The Effects of Cardiovascular Drugs on Bone

Mariëtte Schoofs

STELLINGEN

Behorende bij het proefschrift "Effects of Cardiovascular Drugs on Bone"

1 Langdurig gebruik van thiazide diuretica verlaagt het risico op heupfracturen. Deze risicoreductie verdwijnt binnen enkele maanden na het staken. (dit proefschrift)

2 Statinen geven een hogere botdichtheid en reduceren het risico op fracturen. (dit proefschrift)

3 Remming van het β-adrenerge systeem leidt tot een hogere botdichtheid, maar niet tot een lager risico op alle niet-vertebrale fracturen. (dit proefschrift)

4 Het E*2, E*3, en E*4 polymorfisme van het apolipoproteïne E gen zijn niet geassocieerd met osteoporose. (dit proefschrift)

5 Het gebruik van multiple imputation als methode om met missende variabelen om te gaan, zou de standaard moeten worden in epidemiologisch onderzoek. (dit proefschrift)

6 Het slagen van de kenniseconomie wordt in hoge mate bepaald door het durven investeren.

7 De rigiditeit van de flexwet zorgt, in plaats van het beoogde creëren van vaste aanstellingen, eerder tot het verlies van tijdelijke aanstellingen.

8 De uitdaging voor een internist is niet alleen het instellen van de juiste therapie, maar het motiveren van de patiënt deze therapie te volgen en zijn leefstijl aan te passen.

9 Hoe beter een roeier wordt, hoe meer hij achteruit kijkt bij het vooruit gaan, terwijl een wetenschapper – als hij meer ervaring krijgt - juist minder achterom hoeft te kijken om vooruit te komen.

10 Cogito, ergo sum. Het omgekeerde lijkt minder toepasselijk.

11 De 14-eeuwse epidemie "de Zwarte Dood" zorgde in Europa voor een positieve selectiedruk op de Δ32 deletie variant van het CCR5 gen[1] dat de kans op infectie met HIV verlaagt en resistentie tegen HIV veroorzaakt. Als deze pestepidemie zich destijds ook op grote schaal had uitgebreid over de rest van de wereld was de omvang van de huidige HIV-epidemie wellicht minder groot geweest.

M.W.C.J. Schoofs, Rotterdam 15 juni 2005

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[1] Stephens JC, Reich DE, Goldstein DB, *et al*. Dating the origin of the CCR5-Delta32 AIDS-resistance allele by the coalescence of haplotypes. *Am J Hum Genet* 1998; 62(6):1507-15.

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Copromotor:	Dr. M. C. J. M. Sturkenboom

Aan mijn ouders

Contents

Chapter 1

Introduction

Osteoporosis is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue. The consequence of osteoporosis is an increased susceptibility to fracture.[1] Since the population is ageing, incidence and prevalence of diseases of the elderly are rising as well. Fractures are associated with substantial disability and increased death rates and they are also an important constituent of total costs for health care both in Europe and in the United States.[2,4] Prevention and treatment of osteoporosis is therefore an important issue.

Bone structural integrity is maintained by removal of old bone by osteoclasts and synthesis of new bone in its place by osteoblasts. This process, called bone remodeling, takes place in a temporary group of cells, the bone multicellular unit (BMU). Osteoclasts develop from hematopoeitic progenitors; and osteoblasts are derived from mesenchymal stem cells, also known as marrow stromal fibroblasts. Circulating hormones together with locally produced cytokines and growth factors modulate the replication and differentiation of osteoclast and osteoblast progenitors.[5] In normal adult bone there is a steady state situation with regard to bone resorption and bone formation, in which the amount of bone removed by a BMU is not totally replaced with new bone. This failure to completely reform bone within a BMU leads to a gradual loss of bone with age. In women, just after menopause, the rate of resorption is generally unchanged, but the rate of bone formation is decreased and therefore postmenopausal women have a rapid loss of bone of about 2–3% per year. The rate of bone loss decreases 6–10 years after menopause.[6]

Therapies that prevent or reverse osteoporosis act at least in part by preventing osteoblast apoptosis and/or stimulating osteoclast apoptosis.[5] Most drugs that are currently available to treat osteoporosis are inhibitors of bone resorption.[7] Agents that stimulate bone formation are very recently introduced on the market, but not yet widely used.[8,9] Since osteoporosis is an age-related disease, elderly people are most at risk for osteoporosis. Comorbidity is common among elderly, and so is use of medication. All medication can have adverse effects and the most well-known example of drugs with adverse effects on bone are glucocorticoids.[10] However, unintended effects of drugs can also be beneficial to bone. An old example of a drug with such beneficial effects are thiazide diuretics.[11] Recently, also beneficial effects of HMG CoA reductase inhibitors and ß-blockers on bone have been suggested.[12,13]

In this thesis we investigate effects of use of these (cardiovascular) drugs on bone. Since incidence of cardiovascular diseases increases with age, just as the incidence of osteoporosis, cardiovascular drugs are widely used in people at risk for osteoporosis. Cardiovascular disease can be treated with several different types of medication. Insight in quality and quantity of beneficial effects on bone of these drugs can help in making a choice between the available drugs to treat cardiovascular diseases. Furthermore, research on the association between the use of these drugs and bone outcomes, such as fracture risk and bone mineral density, may answer some of the questions on the potential role of these medicines in prevention and treatment of osteoporosis.

Thiazide diuretics

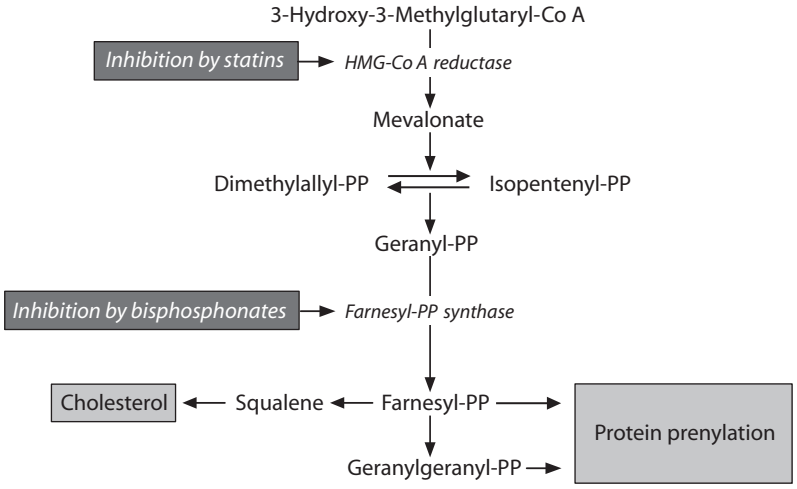
Thiazide diuretics were marketed in the 1950's as antihypertensive agents. They lower blood pressure by 3 mechanisms: 1) sodium diuresis, producing reduced intracellular sodium concentrations within vascular smooth muscle cells and reduced reactivity of vascular smooth muscle to sympathetic stimuli. 2) Diuresis leads to hypovolaemia and haemodynamic changes. 3) Direct vasodilating action on arterioles.[14]

Because thiazides induce sodium diuresis, an unintended effect is reduction of calcium excretion in the kidney. This can create a positive calcium balance.[15,16] Thiazides may cause metabolic acidosis and inhibit bone resorption.[17] A third unintended effect of thiazides is direct inhibition of osteocalcin secretion of osteoblast-like cells. This has been demonstrated in *in vitro* studies.[18]

Statins

Statins were introduced in the 1980's as cholesterol-lowering agents. By inhibiting the conversion of HMG CoA to mevalonate, they inhibit cholesterol synthesis. This does not only lead to lower de novo cholesterol synthesis and lower plasma cholesterol levels, but also increases receptor-mediated catabolism of low density lipoprotein (LDL).[19]

Figure 1



Statins act early in the mevalonate pathway by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase. Nitrogen-containing bisphosphonates interfere with protein prenylation by inhibiting synthesis of famesyl pyrophosphate and geranylgeranyl pyrophosphate. (After: Cummings et al. JAMA 2000; 283: 3255-7.)

Figure 1 shows the mevalonate pathway of the cholesterol synthesis. Statins inhibit this pathway, just as nitrogen-containing bisphosphonates, drugs that are prescribed to treat osteoporosis. Inhibition of the mevalonate pathway by bisphosphonates leads to inhibition of protein prenylation of small glutamyl transpeptidases (GTPases) and can interfere with osteoclast function.[20] The exact mechanism by which statins may have effects on

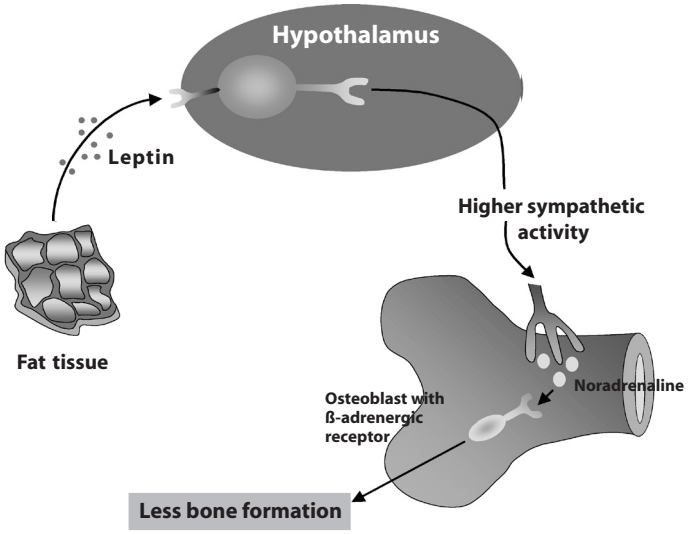
bone is not yet known. It is possible that inhibition of the mevalonate pathway further upstream compared to bisphosphonates may exert the same effects as bisphosphonates. Another possibility is stimulation of bone morphogenetic protein-2 (BMP-2), as was shown in previous studies, which is involved in osteoblast differentiation and bone formation.[21, 22]

ß-blockers

These drugs are competitive antagonists of adrenalin and noradrenalin at ß-adrenergic receptors. They are used in cardiovascular medicine to slow the heart rate and to reduce myocardial contractility.

There is accumulating evidence that the sympathetic nervous system is involved in regulation of bone metabolism. Animal studies showed sympathetic innervation of bone[23, 24] and adrenergic receptors have been found on both osteoblasts and osteoclasts.[25] In sympathetic nerve fibers in bone, neuropeptides are present and some of these neuropeptides can modulate bone resorption.[26, 27] Leptin is a protein hormone with important effects in regulating body weight, metabolism and reproductive function. Leptin is also involved in bone metabolism. Figure 2 shows that blockade of the sympathetic nervous pathway is inhibiting the negative effects of leptin on bone.

Figure 2 — Molecular connections between fat and bone mass.



Leptin is a hormone that is released from fat cells in proportion to body fat, and travels in the blood to the brain. Leptin acts on hypothalamic neurons (although their identity is unknown) to regulate bone mass. The hypothesis is that this stimulates the activity of sympathetic (involuntary) nerves that penetrate the bone, there releasing noradrenaline. This neurotransmitter in turn binds to the ß2-adrenergic receptors on bone-forming cells (osteoblasts), inhibiting their activity. So leptin reduces bone mass. (After: Flier JS. Physiology: is brain sympathetic to bone? Nature 2002; 420 (6916): 619, 21-22.)

Issues in pharmaco-epidemiology

Pharmaco-epidemiology studies the association between use of medicines and occurrence of diseases. Over the last few years several studies on associations between bone mineral density, fractures and use of thiazide diuretics, statins and ß-blockers have been published. Since exposure to these drugs is time-dependent, e.g. people can start, stop and restart taking medication over time, pharmaco-epidemiologic studies are often complicated by limited data on precise exposure status.

There are three sources of drug exposure data:

- *Information on drug use derived from patient (or proxy) interviews*
- *Information on drug exposure derived from prescription databases of general practitioners*
- *Information on drug exposure derived from pharmacy databases*

The accuracy of the exposure data is not the same for these three sources. Information from interviews can be subject to recall bias; patients tend to remember their previous use of drugs more accurately than healthy persons. Information from prescriptions of general practitioners is more objective, but does not cover drugs that are prescribed by medical specialists. Furthermore, we do not know whether patients actually fill these prescriptions at the pharmacy. Finally, pharmacy database-derived exposure information is probably the most accurate source. Although we do not know whether the filled prescriptions are actually taken by the patients, we assume that if a person is compliant when he regularly comes back at the anticipated moment to the pharmacy to fill a prescription for chronic medication.

Previous studies on the effects of cardiovascular drugs on bone were often carried out in populations with limited data on potential confounding factors. Confounding by indication is an important issue. This is the case when the underlying condition that results in drug use is the explanation of the association, and not the drug itself. To examine confounding by indication, detailed data on cofactors such as cardiovascular disease, body mass index (BMI) and presence of hypertension are necessary.

Aim and outline of this thesis

In this thesis, we examine various associations between three important cardiovascular drugs and bone.

Fracture incidence is the most clinically relevant feature of osteoporosis. The risk to fracture a bone is determined by several factors: Bone mineral density (BMD), microarchitecture of bone, bone structure and risk of falling. In this thesis we investigate the association between use of thiazides, ß-blockers and statins and fracture risk, but also the association between medication use and (change in) BMD and the association between medication use and hip bone structure to gain more insight in the causal chain of the association between use of medication and its effects on bone.

We performed all our studies in the cohort of the Rotterdam Study. This is a prospective, population-based cohort study on determinants and occurrence of diseases in the elderly. The study started in 1990 and included 7983 persons of 55 years and older.

Detailed data on many potential confounders was available and since all pharmacies in the research neighborhood are computerized and linked to one network, we have detailed exposure data on drug use for all participants.

In ***Chapter 2*** we discuss the risk of hip fracture with use of thiazide diuretics. In ***Chapter 3*** the association between statin use and bone is presented. ***Chapter 3.1*** discusses the association between bone mineral density and bone structural geometry of the femoral neck and use of statin. ***Chapter 3.2*** focuses on vertebral fracture risk for statin users and in ***Chapter 3.3*** the association between risk for all nonvertebral fractures and statin use is discussed. In ***Chapter 4*** a polymorphism of the ApoE gene is examined in relation to BMD and fracture risk. The study described in ***Chapter 5*** aims at investigating the association between use of ß-blockers and BMD, fracture risk and bone structure. ***Chapter 6*** is a general discussion of the studies and relevant methodological aspects will be considered. The main findings are placed in context of clinical practice. Furthermore, suggestions are made for future research in this field.

References

1. **Anonymous.** Consensus development conference: diagnosis, prophylaxis, and treatment of osteoporosis. Am J Med 1993; 94(6): 646-50.
2. CBO: Tweede herziene richtlijn Osteoporose. Van Zuiden Communications; 2002.
3. **Orsini LS, Rousculp MD, Long SR, Wang S.** Health care utilization and expenditures in the United States: a study of osteoporosis-related fractures. Osteoporos Int 2004.
4. Report on Osteoporosis in the European Community. Brussels; 1998.
5. **Jilka RL.** Biology of the basic multicellular unit and the pathophysiology of osteoporosis. Med Pediatr Oncol 2003; 41(3): 182-5.
6. **Wheeless CR.** Wheeless' book of orthopaedics; 1996.
7. **Russell RG, Rogers MJ, Frith JC, et al.** The pharmacology of bisphosphonates and new insights into their mechanisms of action. J Bone Miner Res 1999; 14 Suppl 2: 53-65.
8. **Marie PJ.** Strontium ranelate: a novel mode of action optimizing bone formation and resorption. Osteoporos Int 2004.
9. **Miyakoshi N.** Effects of parathyroid hormone on cancellous bone mass and structure in osteoporosis. Curr Pharm Des 2004; 10(21): 2615-27.
10. **Tamura Y, Okinaga H, Takami H.** Glucocorticoid-induced osteoporosis. Biomed Pharmacother 2004; 58(9): 500-4.
11. **Wasnich RD, Benfante RJ, Yano K, Heilbrun L, Vogel JM.** Thiazide effect on the mineral content of bone. N Engl J Med 1983; 309(6): 344-7.
12. **Pasco JA, Henry MJ, Sanders KM, Kotowicz MA, Seeman E, Nicholson GC.** Beta-adrenergic blockers reduce the risk of fracture partly by increasing bone mineral density: Geelong Osteoporosis Study. J Bone Miner Res 2004; 19(1): 19-24.
13. **Chan KA, Andrade SE, Boles M, et al.** Inhibitors of hydroxymethylglutaryl-coenzyme A reductase and risk of fracture among older women. Lancet 2000; 355(9222): 2185-8.
14. **Grahame-Smith DG, Aronson JK.** Oxford textbook of clinical pharmacology and drug therapy. 2nd ed. New York: Oxford University Press Inc.; 1994.
15. **Duarte CG, Winnacker JL, Becker KL, Pace A.** Thiazide-induced hypercalcemia. N Engl J Med 1971; 284(15): 828-30.
16. **Adams JS, Song CF, Kantorovich V.** Rapid recovery of bone mass in hypercalciuric, osteoporotic men treated with hydrochlorothiazide. Ann Intern Med 1999; 130(8): 658-60.
17. **Arnett TR, Spowage M.** Modulation of the resorptive activity of rat osteoclasts by small changes in extracellular pH near the physiological range. Bone 1996; 18(3): 277-9.

18. **Lajeunesse D, Delalandre A, Guggino SE.** Thiazide diuretics affect osteocalcin production in human osteoblasts at the transcription level without affecting vitamin D3 receptors. J Bone Miner Res 2000; 15(5): 894-901.
19. **Tobert JA.** New developments in lipid-lowering therapy: the role of inhibitors of hydroxymethylglutaryl-coenzyme A reductase. Circulation 1987; 76(3): 534-8.
20. **Cummings SR, Bauer DC.** Do statins prevent both cardiovascular disease and fracture? Jama 2000; 283(24): 3255-7.
21. **Sugiyama M, Kodama T, Konishi K, Abe K, Asami S, Oikawa S.** Compactin and simvastatin, but not pravastatin, induce bone morphogenetic protein-2 in human osteosarcoma cells. Biochem Biophys Res Commun 2000; 271(3): 688-92.
22. **Ohnaka K, Shimoda S, Nawata H, et al.** Pitavastatin enhanced BMP-2 and osteocalcin expression by inhibition of Rho-associated kinase in human osteoblasts. Biochem Biophys Res Commun 2001; 287(2): 337-42.
23. **Takeda S, Elefteriou F, Levasseur R, et al.** Leptin regulates bone formation via the sympathetic nervous system. Cell 2002; 111(3): 305-17.
24. **Sisask G, Bjurholm A, Ahmed M, Kreicbergs A.** The development of autonomic innervation in bone and joints of the rat. Journal of the Autonomic Nervous System 1996; 59(1-2): 27-33.
25. **Togari A.** Adrenergic regulation of bone metabolism: possible involvement of sympathetic innervation of osteoblastic and osteoclastic cells. Microsc Res Tech 2002; 58(2): 77-84.
26. **Hohmann EL, Levine L, Tashjian AH, Jr.** Vasoactive intestinal peptide stimulates bone resorption via a cyclic adenosine 3′,5′-monophosphate-dependent mechanism. Endocrinology 1983; 112(4): 1233-9.
27. **Lerner UH, Lundberg P, Ransjo M, Persson P, Hakanson R.** Helodermin, helospectin, and PACAP stimulate cyclic AMP formation in intact bone, isolated osteoblasts, and osteoblastic cell lines. Calcified Tissue International 1994; 54(4): 284-9.

Chapter 2

Thiazide diuretics and the risk for hip fracture

Abstract

Background

Since the majority of hip fractures are related to osteoporosis, treatment of accelerated bone loss can be an important strategy to avoid occurrence of hip fractures. Thiazides have been associated with reduced age-related bone loss by decreasing urinary calcium excretion. In this study we examined the association between dose and duration of use of thiazide diuretics and the risk for hip fracture, and studied the consequences of discontinuation.

Methods

The Rotterdam Study is a prospective cohort study that started in 1991 and included 7983 men and women of 55 years and older. For the current study, we included all individuals who were alive at June 1st 1991 and at risk for a first hip fracture at that date. Hip fractures were reported by the general practitioners and verified by trained research assistants. Exposure to thiazides was divided into seven mutually exclusive categories: never use; current use for 1–42 days; current use for 43–365 days; current use for more than 365 days; discontinuation of use since 1–60 days; discontinuation since 61–120 days; discontinuation since more than 120 days. Potential confounders were measured at baseline.

Results

7891 individuals aged 55 years and over were included in the study. 281 cases of hip fracture occurred. Relative to non-use, current use of thiazides for more than 365 days was significantly associated with a lower risk for hip fracture (hazard ratio: 0.46, 95% CI: 0.21–0.96). There was no clear dose-dependency. This lower risk disappeared approximately four months after discontinuation of thiazides.

Conclusions

Thiazide diuretics protect against hip fracture but this protective effect disappears in four months after discontinuation.

Hip fractures are associated with substantial morbidity and mortality. The costs of surgery and rehabilitation are a burden on public health resources, especially because the incidence of hip fracture increases.[1,2] The majority of hip fractures is related to osteoporosis, and treatment of accelerated bone loss may therefore be an important strategy to avoid the occurrence of hip fractures.[3]

Thiazide diuretics are widely used as antihypertensive agents. They are cheap, effective and have few important adverse effects.[4] Thiazides are considered to protect against age-related bone loss by reducing urinary calcium excretion.[5] This bone sparing effect could potentially lead to a reduced fracture incidence in patients treated for hypertension. During the past years, several epidemiological studies have been performed regarding the effect of thiazides on bone mineral density and fracture incidence. Although bone mineral density was found to be increased in thiazide users, the difference was often small.[6–12] Thiazides were found to have a protective effect on hip fracture in the majority of studies,[9,12–17] but occasionally an increased risk was found.[18] Most of the studies, however, had limitations. Some studies had detailed drug-dispensing data but limited information on potential confounders and effect modifiers.[15,18,19] Other studies consisted of small patient populations or used only baseline interview data on thiazide use[9,12,16,20] without taking into account the timing of thiazide use.[14,20,21] Detailed information on duration and dose of thiazide use was often absent, or unreliable because there were no data of use on a day-to-day basis. Because of these limitations it is still not clear how long thiazides have to be taken in order to have an effect on fracture incidence and how long this effect persists after discontinuation of thiazide use.

We conducted a prospective population-based cohort study using detailed drug dispensing information, as well as extensive information on potential risk factors to examine the association between current and past use of thiazides and the incidence of hip fractures in men and women aged 55 years and over. We also studied the effect of discontinuation of thiazides on fracture risk.

Methods

Study population

This study was conducted as part of the Rotterdam Study, a prospective population-based cohort study on the occurrence and determinants of disease and disability in the elderly.[22] In brief, in 1990 all inhabitants of Ommoord, a suburb of Rotterdam in the Netherlands, aged 55 years or older and living for at least one year in the district, were invited to participate in the study. Of the 10,275 eligible persons, 7,983 (78%) participated. Participants gave informed consent and permission to retrieve information from medical records. At baseline, between 1990 and 1993, trained interviewers administered an extensive questionnaire covering, among other topics, socio-economic background and medical history during a home interview. During subsequent visits to the study center, additional interviewing, laboratory assessments and clinical examinations were

performed. Information on vital status is obtained at regular time intervals from the municipal authorities in Rotterdam. The Medical Ethics Committee of the Erasmus MC has approved the study.

For the present study all participants were followed from June 1, 1991 until they either had an incident hip fracture, died, or reached the end of the study at December 31, 1999, whichever came first.

