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Bone Mineral Density in Patients with Apolipoprotein E Type 2/2 and 4/4 Genotype

T. ŠTULC, R. ČEŠKA, A. HOŘÍNEK, J. ŠTĚPÁN

Third Department of Internal Medicine, First Faculty of Medicine, Charles University, Prague, Czech Republic

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Summary

The peak bone mass and the rate of bone loss are in part genetically determined. It has been suggested that bone mineral density (BMD) may be related to allelic variation in the apolipoprotein E (ApoE) gene locus. ApoE is important in the receptor-mediated clearance of chylomicron particles from the plasma, Apo E4 having the highest and Apo E2 the lowest receptor affinity. Chylomicrons are the main carrier of vitamin K in the plasma; vitamin K plays an important role in the carboxylation of osteocalcin. We have tested the hypothesis that persons with E4 variant would have lower BMD and increased bone turnover than those with E2 variant. A total of 18 ApoE 2/2 and ApoE 4/4 homozygotes were selected from 873 patients who were examined for the ApoE genotype. BMD in lumbar vertebral, femoral neck and distal forearm was measured and plasma concentrations of osteocalcin and C-terminal fragments of collagen (CTx) were determined. BMD values (expressed as T-score) at the three specified sites were –0.12±1.72, –0.52±1.32 and –0.52±0.81 in ApoE 2/2 group and –0.24±1.22, 0.00± 0.84 and –0.17±1.07 in the ApoE 4/4 group. Plasma osteocalcin and CTx were within normal limits in both groups. In conclusion, we did not observe any association of ApoE genotype with BMD and biochemical markers of bone metabolism in ApoE 2/2 and ApoE 4/4 homozygotes.

Key words

Bone mineral density • Osteoporosis • Bone turnover • Apolipoprotein E • Vitamin K

Introduction

Osteoporosis is a common disorder and osteoporotic fractures represent an important health problem in elderly patients. The risk of low bone mass and increased bone loss are at least in part genetically determined (Pocock *et al.* 1987). This has led to a search for genetic markers associated with variations in bone mineral density (BMD) to identify patients at risk of osteoporosis and to understand the processes involved in bone loss (Morrison *et al.* 1994, Sano *et al* 1995).

Vitamin K is an important factor for normal bone metabolism. It is involved in the carboxylation of several bone matrix proteins (Vermeer 1990) and low serum concentrations of vitamin K have been linked to the increased risk of bone fracture (Szulc *et al.* 1993). Vitamin K is a water-insoluble compound and it is transported in the plasma as a part of lipoproteins, especially chylomicrons; more than a half of plasma vitamin K in the fasting state is associated with triglyceride-rich particles and this proportion is further

Fax +420 2 293 894
E-mail: physres@biomed.cas.cz
http://www.lf2.cuni.cz/physiolres/physiolres.htm

increased during postprandial period (Kohlmeier *et al.* 1996). Chylomicrons readily enter the interstitial space in the bone marrow, where they serve as a source of vitamin K for osteoblast precursor cells and adjacent mature osteoblasts. ApoE is a major protein component of chylomicrons and is involved in their receptor-mediated uptake (Mahley 1988), Physiologically, it appears in three allelic variants (E2, E3, E4) (Utermann 1987). Population frequencies of E2 and E4 variants are low which results in a very low incidence of E2/2 an E4/4 homozygotes (Hallman *et al* 1991). Individual allelic variants have different receptor affinities (in the order E2<E3<E4, Weintraub *et al* 1987). Patients with various ApoE genotypes therefore differ in their plasma clearance rates of triglyceride-rich particles and thus also in plasma triglyceride and vitamin K concentrations (in the order E4<E3<E2, Saupe *et al* 1993). On the basis of some preliminary data, an association of the ApoE genotype with several age-related degenerative disorders, namely atherosclerosis (Hořejší and Češka 2000, Vogt *et al* 1997, Davignon *et al* 1988), Alzheimer dementia (Ganguli *et al* 1995, Yaffe *et al* 1997) and bone loss (Kohlmeier *et al.* 1998, Shiraki *et al* 1997), has been suggested. Until now, however, neither of these issues has been resolved unequivocally (Suzuki *et al.* 1998).

Because mixing E2/2 and E2/3 or E4/4 and E3/4 patients together as representatives of the "E2" or "E4" genotype may lead to confusion due to the interference of the E3 isoform, we have compared bone mineral density (BMD) and biochemical markers of bone remodeling between E2/2 and E4/4 homozygotes identified from a large population.

Methods

Patients

The ApoE genotype was determined in 873 patients who had been referred to the outpatient lipid clinic. Among these patients, 24 homozygotes E/2/2 and E4/4 were detected (i.e. 2.75 %) and offered bone examination. Out of these, 18 patients volunteered to participate in this study.

Bone densitometry

BMD was measured by dual energy X-ray absorptiometry using Hologic QDR 4500 (Hologic, Inc., Waltham, MA, USA). Images of left femoral neck, left forearm and postero-anterior image of L1-L4 vertebrae were obtained and BMD [g/cm^2] was calculated. The results are expressed as a T-score and (after adjustment for age and body weight) as the Z-score.

