findings of several recent studies, which examined the presence of ApoE ε4 as a predictor of coronary heart disease and mortality in men, confirm this explanation [33–37]. Scuteri et al. [33] found that ApoE ε4 was a strong independent predictor of coronary events in men, but not in women. In a prospective study including 3052 men, Humphries et al. [34] demonstrated an interaction between ApoE ε4 and smoking on coronary heart disease. They found that smokers who were carriers of the ApoE ε4 allele showed an increased risk of coronary heart disease compared to non-smoking ApoE ε4 carriers. The finding that in our study sample, only 17.1% of the male ApoE ε4 carriers were smokers versus 27.8% of the men without the ApoE ε4 allele (see Table 2), also suggests that the male smoking ApoE ε4 carriers died before the start of this study. A second explanation may be that, on the one hand, the presence of ApoE ε4 results in lower BMD values, but one the other hand reduces age-related bone loss. Although the results of this study may support this explanation, the analyses are based on cross-sectional data. Longitudinal data are now being collected to confirm this hypothesis.

In line with the results of Heikkinen et al. [13], we did not find significant differences in biochemical markers of bone turnover when ApoE ε4 carriers were compared with non-ε4 carriers, in either men or women. In contrast Shiraki et al. [8] reported higher OC levels when ApoE ε4 was present.

In this study women with an ApoE ε4 allele had a more than a twofold increased risk of a severe vertebral deformity, but in line with the results of Booth et al. [14] and von Mühlen et al. [15] there was no significant association between the ApoE ε4 phenotype and hip or any fracture in either women or men. These findings contrast with two previous studies [7,9]. In a group of hemodialysis patients, Kohlmeier et al. [7] demonstrated an overrepresentation of the ApoE ε4 allele in patients with a history of fracture when compared to controls. In the Study of Osteoporotic Fractures (SOF), Cauley et al. [9] reported that women with ApoE ε4 had an increased risk of hip fracture and wrist fracture. The discrepancy in results between the studies may be explained by differences in source population and power. In the SOF study, in total 60 hip fractures were reported, whereas in our study only 17 women reported a hip fracture. This number might have been too small to detect significant associations.

The mechanism through which ApoE ε4 may influence BMD and risk of vertebral deformities is unclear. Kohlmeier et al. [38] suggested that the presence of ApoE ε4 may impair the transport of vitamin K to the bone. Because of the rapid clearance rate of chylomicrons from blood into the liver, people with ApoE ε4 have lower plasma levels of vitamin K which is necessary for the γ-carboxylation of three bone matrix proteins, undercarboxylation of osteocalcin has been shown to predict hip fracture risk [39]. Another possibility is that ApoE ε4 may be linked to BMD through increased plasma cholesterol. ApoE ε4 was found to be associated with elevated plasma cholesterol

concentrations [40,41]. Moreover, growing evidence suggests that osteoporosis is associated with arteriosclerosis and vascular calcification (for review, see [42]).

Our study has several limitations. First, the respondents of this study are a selective group of relatively healthy older men and women, because the frailest respondents of the LASA study were not able to visit the hospital or health care center. If these non-responders were more often carriers of ApoE ε4, an underestimation of the associations might have occurred. Second, the number of severe vertebral deformities and fractures might have been too low to detect significant differences in ApoE status and to examine effect modification by age.

In conclusion, this large community-based study confirms the importance of ApoE ε4 as a possible genetic risk factor related to bone mineral density and vertebral deformities, and demonstrates that this effect is gender-related, and depends on age in men only. This may partly explain differences between men and women in the development of osteoporosis. The identification of further genetic risk factors may be useful for both the prediction of fractures and for the elucidation of biological mechanisms underlying osteoporosis.

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