Exposure definition

In the research area, there are seven fully computerized pharmacies, which are all linked to one network. During the study, all participants filled their prescriptions in one of these seven pharmacies. Data on all dispensed drugs since January 1, 1991 is available in computerized format on a day-to-day basis. The data consists of information on the date of prescribing, the total amount of drug units per prescription, the prescribed daily number of units, product name of the drugs and the Anatomical Therapeutical Chemical (ATC)-code.[23]

The exposure of interest included plain thiazides and thiazides in combination with potassium and potassium-sparing agents. Although formally not a thiazide, we included chlorthalidone because it has a similar effect on calcium excretion as thiazide diuretics. In a previous study no difference was found between these two diuretics. Therefore, we did not distinguish between these types.[12]

When a hip fracture occurred, that date was defined as the index date and the cumulative duration of use of thiazides on that date was calculated for each cohort member. Current use was defined as use of thiazides at the index date and expressed as the number of consecutive days of use. Past use was defined as use of thiazides after baseline and before, but not on the index date itself. Past use was expressed as the number of days since discontinuation of the last episode of use. To study the effect of duration of thiazide use, exposure at the index date was divided into seven mutually exclusive categories: never use, current use for 1–42 days; current use for 43–365 days; current use for more than 365 days; discontinuation of use since 1–60 days; discontinuation of use since 61–120 days and discontinuation of use since more than 120 days. These categories were defined *a priori*, so before performing this study. We selected the first duration interval of 42 days, because in the first six weeks of thiazide use the decrease in circulating volume can cause dizziness and relative cerebral ischaemia. We anticipated that this might be associated with a transiently increased risk for falls that should be distinguished from a potentially protective effect after prolonged use. After 42 days, the circulating volume in most patients is within normal limits.[24] The interval of duration of use for more than 365 days was chosen because trials on incidence of nonvertebral fractures with use of anti-osteoporotic agents, such as bisphosphonates, all had at least one year of follow-up too. Finally, the time interval of 60 days after discontinuation of use was chosen because this interval was employed before in an earlier study.[15]

We expressed the prescribed daily dose during current use at the index date as a proportion of the defined daily dose.[25] The defined daily dose of thiazide diuretics equals the standard recommended adult daily dose for treatment of hypertension in the Netherlands.

To reduce potential misclassification of exposure at baseline we ensured potential pharmacy data of at least five months before baseline for all participants.

Outcome definition

General practitioners of the study participants report all fatal and non-fatal events, such as fractures, through a computerized system. These data cover about 80% of the population and for participants not covered, research physicians performed annual checks on the complete medical records of all general practitioners in the Rotterdam Study.

All fractures that occurred during the study period were independently coded by two research physicians according to the International Classification of Diseases, 10th revision (ICD-10).[26] A medical expert in the field (CdL), unaware of the patient's history and medication use (including thiazides), reviewed all coded events for a final classification. Fractures with ICD-codes S72.0, S72.1 and S72.2 were included, but pathological hip fractures (M84.4) and fractures in prosthetic hips (M96.6) were excluded.

Co-factors

The following baseline patient characteristics were individually assessed as potential confounders: age, gender, score on the Mini Mental State Examination (MMSE <26 points),[27] use of a walking aid, any fracture in the past five years, history of hysterectomy, thyroid disease, frequency of falling ($\geq$ once/month), current smoking, intake of alcohol (>2 gram/day), and dizziness (all determined by interview). Participants were interviewed about a previous diagnosis of Parkinson's disease or anti-parkinsonian drug use and screened for symptoms of parkinsonism by study physicians at the research center. Diabetes mellitus was defined as the use of glucose-lowering medication or a random or post load serum glucose level $\geq$11.1 mmol/l. Hypertension (systolic blood pressure >160 mmHg and/or diastolic blood pressure >95 mmHg or use of any antihypertensive drug), visual impairment in one or both eyes, and body mass index (kg/m^2) were measured at the research center. Presence of peripheral arterial disease was measured (as described previously[28]) by single systolic blood pressure calculation both at the left and the right posterior tibial artery. The ratio of the systolic blood pressure at the ankle to the systolic blood pressure at the arm (AAI) was calculated for each leg. Peripheral arterial disease was considered present when the AAI was lower than 0.9 on at least one side. Lower limb disability was assessed using a modified version of the Stanford Health Assessment Questionnaire[29] and by calculating the mean score of answers to questions concerning rising, walking, bending and getting in and out of a car.[30] A score of zero indicates no disability, a score between zero and one indicates mild disability and a score of more than one indicates severe disability. Intake of calcium was adjusted for the total caloric intake according to the method of Willett.[31] Bone mineral density measurements of the femoral neck were performed by dual energy X-ray absorptiometry (DPX-L densitometer, Lunar Corp, Madison, Wisconsin) as described previously.[32] Stratification on bone mineral density was done by dividing the population at the median observation (0.71 g/cm^2).

Use of other medications such as corticosteroids, benzodiazepines, antidepressants, antihistamines, opioids, antacids, antipsychotics, statins and estrogens was analyzed as a potential confounder.

Statistical analysis

Since exposure to thiazides may vary over time, we calculated risks for hip fracture with a Cox proportional hazards model[33] with the exposure represented by time-varying covariates. The model compares the exposure to thiazides on the index date of each case with an incident hip fracture, with all other participants in the cohort who are alive and at risk for hip fracture at the index date. We also used time-dependent categorical variables to compare duration of current use and time since last use. Hereto, the seven above mentioned exposure categories of continuous use in number of days are represented in the model by six dummy variables with 'never use' as a reference category. Use of other prescribed drugs was also analyzed as a time-dependent categorical variable. We did a trend analysis on the exposure categories for current use.

To adjust for potential confounders, co-factors that were associated with the occurrence of hip fracture were included in the age- and gender-adjusted model if this caused a change in the point estimate of more than 5 percent. Because bone mineral density is a potential intermediate factor in the cause-effect relationship of thiazides and hip fractures, we selected only participants who did not use thiazides at baseline to study effect modification by femoral neck bone mineral density. To study whether there was a daily dose-effect relationship for current users, we divided daily dose into equal or less than 1.0 defined daily dosage and higher than 1.0 defined daily dosage and tested the effect of low dose use and high dose use against no use in separate regression analyses. To test an earlier suggestion of effect modification by low calcium intake,[13] we also ran separate regression analyses for participants with the lowest tertile of calcium intake at baseline ($\leq$ 958 mg / day) versus participants with higher intakes.

Multiple imputation was used to impute missing information for confounding variables. Five imputation values were calculated on the basis of the posterior predictive distribution of the missing values and five complete data sets were created. On each complete set, the statistical analyses were performed and the point estimates of the five data sets were combined to form one summary statistic as the average of the five components. The variance of the summary statistic is calculated from the within-imputation variance and the between-imputation variance. The combined variance accounts for the uncertainty introduced by estimating the missing values.[34] All analyses were performed with SAS (procedures MI, MIANALYZE and PHREG, Statistical Analysis System version 8, Cary, NC).

Results

Eighty-four participants died and eight participants had an incident hip fracture before June 1, 1991. Therefore, the study population included 7,891 participants. During a total follow-up of 58,009 person-years, 281 cases of hip fracture occurred. Table 1 shows baseline characteristics for the cases and the total cohort from which they arise and the percentage of missing values for the variables. When included in a Cox proportional hazards model with age and gender, body mass index, lower limb disability, current smoking, and use of estrogens caused a change in the point estimate of 5% or more. These variables were therefore included in the final model. At baseline, thiazide users were more often

disabled, had more often a history of dizziness, diabetes mellitus, frequent falling, and previous fractures in the last five years, but these factors did not change the point estimate by 5% or more.

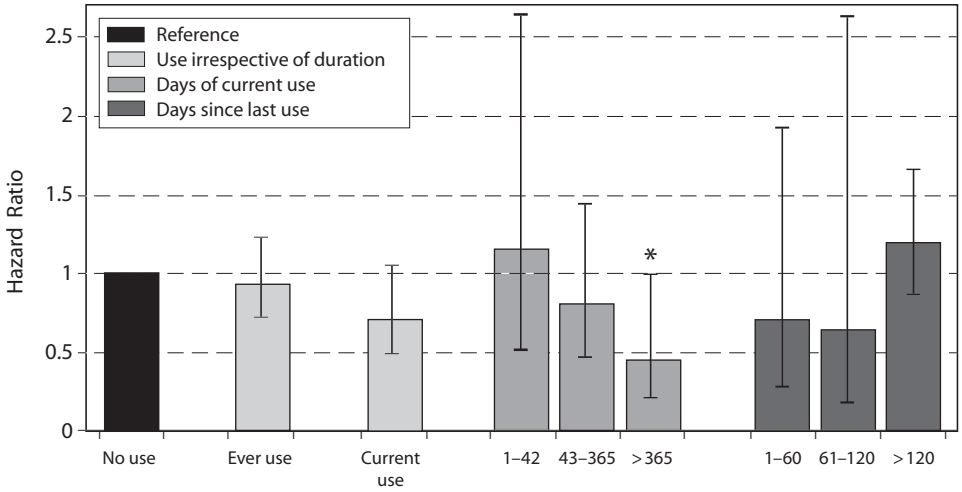
The risk for hip fracture for ever use of thiazides (yes/no) was decreased but did not reach statistical significance (hazard ratio = 0.94, 95% CI = 0.72–1.24). The same holds for current use of thiazides, irrespective of duration of use (hazard ratio = 0.71, 95% CI =

Table 1 — Baseline characteristics of cases and total study population.

	Cases (N = 281)	**Cohort** (N = 7891)	**Missing values** (%)
Gender			0
Male	60	3071	
Female	221	4820	
Age (years)	78.24 (8.61)	68.93 (9.90)	0
55–64	21	3022	
65–74	66	2592	
75–84	121	1658	
≥ 85	73	619	
Femoral neck BMD (g/cm^2)	0.71 (0.124)	0.84 (0.137)	26
< 0.78	106	1972	
0.78–0.89	36	1993	
> 0.89	11	1845	
BMI (kg/m^2)	25.74 (3.60)	26.28 (3.74)	13
Weight (kg)	68.39 (11.12)	72.99 (11.96)	12
Lower limb disability			3
None	47	3584	
Mild	109	2850	
Severe	109	1207	
MMSE < 26	89	1102	10
Hypertension	102	2612	10
Parkinson's disease	6	75	3
Thyroid disease	31	691	8
Diabetes mellitus	39	802	15
Visual impairment	40	523	19
Peripheral arterial disease	74	1210	19
History of fracture	58	1074	7
History of hysterectomy	16	456	6
Use of a walking aid	82	839	8
Dizziness	56	1261	13
Current smoking	58	1725	4
Recent falling	78	1336	3
Estrogen use at baseline	2	101	27
Calcium intake (mg/day)			
Upper two tertiles (> 958)	82	3618	31
Lower tertile (≤ 958)	44	1804	
Alcohol intake (g/day)	8.2 (12.5)	10.3 (15.2)	31

Values in parentheses are standard deviations.
BMD = Bone mineral density; BMI = Body mass index; MMSE = Mini Mental State Examination.

Figure 1 — Hazard ratios and 95% CIs for hip fracture with use of thiazide diuretics.



All estimates were adjusted for age, gender, body mass index, lower limb disability, current smoking and estrogen use.
* p = 0.05
Trend of current use p < 0.05.

Table 2 — Hazard ratios of thiazide use on hip fracture.

	Number of cases	Hazard ratio* (95% CI)
Never use	202	1.00 (reference)
Ever use	79	0.94 (0.72–1.24)
Current use	26	0.71 (0.47–1.06)
Duration of current use		
1–42 days	6	1.17 (0.52–2.63)
43–365 days	13	0.81 (0.46–1.42)
>365 days	7	0.46 (0.21–0.96)
Daily dose+		
≤1.0 DDD	3	0.29 (0.09–0.90)
>1.0 DDD	4	0.85 (0.32–2.32)

* = Adjusted for age, gender, lower limb disability, body mass index, use of estrogens and current smoking.
+ = DDD: defined daily dosage in current users with >365 days of thiazide use.

0.47–1.06). When using duration of current thiazide use (in months) as a continuous variable, there was a significant inverse association between increase of duration of use and risk of hip fracture (hazard ratio = 0.99; 95% CI 0.97–0.99). Table 2 shows that with increasing duration of consecutive use among current users the adjusted risk for hip fracture was significantly reduced to 0.46 for persons exposed for more than one year. Although the risk was lower among persons taking the lowest dose, the difference was not significant. We assessed whether the protective effect of thiazides persisted after discontinuation of

thiazide use and found a non-significant risk reduction up to 120 days, after which the hazard ratio returned to 1.0 (Figure 1).

The strongest protective effect was found in participants older than 80 years but the difference with participants below 80 years was not statistically significant. No differences in the effect of long-term thiazide use for participants with a lower or higher bone mineral density were demonstrated. A somewhat larger risk reduction for participants with a higher intake of calcium was shown (Table 3). Due to a low number of men with hip fracture, there was insufficient power to study effect modification by gender.

Table 3 — Risk for hip fracture for subjects with current thiazide use for more than 365 days: effect modification of age, bone mineral density[+], and calcium intake.

Stratum	**Number of cases** (Exposed / Total)	**Hazard ratio*** (95% CI)
≤ 80 years	4/158	0.53 (0.19–1.43)
> 80 years	3/123	0.38 (0.12–1.21)
BMD ≤ 0.71 g/cm^2	2/82	0.67 (0.09–4.87)
BMD > 0.71 g/cm^2	2/71	0.62 (0.09–4.55)
Calcium intake[†] ≤ 958 mg/day	6/199	0.57 (0.13–2.45)
Calcium intake[†] > 958 mg/day	1/82	0.21 (0.03–1.50)

BMD = Bone mineral density.

* = The estimates were adjusted for age (days), gender, lower limb disability, current smoking, body mass index, and use of estrogens.

[+] = For investigation of effect modification of bone mineral density, subjects with thiazide use at baseline were excluded.

[†] = The calcium intake was adjusted for total caloric intake.

To adjust for potential misclassification of duration of thiazide use at the start of the study, we also did an analysis in which we excluded all participants (35 cases, 849 controls) who used thiazides at baseline (self-reported users and pharmacy data-derived users) and cases with less than 365 days of follow-up. We still found a 30% reduction in incident hip fractures, but because of smaller numbers of participants this was no longer statistically significant (hazard ratio = 0.72, 95% CI = 0.29–2.15).

Discussion

In this study, long-term current use of thiazides was associated with a lower risk for hip fractures. Although this was already visible after short-term use, the risk reduction only reached statistical significance after one year of continuous intake. This protective effect occurred independently of thiazide dose. After discontinuation of thiazide treatment, the protective effect disappeared after four months. Because of the large female preponderance in cases, we were not able to study effect modification by gender.

With the exception of one case-control study in which thiazides were associated with an increased risk for hip fracture,[18] several other observational studies found that thiazide use is associated with a lower incidence of hip fractures.[12,13,15,16,19] Very few studies have investigated the duration of the protective effect of thiazides. In a previous study,[15] a decreased risk for fracture for up to two months after discontinuation was found, and in a case-control study an increased risk for hip fracture for any past use was found.[13] We assessed how long the protective effect of thiazides lasted after discontinuation of use in a more precise way, and found a risk reduction up to four months after discontinuation of thiazide use.

In several studies published over the past decades, the mechanisms by which thiazides might protect against hip fracture have been discussed. First, thiazides can reduce renal calcium excretion, thereby creating a positive calcium balance.[5,35] Second, by inducing a metabolic alkalosis thiazides can inhibit bone resorption.[36,37] Furthermore, *in vitro* studies showed that thiazides directly inhibit osteocalcin secretion of osteoblast-like cells.[38,39] Transbol et al.[21] found an effect of thiazides on bone mineral density only in the first six months of use, whereafter the mineral density of thiazide users was not significantly different from placebo users. Other studies, including a randomized controlled trial, found protective effects of thiazides on bone mineral density,[8,9,11] but this effect was often small. In our study, we could not study the effect of thiazides on bone mineral density because we had only cross-sectional data. However, if we adjusted for a cross-sectionally measured bone mineral density in 1993, two years after start of the study, the effect remained. When we investigated effect modification by calcium intake we observed a trend towards a larger thiazide effect for participants with a higher calcium intake. This might suggest that creating a positive calcium balance is not the only mechanism by which thiazides do affect fracture risk, because participants with moderate to high calcium intake do benefit from thiazides as well.

Several aspects of validity need to be discussed. Selection bias is unlikely to have occurred, as our study was prospective and population-based. Although non-participants of the Rotterdam Study were slightly older (on average 73 vs. 70 years of age), it is very unlikely that participation was conditional on the exposure to thiazides. Information bias is also unlikely to play a role, as exposure data were gathered before disease onset. We used pharmacy records to overcome the problem of potential misclassification of exposure that was the main concern in earlier studies, which defined exposure as use of thiazides at a baseline interview. Because of independent and reliable information regarding drug exposure, in particular when it comes to duration of use and past use, we were able to investigate the effect of thiazides for different periods of use. It is also not likely that confounding explains our results as we adjusted for many known confounders. It has been known for quite some time that thiazides can enhance calcium metabolism, but it is unlikely that general practitioners, during the study period, prescribed thiazides preferentially to patients to lower their hip fracture risk. Confounding by indication cannot be an explanation for our results, because thiazides were somewhat more frequently prescribed to patients at a higher risk for hip fracture. This would rather tend to take away the protec-

tive effect and even a spurious risk increase might be found. Because there were few male cases in our study, the effects of thiazides on hip fracture risk in women probably dominate our results. Hence, it is possible that these effects are somewhat different in men.

Hypertension is a very common medical problem that often requires long-term treatment. Thiazides are still advised as first choice antihypertensive agents, but the prescription rate of thiazides has decreased over the past decade.[40] Recently a randomized trial showed that thiazide-type diuretics are superior in preventing major forms of cardiovascular disease compared to angiotensin-converting enzyme (ACE) inhibitors and calcium channel blockers.[41] Thiazides are cheap and have few adverse effects.[42] Our results demonstrate that with long-term thiazide use, a significant reduction in hip fracture incidence can be observed.

References

1. **Cummings SR, Melton LJ.** Epidemiology and outcomes of osteoporotic fractures. Lancet 2002; 359 (9319): 1761-7.
2. **Melton LJ, 3rd.** Hip fractures: a worldwide problem today and tomorrow. Bone 1993; 14 (Suppl 1): S1-8.
3. **Cummings SR, Kelsey JL, Nevitt MC, O'Dowd KJ.** Epidemiology of osteoporosis and osteoporotic fractures. Epidemiol Rev 1985; 7: 178-208.
4. **Moser M.** Why are physicians not prescribing diuretics more frequently in the management of hypertension? JAMA 1998; 279 (22): 1813-6.
5. **Duarte CG, Winnacker JL, Becker KL, Pace A.** Thiazide-induced hypercalcemia. N Engl J Med 1971; 284 (15): 828-30.
6. **Morton DJ, Barrett-Connor EL, Edelstein SL.** Thiazides and bone mineral density in elderly men and women. Am J Epidemiol 1994; 139 (11): 1107-15.
7. **Reid IR, Ames RW, Orr-Walker BJ, et al.** Hydrochlorothiazide reduces loss of cortical bone in normal postmenopausal women: a randomized controlled trial. Am J Med 2000; 109 (5): 362-70.
8. **Wasnich RD, Benfante RJ, Yano K, Heilbrun L, Vogel JM.** Thiazide effect on the mineral content of bone. N Engl J Med 1983; 309 (6): 344-7.
9. **Wasnich RD, Ross PD, Heilbrun LK, Vogel JM, Yano K, Benfante RJ.** Differential effects of thiazide and estrogen upon bone mineral content and fracture prevalence. Obstet Gynecol 1986; 67 (4): 457-62.
10. **LaCroix AZ, Ott SM, Ichikawa L, Scholes D, Barlow WE.** Low-dose hydrochlorothiazide and preservation of bone mineral density in older adults. A randomized, double-blind, placebo-controlled trial. Ann Intern Med 2000; 133 (7): 516-26.
11. **Sigurdsson G, Franzson L.** Increased bone mineral density in a population-based group of 70-year-old women on thiazide diuretics, independent of parathyroid hormone levels. J Intern Med 2001; 250 (1): 51-6.
12. **Cauley JA, Cummings SR, Seeley DG, et al.** Effects of thiazide diuretic therapy on bone mass, fractures, and falls. The Study of Osteoporotic Fractures Research Group. Ann Intern Med 1993; 118(9): 666-73.
13. **Felson DT, Sloutskis D, Anderson JJ, Anthony JM, Kiel DP.** Thiazide diuretics and the risk of hip fracture. Results from the Framingham Study. JAMA 1991; 265 (3): 370-3.
14. **Hale WE, Stewart RB, Marks RG.** Thiazide and fractures of bones. N Engl J Med 1984; 310(14): 926-7.
15. **Herings RM, Stricker BH, de Boer A, Bakker A, Sturmans F, Stergachis A.** Current use of thiazide diuretics and prevention of femur fractures. J Clin Epidemiol 1996; 49(1): 115-9.
16. **LaCroix AZ, Wienpahl J, White LR, et al.** Thiazide diuretic agents and the incidence of hip fracture. N Engl J Med 1990; 322(5): 286-90.
17. **Rashiq S, Logan RF.** Role of drugs in fractures of the femoral neck. Br Med J (Clin Res Ed) 1986; 292 (6524): 861-3.
18. **Heidrich FE, Stergachis A, Gross KM.** Diuretic drug use and the risk for hip fracture. Ann Intern Med 1991; 115 (1): 1-6.