Markers of bone turnover

Plasma osteocalcin was measured using radioimmunoanalysis (Brahms, Germany); serum CTx was measured by the ELISA method (Osteometer, USA). The concentrations of osteocalcin and CTx were expressed in μg/l and pmol/l, respectively.

Lipid parameters

Serum total cholesterol (TC), triglycerides (TG) and HDL-cholesterol (HDL) were measured using an automated analyzer after an overnight fast. All values are expressed in mmol/l.

Genotype analysis

DNA was isolated from whole blood using a DNA isolation kit (Ready Amp Genomic DNA Purification System, Promega, USA) and amplified by PCR using primers described by Hixson and Vernier (1990). The ApoE genotype was determined the method of by restriction isoform typing using Cfo I restriction enzyme.

Statistics

All results are expressed as mean ± S.D. The differences between groups were tested at p=0.05 using two-tailed Student's t-test.

Table 1. Lipid values and bone turnover markers in ApoE 2/2 and 4/4 patients

ApoE genotype	***2/2***	***4/4***
Number of patients	**7**	**11**
Lipid values		
Total cholesterol (mmol/l)	10.71±5.45	7.60±1.93
Triglycerides (mmol/l)	8.35±10.03	4.98±1.53
HDL-cholesterol (mmol/l)	1.26±0.46	1.62±0.38
Bone turnover markers		
Osteocalcin (μg/l)	9.75±2.62	8.47±4.16
CTx (pmol/l)	4720±2788	3168±2493

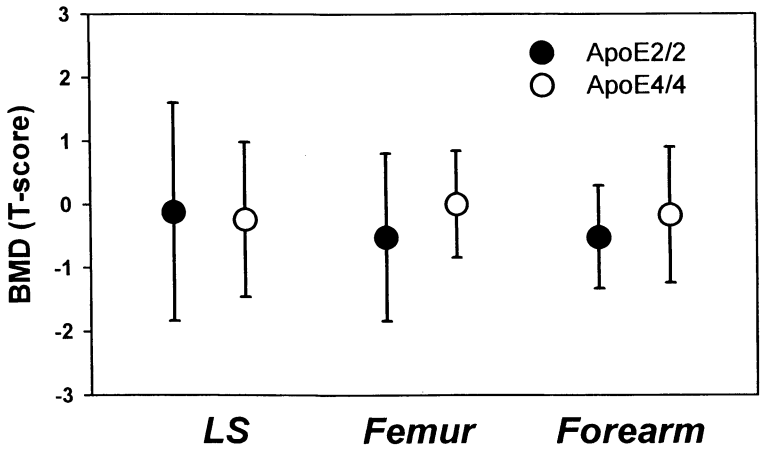

Fig. 1. *BMD values of lumbar spine (LS), femur and forearm in E2/2 and 4/4 patients, expressed as the T-score.*

Results

ApoE genotype frequencies

The genotype of ApoE was analyzed in a total of 873 patients; the relative frequencies of individual genotypes were 0.011 for E2/2, 0.111 for E2/3, 0.013 for E2/4, 0.637 for E3/3, 0.212 for E3/4 and 0.016 for E4/4.

Patient characteristics

A total of 18 patients were examined (8 women, 10 men). Seven patients had E2/2 genotype (1 woman, 6 men), 11 patients had E4/4 genotype (7 women, 4 men). Five out of 8 women were postmenopausal (1 in the E2/2 group, 4 in the E4/4 group). The average age was 53.6±12.3 and 46.5±13.9 years; the average weight was 85.3±8.7 and 72.6±11.9 kg for E2/2 and E4/4 groups, respectively. Plasma lipid concentrations of both groups are given in Table 1. Higher values of plasma cholesterol and triglyceride concentrations in the ApoE 2/2 group are due to markedly elevated values of these parameters in one patient with severe combined hyperlipidemia and also to the presence of four patients with dysbeta-lipoproteinemia (type III hyperlipoproteinemia).

Bone mineral density

BMD values are shown in Figures 1 and 2. The results were compared between both homozygote groups and to the reference population value. Neither difference was significant at $p<0.05$.

Markers of bone turnover are given in Table 1. The osteocalcin values were within normal limits in all patients. In three patients (two E2/2, one E4/4) CTx values were slightly elevated, suggesting an increased bone turnover. No significant differences in osteocalcin and CTx concentrations were found between E2/2 and E4/4 patients.