19. **Ray WA, Griffin MR, Downey W, Melton LJ.** Long-term use of thiazide diuretics and risk of hip fracture. Lancet 1989; 1 (8640): 687-90.
20. **Adland-Davenport P, McKenzie MW, Notelovitz M, McKenzie LC, Pendergast JF.** Thiazide diuretics and bone mineral content in postmenopausal women. Am J Obstet Gynecol 1985; 152 (6 Pt 1): 630-4.
21. **Transbol I, Christensen MS, Jensen GF, Christiansen C, McNair P.** Thiazide for the postponement of postmenopausal bone loss. Metabolism 1982; 31 (4): 383-6.
22. **Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA.** Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol 1991; 7 (4): 403-22.
23. **WHO.** Anatomical Therapeutical Chemical (ATC) Classification Index. In. Oslo: World Health Organization Collaborating Centre for Drug Statistics Methodology; 1993.
24. **Anonymous.** Tractus Uropoeticus. In: van der Kuy A, ed. Farmacotherapeutisch Kompas. Amstelveen: Commissie Farmaceutische Hulp of the College voor zorgverzekeringen; 2000: 523.
25. **WHO.** Anatomical Therapeutic Classification (ATC) Index: Including Defined Daily Doses (DDD) for Plain Substances. In. Oslo; 1992.
26. **WHO.** International Statistical Classification of Diseases and Related Health Problems, 10th revision. In. Geneva; 1992.
27. **Folstein MF, Folstein SE, McHugh PR.** "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975; 12 (3): 189-98.
28. **Meijer WT, Hoes AW, Rutgers D, Bots ML, Hofman A, Grobbee DE.** Peripheral arterial disease in the elderly: the Rotterdam Study. Arterioscler Thromb Vasc Biol 1998; 18 (2): 185-92.
29. **Pincus T, Summey JA, Soraci SA, Jr., Wallston KA, Hummon NP.** Assessment of patient satisfaction in activities of daily living using a modified Stanford Health Assessment Questionnaire. Arthritis Rheum 1983; 26 (11): 1346-53.
30. **Burger H, de Laet CE, van Daele PL, et al.** Risk factors for increased bone loss in an elderly population: the Rotterdam Study. Am J Epidemiol 1998; 147 (9): 871-9.
31. **Willett WC, Sampson L, Stampfer MJ, et al.** Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 1985; 122 (1): 51-65.
32. **Burger H, van Daele PL, Algra D, et al.** The association between age and bone mineral density in men and women aged 55 years and over: the Rotterdam Study. Bone Miner 1994; 25 (1): 1-13.
33. **Cox D.** Regression models and life tables. J Royal Stat Society 1972: 187-220.
34. **Twisk J, de Vente W.** Attrition in longitudinal studies. How to deal with missing data. J Clin Epidemiol 2002; 55 (4): 329-37.
35. **Adams JS, Song CF, Kantorovich V.** Rapid recovery of bone mass in hypercalciuric, osteoporotic men treated with hydrochlorothiazide. Ann Intern Med 1999; 130 (8): 658-60.
36. **Arnett TR, Spowage M.** Modulation of the resorptive activity of rat osteoclasts by small changes in extracellular pH near the physiological range. Bone 1996; 18 (3): 277-9.
37. **Peh CA, Horowitz M, Wishart JM, Need AG, Morris HA, Nordin BE.** The effect of chlorothiazide on bone-related biochemical variables in normal post-menopausal women. J Am Geriatr Soc 1993; 41 (5): 513-6.
38. **Lajeunesse D, Delalandre A, Guggino SE.** Thiazide diuretics affect osteocalcin production in human osteoblasts at the transcription level without affecting vitamin D3 receptors. J Bone Miner Res 2000; 15 (5): 894-901.
39. **Aubin R, Menard P, Lajeunesse D.** Selective effect of thiazides on the human osteoblast-like cell line MG-63. Kidney Int 1996; 50 (5): 1476-82.
40. **Onder G, Gambassi G, Landi F, et al.** Trends in antihypertensive drugs in the elderly: the decline of thiazides. J Hum Hypertens 2001; 15 (5): 291-7.
41. Major outcomes in high-risk hypertensive patients randomized to angiotensin-converting enzyme inhibitor or calcium channel blocker vs diuretic: The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). Jama 2002; 288 (23): 2981-97.
42. **Savage PJ, Pressel SL, Curb JD, et al.** Influence of long-term, low-dose, diuretic-based, antihypertensive therapy on glucose, lipid, uric acid, and potassium levels in older men and women with isolated systolic hypertension: The Systolic Hypertension in the Elderly Program. SHEP Cooperative Research Group. Arch Intern Med 1998; 158 (7): 741-51.

Chapter 3

Chapter 3.1

Long-term use of statins is associated with bone mineral density

Abstract

Background

Over the past few years, several reports on the association between statins and bone mineral density (BMD) have been published. The results of these studies were contradictory and most studies were small and/or cross-sectional. We studied the association between use of statins, BMD and hip bone structure of the femoral neck in the Rotterdam Study.

Methods

The Rotterdam Study is a prospective cohort study that started in 1991 and included 7983 men and women of 55 years and older. For the current study, we included all individuals for whom BMD measurements were available at the third examination round. BMD was measured by DXA and hip bone structure parameters were estimated from the DXA outputs. Exposure to statins was available on a day-to-day basis from complete medication histories. Potential confounders were measured at baseline.

Results

We included a total of 2644 participants (1132 men and 1512 women), for whom femoral neck BMD measurements and data on potential confounders were available. Hip bone structure data were present for 2597 persons. Mean follow-up was 6.3 years. During the follow-up period, 366 participants used statins on at least one day. The median duration of statin use was 782 days. Mean BMD of long-term users at end of follow-up (>4 years of use) was significantly higher than mean BMD of non-users and rates of bone loss were significantly lower for long-term users. Use of lipophilic statins (all statins except pravastatin) was associated with a higher mean BMD. Bone structure analyses of exposed subjects showed a significantly thicker cortex and greater femoral neck stability compared with non-exposed subject.

Conclusion

Use of statins is associated with a higher mean BMD at the end of follow-up, a lower rate of bone loss and greater femoral neck stability.

Osteoporosis is a skeletal disorder characterized by a low bone mass and micro-architectural deterioration of bone. It is a condition that leads to a substantial number of fractures and subsequent impairment in the elderly. Prevention and treatment of osteoporosis is therefore an important way to decrease morbidity and health expenditures. Osteoporosis is mainly treated with bone resorption inhibitors such as bisphosphonates. Stimulation of bone formation might be an addition to treatment and prevention of osteoporosis.

Statins are cholesterol-lowering agents that inhibit the mevalonate pathway of cholesterol synthesis. This mevalonate pathway is also involved in bone metabolism.[1] *In vitro,* statins have been shown to stimulate bone formation,[2] but observational studies on bone mineral density (BMD) and statin use in humans gave contradictory results.[3–10] Many of these studies, however, had one or more limitations such as a low number of (exposed) subjects and the selection of participants from diseased populations (e.g. steroid-users, diabetics, or persons visiting special clinics).[3,6,7] Exposure to statins in these studies was often based on self-reported data from participants or retrospective review of medical records.[3,4,7,9–11] Reliable information on precise duration of use was therefore often not available and most of the previous studies on this subject did not examine the association between duration of statin use and BMD.

Statins are used to treat persons at a higher risk of cardiovascular disease and because it is speculated that cardiovascular disease and osteoporosis are somehow related to each other,[12] it is important to adjust for confounders that reflect cardiovascular disease risk. However, in the available cross-sectional and retrospective medical record studies, data on (potential) confounders is limited.

Analyzing bone structural geometry, derived from dexa scans, might provide more insight into a potential association between statin use and fracture risk. Therefore, the objective of this study was to examine the association between (duration of) statin use and BMD and structure of the femoral neck in a prospective cohort study with data on potential confounders, and detailed information on exposure to statins and other drugs.

Methods

Study population

This study was conducted as part of the Rotterdam Study, a prospective, population-based cohort study on the occurrence and determinants of disease and disability in elderly persons.[13] In 1990, all inhabitants of Ommoord, a suburb of Rotterdam in the Netherlands, who were 55 years of age or older and had lived for at least 1 year in the district were invited to participate in the study. Of the 10,275 eligible persons, 7,983 (78%) participated. Participants gave informed consent and permission to retrieve information from medical records. At baseline, between 1990 and 1993, trained interviewers administered an extensive questionnaire covering socioeconomic background and medical history, among other topics, during a home interview. During subsequent visits to the study center, additional interviewing, laboratory assessments, and clinical examinations

were performed. Information on vital status is obtained at regular time intervals from the municipal authorities in Rotterdam. The Medical Ethics Committee of the Erasmus Medical Center, Rotterdam, the Netherlands, approved the study. Follow-up visits took place between 1993 and 1994 and between 1997 and 1999.

For the present study, all participants who had their BMD measured at the third visit were included in the study.

Bone mineral density measurements

BMD (g/cm^2) was measured during baseline visit and during the third examination, by dual energy X-ray absorptiometry (DXA, Lunar DPX-L densitometer) as described previously.[14] During the study, all measurements were done using the same densitometer and the same standard protocols. All scans (n = 13.391) were reanalyzed using DPX-IQ v.4.7d software to adjust for software upgrades of the densitometer during the study. To increase the accuracy in follow-up BMD measurements, the search and template tools in the compare mode of the DPX-IQ software were used to position the femoral neck region-of-interest in scans of the same individual. Additional retrospective calibrations using phantom measurements were performed to adjust for software changes not corrected by the DPX-IQ reanalysis.[15]

Rates of loss of BMD were expressed as the percentage rate of change in BMD per year, calculated as the BMD difference between baseline and the third examination period divided by the baseline BMD and divided by follow-up time in years.

Hip structural analysis

Hip bone structure was derived from DXA scans of the narrow-neck region across the narrowest point of the femoral neck by hip structural analysis software developed by Thomas J. Beck (Figure 1).[16] Bone width and cross-sectional moment of inertia (CSMI)

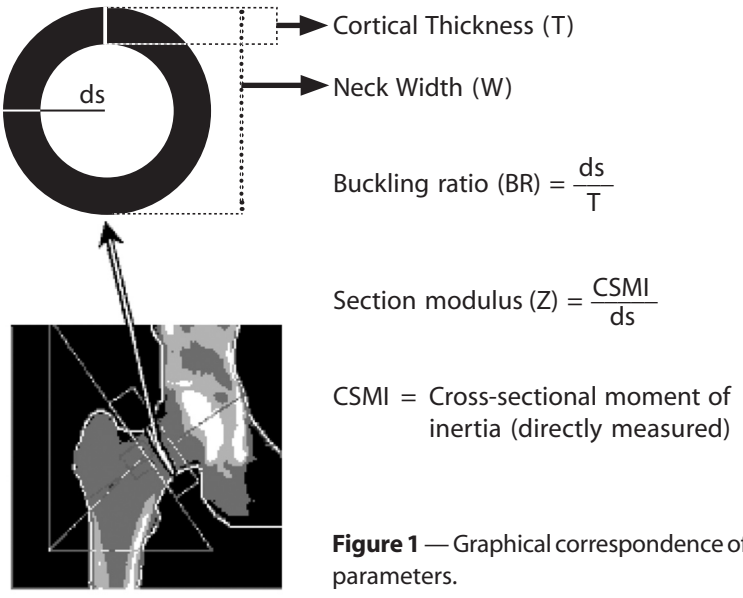


Figure 1 — Graphical correspondence of the hip bone structure parameters.

were measured directly from mineral mass distributions using algorithms described previously.[17] In addition, estimates of cortical thickness and endocortical diameters were obtained modeling the NN region as a circular annulus, which assumes a proportion of cortical/trabecular bone of 60/40. The cross-sectional area quantifies the total surface area of bone in the cross section after excluding soft tissue spaces. Section modulus, an index of bending strength, was calculated as CSMI/ds, where ds is the maximum distance from the center of mass to the medial or lateral surface. Buckling ratio, an index of bone instability, was computed as ds divided by estimated mean cortical thickness. A higher buckling ratio means higher instability.

Exposure

In the research area, there are seven fully computerized pharmacies, which are all linked to one network. During the study, all participants filled their prescriptions in one of these seven pharmacies. Information on all dispensed drugs since 1 January 1991 is available in computerized format on a day-to-day basis. The data consists of information on the date of prescribing, the total amount of drug units per prescription, the prescribed daily number of units, product name of the drugs and the Anatomical Therapeutic Chemical (ATC)-code.[18]

Statin use was assessed during the period between the first and third examination round and classified as use or non-use. We then assessed cumulative statin use during the follow-up period and categorized statin use on the basis of duration of exposure. We defined four mutually exclusive intervals: non-use, ≤ 2 years of use, >2 years and ≤ 4 years of use, and >4 years of use.

We distinguished users of pravastatin (a hydrophilic statin) from users of other (lipophilic) statins, because pravastatin was previously reported not to induce BMP-2.[19,20]

To examine dose-effects, we performed stratified analyses on the median daily dose among statin users.

Co-factors

During a baseline home interview, trained interviewers gathered information on medical history, risk factors for chronic diseases, medication use and habitual diet. Amongst others, information was gathered on potential risk factors such as smoking and age at menopause. Lower limb disability was assessed using a modified version of the Stanford Health Assessment Questionnaire[21] and by calculating the mean score of answers to questions concerning rising, walking, bending and getting in and out of a car.[14] A score of more than one indicates disability.

After the home interview, the participants were invited to visit the research center for clinical examinations and laboratory assessments. Blood samples were drawn and analyzed. Cognitive impairment was measured using the Mini Mental State Examination.[22] We computed the 5-year Framingham cardiovascular disease risk score for all participants with help of a previously published algorithm.[23] This score predicts 5-year cardiovascular disease risk and can be used as a measure of indication for statin therapy.

Information on use of other medications, such as thiazide diuretics and ß-blockers, was also derived from the computerized pharmacy database.

Statistical analysis

Demographic and clinical characteristics of statin users versus non-users were compared with Student's t-test and Pearson's Chi-square.

To examine the association between bone mineral density and cumulative statin use we used a linear regression model. Regression coefficients were computed using bone mineral density as dependent variable and statin use in years as independent variable. In subsequent models, we additionally adjusted for age, gender and follow-up time. Other potential confounders, such as risk factors for cardiovascular disease and osteoporosis were included in the model if they were biologically plausible and/or caused a change in the point estimate of more than 10%.

We performed analyses of covariance to compute crude and adjusted means of bone mineral density and bone structure parameters for categories of statin use. The category reflecting no use of statins was used as the reference category for significance tests. All analyses were conducted using SPSS 11.5 for Windows (SPSS Inc., Chicago, IL).

Results

In our study, we included a total of 2644 participants (men and women), for whom femoral neck BMD measurements and data on potential confounders were available. Hip bone structure data were present for 2597 persons. Mean follow-up was 6.3 years. During the study, 366 participants used statins (48 the non-lipophilic pravastatin only, 284 lipophilic statins only, and 34 both types) on at least one day of whom 156 were past users at time of the third visit. The median duration of statin use was 782 days.

Participants who were included in this study were on average younger and healthier than participants of the total cohort (Table 1).

In a linear regression analysis, we observed a significant association between BMD of the femoral neck and increasing duration of statin use. Per year increase in statin use, BMD of femoral neck increased 0.009 g/cm^2 ($p < 0.001$) as estimated by a univariate linear regression analysis (Table 2). After adjustment for age, gender and duration of follow-up, the positive association remained significant. Since use of statins was associated with use of ß-blockers and thiazide diuretics (odds ratio (OR) 3.6; 3.0–4.4 and OR 1.5; 1.2–1.8, respectively), and use of ß-blockers and thiazides is also associated with an increased BMD,[24,25] we adjusted for the cumulative number of days of ß-blocker and thiazide use. After additional adjustments for body mass index, diabetes mellitus, baseline BMD and 5-yr cardiovascular disease risk, there was still a significant beta-coefficient of 0.002 g/cm^2 per year of statin use. Additional adjustment for smoking, lower limb disability, use of a walking aid, use of glucocorticoids or hormone replacement therapy, did not change the estimates. Baseline BMD was not associated with baseline serum cholesterol, and there was no significant trend between tertiles of cholesterol levels and baseline BMD. Adjustment for baseline serum cholesterol levels did not change the estimates, but we included cholesterol levels in the model to deal with potential confounding by indication.

Any use of statins during follow-up was associated with a significantly higher mean BMD at the end of follow-up; non-users had a mean BMD of 0.840 g/cm^2 (CI 95% 0.834–0.846) and statin users had a mean BMD of 0.866 g/cm^2 (0.851–0.880). After adjustments

for age and gender, mean BMD for statin users was still significantly higher than BMD of non-users (0.841 and 0.858, respectively), but in the full model the differences were not significantly different any more (0.843 and 0.846). We divided statin users in users of pravastatin and users of lipophilic statins, and excluded persons who used both types.

Table 1 — Baseline characteristics for the current study population and for the total cohort of the Rotterdam Study.

	Study population	**Rotterdam Study**
Number	2644	7983
Age (year)	65.8 (6.6)	68.1 (8.0)
Gender (women)	1512 (57.2%)	4878 (61.1%)
Previous nonvertebral fracture	380 (14.4%)	1060 (13.3%)
Femoral neck BMD (g/cm^2)	0.88 (0.14)	0.87 (0.14)
Serum cholesterol (mmol/l)	6.7 (1.2)	6.6 (1.2)
Use of a walking aid	75 (2.8%)	845 (10.6%)
Lower limb disability	101 (3.8%)	1237 (15.5%)
Body mass index (kg/m^2)	26.3 (3.5)	26.3 (3.7)
MMSE	28.1 (1.5)	27.1 (3.4)
Current smoking	569 (21.5%)	1725 (21.6%)
Diabetes mellitus	174 (6.6%)	811 (10.2%)
Framingham 5-yr CVD risk (%)	14 (9)	15 (1)
Hypertension	743 (28.1%)	2595 (32.5%)
History of myocardial infarction	127 (4.8%)	483 (6.1%)
Alcohol intake (g/day)	11.0 (15.3)	10.4 (15.2)
Use of hormone replacement therapy	52 (2.0%)	101 (1.3%)
Use of diuretics[#]	261 (9.9%)	1316 (16.5%)
Use of ß-blockers[#]	371 (14.0%)	1134 (14.2%)
Use of lipid-lowering drugs[#]	76 (2.9%)	175 (2.2%)

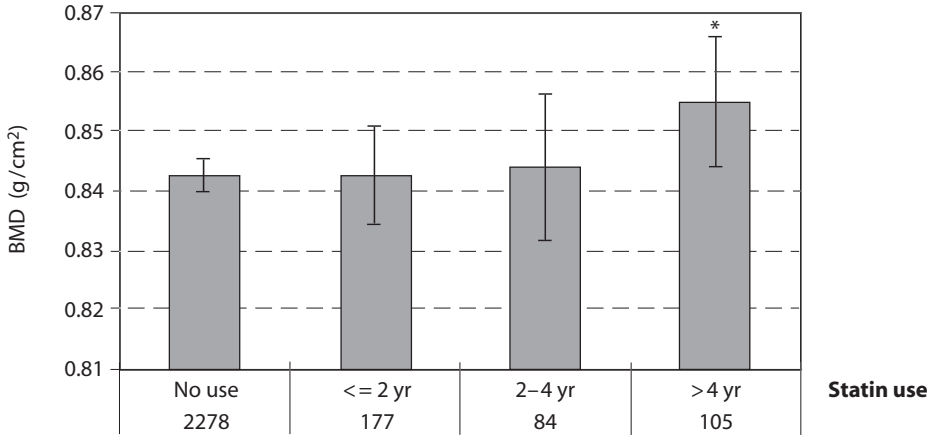
Values are means with standard deviations or numbers with percentages. Some variables have missing values.
[#] = Determined from baseline interview. Not further specified.

Table 2 — Difference in BMD per year of statin use, compared with non-users.

	Crude		*Model 1*		*Model 2*	
	g/cm^2 (95% CI)	p-value	g/cm^2 (95% CI)	p-value	g/cm^2 (95% CI)	p-value
All statins	0.009 (0.005 ; 0.013)	<0.001	0.007 (0.004 ; 0.011)	<0.001	0.002 (0.001 ; 0.004)	0.04
Pravastatin	0.007 (-0.005 ; 0.012)	0.27	-0.001 (-0.011 ; 0.010)	0.93	-0.004 (-0.005 ; 0.005)	0.88
Lipophilic statins	0.008 (0.004 ; 0.012)	<0.001	0.008 (0.004 ; 0.011)	<0.001	0.002 (0.002 ; 0.004)	0.03

Model 1 is adjusted for age, gender and duration of follow-up.
Model 2 is adjusted for age, gender, duration of follow-up, body mass index, baseline BMD, total serum cholesterol, diabetes mellitus, Framingham 5-yr cardiovascular disease risk, and use of ß-blockers or thiazide diuretics.

Figure 2 — Mean femoral neck bone mineral density according to duration of statin use.

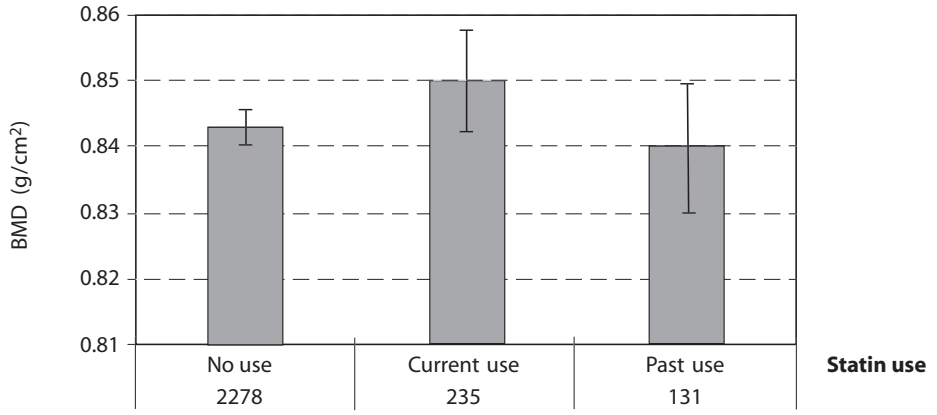


Means are adjusted for age, gender, follow-up time, body mass index, total serum cholesterol, Framingham 5-yr CVD risk, diabetes mellitus, and baseline BMD.
Numbers of subjects per group are mentioned below the bars.
* $p < 0.05$ in comparison with non-users.

Use of lipophilic statins was compared with no use and a difference in mean BMD after adjustments was observed (non-users 0.842; 0.840–0.845; lipophilic statin users 0.848; 0.841–0.855), but this difference was not significant. Pravastatin users (0.829; 0.813–0.845) had a lower mean BMD than non-users, but this difference was also not significant.

After categorization of the duration of statin use, a higher mean femoral neck BMD was observed with increasing duration of exposure (Figure 2). Femoral neck BMD in long-term

Figure 3 — Mean femoral neck bone mineral density for non-users, current users, and past users.



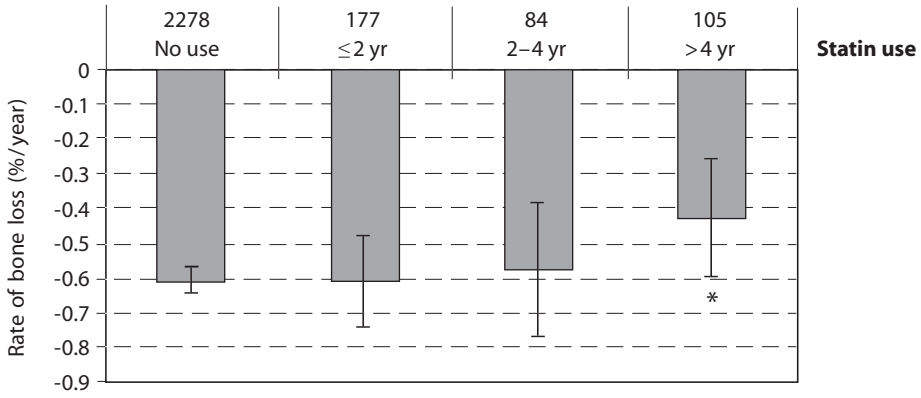
Means are adjusted for age, gender, follow-up time, body mass index, total serum cholesterol, Framingham 5-yr CVD risk, diabetes mellitus, and baseline BMD.
Numbers of subjects per group are mentioned below the bars.

(>4 years) statin users was significantly higher (adjusted mean 0.855; 0.844–0.867 g/cm^2) than mean BMD of non-users (0.843; 0.840–0.845, p = 0.03). The trend between duration of use and mean BMD was not significant (p = 0.08). However, the test for trend between duration of use and mean BMD in lipophilic statin users was significant (p = 0.02). Long-term lipophilic statin users had a mean BMD of 0.852 (0.842–0.865). Femoral neck BMD did not differ between users of pravastatin (mean BMD for >4 yrs of use: 0.845; 0.795–0.895) and non-users. Mean BMD for current use of statins, irrespective of duration of use, was higher (0.850; 0.843–0.858) than BMD of past users (0.840; 0.830–0.850), but not significantly different (Figure 3).

When we divided statin users in low-dose users and high-dose users on basis of the median dose (DDD = 0.72), and compared BMD of these groups with each other, there was no significant difference. There was no interaction between gender and use of statins (data not shown).

Persons who used statins for more than 4 years had lower rates of bone loss compared to non-users, and the difference was significant (p = 0.04) (Figure 4). Long-term users had a mean loss of 0.43% per year and non-users lost approximately 0.61% per year. The p-value for trend for mean rate of loss and duration of use of statins was 0.08.

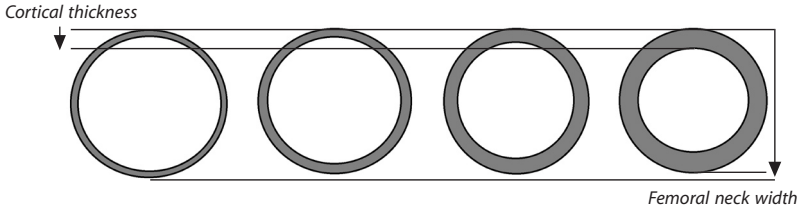
Figure 4 — Mean annual rate of bone loss according to duration of statin use.



Means are adjusted for age, gender, follow-up time, body mass index, total serum cholesterol, Framingham 5-yr CVD risk, diabetes mellitus, and baseline BMD.
Numbers of subjects per group are mentioned above the bars.
* p < 0.05 in comparison with non-users.

Hip bone structure analyses showed a significantly thicker cortex and larger cross-sectional area for statin users (Figure 5). Endosteal diameter and femoral neck width were smaller for long-term statin users, but not significantly different from non-users. Femoral neck stability, reflected in a lower buckling ratio, was significantly greater for long-term statin users. Section modulus was not significantly different in statin users.

Figure 5 — Hip structural analysis parameters categorized by duration of statin use.



	Non-users	≤ 2 years	2–4 years	>4 years	
Cortical thickness (mm)	1.37	↑ 1.1%	↑ 3.5%	↑ 5.3%	*#
Femoral neck width (cm)	3.13	↓ 0.8%	↓ 0.4%	↓ 0.6%	
Endosteal diameter (cm)	2.86	↑ 1.0%	↓ 0.8%	↓ 1.1%	
Cross-sectional area (cm^2)	2.14	↑ 0.2%	↑ 2.8%	↑ 4.6%	*#
Section modulus (cm^3)	1.17	↓ 2.6%	↑ 2.3%	↑ 2.9%	
Buckling ratio	13.17	↓ 3.1%	↓ 4.6%	↓ 6.6%	*#

The figure represents a caricature of the femoral neck cross sections (not to scale).

Means are adjusted for age, gender, follow-up time, height, weight, total serum cholesterol, Framingham 5-yr CVD risk, and diabetes mellitus.
* $p < 0.05$ in comparison with non-users; # $p < 0.05$ for trend.

Discussion

In this study we found a significant positive association between statin use and bone mineral density. Statin use during the study was associated with a higher mean bone mineral density at the end of follow-up and this association had a duration-dependent character. After 4 years of statin use, there was a significant difference in BMD compared with non-users. Long-term statin users had lower rates of bone loss than non-users. The higher mean BMD in statin users is therefore probably a reflection of lower rates of bone loss. Cortical thickness was significantly greater in long-term users. The increase in cortical thickness among statin users is probably the consequence of both periosteal apposition and inhibition of endosteal bone resorption (or endosteal bone apposition), because endosteal diameter was smaller and femoral neck width was larger, although not significantly different from non-users. Measures of bone stability and bending strength, the buckling ratio and section modulus, were both compatible with better hip bone structure for statin users. Unlike the section modulus, the buckling ratio was significantly increased for statin users. Something similar was previously observed with use of alendronate, a bisphosphonate.[26] Alendronate users lost less cortical thickness after 3 years of use compared with non-users. However, for alendronate users, the cross-sectional area did not change over 3 years. In our study statin users had a larger cross-sectional area, which is linearly related to bone mass. Unfortunately, we could not determine change in hip structural geometry over the follow-up period for individuals, so we cannot determine whether statins acted as bone resorption inhibitors or as stimulators of bone formation. Long-term users did not gain, but lost BMD during the study period, although less than non-users. Inhibition

of bone resorption by statin use may be the reason of the lower rate of bone loss. On the other hand, it may be that age-related bone loss dominated over a potential bone-forming influence of statins, resulting in a netto bone loss.

Pravastatin, a hydrophilic statin, was not associated with an increased BMD, in contrast to the use of lipophilic statins. This could be in line with a previous study in which was demonstrated that pravastatin did not induce BMP-2 expression and may therefore have a lower potential for beneficial effects on bone.[20] However, there were only few persons in our study that exclusively used pravastatin. We did not have power to detect differences between the two types of statins.

Our results are in line with previous observational studies that found an association between statin use and BMD.[3–6,10] However, there were also reports that could not confirm this association.[7–9] These studies all included a low number of statin users (140 exposed persons at most) and two of these studies had selected participants from a special patient population.[7, 8] Wada and colleagues[7] investigated only lumbar spine BMD and not femoral neck BMD, and more than 80% of their participants took pravastatin.

In the Rotterdam Study, all participants were selected from a general population of elderly and before assessment of the exposure. Selection bias is therefore unlikely. The population for this study is on average younger and healthier because they had to survive until the third examination round in order to be included in this study. We do, however, not think that this is of influence on the association between statin use and BMD. Healthier persons may have a higher BMD at baseline, we therefore adjusted for this baseline BMD in the analyses. Rates of bone loss are probably not influenced by baseline BMD and since we also found a significant association between statin use and rates of bone loss, we do not think that our results are biased.

All information on co-factors and potential confounders was gathered during the first visit and independent of the exposure and outcome. Computerized pharmacy databases provide more detailed information on medication use than medical records or self-reported use. In contrast with other studies that determined exposure by interview or from medical records, we had very detailed information on statin use, which made it possible to study the association between increasing duration of use and BMD and limited the chance of misclassification of exposure. If patients regularly refill their prescriptions of medication, non-compliance is unlikely. Confounding by indication would happen whenever the indication for statin therapy (high serum cholesterol levels and/or cardiovascular disease) is associated with a higher BMD. We adjusted for a large number of cardiovascular disease risk factors and serum cholesterol levels. Although we cannot exclude residual confounding, we do not think confounding (by indication) can be an explanation for our results.

In conclusion, our results strongly suggest that statin use may protect against bone loss. With accumulating evidence for potential positive effects of statin use on bone, the need for randomized controlled trials on statin use and bone-effects is evident. Not only should these studies investigate the association between statin use and BMD, but the association between statin use and fracture risk needs to be examined as well.

References

1. **Fisher JE, Rogers MJ, Halasy JM, et al.** Alendronate mechanism of action: geranylgeraniol, an intermediate in the mevalonate pathway, prevents inhibition of osteoclast formation, bone resorption, and kinase activation in vitro. Proc Natl Acad Sci U S A 1999; 96 (1): 133-8.
2. **Mundy G, Garrett R, Harris S, et al.** Stimulation of bone formation in vitro and in rodents by statins. Science 1999; 286 (5446): 1946-9.
3. **Chung YS, Lee MD, Lee SK, Kim HM, Fitzpatrick LA.** HMG-CoA reductase inhibitors increase BMD in type 2 diabetes mellitus patients. J Clin Endocrinol Metab 2000; 85 (3): 1137-42.
4. **Edwards CJ, Hart DJ, Spector TD.** Oral statins and increased bone-mineral density in postmenopausal women. Lancet 2000; 355 (9222): 2218-9.
5. **Funkhouser HL, Adera T, Adler RA.** Effect of HMG-CoA reductase inhibitors (statins) on bone mineral density. J Clin Densitom 2002; 5 (2): 151-8.
6. **Montagnani A, Gonnelli S, Cepollaro C, et al.** Effect of simvastatin treatment on bone mineral density and bone turnover in hypercholesterolemic postmenopausal women: a 1-year longitudinal study. Bone 2003; 32 (4): 427-33.
7. **Wada Y, Nakamura Y, Koshiyama H.** Lack of positive correlation between statin use and bone mineral density in Japanese subjects with type 2 diabetes. Arch Intern Med 2000; 160 (18): 2865.
8. **Rejnmark L, Buus NH, Vestergaard P, Andreasen F, Larsen ML, Mosekilde L.** Statins decrease bone turnover in postmenopausal women: a cross-sectional study. Eur J Clin Invest 2002; 32 (8): 581-9.
9. **Sirola J, Honkanen R, Kroger H, Jurvelin JS, Maenpaa P, Saarikoski S.** Relation of statin use and bone loss: a prospective population-based cohort study in early postmenopausal women. Osteoporos Int 2002; 13 (7): 537-41.
10. **Solomon DH, Finkelstein JS, Wang PS, Avorn J.** Statin lipid-lowering drugs and bone mineral density. Pharmacoepidemiol Drug Saf 2004.
11. **Pasco JA, Kotowicz MA, Henry MJ, Sanders KM, Nicholson GC.** Statin use, bone mineral density, and fracture risk: Geelong Osteoporosis Study. Arch Intern Med 2002; 162 (5): 537-40.
12. **Burnett JR, Vasikaran SD.** Cardiovascular disease and osteoporosis: is there a link between lipids and bone? Ann Clin Biochem 2002; 39 (Pt 3): 203-10.
13. **Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA.** Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol 1991; 7 (4): 403-22.
14. **Burger H, de Laet CE, van Daele PL, et al.** Risk factors for increased bone loss in an elderly population: the Rotterdam Study. Am J Epidemiol 1998; 147 (9): 871-9.
15. **Fang Z, Smith JA, Kleppinger A, Reisine ST, Emerson D, Kulldorff M.** Retrospective evaluation and adjustment of dual energy X-ray absorptiometry measurements for bone mineral density research studies. J Clin Densitom 2002; 5 (4): 421-33.
16. **Beck TJ, Looker AC, Ruff CB, Sievanen H, Wahner HW.** Structural trends in the aging femoral neck and proximal shaft: analysis of the Third National Health and Nutrition Examination Survey dual-energy X-ray absorptiometry data. J Bone Miner Res 2000; 15 (12): 2297-304.
17. **Beck TJ, Ruff CB, Warden KE, Scott WW, Jr., Rao GU.** Predicting femoral neck strength from bone mineral data. A structural approach. Invest Radiol 1990; 25 (1): 6-18.
18. **WHO.** Anatomical Therapeutical Chemical (ATC) Classification Index. In. Oslo: World Health Organization Collaborating Centre for Drug Statistics Methodology; 1993.
19. **Watanabe S, Fukumoto S, Takeuchi Y, Fujita H, Nakano T, Fujita T.** Effects of 1-year treatment with fluvastatin or pravastatin on bone. Am J Med 2001; 110 (7): 584-7.
20. **Sugiyama M, Kodama T, Konishi K, Abe K, Asami S, Oikawa S.** Compactin and simvastatin, but not pravastatin, induce bone morphogenetic protein-2 in human osteosarcoma cells. Biochem Biophys Res Commun 2000; 271 (3): 688-92.
21. **Pincus T, Summey JA, Soraci SA, Jr., Wallston KA, Hummon NP.** Assessment of patient satisfaction in activities of daily living using a modified Stanford Health Assessment Questionnaire. Arthritis Rheum 1983; 26 (11): 1346-53.
22. **Folstein MF, Folstein SE, McHugh PR.** "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975; 12 (3): 189-98.

23. **Anderson KM, Odell PM, Wilson PW, Kannel WB.** Cardiovascular disease risk profiles. Am Heart J 1991; 121 (1 Pt 2): 293-8.
24. **Schoofs MW, van der Klift M, Hofman A, et al.** Thiazide diuretics and the risk for hip fracture. Ann Intern Med 2003; 139 (6): 476-82.
25. **Pasco JA, Henry MJ, Sanders KM, Kotowicz MA, Seeman E, Nicholson GC.** Beta-adrenergic blockers reduce the risk of fracture partly by increasing bone mineral density: Geelong Osteoporosis Study. J Bone Miner Res 2004; 19 (1): 19-24.
26. **Greenspan SL, Bhattacharya R, Beck TJ, Resnick NM, Parker RA.** The effect of hormone replacement therapy, alendronate, or the combination of both on hip structural geometry: a 3-year, randomized, double-blind, placebo-controlled, NIH-funded clinical trial. J Bone Miner Res 2003; 18 (Suppl 2): S92 (Abstract F356).

Chapter 3.2

HMG-CoA reductase inhibitors and the risk of vertebral fracture

Abstract

Introduction

Statins are cholesterol-lowering agents that potentially could affect bone. Previous studies on statin use and fracture risk reported contradictory results and did not include both symptomatic and non-symptomatic vertebral fractures.

Methods

To examine the association of statin use and vertebral fractures and lumbar spine bone mineral density, we performed a prospective population-based cohort study in men and women (N = 3469) aged 55 years or older, for whom both baseline and follow-up spinal X-rays were available. Statin use was obtained from detailed computerized pharmacy data and the total number of days of exposure before second X-ray was calculated. A multivariate logistic regression model was fitted to calculate odds ratios and confidence intervals.

Results

During a mean follow-up of 6.5 years, 176 incident vertebral fractures occurred. There were 508 statin users and 16 exposed cases. The adjusted relative risk for incident vertebral fracture in users of statins (compared to non-users) was 0.58 (95% confidence interval 0.34–0.99). The relative risk decreased upon higher cumulative use to 0.52 (0.28–0.98) for use for more than 365 days during the study period. Use of (the hydrophilic statin) pravastatin, and use of non-statin cholesterol lowering drugs was not significantly associated with vertebral fracture risk. Statin use was not significantly associated with lumbar spine BMD.

Conclusion

Statin use is associated with a lower risk of vertebral fracture. Randomized clinical trials in a population at risk for fracture are needed to examine this association.

Statins are 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors that are used to treat hypercholesterolemia. By inhibition of the enzyme that catalyses the conversion of HMG CoA to mevalonate in the cholesterol synthesis, statins lower serum low-density lipoprotein (LDL) cholesterol levels. Inhibition of the mevalonate pathway may also have positive effects on bone.[1] Statins were shown to increase expression of bone morphogenetic protein-2 (BMP-2) in bone cells, thereby increasing bone formation.[1–4] Statins may also play a role in reducing bone resorption by inhibiting osteoclast differentiation.[1,5]

Studies on use of statins and fractures or bone mineral density (BMD) have reported contradictory results. In most studies an association between statin use and lower fracture incidence was found,[6–10] but not all reports confirmed this association.[11–14] The studies on lumbar spine BMD and statin use were contradictory as well.[10,15–21] One study also examined the association between statin use and symptomatic vertebral fractures,[12] and another study included data on morphometric vertebral fractures in women with low bone mass and statin use,[22] but to our knowledge there has been no report of the incidence of all (non-)symptomatic vertebral deformities among statin users in the general population. Vertebral fractures are the most common and typical fractures in osteoporosis patients and are associated with increased morbidity and mortality.[23,24] Most vertebral fractures are not symptomatic and are, in contrast to other fractures, not related to falls. Because comparisons of multiple radiographs, taken at different points in time, are essential to study the incidence of vertebral fractures, this type of fracture is not well studied. Therefore, we examined the association between use of statins and the incidence of vertebral fractures in a prospective population-based cohort study with baseline and follow-up radiographs of the lateral spine available.

Methods

Study population

The Rotterdam Study is a population based cohort study designed to assess the occurrence and determinants of diseases in an ageing population. The cohort includes 3105 men and 4878 women aged 55 years and over (78% of the eligible population), who lived for at least one year in a defined district in Rotterdam in the Netherlands.[25] All participants gave written informed consent to retrieve all relevant medical information from treating physicians. The Medical Ethics Committee of the Erasmus MC approved the study. Baseline measurements were obtained from 1990 to 1993 and consisted of a home interview and research center visits for physical examinations. The third examination phase took place from 1997 until 1999. For the present study, all participants were included for whom spinal radiographs were available both at baseline and at the third examination.

Exposure assessment

In the research area, there are seven fully computerized pharmacies, which are all linked to one network. During the study, all participants filled their prescriptions in one of

these seven pharmacies. Information on all dispensed drugs since 1 January 1991 is available in computerized format on a day-to-day basis. The data consist of information on the date of prescribing, the total amount of drug units per prescription, the prescribed daily number of units, product name of the drugs and the Anatomical Therapeutic Chemical (ATC)-code.[26]

Because we studied all vertebral deformities, including non-symptomatic fractures, we did not have an exact date of occurrence of fracture. Therefore, we cumulated the total number of days of statin use between the two radiographs. For participants (n = 566) who had their first radiograph taken before 1 January 1991, we cumulated the number of days of statin use from that date onwards. Patients were classified as statin users if they received at least one prescription for statins between the baseline and follow-up radiograph. To investigate effects of increasing cumulative exposure we defined, *a priori*, three mutually exclusive intervals of statin use: no use, short-term use (1–365 days), and long-term use (>365 days). We expressed the mean prescribed daily dose during the study period as a proportion of the defined daily dose. One defined daily dose of statins equals the standard recommended adult daily dose for treatment of hypercholesterolemia in the Netherlands. Because, in contrast to lipophilic statins (all statins except pravastatin. In our study; atorvastatin, simvastatin, fluvastatin, lova-statin), the hydrophilic pravastatin was previously shown not to induce bone morphogenetic protein-2.[4] Therefore we distinguished pravastatin use from use of all other (lipophilic) statins in separate analyses.

Outcome assessment

Vertebral deformities were assessed as described previously.[27] In short, all radiographs of the third examination phase were evaluated morphometrically in Sheffield by the McCloskey-Kanis method.[28] If a vertebral fracture was detected, the baseline radiograph was evaluated as well. If the fracture was already present at baseline it was considered a prevalent fracture. However, if the specific vertebra was determined to be normal at baseline, it was considered an incident fracture. All vertebral deformities were confirmed by visual interpretation by an expert in the field, to rule out artifacts and other etiologies, such as pathological fractures.

Lumbar spine (L2-L4) bone mineral density was measured by dual energy X-ray absorptiometry (DXA, Lunar DPX-L densitometer) during the third examination phase as described previously.[29]

Co-factors

During a baseline home interview, trained interviewers gathered information on medical history, risk factors for chronic diseases, medication use and habitual diet. Amongst others, information was gathered on potential risk factors such as smoking habits and age at menopause. Lower limb disability was assessed using a modified version of the Stanford Health Assessment Questionnaire[30] and by calculating the mean score of answers to questions concerning rising, walking, bending and getting in and out of a car.[31] A score of more than one indicates disability.

After the home interview, the participants were invited to visit the research center for clinical examinations and laboratory assessments. Non-fasting blood samples were

drawn. Cognitive impairment was measured using the Mini Mental State Examination.[32] We computed the 5-year Framingham cardiovascular disease risk score for all participants with help of a previously published algorithm.[33] This score predicts 5-year cardiovascular disease risk and can be used as a measure of indication for statin therapy. Use of other medication was extracted from the pharmacy database.

Statistical analysis

Demographic and clinical characteristics of participants with and without vertebral fractures, and statin users versus non-users were compared with Student's t-test and Pearson's Chi-square.

To examine the association between vertebral fractures and statin use we used a logistic regression model with incident vertebral fractures as the dependent variable and with exposure to statins as an independent variable. In subsequent models we adjusted for age, gender and the number of days of available pharmacy data. Other co-factors were also included if they caused a change in the risk estimate of ever use of statins of at least 10%, or were biologically plausible. Tests of significance for the ordered variable of the categories of statin use were considered to be tests for trends of increasing duration of use. Effect modification by age, gender and bone mineral density at baseline was investigated by stratified analysis.

The association between lumbar spine BMD and statin use was investigated with analyses of variance. We computed crude and adjusted means of bone mineral density for categories of statin use. The category reflecting no use of statins was used as the reference category for significance tests.

Multiple imputation was used to impute missing information for confounding variables. Five imputation values were calculated on the basis of the posterior predictive distribution of the missing values and five complete data sets were created. On each complete set, the statistical analyses were performed and the point estimates of the five data sets were combined to form one summary statistic as the average of the five components. The variance of the summary statistic is calculated from the within-imputation variance and the between-imputation variance. The combined variance accounts for the uncertainty introduced by estimating the missing values.[34] In the original dataset there were no missing values for age, gender and length of follow-up, and 170 (4.9%), 235 (7.3%) and 19 (0.5%) missing values for diabetes mellitus, cardiovascular disease risk and body mass index, respectively. SPSS 11.0 for Windows (SPSS Inc., Chicago, IL) and SAS (procedures mi, mianalyze, and logistic; Statistical Analysis System version 8.2, Cary, NC) were used for the analyses.

Results

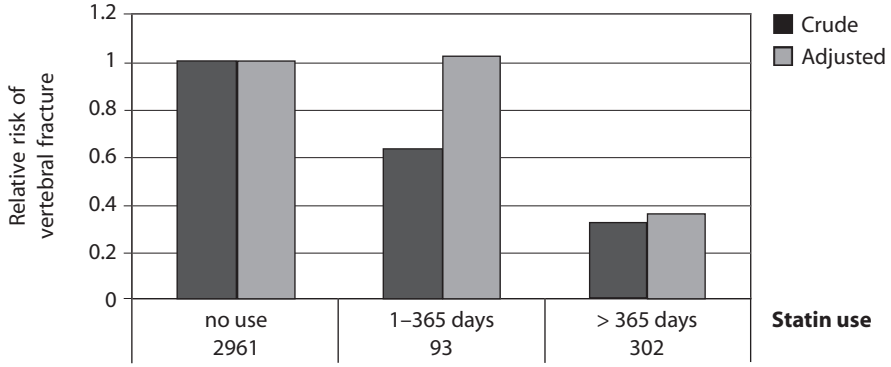
At the third examination phase, nearly 2000 participants of the original 7983 had died and 1260 participants were too old, disabled to visit the center or refused to come. For 3469 of 4730 participants of this examination phase both baseline and follow-up radiographs were available (1498 men and 1971 women) and for 3525 participants (1512 men and 2013 women) lumbar spine bone mineral density BMD measurements were available.

Table 1 — Baseline characteristics of the study population.

	Incident vertebral fracture N = 176	No incident vertebral fracture N = 3293	p-value	Statin users N = 508	Non users N = 2961	p-value
Days of available pharmacy	2797 (279)	2819 (304)		2846 (281)	2814 (307)	*
Age in years (SD)	67.9 (6.9)	65.4 (6.6)	**	64.3 (5.6)	65.7 (6.7)	**
Women	129 (74%)	1842 (56%)	**	288 (57%)	1683 (57%)	
Prevalent vertebral fracture	48 (27%)	205 (6%)	**	30 (6%)	223 (7.5%)	
Prevalent nonvertebral fracture	40 (23%)	417 (13%)	**	77 (15%)	380 (13%)	
Cholesterol level in mmol/l	6.9 (1.3)	6.7 (1.2)		7.6 (1.3)	6.5 (1.1)	**
Body mass index in kg/m^2	25.7 (3.7)	26.4 (3.5)	*	26.4 (3.4)	26.3 (3.6)	
BMD lumbar spine in g/cm^2	0.96 (0.17)	1.11 (0.19)	**	1.09 (0.18)	1.10 (0.19)	
Current smoking	49 (28%)	681 (21%)	*	115 (23%)	615 (21%)	
Diabetes mellitus	12 (7 %)	224 (7%)		48 (10%)	188 (7%)	*
Use of estrogens†	1 (1.1%)	65 (4.9%)		9 (3.9%)	57 (4.8%)	
Hypertension	49 (28%)	948 (29%)		201 (40%)	796 (27%)	**
Thyroid disease	16 (9%)	285 (9%)		34 (7%)	267 (9%)	
Mini Mental State Examination Score	27.9 (1.8)	28.1 (1.5)		28.2 (1.4)	28.1 (1.6)	
5-year Cardiovascular Disease Risk	0.13 (0.09)	0.13 (0.08)		0.15 (0.09)	0.12 (0.08)	**
History of myocardial infarction	8 (4.5%)	163 (5.0%)		61 (12.1%)	110 (3.7%)	

Data are means (SD) or n (%). Statin use was defined as use of stations in at least one day during the study period.
* = $p < 0.05$.
** = $p < 0.001$.
† = Use of estrogens was analyzed in women only ($p = 0.09$).