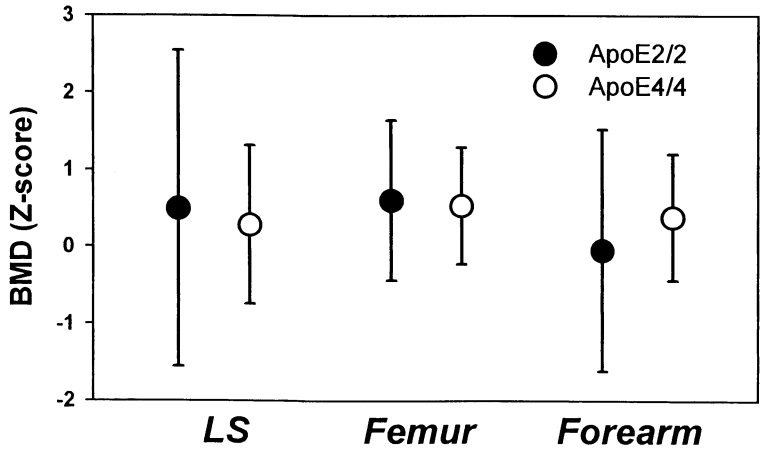

Fig. 2. *BMD values of lumbar spine (LS), femur and forearm in E 2/2 and 4/4 patients after adjustment for age (Z-score).*

Discussion

In the present study, we did not observe any significant differences in BMD and markers of bone turnover between E2/2 and E4/4 patient groups; there was not even a trend toward higher values of BMD in the E2/2 patients. The lack of association of E2/2 genotype with higher BMD contrasts with the finding of substantially elevated plasma triglyceride levels in this patient group, which is consistent with the proposed

mechanism of increased vitamin K saturation. Our results are in disagreement with the previously published results concerning the association of ApoE genotype with BMD (Shiraki *et al.* 1997, Kohlmeier *et al.* 1998, Cauley *et al.* 1999). On the other hand, Suzuki *et al.* (1998) failed to detect significant association between the two variables.

Because of the low incidence of E4/4 and E2/2 homozygotes, the studies published to date have analyzed E4/4 + E3/4 and E2/2 + E2/3 patients together. However, the nature of the interaction between various ApoE allelic variants in heterozygous setting is hitherto unknown and the combination of homozygotes with heterozygotes introduces a potentially confusing factor to the analysis. To eliminate this problem we decided to perform this study in E2/2 and E4/4 homozygotes only. The studied subjects were selected from the group of 873 individuals who were examined for ApoE genotype in our laboratory, which is one of the largest groups published to date (Vrablík *et al.* 1999). Only a small number of E2/2 and E4/4 homozygotes impaired the power of the analysis. However, there was not even a trend towards higher values of BMD in the E2/2 patients. Despite the relatively low number of patients, such a result could hardly be expected if there were any significant association between ApoE genotype and BMD.

One of the possible reasons for disagreement of the present results with those published previously concerns the selection of patients. Subjects with ApoE4 variant have been reported to show an association with some involutional diseases, such as the Alzheimer disease (Ganguli *et al* 1995, Yaffe *et al.* 1997), atherosclerosis (Davignon *et al.* 1988, Vogt *et al.* 1997) or osteoporosis (Shiraki *et al.* 1997, Kohlmeier *et al.* 1998). Most studies published to date concentrated therefore on subgroups at particular risk of these diseases. Shiraki *et al.* (1997) reported a relationship between low BMD and ApoE4/4 and 4/- genotypes in 284 postmenopausal Japanese women (mean age 64 years). Kohlmeier *et al.* (1998) analyzed ApoE genotype in 219 hemodialysed patients (mean age 65 years). They reported an association of both retrospective and prospective risk of bone fracture with ApoE variants, E2 being at the lowest and E4 at the highest risk. In a prospective community-based Pittsburgh Study of Osteoporotic Fracture (Cauley *et al.* 1999) among elderly women (aged over 65, mean age 71 years) those with the E4 variant had significantly higher prospective risk of hip fracture compared to those without E4 variant. In our study, patients were relatively young (mean age 49.3 years) and only 4 out of 8 women were postmenopausal; the patients were without any disabling disease and all of them were ambulatory. One possible explanation for the lack of association between ApoE genotype and BMDis that subjects included in our study were younger and exhibited better overall health status compared to those in some other studies. Elderly and/or diseased patients are often of suboptimal nutritional status and therefore at increased risk of bone disease due to deficiency of calcium, vitamin D (Chapuay *et al.* 1992) and also of vitamin K (Szulc *et al.* 1993). A minor contribution of various ApoE variants to the overall BMD and bone turnover thus possibly remains obscured in relatively young and healthy subjects and only becomes manifest in elderly and/or diseased patients at higher risk of accelerated bone loss. Interesting in this respect is the recently published report by Suzuki *et al.* (1998), who did not observe significant association of ApoE genotype and BMD in postmenopausal Japanese women (aged over 65 years). More extensive studies in various patient groups are needed to clarify the relation of the ApoE genotype to bone metabolism.

In conclusion, we did not observe any association of ApoE genotype with BMD and biochemical markers of bone metabolism in ApoE2/2 and ApoE4/4 homozygotes.

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Reprint requests
T. Štulc, M.D., Third Department of Internal Medicine, U nemocnice 1, 128 21 Prague 2, Czech Republic. Fax: 420-2-2496 2946. E-mail: tstulc@lf1.cuni.cz