Figure 1 — Relative risk of vertebral fracture with increasing lipophilic statin use.



Numbers under the bars are reflecting the total number of participants in that category. All estimates were ad-justed for age at baseline, gender, number of days of available pharmacy data, diabetes mellitus, body mass index and natural logarithm of 5-year cardiovascular disease risk.

During the study period with a mean follow-up of 6.50 years (3–9 years) 176 individuals suffered a new vertebral fracture. Of these, 48 occurred in participants with a vertebral fracture present at baseline. In total, 508 participants of the vertebral fracture analyses used a statin on one day or more during the study period and among cases there were 16 exposed participants (5 short-term and 11 long-term users). There were 359 subjects who used simvastatin, 63 subjects who used fluvastatin, 70 who used atorvastatin and 106 who used pravastatin. Some subjects took more than one type of statin. Out of the 3525 participants with bone mineral density BMD measurements, 511 were statin users.

Characteristics of participants with and without vertebral fractures and of participants who did and did not use statins during follow-up, respectively, are described in Table 1. As expected, participants with a vertebral fracture were older, more often female and more often had a history of fractures. They had a lower bone mineral density in the lumbar spine and a lower body mass index. Statin users were younger and had more often a history of hypertension and diabetes mellitus and were more often smokers. The 5-year cardiovascular disease risk was significantly higher for statin users, but it did not differ between fracture cases and non-cases.

Use of statins on one or more days of the period between the two radiographs was associated with a lower incidence of vertebral fractures. The incidence of vertebral fractures was approximately 40% decreased in statin users (Table 2). After adjustments for age, gender, length of available pharmacy data between the radiographs, diabetes mellitus, body mass index, and the natural logarithm of the 5-year risk of cardiovascular disease, a proxy for indication for statin therapy, statin use was still associated with a significant risk reduction (odds ratio [OR] 0.58; 95% confidence interval [CI 95%], 0.34–0.99). Additional adjustments for prevalent vertebral fractures (present at baseline), baseline lumbar spine bone mineral density, use of other medications (thiazides, estrogens), lower limb disability, smoking or presence of other diseases, such as thyroid disease, cholesterol levels, and MMSE score did not change the association essentially.

Table 2 — Risk of incident vertebral fracture with use of statins.

	Total per group	Crude OR (95% CI)	Adjusted OR (95% CI)
No use of statins	2961	1.00 (reference)	1.00 (reference)
Any statin use during study period	508	0.57 (0.34–0.96)	0.58 (0.34–0.99)
Any pravastatin use during study period†	62	1.21 (0.43–3.37)	1.26 (0.44–3.53)
Any lipophilic statin use during study period†	395	0.50 (0.27–0.93)	0.52 (0.27–0.97)
Duration of any statin use			
No use	2961	1.00 (reference)	1.00 (reference)
Any statin during 1–365 days	120	0.76 (0.31–1.89)	0.78 (0.31–1.95)
Any statin during > 365 days	388	0.51 (0.28–0.95)	0.52 (0.28–0.98)
P for trend		0.03	0.04
Duration of lipophilic statin use†			
No use	2961	1.00 (reference)	1.00 (reference)
Lipophilic statin during 1–365 days	93	0.63 (0.50–2.06)	1.02 (0.40–2.57)
Lipophilic statin during > 365 days	302	0.32 (0.13–0.80)	0.36 (0.16–0.84)
P for trend		0.01	0.02

All estimates were adjusted for age at baseline, gender, number of days of available pharmacy data, diabetes mellitus, body mass index and natural logarithm of 5-year cardiovascular disease risk.

† For the analyses on type of statins, we excluded all participants that had used both prevastatin and a lipophilic statin during the study period (N = 51, one incident vertebral fracture). For analyses on lipo-philic statins we excluded pravastatin users (N = 62) and for analyses on pravastatin, lipophilic statin users (N = 395) were excluded.

When we further examined the association between statin use and vertebral fractures in terms of duration of statin use, we found a statistically significant risk reduction associated with long-term statin use (OR 0.51; CI 95%, 0.28–0.95). After adjustments, the OR for long-term use did not change essentially. There was a significant trend in decreasing ORs with increasing duration of use. In contrast with users of lipophilic statins (OR 0.52; CI 95%, 0.27–0.97) (5 short-term exposed cases, 6 long-term exposed cases), participants that used pravastatin (4 exposed cases) did not have a reduced risk of vertebral fracture (OR 1.26; CI 95%, 0.44–3.53). The median dose of statin users was 0.7 DDD (defined daily dosages). Most statin users had a mean dose over the study period below 1 DDD. Therefore the variation in doses taken by the participants was too low to study dose-effects.

When we examined use of other lipid lowering drugs (e.g. fibrates and nicotinic acids), we did not observe a significant association between use and vertebral fracture incidence (adjusted OR 0.85; CI 95%, 0.37–1.97). Among participants who did not take any cholesterol-lowering drug during the study period, cholesterol levels at baseline, categorized in tertiles, were not significantly associated with vertebral fractures (highest versus lowest level: adjusted OR 1.19; CI 95%, 0.78–1.83, trend p = 0.44).

Stratification on age at baseline ($\leq$65 yrs vs. >65 yrs), gender, and on lumbar spine bone mineral density at baseline ($\leq$1.09 g/cm^2 vs. >1.09 g/cm^2) did not reveal effect modification by these factors.

We conducted additional analyses to examine the potential effect of misclassification of exposure. All participants who used statins or other lipid lowering drugs at baseline

were excluded. These participants may have used statins before baseline and therefore could have been assigned to the wrong duration category. Exclusion of these subjects (n = 105) did not change the risk estimates essentially (any use OR 0.62, CI 95% 0.35–1.11; long-term use OR 0.52; CI 95% 0.28–0.99). After exclusion of subjects with a prevalent vertebral fracture at baseline, no essential changes in the estimates were observed.

Lumbar spine bone mineral density BMD was not associated with statin use (Table 3). Although mean BMD was highest among persons with the longest duration of statin use, there was no significant trend for increasing BMD with increasing duration of use of either all statins or lipophilic statins.

Table 3. Mean lumbar spine bone mineral density for categories of statin use.

	Number	Mean BMD (95% CI)
No statin use	3014	1.129 (1.122–1.136)
≤ 2 years of statin use	238	1.114 (1.090–1.139)
2–4 years of statin use	123	1.112 (1.078–1.146)
> 4 years of statin use	150	1.135 (1.104–1.166)
No statin use	3014	1.129 (1.122–1.135)
≤ 2 years of lipophilic statin use	190	1.114 (1.087–1.142)
2–4 years of lipophilic statin use	84	1.127 (1.085–1.168)
> 4 years of lipophilic statin use	122	1.141 (1.106–1.175)

All estimates were adjusted for age at baseline, gender, number of days of available pharmacy data, diabetes mellitus, body mass index and natural logarithm of 5-year cardiovascular disease risk.
For the analyses on lipophilic statins, we excluded all participants that had used both pravastatin and a lipophilic statin during the study period (N = 53).

Discussion

Our results show that use of statins is associated with a decreased risk of incident vertebral fractures. When statins are used for one year or more, this risk is reduced by approximately 50%. Pravastatin, in contrast to other statins, seems to lack this protective effect, although we emphasize that absolute numbers of fracture cases were low. We did not observe a significant relationship between statin use and lumbar spine bone mineral density.

Our results are in line with most other observational studies of statin use and (nonvertebral) fracture incidence. Several studies detected a trend towards a lower risk of fracture of approximately 40 to 60%.[7–10] At variance with those studies, van Staa and colleagues did not confirm this association with fractures, but reported an odds ratio for vertebral fractures of 1.15 (95% CI, 0.62–2.14).[12] However, vertebral fractures as assessed in that study were symptomatic in contrast to our study in which we examined all participants for vertebral deformities with follow-up radiographs. Because only one-third of all vertebral fractures come to medical attention,[35] it is possible that the abovementioned study was influenced by diagnostic bias. In secondary analyses of two randomized trials on statin use and mortality, no association was found between statin use and fractures re-

ported as adverse events.[11,13] When we recalculated the data available from one of these studies, the 4S Study,[13] we found a relative risk of vertebral fracture with simvastatin use of 0.47 for subjects older than 60 years of age, which is in line with our data. Pravastatin use, investigated in the other trial, is reported to have less effect on bone than use of other statins, which was also in line with our study. There have been several studies on the association between (lumbar spine) BMD and statin use. Some studies did not find an association,[10,16,19–21] and other studies did report an association between lumbar spine BMD and statin use.[15,17,18] In our study we did not observe an association between statin use and lumbar spine BMD. In elderly people, osteoarthritis, which is associated with an increased BMD, occurs often in the spine, and because our population consists of elderly we expect that this could have influenced our results.

Previous studies on statin use and risk of fracture were criticized because risk estimates were not adjusted for common confounders such as body mass index, diabetes mellitus, and dementia. In our study, we had extensive information on potential confounders and could therefore adjust for actual confounders. Vertebral fractures, in contrast with other fractures, are not strictly related to falls. Differences in activity levels and fall incidents between statin users and non-users are therefore not a likely explanation for our results.

Because our participants had to survive until the third examination phase, they were possibly healthier than the general population of the same age. This does not necessarily influence the association between statin use and vertebral fractures. Statin use was ascertained from pharmacy records and therefore not subject to recall bias or subject to assumptions about duration of use, such as in studies that only had data on baseline statin use. Misclassification of exposure, however, cannot be excluded, because we do not have an exact fracture date. We expect misclassification of exposure to be randomly distributed among cases and controls, which leads rather to underestimation than to overestimation of an association. Confounding by indication would happen when physicians prescribed statins predominantly to subjects at a lower risk for vertebral fracture. In our study, statin users did not differ much from non-users and we adjusted in our analyses for all covariates that were different between users and non-users. We did not detect an association between cholesterol levels and fractures. Use of other lipid lowering drugs was also not related to fracture incidence, as a confirmation of findings in previous studies.[7,9,36] Because of all abovementioned analyses, confounding by indication is therefore unlikely.

In conclusion, long-term statin use is associated with a 50% lower risk of vertebral fracture. Statins are designed to act in the liver and in future, statins with higher affinity to bone could potentially be interesting in the treatment of osteoporosis and fracture prevention. At present, there is not enough evidence to prescribe statins for the indication of osteoporosis. Randomized trials designed to investigate this subject and carried out in the proper population (e. g. patients at high risk for fracture) could be helpful to solve this issue.

References

1. **Mundy G, Garrett R, Harris S, Chan J, Chen D, Rossini G, Boyce B, Zhao M, Gutierrez G.** Stimulation of bone formation in vitro and in rodents by statins. Science 1999; 286 (5446): 1946-9.
2. **Song C, Guo Z, Ma Q, Chen Z, Liu Z, Jia H, Dang G.** Simvastatin induces osteoblastic differentiation and inhibits adipocytic differentiation in mouse bone marrow stromal cells. Biochemical and Biophysical Research Communications 2003; 308 (3): 458-462.
3. **Ohnaka K, Shimoda S, Nawata H, Shimokawa H, Kaibuchi K, Iwamoto Y, Takayanagi R.** Pitavastatin enhanced BMP-2 and osteocalcin expression by inhibition of Rho-associated kinase in human osteoblasts. Biochem Biophys Res Commun 2001; 287 (2): 337-42.
4. **Sugiyama M, Kodama T, Konishi K, Abe K, Asami S, Oikawa S.** Compactin and simvastatin, but not pravastatin, induce bone morphogenetic protein-2 in human osteosarcoma cells. Biochem Biophys Res Commun 2000; 271 (3): 688-92.
5. **Woo JT, Kasai S, Stern PH, Nagai K.** Compactin suppresses bone resorption by inhibiting the fusion of prefusion osteoclasts and disrupting the actin ring in osteoclasts. J Bone Miner Res 2000; 15(4): 650-62.
6. **Bauer DC.** HMG CoA Reductase Inhibitors and the Skeletal: A Comprehensive Review. Osteoporos Int 2003; 14 (4): 273-282.
7. **Chan KA, Andrade SE, Boles M, Buist DS, Chase GA, Donahue JG, Goodman MJ, Gurwitz JH, LaCroix AZ, Platt R.** Inhibitors of hydroxymethylglutaryl-coenzyme A reductase and risk of fracture among older women. Lancet 2000; 355 (9222): 2185-8.
8. **Meier CR, Schlienger RG, Kraenzlin ME, Schlegel B, Jick H.** Statin drugs and the risk of fracture. JAMA 2000; 284 (15): 1921-2.
9. **Wang PS, Solomon DH, Mogun H, Avorn J.** HMG-CoA reductase inhibitors and the risk of hip fractures in elderly patients. JAMA 2000; 283 (24): 3211-6.
10. **Pasco JA, Kotowicz MA, Henry MJ, Sanders KM, Nicholson GC.** Statin use, bone mineral density, and fracture risk: Geelong Osteoporosis Study. Arch Intern Med 2002; 162 (5): 537-40.
11. **Reid IR, Hague W, Emberson J, Baker J, Tonkin A, Hunt D, MacMahon S, Sharpe N.** Effect of pravastatin on frequency of fracture in the LIPID study: secondary analysis of a randomised controlled trial. Long-term Intervention with Pravastatin in Ischaemic Disease. Lancet 2001; 357 (9255): 509-12.
12. **van Staa TP, Wegman S, de Vries F, Leufkens B, Cooper C.** Use of statins and risk of fractures. JAMA 2001; 285 (14): 1850-5.
13. **Pedersen TR, Kjekshus J.** Statin drugs and the risk of fracture. 4S Study Group. JAMA 2000; 284 (15): 921-2.
14. **LaCroix AZ, Cauley JA, Pettinger M, Hsia J, Bauer DC, McGowan J, Chen Z, Lewis CE, McNeeley SG, Pasaro MD, Jackson RD.** Statin use, clinical fracture, and bone density in postmenopausal women: results from the Women's Health Initiative Observational Study. Ann Intern Med 2003; 139 (2): 97-104.
15. **Funkhouser HL, Adera T, Adler RA.** Effect of HMG-CoA reductase inhibitors (statins) on bone mineral density. J Clin Densitom 2002; 5 (2): 151-8.
16. **Sirola J, Honkanen R, Kroger H, Jurvelin JS, Maenpaa P, Saarikoski S.** Relation of statin use and bone loss: a prospective population-based cohort study in early postmenopausal women. Osteoporos Int 2002; 13 (7): 537-41.
17. **Montagnani A, Gonnelli S, Cepollaro C, Pacini S, Campagna MS, Franci MB, Lucani B, Gennari C.** Effect of simvastatin treatment on bone mineral density and bone turnover in hypercholesterolemic postmenopausal women: a 1-year longitudinal study. Bone 2003 32 (4): 427-433.
18. **Edwards CJ, Hart DJ, Spector TD.** Oral statins and increased bone-mineral density in postmenopausal women. Lancet 2000; 355 (9222): 2218-9.
19. **Rejnmark L, Buus NH, Vestergaard P, Andreasen F, Larsen ML, Mosekilde L.** Statins decrease bone turnover in postmenopausal women: a cross-sectional study. Eur J Clin Invest 2002; 32 (8): 581-9.
20. **Chung YS, Lee MD, Lee SK, Kim HM, Fitzpatrick LA.** HMG-CoA reductase inhibitors increase BMD in type 2 diabetes mellitus patients. J Clin Endocrinol Metab 2000; 85 (3): 1137-42.
21. **Wada Y, Nakamura Y, Koshiyama H.** Lack of positive correlation between statin use and bone mineral density in Japanese subjects with type 2 diabetes. Arch Intern Med 2000; 160 (18): 2865.

22. **Bauer DC, Mundy GR, Jamal SA, Black DM, Cauley JA, Ensrud KE, van der Klift M, Pols HA.** Use of statins and fracture: results of 4 prospective studies and cumulative meta-analysis of observational studies and controlled trials. Arch Intern Med 2004; 164 (2): 146-52.
23. **Nevitt MC, Ettinger B, Black DM, Stone K, Jamal SA, Ensrud K, Segal M, Genant HK, Cummings SR.** The association of radiographically detected vertebral fractures with back pain and function: a prospective study. Ann Intern Med 1998; 128 (10): 793-800.
24. **Ensrud KE, Thompson DE, Cauley JA, Nevitt MC, Kado DM, Hochberg MC, Santora AC, 2nd, Black DM.** Prevalent vertebral deformities predict mortality and hospitalization in older women with low bone mass. Fracture Intervention Trial Research Group. J Am Geriatr Soc 2000; 48 (3): 241-9.
25. **Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA.** Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol 1991; 7 (4): 403-22.
26. **WHO.** Anatomical Therapeutic Classification (ATC) Index: Including Defined Daily Doses (DDD) for Plain Substances. Oslo; 1992.
27. **Van der Klift M, De Laet CE, McCloskey EV, Hofman A, Pols HA.** The incidence of vertebral fractures in men and women: the Rotterdam Study. J Bone Miner Res 2002; 17 (6): 1051-6.
28. **McCloskey EV, Spector TD, Eyres KS, Fern ED, O'Rourke N, Vasikaran S, Kanis JA.** The assessment of vertebral deformity: a method for use in population studies and clinical trials. Osteoporos Int 1993; 3 (3): 138-47.
29. **Burger H, van Daele PL, Algra D, van den Ouweland FA, Grobbee DE, Hofman A, van Kuijk C, Schutte HE, Birkenhager JC, Pols HA.** The association between age and bone mineral density in men and women aged 55 years and over: the Rotterdam Study. Bone Miner 1994; 25 (1): 1-13.
30. **Pincus T, Summey JA, Soraci SA, Jr., Wallston KA, Hummon NP.** Assessment of patient satisfaction in activities of daily living using a modified Stanford Health Assessment Questionnaire. Arthritis Rheum 1983; 26 (11): 1346-53.
31. **Burger H, de Laet CE, van Daele PL, Weel AE, Witteman JC, Hofman A, Pols HA.** Risk factors for increased bone loss in an elderly population: the Rotterdam Study. Am J Epidemiol 1998; 147 (9): 871-9.
32. **Folstein MF, Folstein SE, McHugh PR.** "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975; 12 (3): 189-98.
33. **Anderson KM, Odell PM, Wilson PW, Kannel WB.** Cardiovascular disease risk profiles. Am Heart J 1991; 121 (1 Pt 2): 293-8.
34. **Twisk J, de Vente W.** Attrition in longitudinal studies. How to deal with missing data. J Clin Epidemiol 2002; 55 (4): 329-37.
35. **Cooper C, Atkinson EJ, O'Fallon WM, Melton LJ, 3rd.** Incidence of clinically diagnosed vertebral fractures: a population-based study in Rochester, Minnesota, 1985-1989. J Bone Miner Res 1992; 7 (2): 221-7.
36. **Meier CR, Schlienger RG, Kraenzlin ME, Schlegel B, Jick H.** HMG-CoA reductase inhibitors and the risk of fractures. Jama 2000; 283 (24): 3205-10.

Chapter 3.3

Long-term statin use is associated with a reduced incidence of nonvertebral fractures

Abstract

Background

Statins, also known as hydroxy-methyl coenzyme A (HMG-CoA) reductase inhibitors, might be associated with a decreased fracture incidence because they inhibit the mevalonate pathway a step further upstream as nitrogen-containing bisphosphonates do. We examined the association between statin use and risk of nonvertebral fracture.

Methods

The Rotterdam Study is a prospective cohort study that started in 1991 and included 7983 men and women of 55 years and older. For the current study we included all persons that were alive at July 1, 1991 and follow-up ended when a fracture occurred, persons died or reached the end of the study at December 31, 2001. Exposure to statins was available on a day-to-day basis and derived from pharmacy databases. Potential confounders were measured at baseline. Risks for nonvertebral fracture were estimated with a Cox proportional hazards model with exposure defined as time-dependent covariates.

Results

We included 7892 persons and the mean duration of follow-up was 8.12 years. During the study 1218 nonvertebral fractures occurred. There were 1069 persons that used a statin at any time during the follow-up. Adjusted risk for nonvertebral fracture was lower for current statin users compared to non-users (HR 0.81, 95% confidence interval 0.61–1.07). When statins were continuously used for at least 2 years the risk of nonvertebral fracture was significantly reduced to 0.57 (0.37–0.90).

Conclusions

Statin use was significantly associated with a lower risk of nonvertebral fracture in a duration-dependent relation.

Statins, also known as hydroxy-methyl coenzyme A (HMG CoA) reductase inhibitors, were introduced in the 1980's as cholesterol-lowering agents. By inhibiting the conversion of HMG CoA to mevalonate, they lower de novo cholesterol synthesis and plasma cholesterol levels, but also increase receptor-mediated catabolism of low density lipoprotein (LDL).[1]

In the years after introduction on the market, it was suggested that statins may have pleiotropic effects,[2–5] probably because mevalonate is also a precursor of many non-steroidal isoprenoid compounds. Since a report on actions of statins on bone of rodents in 1999,[6] several observational and experimental studies on effects of statins on bone were undertaken. It has been demonstrated that statins increase expression of bone morphogenetic protein-2 (BMP-2) in osteoblasts, thereby increasing bone formation.[6–9] Studies on statins and effects on bone mineral density (BMD) sometimes reported an increased BMD in users,[10–14] but there were also reports that did not show an association.[15–17] If statins increase BMD, they might also affect the incidence of bone fracture. Conflicting results on statin use and fracture incidence were published over the last years. Beneficial effects[18–21] as well as adverse effects or absent associations[22–25] were reported. One of the potential explanations for differences in statin response among the previous studies could be the type of statins that were used. For instance, the hydrophilic statin pravastatin does not induce BMP-2, in contrast with other (lipophilic) statins.[9] In addition, it has been suggested that lipophilic statins could also stimulate osteoblast mineralization in a BMP-2 independent manner.[26]

Accuracy of information on exposure and case status, and detailed information on potential confounders, are of vital importance to address a research question that gave such conflicting results in the past. Since statin use is strongly associated with cardiovascular disease, and there is a link between cardiovascular disease and osteoporosis,[27] adjustment for cardiovascular disease might be of vital importance. Statin use is also associated with use of other (cardiovascular) drugs that may be beneficial to bone,[28,29] so adjustment for use of these other drugs is necessary.

Therefore we examined the association between statin use and risk of nonvertebral fracture in a prospective cohort study with detailed information on medication use, fracture incidence and potential confounders.

Methods

Study population

The Rotterdam Study is a population-based cohort study designed to assess the occurrence and determinants of diseases in an ageing population. The cohort includes 3105 men and 4878 women aged 55 years and over in 1991 (78% of the eligible population), who lived for at least one year in a defined district in Rotterdam in the Netherlands.[30] All participants gave written informed consent to retrieve all relevant medical information from treating physicians. The Medical Ethics Committee of the Erasmus Medical Center approved the study. Baseline measurements were obtained from 1990 to 1993

and consisted of a home interview and research center visits for physical examinations. A second examination round took place between 1995 and 1997, and the third examination phase took place from 1997 until 1999. For the entire cohort, information on vital status is obtained continuously from the municipal authorities in Rotterdam. For subjects who moved outside the research area, mortality data are obtained from general practitioners (GPs).

Outcome assessment

GPs in the research area reported all relevant fatal and non-fatal events, such as fractures, through a computerized system. This system covers approximately 80% of the population and for participants not covered, research physicians performed annual checks on the complete medical records of all general practitioners in the Rotterdam Study. All follow-up information was checked in GPs' patient records by research physicians and independently coded according to the International Classification of Diseases, 10th revision (ICD-10).[31] A medical expert in the field reviewed all coded events for a final classification. For the analyses in this study, we included all fractures that occurred in the study population during the follow-up period, except for vertebral fractures and pathological and post-procedural fractures. Patients were followed until the first fracture. For patients with more than one type of fracture, the follow-up ended on the day of the fracture of interest, regardless of a previous other type of fracture. Information on mortality and on incident nonvertebral fractures was collected from baseline until the end of the study period on December 31, 2001.

Exposure assessment

All participants fill their drug prescriptions at one of the seven computerized pharmacies in the research area. These pharmacies are linked to one network and drug-dispensing data is available for all subjects from January 1, 1991 onwards. The data consist of information on the date of prescribing, the total amount of drug units per prescription, the prescribed daily number of units, product name of the drugs and the Anatomical Therapeutic Chemical (ATC)-code.[32]

When a nonvertebral fracture occurred, that date was defined as the index date and the number of days of statin use up to that date was calculated for each cohort member. Exposure status was updated for all cohort members on every index date. To investigate the duration-effect relationship for statin use and fractures, a categorical variable reflecting no use, use $\leq$2 years, and use >2 years was created.

The dosage was expressed as the prescribed mean daily dose during the study period in defined daily dose equivalents.[33] The defined daily dose (DDD) equals the standard recommended adult daily dose for treatment of the main indication. The dose-effect relationship was studied by categorizing daily dose below and above the median. To differentiate between effects of lipophilic statins (all statins except pravastatin) and the hydrophilic statin, pravastatin, we performed stratified analyses.

To investigate confounding by indication, we examined the association between non-statin lipid lowering drugs (nicotinic acids and fibrates) and risk of fracture.

To reduce potential misclassification of exposure at baseline we ensured potential pharmacy data of at least six months before baseline for all participants. Therefore the follow-up for fracture analyses started at July 1,1991 and all cohort members were followed until the first fracture of interest occurred, or until participants died or reached the end of the study.

Co-factors

During a baseline home interview, trained interviewers gathered information on medical history, risk factors for chronic diseases, medication use, walking aid use, and habitual diet. Amongst others, information was gathered on potential risk factors such as smoking habits and age at menopause. Previous fractures were defined as a fracture in the 5 years before baseline. Lower limb disability was assessed using a modified version of the Stanford Health Assessment Questionnaire[34] and by calculating the mean score of answers to questions concerning rising, walking, bending and getting in and out of a car.[35] A score of more than one indicates disability.

After the home interview, the participants were invited to visit the research center for clinical examinations. Amongst others, body mass index (BMI) and blood pressure were measured. Hypertension was defined as a systolic blood pressure $\geq$160 mmHg and/or diastolic blood pressure $\geq$100 mmHg and/or use of antihypertensive medication. Nonfasting blood samples were drawn. Cognitive impairment was measured using the Mini Mental State Examination (MMSE).[36] We computed the 5-year Framingham cardiovascular disease risk score for all participants at baseline with an algorithm.[37] This score predicts 5-year cardiovascular disease risk. ApoE genotyping was performed as described previously.[38] The ApoE*4 allele was regarded as a risk allele.

Information on use of other medications was also derived from the computerized pharmacy database. We considered the number of days of exposure to thiazide diuretics, ß-blockers, and hormone replacement therapy, and the number of prescriptions during follow-up for glucocorticoids as potential confounders.

Statistical analysis

We calculated the relative risks of fracture (and 95 percent confidence intervals) with the use of a Cox proportional-hazards model; the cumulative use of each drug was represented by a time-dependent covariate. In the Cox model, age in days was used as the time axis to ensure optimal adjustment for age and we additionally adjusted for follow-up time to control for differences in prescription behavior over calendar time.[39] Apart from a time-dependent comparison in which any use was compared with no use, we created three time-dependent mutually exclusive categorical variables: non-use, short-term use (less than 2 years of use), and long-term use (2 years or more). These cut-off points were chosen to ensure an adequate number of subjects in each group.

Use of other medication, such as thiazides and ß-blockers, was also represented by time-dependent variables.

Multiple imputation was used to impute missing information for confounding variables. Five imputation values were calculated on the basis of the posterior predictive

Table 1 — Baseline characteristics of fracture cases and the total cohort.

	Nonvertebral fracture cases	Total cohort	HR [95% CI]	Missing values
Number	1218	7892		
Age (yr)	73.1 (9.8)	69.9 (9.9)	1.06 [1.05–1.06]	0%
Gender (women)	858 (80.3%)	4827 (61.1%)	2.46 [2.15–2.82]	0%
Previous nonvertebral fracture	223 (20.9%)	1058 (13.4%)	1.82 [1.58–2.09]	7%
Femoral neck BMD (g/cm^2)	0.80 (0.13)	0.87 (0.14)	0.02 [0.01–0.40]	26%
Lowest tertile	0.70 (0.07)	0.72 (0.06)	Reference	
Middle tertile	0.86 (0.03)	0.86 (0.03)	0.51 [0.44–0.60]	
Highest tertile	1.01 (0.07)	1.02 (0.09)	0.33 [0.27–0.39]	
Serum cholesterol (mmol/l)	6.60 (1.3)	6.61 (1.2)	0.95 [0.91–1.00]	11%
Use of a walking aid	177 (16.6%)	840 (10.6%)	2.56 [2.19–3.00]	8%
Lower limb disability	244 (22.8%)	963 (14.1%)	2.34 [2.04–2.69]	3%
Body mass index (kg/m^2)	26.2 (3.7)	26.3 (3.7)	0.99 [0.97–1.01]	13%
MMSE	26.8 (3.4)	27.1 (3.2)	0.92 [0.91–0.94]	10%
Current smoking	208 (19.5%)	1714 (21.7%)	0.91 [0.79–1.04]	4%
Diabetes mellitus	111 (10.4%)	802 (10.2%)	1.18 [0.98–1.42]	1.4%
Prevalent myocardial infarction	42 (3.9%)	479 (6.1%)	0.75 [0.57–0.99]	3%
Framingham 5-yr CVD risk	0.15 (0.10)	0.15 (0.10)	2.48 [1.32–4.69]	16%
Hypertension	396 (37.0%)	2576 (32.5%)	1.28 [1.14–1.44]	10%
Alcohol intake (g/day)	8.6 (13.0)	10.4 (15.2)	0.99 [0.98–0.99)	31%
Use of hormone replacement therapy	13 (1.2%)	101 (1.3%)	0.97 [0.60–1.56]	0.2%
Use of diuretics	210 (19.6%)	1283 (16.2%)	1.47 [1.27–1.70]	0.2%
Use of ß-blockers	127 (11.9%)	1130 (14.3%)	0.83 [0.70–0.99]	0.2%

Values are means with standard deviations or numbers with percentages.

distribution of the missing values and five complete data sets were created. On each complete set, the statistical analyses were performed and the point estimates of the five data sets were combined to form one summary statistic as the average of the five components. The variance of the summary statistic is calculated from the within-imputation variance and the between-imputation variance. The combined variance accounts for the uncertainty introduced by estimating the missing values.[40] Analyses were performed with SAS (Statistical Analysis System version 8, Cary, NC) and with SPSS 11.5 for Windows (SPSS Inc., Chicago, IL)

Results

At the start of the study, July 1st 1991, 7892 persons of the original cohort were still alive and therefore included in our study population. The mean duration of follow-up (for nonvertebral fracture analyses) was 8.12 years. During the study, 1218 nonvertebral fractures, 352 hip fractures and 315 wrist fractures occurred. There were 1069 persons who used a statin at any time during the follow-up, and 803 persons used a statin for at least 2 years. Pravastatin was used by 216 participants and 959 participants used other statins (106 individuals took both types of statins during the study). Non-statin lipid lowering drugs were used by 206 persons, of which 60 were users for at least 2 years.

Table 1 shows baseline characteristics of the cases and of the total cohort from which they arise. Univariate risks of nonvertebral fracture for the baseline characteristics are showed. The percentage missing values for baseline characteristics before imputation are also shown in Table 1.

The unadjusted risk of nonvertebral fracture was significantly reduced by 35% for persons who were current users of statins (Table 2). Categorization of duration of statin use showed that risk of fracture was lower when duration of use was longer; the risk of non-

Table 2 — Risk of nonvertebral fracture.

	Crude Hazard ratio (95% CI)	**Adjusted*** (95% CI)
Statins		
No use	1 (reference)	1 (reference)
Current use	0.65 (0.50 – 0.86)	0.81 (0.61 – 1.07)
Current use		
1 day – 2 years	0.81 (0.58 – 1.14)	1.06 (0.76 – 1.50)
>2 years	0.48 (0.31 – 0.75)	0.57 (0.37 – 0.90)
Non-statin lipid lowering drugs		
No use	1 (reference)	1 (reference)
Current use	1.45 (0.78 – 2.70)	1.44 (0.78 – 2.70)
Current use		
1 day – 2 years	1.12 (0.47 – 2.70)	1.09 (0.45 – 2.65)
>2 years	2.06 (0.86 – 4.96)	2.13 (0.88 – 5.14)

* = Adjusted for age, gender, follow-up period, previous fractures in the 5 years before baseline, body mass index, 5-yr cardiovascular disease risk, baseline BMD, and use of thiazides or ß-blockers.

vertebral fracture was significantly decreased with approximately 50% for persons with a continuous use of at least 2 years before the index date, compared to non-users. Users of non-statin lipid lowering drugs did not have a lower risk for fracture. Neither current use of non-statin lipid lowering drugs, nor duration of use was associated with fracture risk.

After adjustments for age, gender and calendar time, risk of fracture with current use of statins was 0.59 (95% confidence interval (CI) 0.38–0.91). Additional adjustments for a previous fracture, BMI, and baseline BMD gave a change in the risk estimate of, respectively, 1.2%, -0.1%, and 4%. The, for these factors and age, gender and calendar time adjusted hazard ratio was 0.83 (0.63–1.09). Finally, we included use of thiazides or ß-blockers and 5-yr cardiovascular disease risk in the model and the risk for fracture with current use of statins was 0.81 (0.61–1.07) (Table 2). Further adjustments for diabetes mellitus, cholesterol levels at baseline, lower limb disability, and presence of the ApoE*4 allele, did not change the estimates. Long-term use (>2 years) of statins was, after adjustments, associated with an approximately 40% lower risk for nonvertebral fractures. Long-term use of statins was, not significantly, associated with a lower risk of hip and wrist fracture (HR 0.16; 0.02–1.13, and HR 0.62; 0.27–1.42, respectively).

Crude risk of nonvertebral fracture with current use of pravastatin or with current use of lipophilic statins was, respectively, not significantly 44% and 33% lower than risk of non-users. After adjustments, risk for nonvertebral fracture was lower for both pravastatin and lipophilic statin users (respectively 30% and 14%, Table 3). The difference between the two types of statins was not significant.

Table 3 — Risk of nonvertebral fracture for pravastatin users and other statin users.

	Pravastatin Hazard ratio (95% CI)	**Lipophilic statins** Hazard ratio (95% CI)
No statin use	1 (reference)	1 (reference)
Current use	0.70 (0.33 – 1.48)	0.86 (0.64 – 1.16)
Duration of current use		
1–730 days	0.92 (0.38 – 2.21)	1.06 (0.73 – 1.53)
> 730 days	0.44 (0.11 – 1.78)	0.65 (0.41 – 1.05)

All hazard ratios are adjusted for age, gender, body mass index, baseline BMD, previous fracture in the 5 years before baseline, and use of ß-blockers or thiazide diuretics.

Stratifying on median age of the cases (younger than 72 years and older than 72 years) did not result in different estimates for the two groups (Data not shown). Stratifying on gender resulted in a risk of nonvertebral fracture with long-term statin use of 0.37 (0.12–1.15) for men and 0.68 (0.42–1.12) for women.

Because there were a substantial number of individuals who had missing values for baseline BMD, we performed a separate analysis in which we excluded all participants with missing values for baseline BMD. This did not result in substantially different estimates (HR for long-term use 0.49; 0.49–0.82).

Discussion

In this study we showed a significant association between use of statins and risk of nonvertebral fracture. This association was duration-dependent. We could not detect any difference in risk of fracture with either pravastatin or lipophilic statin use. We had, however, low power to detect such a difference, because there were not many pravastatin users. For both men and women, risk of nonvertebral fracture was decreased with long-term duration of statin use. There was no significant difference between sexes.

Our results are in line with previous studies in other populations.[18,19,21,41] Previously we reported a study on the association between statin use and vertebral fractures in the Rotterdam Study that also showed a beneficial effect of statin use.[42] The fact that we found a duration-dependent association is biologically plausible, in view of the fact that statins are thought to influence bone metabolism, which is a slow process. Other investigators also found a duration-dependent effect of statins.[18,21,43] However, some studies did not detect an association between fracture incidence and statin use.[22–25] Although several of these studies showed decreased risks,[23–25] there was no significant association between statin use and fracture risk. Ray and colleagues[44] found an association between statin use and fracture risk, but attributed the results to a 'healthy user'-effect; persons that take statins might be more aware of their health status and have healthier lifestyles than non-users. Since BMD may be a reflection of co-morbidity and general health, lower BMD is often observed in persons with health problems. In our study, adjusting for baseline BMD increased our age- and gender-adjusted estimate by 4%. This shows that baseline BMD might be a confounder, and adjusting for BMD might be important to, at least partially, adjust for a potential healthy user effect. In previous publications, confounding by BMI was mentioned as an explanation for the association between statins and fractures. Statins were thought to be more often prescribed in persons with a higher body mass. In our study it is highly unlikely that differences in BMI can explain the association between statins and fracture risk. To deal with confounding by BMI, we adjusted for this factor. The risk estimate changed less than 1% after inclusion in the model, which does not indicate major confounding by BMI. It is suggested that carriers of the ApoE*4 allele have higher serum cholesterol levels[45] and a higher risk of fracture.[46] We examined whether presence of the ApoE*4 allele was associated with use of statins. Although we did not find an association between ApoE*4 and bone in a previous study,[47] the beneficial effects of statins on bone could be attenuated by the fact that persons using statins were having a higher risk of fracture. Adjustment for the ApoE*4 allele did not change the estimates and was therefore not included in the final model.

Potentially, differences between the studies can be explained by the fact that only after at least 2 years the association between statin use and fracture risk becomes significant. Van Staa and colleagues did take duration into account, but they used a cutoff of 1 year of cumulative use.[25] This might have attenuated the risk reduction. Not only duration of use, but also accuracy of defining exposure was different among the studies. Exposure information derived from interview[22] might not be as accurate with respect to duration and time that has passed since discontinuation of the drugs, as pharmacy database-derived information. Furthermore, interview data are susceptible to recall bias.

Two reanalyses of clinical trials did not find significant associations between statin use and fractures.[23,24] These studies, however, had both less than 20% women included. Baseline age was approximately 7 years younger in the study of Reid and colleagues and mean follow-up was 2 years shorter than baseline age and follow-up of our study.[24] In the study of Pedersen and colleagues, baseline age was also lower and mean follow-up was 3 years shorter.[23] In both studies there were less than 160 fractures in persons that were older than 60 years. The power to detect a significant difference might have been too low to find a significant association with statin use in those studies.

The strength of this study was the detailed information on exposure, fracture incidence and data on (potential) confounders. Statin use was ascertained from pharmacy records without knowledge of the outcome and therefore not subject to information bias or recall bias of participants. Participants had to collect the medications at the pharmacy in order to be classified as exposed; therefore our exposure data is potentially more accurate than self-reported statin use or exposure data generated from general practitioner prescriptions. These detailed pharmacy dispensing data also allowed us to study duration of statin use. In the Netherlands, the general practitioner is the 'gate-keeper' for all health care that is provided.[48] Information on fractures that occurred in our participants was therefore complete and detailed and highly unlikely to be susceptible to misclassification. Cofactors that were mentioned in previous studies for their potential confounding influence were included in the model. Since there may be a link between cardiovascular disease and osteoporosis,[27,49] we adjusted for a number of cardiovascular disease risk factors and baseline serum cholesterol levels, to deal with confounding by indication. Furthermore, we also examined risk of fracture for users of non-statin lipid lowering drugs. There was no association between use of these drugs and fracture risk. Confounding by indication as an explanation for our results is unlikely.

In conclusion, we demonstrated a significant association between statin use and non-vertebral fracture risk. The mechanism by which statins might influence this risk deserves further research. Randomized trials designed to examine risk of fracture with statin use can contribute more evidence for a causal relation and need to be carried out before recommendation of statins for (prevention of) osteoporosis can be given.

References

1. **Tobert JA.** New developments in lipid-lowering therapy: the role of inhibitors of hydroxymethylglutaryl-coenzyme A reductase. Circulation 1987; 76 (3): 534-8.
2. **Lefer DJ.** Statins as Potent Antiinflammatory Drugs. Circulation 2002; 106 (16): 2041-2.
3. **McFarlane SI, Muniyappa R, Francisco R, Sowers JR.** Clinical review 145: Pleiotropic effects of statins: lipid reduction and beyond. J Clin Endocrinol Metab 2002; 87 (4): 1451-8.
4. **Waldman A, Kritharides L.** The Pleiotropic Effects of HMG-CoA Reductase Inhibitors: Their Role in Osteoporosis and Dementia. Drugs 2003; 63 (2): 139-52.
5. **Chan KKW, Oza AM, Siu LL.** The Statins as Anticancer Agents. Clin Cancer Res 2003; 9 (1): 10-9.
6. **Mundy G, Garrett R, Harris S, et al.** Stimulation of bone formation in vitro and in rodents by statins. Science 1999; 286 (5446): 1946-9.
7. **Song C, Guo Z, Ma Q, et al.** Simvastatin induces osteoblastic differentiation and inhibits adipocytic differentiation in mouse bone marrow stromal cells. Biochemical and Biophysical Research Communications 2003; 308 (3): 458-62.

8. **Ohnaka K, Shimoda S, Nawata H, et al.** Pitavastatin enhanced BMP-2 and osteocalcin expression by inhibition of Rho-associated kinase in human osteoblasts. Biochem Biophys Res Commun 2001; 287 (2): 337-42.
9. **Sugiyama M, Kodama T, Konishi K, Abe K, Asami S, Oikawa S.** Compactin and simvastatin, but not pravastatin, induce bone morphogenetic protein-2 in human osteosarcoma cells. Biochem Biophys Res Commun 2000; 271 (3): 688-92.
10. **Funkhouser HL, Adera T, Adler RA.** Effect of HMG-CoA reductase inhibitors (statins) on bone mineral density. J Clin Densitom 2002; 5 (2): 151-8.
11. **Montagnani A, Gonnelli S, Cepollaro C, et al.** Effect of simvastatin treatment on bone mineral density and bone turnover in hypercholesterolemic postmenopausal women: a 1-year longitudinal study. Bone 2003; 32 (4): 427-33.
12. **Edwards CJ, Hart DJ, Spector TD.** Oral statins and increased bone-mineral density in postmenopausal women. Lancet 2000; 355 (9222): 2218-9.
13. **Chung YS, Lee MD, Lee SK, Kim HM, Fitzpatrick LA.** HMG-CoA reductase inhibitors increase BMD in type 2 diabetes mellitus patients. J Clin Endocrinol Metab 2000; 85 (3): 1137-42.
14. **Lupattelli G, Scarponi AM, Vaudo G, et al.** Simvastatin increases bone mineral density in hypercholesterolemic postmenopausal women. Metabolism 2004; 53 (6): 744-8.
15. **Rejnmark L, Buus NH, Vestergaard P, Andreasen F, Larsen ML, Mosekilde L.** Statins decrease bone turnover in postmenopausal women: a cross-sectional study. Eur J Clin Invest 2002; 32 (8): 581-9.
16. **Sirola J, Honkanen R, Kroger H, Jurvelin JS, Maenpaa P, Saarikoski S.** Relation of statin use and bone loss: a prospective population-based cohort study in early postmenopausal women. Osteoporos Int 2002; 13 (7): 537-41.
17. **Wada Y, Nakamura Y, Koshiyama H.** Lack of positive correlation between statin use and bone mineral density in Japanese subjects with type 2 diabetes. Arch Intern Med 2000; 160 (18): 2865.
18. **Meier CR, Schlienger RG, Kraenzlin ME, Schlegel B, Jick H.** HMG-CoA reductase inhibitors and the risk of fractures. Jama 2000; 283 (24): 3205-10.
19. **Wang PS, Solomon DH, Mogun H, Avorn J.** HMG-CoA reductase inhibitors and the risk of hip fractures in elderly patients. JAMA 2000; 283 (24): 3211-6.
20. **Pasco JA, Kotowicz MA, Henry MJ, Sanders KM, Nicholson GC.** Statin use, bone mineral density, and fracture risk: Geelong Osteoporosis Study. Arch Intern Med 2002; 162 (5): 537-40.
21. **Chan KA, Andrade SE, Boles M, et al.** Inhibitors of hydroxymethylglutaryl-coenzyme A reductase and risk of fracture among older women. Lancet 2000; 355 (9222): 2185-8.
22. **LaCroix AZ, Cauley JA, Pettinger M, et al.** Statin use, clinical fracture, and bone density in postmenopausal women: results from the Women's Health Initiative Observational Study. Ann Intern Med 2003; 139 (2): 97-104.
23. **Pedersen TR, Kjekshus J.** Statin drugs and the risk of fracture. 4S Study Group. JAMA 2000; 284 (15): 1921-2.
24. **Reid IR, Hague W, Emberson J, et al.** Effect of pravastatin on frequency of fracture in the LIPID study: secondary analysis of a randomised controlled trial. Long-term Intervention with Pravastatin in Ischaemic Disease. Lancet 2001; 357 (9255): 509-12.
25. **van Staa TP, Wegman S, de Vries F, Leufkens B, Cooper C.** Use of statins and risk of fractures. JAMA 2001; 285 (14): 1850-5.
26. **Izumo N, Fujita T, Nakamuta H, Koida M.** Lipophilic statins can be osteogenic by promoting osteoblastic calcification in a Cbfa1- and BMP-2-independent manner. Methods Find Exp Clin Pharmacol 2001; 23 (7): 389-94.
27. **Burnett JR, Vasikaran SD.** Cardiovascular disease and osteoporosis: is there a link between lipids and bone? Ann Clin Biochem 2002; 39 (Pt 3): 203-10.
28. **Schoofs MW, van der Klift M, Hofman A, et al.** Thiazide diuretics and the risk for hip fracture. Ann Intern Med 2003; 139 (6): 476-82.
29. **Pasco JA, Henry MJ, Sanders KM, Kotowicz MA, Seeman E, Nicholson GC.** Beta-adrenergic blockers reduce the risk of fracture partly by increasing bone mineral density: Geelong Osteoporosis Study. J Bone Miner Res 2004; 19 (1): 19-24.
30. **Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA.** Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol 1991; 7 (4): 403-22.

31. **WHO.** International Statistical Classification of Diseases and Related Health Problems, 10th revision. In. Geneva; 1992.
32. **WHO.** Anatomical Therapeutical Chemical (ATC) Classification Index. In. Oslo: World Health Organization Collaborating Centre for Drug Statistics Methodology; 1993.
33. **WHO.** Anatomical Therapeutic Classification (ATC) Index: Including Defined Daily Doses (DDD) for Plain Substances. In. Oslo; 1992.
34. **Pincus T, Summey JA, Soraci SA, Jr., Wallston KA, Hummon NP.** Assessment of patient satisfaction in activities of daily living using a modified Stanford Health Assessment Questionnaire. Arthritis Rheum 1983; 26 (11): 1346-53.
35. **Burger H, de Laet CE, van Daele PL, et al.** Risk factors for increased bone loss in an elderly population: the Rotterdam Study. Am J Epidemiol 1998; 147 (9): 871-9.
36. **Folstein MF, Folstein SE, McHugh PR.** "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975; 12 (3): 189-98.
37. **Anderson KM, Odell PM, Wilson PW, Kannel WB.** Cardiovascular disease risk profiles. Am Heart J 1991; 121 (1 Pt 2): 293-8.
38. **van Duijn CM, de Knijff P, Cruts M, et al.** Apolipoprotein E4 allele in a population-based study of early-onset Alzheimer's disease. Nat Genet 1994; 7 (1): 74-8.
39. **Cox D.** Regression models and life tables. J Royal Stat Society 1972: 187-220.
40. **Twisk J, de Vente W.** Attrition in longitudinal studies. How to deal with missing data. J Clin Epidemiol 2002; 55 (4): 329-37.
41. **Bauer DC, Mundy GR, Jamal SA, et al.** Use of statins and fracture: results of 4 prospective studies and cumulative meta-analysis of observational studies and controlled trials. Arch Intern Med 2004; 164 (2): 146-52.
42. **Schoofs MW, Sturkenboom MC, van der Klift M, Hofman A, Pols HA, Stricker BH.** HMG-CoA reductase inhibitors and the risk of vertebral fracture. J Bone Miner Res 2004; 19 (9): 1525-30.
43. **Rejnmark L, Olsen ML, Johnsen SP, Vestergaard P, Sorensen HT, Mosekilde L.** Hip fracture risk in statin users-a population-based Danish case-control study. Osteoporos Int 2004.
44. **Ray WA, Daugherty JR, Griffin MR.** Lipid-lowering agents and the risk of hip fracture in a Medicaid population. Inj Prev 2002; 8 (4): 276-9.
45. **Sheehan D, Bennett T, Cashman K.** Apolipoprotein E gene polymorphisms and serum cholesterol in healthy Irish adults: a proposed genetic marker for coronary artery disease risk. Ir J Med Sci 2000; 169 (1): 50-4.
46. **Cauley JA, Zmuda JM, Yaffe K, et al.** Apolipoprotein E polymorphism: A new genetic marker of hip fracture risk--The Study of Osteoporotic Fractures. J Bone Miner Res 1999; 14 (7): 1175-81.
47. **Schoofs MW, van der Klift M, Hofman A, et al.** ApoE gene polymorphisms, BMD, and fracture risk in elderly men and women: the Rotterdam study. J Bone Miner Res 2004; 19 (9): 1490-6.
48. **Wensing M, Mainz J, Ferreira P, et al.** General practice care and patients' priorities in Europe: an international comparison. Health Policy 1998; 45 (3): 175-86.
49. **Samelson EJ, Cupples LA, Hannan MT, et al.** Long-term effects of serum cholesterol on bone mineral density in women and men: the Framingham Osteoporosis Study. Bone 2004; 34 (3): 557-61.

Chapter 4

ApoE gene polymorphisms and Osteoporosis

Abstract

Introduction

The E*4 allele of the E*2, E*3, E*4 protein isoform polymorphism in the gene encoding Apolipoprotein E (ApoE) has previously been associated with an increased fracture risk. We investigated the association between the ApoE polymorphism and bone mineral density (BMD), bone loss and incident fractures as part of the Rotterdam Study, a prospective population-based cohort study of diseases in the elderly.

Methods

The study population consisted of 5857 subjects (2560 men; 3297 women) for whom data on ApoE genotypes, confounding variables and follow-up of nonvertebral fractures were available. Data on femoral neck BMD and lumbar spine BMD were available for 4814 participants. Genotype analyses for bone loss and BMD were performed using ANOVA. Incident fractures were analyzed using a Cox proportional-hazards model and logistic regression. All relative risks were adjusted for age and body mass index.

Results and Conclusions

The genotype distribution of the study population was in Hardy-Weinberg equilibrium ($p = 0.98$) and did not differ by gender. At baseline, mean BMD of lumbar spine and femoral neck did not differ between the ApoE genotypes for men and women. Bone loss defined as annualized percent change in BMD at the hip and lumbar spine (mean follow-up 2.0 yr), did not differ by ApoE genotype for women and men. During a mean follow-up of 6.6 years, 708 nonvertebral fractures (198 hip fractures, 179 wrist fractures) and 149 incident vertebral fractures occurred. No consistent differences in the distribution of alleles could be observed between subjects with or without these fractures.

Our data from this, to our knowledge, largest study performed on the association between ApoE and osteoporosis, do not support the hypotheses that the ApoE*4 risk allele is associated with BMD, increased bone loss, or an increased risk of osteoporotic fractures.

Osteoporosis is a multifactorial disorder, characterized by low bone mineral density and microarchitectural deterioration of bone tissue.[1] Twin and family studies showed that bone density and bone turnover are affected by genetic factors. It was estimated that 50–80% of the variability in BMD is explained by genetic factors.[2–4] Several approaches to identify gene variants that predict the risk for osteoporosis have been proposed, including genome searches and candidate gene studies. Of these, candidate gene studies have been shown to be able to identify true genetic risk factors, such as the collagen I alpha 1 Sp1 polymorphism.[5,6] Candidate genes are chosen based on their known involvement in bone biology. A recently emerged pathway involved in bone metabolism is that of apolipoprotein E (ApoE). A polymorphism in the gene encoding the Apolipoprotein E has previously been reported to be associated with an increased risk of both cardiovascular disease and dementia, including Alzheimer's disease.[7–9]

ApoE is a protein of which three isoforms exist, the E*2, E*3 and E*4 isoforms. The primary sequence of these proteins is identical except at amino acids 112 and 158, where there can be cysteines (E*2), arginines (E*4) or cysteine at position 112 and arginine at position 158 (E*3).[10] It is thought that ApoE mediates vitamin K transport, which in turn influences bone turnover.[11,12] Another hypothesis is that low density lipoprotein (LDL) levels in subjects with the E*4 allele are increased and that accumulation of oxidized lipids in subendothelial space of bone may lead to inhibition of osteoblast differentiation.[13,14]

Some studies suggest that the polymorphism in this pleiotropic gene and more specifically the E*4 allele, is associated with an increased fracture risk, low bone mineral density (BMD) and increased rates of bone loss.[15–20] Other studies, however, suggested that there is no association between ApoE, bone loss or fracture risk in either men or women.[21–24] While most of the previous studies included a limited number of subjects, some of these studies also had selected populations indicated by a deviation from the Hardy-Weinberg equilibrium.

Therefore, we examined the association between ApoE alleles, bone mineral density, bone loss and incident fractures in the population of the Rotterdam Study, a large prospective cohort study of diseases in the elderly.

Materials and Methods

Study population

The Rotterdam Study is a prospective population-based cohort study of men and women aged 55 and over and has the objective to investigate the incidence of, and risk factors for, chronic disabling diseases. Both the rationale and the study design have been described previously.[25] The focus of the Rotterdam Study is on neurological, cardiovascular, ophthalmologic and locomotor diseases. All 10,275 inhabitants of Ommoord, a district in Rotterdam, the Netherlands, were invited to participate. Of these, 7,983 (78%) participated in the study. The Medical Ethics Committee of the Erasmus Medical Center approved the Rotterdam Study.

There was data on ApoE genotypes available for 6137 subjects of the total 7983 subjects in our cohort. Data on follow-up of nonvertebral fractures and data on confounding variables was present for 5857 (3297 women) of them. In a subset of 4814 individuals, baseline BMD measurements at both the femoral neck and lumbar spine regions were performed. Analyses on incident vertebral fractures were performed in a subset of 2900 individuals (1519 women).

Outcome assessment

Vertebral deformities

Vertebral deformities were assessed as described previously.[26] In short, both at baseline, between 1990 and 1993, and at the second follow-up visit, between 1997 and 1999, a trained research technician obtained lateral radiographs of the thoracolumbar spine of subjects who were able to come to the research center. All follow-up radiographs were evaluated morphometrically in Sheffield by the McCloskey-Kanis method.[27] If a vertebral fracture was detected, the baseline radiograph was evaluated as well. If the fracture was already present at baseline it was considered a prevalent fracture. If, however, the vertebra was determined to be normal at baseline and any of the three vertebral heights (anterior, central or posterior) showed a minimum decrease of at least 4.6 mm and 15% in absolute height on the later film, it was considered an incident fracture. All vertebral fractures were confirmed by visual interpretation by an expert in the field, to rule out artifacts and other etiologies, such as pathological fractures.

Mortality and nonvertebral fractures

For the entire cohort, information on vital status is obtained continuously from the municipal authorities in Rotterdam. For subjects who moved outside the research area, mortality data are obtained from general practitioners (GPs). GPs in the research area (covering 80% of the cohort) reported all relevant fatal and non-fatal events, such as fractures, through a computerized system. Research physicians verified follow-up information by checking GPs' patient records. This is possible because in the Netherlands the GP has a gatekeeper function, which means that the GP retains all medical information of his patients. For the remaining 20% of the population, research physicians collected data from their GPs' patient records. For hospitalized patients, discharge reports and letters from medical specialists were additionally used for verification. All non-fatal events, such as fractures, were coded independently by two research physicians according to the International Classification of Diseases, 10th revision (ICD-10).[28] If there was disagreement, consensus was reached in a separate session. A medical expert in the field reviewed all coded events for a final classification. Data for overall mortality were available until 31st December 1999.

Bone mineral density

Bone mineral density measurements of the femoral neck and lumbar spine were performed by dual energy X-ray absorptiometry (DXA) (Lunar DPX-L densitometer, Madison, Wisconsin, USA) as described previously.[29] Bone mineral density measurements were

repeated at the second center visit, between 1994 and 1995. Rates of bone loss were calculated as yearly percentages of change in BMD.

ApoE gene polymorphism assessment

ApoE genotyping was performed on coded blood samples, without knowledge of the outcome. Genotyping was performed with the use of a polymerase chain reaction, as described previously.[30] The ApoE*4 allele was regarded as the risk allele.

Co-factors

Between 1990 and 1993, an extensive baseline home interview on medical history, risk factors for chronic diseases, medication use and habitual diet was performed on all participants by trained interviewers. Amongst others, information was gathered on fall frequency, smoking habits (defined as current, former or never smoking) and age at menopause. After the home interview, the participants were invited to visit the research center for clinical examination and laboratory assessments. Non-fasting blood samples were drawn. Height (m) and weight (kg) were measured in subjects wearing light clothing without shoes. Body Mass Index was calculated as weight divided by height squared (kg/m^2). Cognitive impairment was measured using the Mini Mental State Examination.[31]

Statistical analyses

Student's t-test and Pearson's Chi-square were used to compare baseline characteristics for ApoE*4 carriers and non-carriers.

Subjects were grouped by allele copy number (0, 1 or 2 copies) for the ApoE*4 allele. We allowed three possible models to explain the association results. These are effects based on a dominant effect, a recessive, or an allele dose-effect. Allele dose was defined as the number of copies of a certain allele in the genotype. In case of a consistent trend, reflected as an allele dose effect, we performed a (multiple) linear regression analysis to quantify the association. In case of a dominant or recessive effect of the test-allele, ANOVA and ANCOVA tests were performed. For dominant effects we compared test-allele carriers versus non-carriers while for recessive effects, subjects homozygous for the test-allele were compared to heterozygous carriers and non-carriers.

To analyze the association between number of ApoE*4 alleles and BMD and yearly changes in BMD, differences between genotype groups were calculated stratified by gender. Then, a general linear model adjusting for age and body mass index was used.

Hazard ratios for incident nonvertebral fractures overall, and hip and wrist fractures in particular were calculated using a Cox Proportional Hazards Model, stratified by gender. Initially, crude analyses were performed, followed by adjustment for age and body mass index. Further adjustment for cognitive impairment, frequent falling, and age at menopause for women was performed to investigate the stability of the estimates.

For the analyses on incident vertebral fractures, a similar approach was used. The only difference was that a logistic regression model was used instead of a Cox Proportional Hazard Model. This was done because follow-up time could not be calculated since we have no information on the date of the event.

Results

We identified 3 alleles (ApoE*2, -3, and -4) and 6 genotypes 22 (0.8%), 23 (13%), 24 (2.6%), 33 (57.9%), 34 (23.3%), 44 (2.4%). In our study population for both men and women, allele frequencies did not deviate from the Hardy-Weinberg equilibrium (HWE) (p-value 0.43 and 0.89, respectively). For the subsets for analyses of bone mineral density and bone loss, the genotype distributions were also in HWE.

In Table 1 baseline characteristics for ApoE*4 carriers and non-carriers are shown. ApoE*4 carriers are younger and have a lower body weight. Serum cholesterol levels of ApoE*4 carriers were higher and their MMSE scores were lower. Baseline characteristics of the total study population and the subset of individuals with bone mineral density measurements available did not differ substantially, except for subjects with BMD data available being somewhat younger. No substantial differences in any of the other variables were observed (data not shown).

Table 1 — Baseline characteristics of non-E*4 carriers compared with E*4 carriers.

	Men		***Women***	
	Non-E*4 carriers	E*4 carriers	Non-E*4 carriers	E*4 carriers
Number	1829	731	2374	923
Age (years)	68.4 (± 8.3)	68.1 (± 7.9)	70.6 (± 9.5)	69.9 (± 9.4)
Weight (kg)	78.4 (± 11.1)	78.0 (± 10.3)	69.9 (± 11.4)	68.7 (± 11.3)*
Body mass index (kg/m^2)	25.7 (± 2.9)	25.6 (± 2.9)	26.9 (± 4.1)	26.6 (± 4.2)
Total serum cholesterol	6.2 (± 1.1)	6.4 (± 1.2)*	6.8 (± 1.2)	6.9 (± 1.2)*
Age at menopause (years)			48.8 (± 5.0)	48.6 (± 5.2)
MMSE score	27.6 (± 2.3)	27.5 (± 2.4)*	27.2 (± 2.7)	26.7 (± 3.2)*
Recent falling	159 (9.6%)	72 (10.9%)	487 (22.5%)	172 (20.7%)
Smoking				
Current	549 (30.4%)	185 (25.8%)	412 (17.8%)	176 (19.6%)
Former	1109 (61.3%)	477 (66.4%)	640 (27.6%)	253 (28.1%)
Never	150 (8.3%)	56 (7.8%)	1268 (54.7%)	470 (52.3%)

Variables are tested for difference from non-carriers using Student's *t*-test.
* p-value = < 0.01.

In Table 2, averages of BMD, as measured at both the femoral neck and lumbar spine, are shown for subjects without the ApoE*4 allele (non-carriers), with one copy (heterozygotes) or with two copies of the ApoE*4 allele (homozygotes). No significant differences in BMD at baseline could be observed.

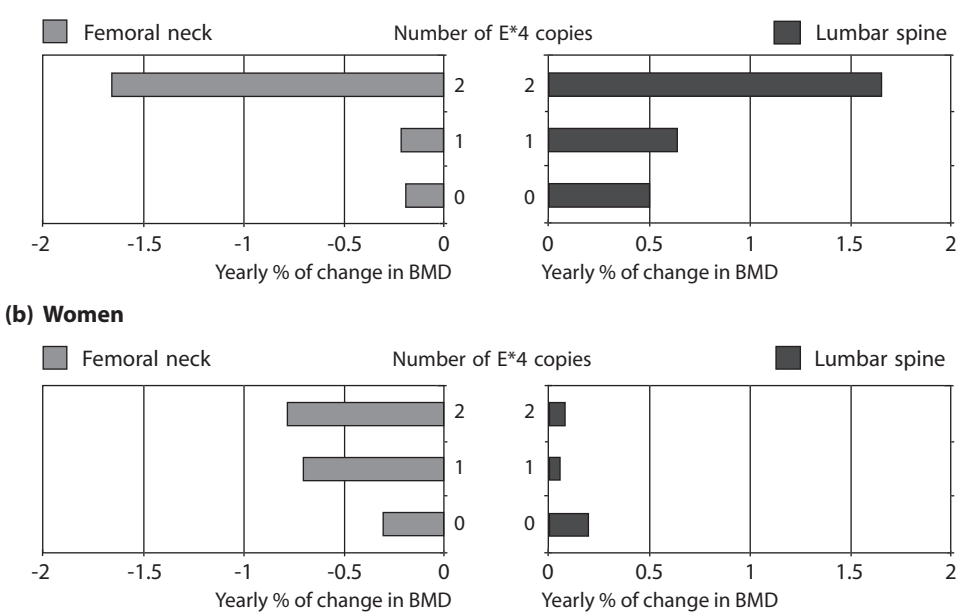
Figure 1 shows average change in BMD per year between the first and second center visit. The mean follow-up between the BMD measurements was 2.0 year (SD = 0.61). An inconsistent association between the ApoE*4 genotype and rate of loss was found for men. In the femoral neck a larger loss was observed for ApoE*4 homozygotes whereas in the lumbar spine, homozygous men actually gained bone.

Table 2 — Mean bone mineral density at baseline (95% CI) (g/cm^2) by ApoE genotype.

	N	Total cohort	N	Men	N	Women
Femoral neck BMD						
All subjects	4814		2220		2594	
Non-carriers	3462	0.84 (0.84 ; 0.85)	1585	0.88 (0.87 ; 0.88)	1877	0.81 (0.80 ; 0.81)
E*4 Heterozygotes	1228	0.84 (0.83 ; 0.84)	573	0.87 (0.86 ; 0.88)	655	0.81 (0.80 ; 0.82)
E*4 Homozygotes	124	0.84 (0.82 ; 0.86)	62	0.88 (0.85 ; 0.91)	62	0.80 (0.78 ; 0.83)
Lumbar spine BMD						
All subjects	4814		2220		2594	
Non-carriers	3462	1.10 (1.09 ; 1.10)	1585	1.16 (1.15 ; 1.17)	1877	1.04 (1.03 ; 1.05)
E*4 Heterozygotes	1228	1.10 (1.08 ; 1.10)	573	1.16 (1.15 ; 1.18)	655	1.03 (1.02 ; 1.04)
E*4 Homozygotes	124	1.11 (1.07 ; 1.14)	62	1.19 (1.14 ; 1.23)	62	1.03 (0.99 ; 1.07)

Values are means with 95% confidence intervals.
Adjustment was made for age and body mass index.

Figure 1 — Mean yearly percentages of change in bone mineral density between the first (1990–1993) and second (1994–1995) center follow-up visits by ApoE genotype.



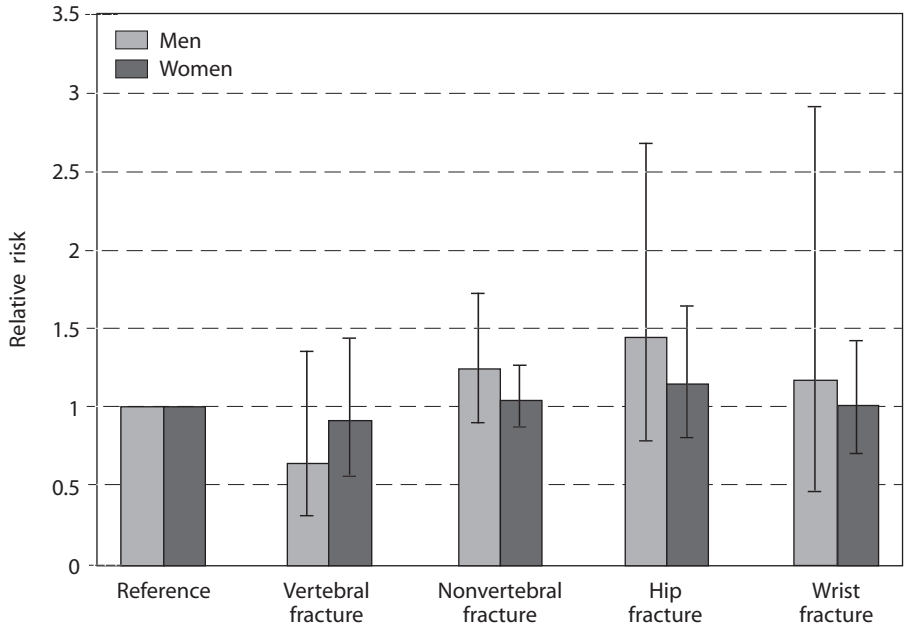
During follow-up, 708 nonvertebral fractures (of which 198 hip fractures and 179 wrist fractures) and 149 incident vertebral fractures occurred in the study population. Table 3 shows the number of fractures by ApoE*4 genotype for all nonvertebral fractures, hip and wrist fractures, and for incident vertebral fractures. Results are shown both for the total

Table 3 — Allele frequencies of ApoE*4 in relation to osteoporotic fractures in men and women.

	Total cohort			Men			Women		
	Non-carriers	E*4 hetero-zygotes	E*4 homo-zygotes	Non-carriers	E*4 hetero-zygotes	E*4 homo-zygotes	Non-carriers	E*4 hetero-zygotes	E*4 homo-zygotes
Vertebral fractures	113 / 2080 (5.4%)	34 / 759 (4.5%)	2 / 61 (3.3%)	34 / 981 (3.5%)	7 / 368 (1.9%)	2 / 32 (6.3%)	79 / 1099 (7.2%)	27 / 391 (6.9%)	0 / 29 (0%)
Nonvertebral fractures	499 / 4203 (11.9%)	196 / 1514 (12.9%)	13 / 140 (9.3%)	110 / 1829 (6.0%)	52 / 665 (7.8%)	3 / 66 (4.5%)	389 / 2374 (16.4%)	144 / 849 (17.0%)	10 / 74 (13.5%)
Hip fractures	139 / 4203 (3.3%)	56 / 1514 (3.7%)	3 / 140 (2.1%)	30 / 1829 (1.6%)	16 / 665 (2.4%)	0 / 66 (0%)	109 / 2374 (4.6%)	40 / 849 (4.7%)	3 / 74 (4.1%)
Wrist fractures	129 / 4203 (3.1%)	45 / 1514 (3.0%)	5 / 140 (3.6%)	15 / 1829 (0.8%)	5 / 665 (0.8%)	2 / 66 (3.0%)	114 / 2374 (4.8%)	40 / 849 (4.7%)	3 / 74 (4.1%)

Shown is the number of cases (%) and the total number of subjects per group.

Figure 2 — Risk of incident fracture (95% CI) for ApoE*4 carriers compared to non-carriers.



Adjustment was made for age and body mass index.

cohort and for men and women separately. Overall, no consistent trends in the frequency for each genotype group could be observed. Genotype frequencies were essentially the same in the subgroup of individuals with data on BMD available (data not shown).

Figure 2 shows the age- and BMI-adjusted risk of incident fractures for ApoE*4 carriers compared to non-carriers for men and women separately. The risk of fracture for ApoE*4 carriers did not significantly deviate from the risk of fracture for non-carriers. Further adjustment for falling, cognitive impairment, femoral neck and lumbar spine BMD, or age at menopause did not alter the estimates (data not shown). Stratification in five-year age categories showed no effect modification by age on the association between ApoE and risk of fracture (data not shown).

All analyses on fractures, BMD and rates of bone loss were repeated for subjects who carry one or two ApoE*2 alleles. No consistent association was found between the ApoE*2 allele and fractures, BMD or rates of bone loss (data not shown).

Discussion

In this large population-based cohort study, no consistent association between the apolipoprotein E gene polymorphism, bone mineral density, and fractures could be observed in either men or women.

To our knowledge, this is the largest population-based study on the association of the ApoE gene polymorphism and bone mass parameters. We had data on ApoE genotypes

as well as data on incident fractures and potential confounders for almost 6000 subjects. Previously, several other studies have investigated the association between ApoE gene polymorphisms and osteoporotic fractures. Some, but not all, showed an increased fracture risk in ApoE*4 carriers. In one of these studies, which was performed in women only, there was a significant deviation from the Hardy-Weinberg equilibrium.[17] This may suggest that the association observed was due to a selection bias. In our study, the allele frequencies were in Hardy-Weinberg equilibrium. Hence, no selection has occurred among genotypes. In other studies the size of the study population, and therefore the number of subjects with a fracture was limited.[19,20] Our findings on ApoE*4 and fractures are in line with two previous studies.[21,22] Furthermore, Pluijm et al. also did not find an increased nonvertebral fracture risk, but in contrast with our study, more vertebral deformities were demonstrated in women.[18]

Several studies showed an association between ApoE*4 and BMD or rates of bone loss.[15,16,18] The sizes of the population in these studies were smaller than the present study and two of them were not in Hardy-Weinberg equilibrium.[15,18] Four previous studies were in line with our results, suggesting that there is no association present between ApoE and BMD or rates of bone loss.[21–24] A meta-analysis of these studies and ours combined might be helpful in estimating the true effect size, if any, of the ApoE polymorphism on BMD and fracture risk.

Our study has some potential limitations. First of all, for the BMD analyses, the study population was restricted to approximately two-thirds of the total study population, due to the fact that subjects had to be able to visit our research center to have their BMD measured. Furthermore, the follow-up for the analyses on rates of bone loss was short. This may have introduced a selection bias. Although the subjects were somewhat younger, they did not differ in any of the other variables studied. In addition, allele frequencies were essentially the same in this subgroup as in the total cohort. When studying vertebral fractures, health selection bias might also play a role. Since in order to have data on incident vertebral fractures, subjects had to be able to visit the research center both at baseline and at the second follow-up. Unfortunately, the only way to establish the presence or absence of incident vertebral fractures is by repeated evaluation of radiographs of the thoracolumbar spine, since two-thirds of all vertebral fractures remains clinically unnoticed. But, again, genotype distributions did not differ from the total study population.

All of the studies on ApoE and osteoporosis, including our own, have focused on the same polymorphism in the coding region of the apolipoprotein E gene. This polymorphism is responsible for the presence of the three isoforms of ApoE (E*2, E*3 and E*4). However, in addition to this particular polymorphism, several other variations in the gene encoding ApoE have been reported.[32,33] These other potentially interesting polymorphisms, such as those in the promoter, may influence the transcription rate and eventually the concentration of ApoE protein in the circulation. Whether or not differences in absolute levels of ApoE are associated with the risk of either low BMD, high fracture risk or both, deserves further investigation.

In conclusion, the results of this study in elderly men and women do not support an association between the ApoE*4 allele and bone mineral density or incident fractures.

References

1. **Kanis JA, Melton LJ, 3rd, Christiansen C, Johnston CC, Khaltaev N.** The diagnosis of osteoporosis. J Bone Miner Res 1994; 9 (8): 1137-41.
2. **Kelly PJ, Harris M.** Genetic regulation of peak bone mass. Acta Paediatr Suppl 1995; 411: 24-9; discussion 30.
3. **Pocock NA, Eisman JA, Hopper JL, Yeates MG, Sambrook PN, Eberl S.** Genetic determinants of bone mass in adults. A twin study. J Clin Invest 1987; 80(3): 706-10.
4. **Gueguen R, Jouanny P, Guillemin F, Kuntz C, Pourel J, Siest G.** Segregation analysis and variance components analysis of bone mineral density in healthy families. J Bone Miner Res 1995; 10 (12): 2017-22.
5. **Efstathiadou Z, Tsatsoulis A, Ioannidis JP.** Association of collagen Ialpha 1 Sp1 polymorphism with the risk of prevalent fractures: a meta-analysis. J Bone Miner Res 2001; 16 (9): 1586-92.
6. **Mann V, Hobson EE, Li B, Stewart TL, Grant SF, Robins SP, Aspden RM, Ralston SH.** A COL1A1 Sp1 binding site polymorphism predisposes to osteoporotic fracture by affecting bone density and quality. J Clin Invest 2001; 107 (7): 899-907.
7. **Weisgraber KH, Mahley RW.** Human apolipoprotein E: the Alzheimer's disease connection. Faseb J 1996; 10 (13): 1485-94.
8. **Contois JH, Anamani DE, Tsongalis GJ.** The underlying molecular mechanism of apolipoprotein E polymorphism: relationships to lipid disorders, cardiovascular disease, and Alzheimer's disease. Clin Lab Med 1996; 16 (1): 105-23.
9. **Wilson PW, Myers RH, Larson MG, Ordovas JM, Wolf PA, Schaefer EJ.** Apolipoprotein E alleles, dyslipidemia, and coronary heart disease. The Framingham Offspring Study. Jama 1994; 272 (21): 1666-71.
10. **Frikke-Schmidt R.** Context-dependent and invariant associations between APOE genotype and levels of lipoproteins and risk of ischemic heart disease: a review. Scand J Clin Lab Invest Suppl 2000; (233): 3-25.
11. **Kohlmeier M, Salomon A, Saupe J, Shearer MJ.** Transport of vitamin K to bone in humans. J Nutr 1996; 126 (4 Suppl): 1192S-6S.
12. **Knapen MH, Hamulyak K, Vermeer C.** The effect of vitamin K supplementation on circulating osteocalcin (bone Gla protein) and urinary calcium excretion. Ann Intern Med 1989; 111 (12): 1001-5.
13. **Parhami F, Garfinkel A, Demer LL.** Role of Lipids in Osteoporosis. Arterioscler Thromb Vasc Biol 2000; 20 (11): 2346-2348.
14. **Parhami F, Morrow AD, Balucan J, Leitinger N, Watson AD, Tintut Y, Berliner JA, Demer LL.** Lipid oxidation products have opposite effects on calcifying vascular cell and bone cell differentiation. A possible explanation for the paradox of arterial calcification in osteoporotic patients. Arterioscler Thromb Vasc Biol 1997; 17 (4): 680-7.
15. **Salamone LM, Cauley JA, Zmuda J, Pasagian-Macaulay A, Epstein RS, Ferrell RE, Black DM, Kuller LH.** Apolipoprotein E gene polymorphism and bone loss: estrogen status modifies the influence of apolipoprotein E on bone loss. J Bone Miner Res 2000; 15 (2): 308-14.
16. **Shiraki M, Shiraki Y, Aoki C, Hosoi T, Inoue S, Kaneki M, Ouchi Y.** Association of bone mineral density with apolipoprotein E phenotype. J Bone Miner Res 1997; 12 (9): 1438-45.
17. **Cauley JA, Zmuda JM, Yaffe K, Kuller LH, Ferrell RE, Wisniewski SR, Cummings SR.** Apolipoprotein E polymorphism: A new genetic marker of hip fracture risk--The Study of Osteoporotic Fractures. J Bone Miner Res 1999; 14 (7): 1175-81.
18. **Pluijm SM, Dik MG, Jonker C, Deeg DJ, Kamp GJ, Lips P.** Effects of Gender and Age on the Association of Apolipoprotein E epsilon4 with Bone Mineral Density, Bone Turnover and the Risk of Fractures in Older People. Osteoporos Int 2002; 13 (9): 701-9.
19. **Kohlmeier M, Saupe J, Schaefer K, Asmus G.** Bone fracture history and prospective bone fracture risk of hemodialysis patients are related to apolipoprotein E genotype. Calcif Tissue Int 1998; 62 (3): 278-81.
20. **Johnston JM, Cauley JA, Ganguli M.** APOE 4 and hip fracture risk in a community-based study of older adults. J Am Geriatr Soc 1999; 47 (11): 1342-5.
21. **von Muhlen DG, Barrett-Connor E, Schneider DL, Morin PA, Parry P.** Osteoporosis and apolipoprotein E genotype in older adults: the Rancho Bernardo study. Osteoporos Int 2001; 12 (4): 332-5.

22. **Booth SL, Tucker KL, Chen H, Hannan MT, Gagnon DR, Cupples LA, Wilson PW, Ordovas J, Schaefer EJ, Dawson-Hughes B, Kiel DP.** Dietary vitamin K intakes are associated with hip fracture but not with bone mineral density in elderly men and women. Am J Clin Nutr 2000; 71 (5): 1201-8.
23. **Heikkinen AM, Kroger H, Niskanen L, Komulainen MH, Ryynanen M, Parviainen MT, Tuppurainen MT, Honkanen R, Saarikoski S.** Does apolipoprotein E genotype relate to BMD and bone markers in postmenopausal women? Maturitas 2000; 34 (1): 33-41.
24. **Stulc T, Ceska R, Horinek A, Stepan J.** Bone mineral density in patients with apolipoprotein E type 2/2 and 4/4 genotype. Physiol Res 2000; 49 (4): 435-9.
25. **Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA.** Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol 1991; 7 (4): 403-22.
26. **Van der Klift M, De Laet CE, McCloskey EV, Hofman A, Pols HA.** The incidence of vertebral fractures in men and women: the Rotterdam Study. J Bone Miner Res 2002; 17 (6): 1051-6.
27. **McCloskey EV, Spector TD, Eyres KS, Fern ED, O'Rourke N, Vasikaran S, Kanis JA.** The assessment of vertebral deformity: a method for use in population studies and clinical trials. Osteoporos Int 1993; 3 (3): 138-47.
28. **WHO.** International Statistical Classification of Diseases and Related Health Problems, 10th revision, vol. 1, Geneva; 1992.
29. **Burger H, van Daele PL, Algra D, van den Ouweland FA, Grobbee DE, Hofman A, van Kuijk C, Schutte HE, Birkenhager JC, Pols HA.** The association between age and bone mineral density in men and women aged 55 years and over: the Rotterdam Study. Bone Miner 1994; 25 (1): 1-13.
30. **van Duijn CM, de Knijff P, Cruts M, Wehnert A, Havekes LM, Hofman A, Van Broeckhoven C.** Apolipoprotein E4 allele in a population-based study of early-onset Alzheimer's disease. Nat Genet 1994; 7 (1): 74-8.
31. **Folstein MF, Folstein SE, McHugh PR.** "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975; 12 (3): 189-98.
32. **Artiga MJ, Bullido MJ, Frank A, Sastre I, Recuero M, Garcia MA, Lendon CL, Han SW, Morris JC, Vazquez J, Goate A, Valdivieso F.** Risk for Alzheimer's disease correlates with transcriptional activity of the APOE gene. Hum Mol Genet 1998; 7 (12): 1887-92.
33. **Bullido MJ, Artiga MJ, Recuero M, Sastre I, Garcia MA, Aldudo J, Lendon C, Han SW, Morris JC, Frank A, Vazquez J, Goate A, Valdivieso F.** A polymorphism in the regulatory region of APOE associated with risk for Alzheimer's dementia. Nat Genet 1998; 18 (1): 69-71.

Chapter 5

Epidemiological support for involvement of the ß-adrenergic system in bone metabolism

Abstract

Background

There is accumulating evidence that the sympathetic nervous system is involved in the regulation of bone metabolism. We examined the association between dose and duration of ß-blocker use and bone mineral density (BMD), bone loss, bone structure and (non)vertebral fracture incidence.

Methods

We performed our study in the cohort of the Rotterdam Study, a population-based cohort study in men and women of 55 years and older. BMD was measured by DXA and hip bone structural parameters were obtained for DXA using hip structural analysis software. Non-vertebral fractures were reported by general practitioners and vertebral fractures were assessed from spinal X-rays. Medication use was available on a day-to-day-basis from pharmacy records.

Results

For 3009 participants, follow-up femoral neck BMD measurements were available and for 7892 and 3469 participants follow-up data were present on nonvertebral fractures and vertebral fractures, respectively. Mean BMD (0.858 g/cm^2; CI 95% 0.851–0.864) of long-term ß-blocker users (>4 yr) was significantly higher than BMD of non-users (0.841; 0.839–0.844) and long-term users had a significantly lower rate of loss of BMD per year (0.43%; 0.32–0.53%) than non-users (0.71%; 0.66–0.76%). Hip structural analyses showed increased cortical thickness and higher stability for the femoral neck for long-term users. Risk for all non-vertebral fractures was not decreased, but there was a significant association between long-term ß-blocker use and frailty fracture risk (HR 0.67; 0.46–0.97). Vertebral fracture risk was lower for long-term users, but not significantly decreased.

Conclusions

In conclusion, we report a significant and duration-dependent association between the use of ß-blockers and BMD, rates of bone loss and risk of frailty fractures.

There is accumulating evidence that the sympathetic nervous system is involved in regulation of bone metabolism. Animal studies showed sympathetic innervation of bone[1,2] and adrenergic receptors have been found on both osteoblasts and osteoclasts.[3] In sympathetic nerve fibers in bone, neuropeptides are present and these neuropeptides modulated bone resorption.[4,5] Sympathectomy in rats impaired bone resorption by inhibiting preosteoclast differentiation.[6] Furthermore, propranolol increased bone formation in mice.[7]

ß-blockers are a common therapy in patients with cardiovascular disease. They block the ß-adrenergic receptor, thereby inhibiting the effect of catecholamines and probably the effect of leptin-dependent regulation of bone metabolism.[1] Therefore, use of ß-blockers could be associated with differences in bone metabolism resulting in differences in bone mineral density (BMD) and fracture incidence. Considering that vertebral and nonvertebral fractures cause major morbidity and mortality, prevention of these fractures is an important aim in an elderly population.

BMD is the most often used parameter of osteoporosis, but mechanical strength of bone is also influenced by the structural geometry in ways that may not be apparent in the density.[8] Therefore, structural properties of (hip) bone also deserve study in relation to ß-blocker use. Comparison of hip structure of users and non-users might provide insight into the mechanism by which ß-blockers might affect fracture risk. In the present study, we examined the association between dose and duration of ß-blocker use and BMD, hip bone structure and the risk of fracture in a prospective cohort study.

Methods

Study population

The Rotterdam Study is a population-based cohort study designed to assess the occurrence and determinants of diseases in an ageing population. The cohort includes 3105 men and 4878 women aged 55 years and over (78% of the eligible population), who lived for at least one year in a defined district in Rotterdam in the Netherlands.[9] All participants gave written informed consent to retrieve all relevant medical information from treating physicians. The Medical Ethics Committee of the Erasmus Medical Center approved the study. Baseline measurements were obtained from 1990 to 1993 and consisted of a home interview and research center visits for physical examinations. A second examination round took place between 1995 and 1997 and the third examination phase took place from 1997 until 1999.

Bone mineral density and bone structure

BMD was measured during baseline visit and the third examination, by dual energy X-ray absorptiometry (DXA, Lunar DPX-L densitometer) as described previously.[10] Rates of loss of BMD were expressed as the percentage of change from baseline BMD per year. Parameters of structural geometry were calculated indirectly from conventional DXA scans of the femoral neck using hip structural analysis software, as described pre-

viously.[11–13] Bone width (outer diameter) and cross-sectional moment of inertia were measured directly from mineral mass distributions. The section modulus, estimated as the cross-sectional moment of inertia divided by bone width, is an index of bending strength. The cortical buckling ratio is an index of bone instability, calculated as the ratio of the radius to the average estimated cortical thickness.

Mortality and nonvertebral fractures

For the entire cohort, information on vital status is obtained continuously from the municipal authorities in Rotterdam. For subjects who moved outside the research area, mortality data are obtained from general practitioners (GPs). GPs in the research area reported all relevant fatal and non-fatal events, such as fractures, through a computerized system. This system covers approximately 80% of the population and for participants not covered, research physicians performed annual checks on the complete medical records of all general practitioners in the Rotterdam Study. All follow-up information was checked in GPs' patient records by research physicians and independently coded according to the International Classification of Diseases, 10th revision (ICD-10).[14] A medical expert in the field reviewed all coded events for a final classification. For analyses on nonvertebral fractures, we included all fractures that occurred in the study population during the follow-up period, except for vertebral fractures and pathological and post-procedural fractures. Patients were followed until the first fracture. For patients with more types of fracture, the follow-up ended on the day of the fracture of interest, regardless of a previous other type of fracture. Frailty fractures were defined as fractures of hip, pelvis and upper humerus and were analyzed separately because of their occurrence at older age. Information on mortality and on incident nonvertebral fractures was collected from baseline until the end of the study period on December 31, 2001.

To reduce potential misclassification of exposure at baseline we ensured potential pharmacy data of at least six months for all participants. Therefore the follow-up for fracture analyses started at July 1,1991 and all cohort members were followed until the first fracture of interest, or until participants died or reached the end of the study period.

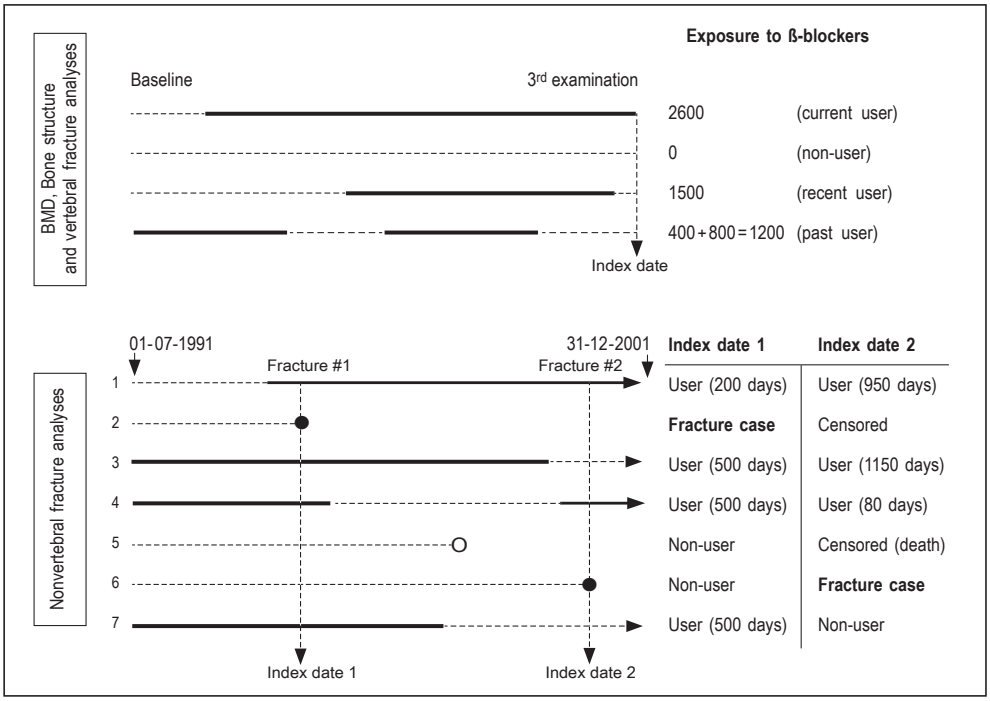
Vertebral deformities

Vertebral deformities were assessed from baseline and follow-up lateral radiographs, as described previously.[15] All vertebral fractures were confirmed by visual interpretation by an expert in the field, to rule out artifacts and other etiologies, such as pathological fractures.

Exposure assessment

All participants fill their drug prescriptions at one of the seven computerized pharmacies in the research area. These pharmacies are linked to one network and drug-dispensing data is available for all subjects from January 1, 1991 onwards. The data consists of information on the date of prescribing, the total amount of drug units per prescription, the prescribed daily number of units, product name of the drugs and the Anatomical Therapeutic Chemical (ATC)-code.[16]

Figure 1 — Exposure definitions for BMD, hip bone structure and fracture analyses.



Solid lines represent use of ß-blockers, dashed lines represent no use.

● = Fracture
O = Death

For BMD and bone structure analyses, exposure to ß-blockers is accumulated during the study period. When ß-blocker use was discontinued >120 days before the third examination, the person was regarded as 'past user'. When discontinuation happened ≤120 days before the end of the study, this person was regarded as 'recent user'.

For fracture analyses, exposure status is updated for every index date (of fracture). After discontinuation, during the first 120 days the participant will be regarded as 'exposed' (participant no. 3). When ß-blocker use is discontinued for >120 days, the participant will be considered 'non-user' (participant no. 7). When a participant discontinued use, and afterwards started using again, only the last episode was taken into account for computation of duration of use (participant no. 4).

Exposure to ß-blockers is explained in Figure 1. Exposure for BMD, bone structure and vertebral fracture analyses was defined as the cumulative number of days of exposure between the first and third examination round. ß-blocker use was also categorized as any or no use, and as four mutually exclusive intervals: non-use, 1 day to ≤2 years of use, >2 years and ≤4 years of use, and >4 years of use.

When a nonvertebral fracture occurred, that date was defined as the index date and the number of days of current use of ß-blockers on that date was calculated for each cohort member. To investigate the duration-effect relationship for ß-blocker use and fractures, a categorical variable was created, as stated above. The total cumulative dose

of ß-blockers ingested before the index date was also calculated, and categorized as described above. For vertebral fracture analyses, we computed the total number of days of ß-blocker use between the first and the last X-ray and categorized ß-blocker use in the same periods as for nonvertebral fracture analyses.

The dosage was expressed as mean of prescribed daily dose equivalents during the study period.[17] The defined daily dose (DDD) of ß-blockers equals the standard recommended adult daily dose for treatment of the main indication. The dose-effect relationship was studied by categorizing daily dose below and above the median. Differences between cardioselective and non-cardioselective ß-blockers were investigated by stratified analysis as well.

Co-factors

During a baseline home interview, trained interviewers gathered information on medical history, risk factors for chronic diseases, medication use and habitual diet. Amongst others, information was gathered on potential risk factors such as use of a walking aid, incidence of falling in the previous year (>once/month), smoking habits, and age at menopause. Lower limb disability was assessed using a modified version of the Stanford Health Assessment Questionnaire[18] and by calculating the mean score of answers to questions concerning rising, walking, bending and getting in and out of a car.[10] A score of more than one indicates disability.

After the home interview, the participants were invited to visit the research center for clinical examinations and laboratory assessments. Hypertension was defined as a systolic blood pressure $\geq$160 mmHg and/or diastolic blood pressure $\geq$100 mmHg and/or use of antihypertensive medication. Blood samples were drawn and analyzed. Cognitive impairment was measured using the Mini Mental State Examination (MMSE).[19] We computed the 5-year Framingham cardiovascular disease risk score for all participants at baseline, with help of an algorithm.[20] This score predicts 5-year cardiovascular disease risk.

Information on use of other medications was also derived from the computerized pharmacy database. We considered the number of days of exposure to thiazide diuretics, statins, and hormone replacement therapy, and the number of prescriptions during follow-up for glucocorticoids as potential confounders. The number of prescriptions of inhaled ß-mimetics was analyzed to investigate potential confounding by contra-indication; use of ß-blockers is contraindicated in asthmatic persons and these persons often use steroids that decrease BMD. Also, BMD and risk of fracture with use of angiotensin converting enzyme-inhibitors (ACE-inhibitors) was examined, as like ß-blockers ACE-inhibitors are prescribed for hypertension.

Statistical analysis

Baseline demographic and clinical characteristics of participants with and without ß-blocker use were compared with Student's t-test and Pearson's Chi-square.

To examine the association between BMD and ß-blocker use, we used a multivariate linear regression model. Regression coefficients were computed using BMD as dependent variable and ß-blocker use in years as independent variable. In subsequent models, we

additionally adjusted for age, gender and duration of follow-up. Other potential confounders, such as risk factors for cardiovascular disease and osteoporosis were included in the model if they were biologically plausible and/or caused a change in the point estimate of more than 10% when included in the model.

We performed ANCOVA to compute crude and adjusted means of BMD and means of rates of loss for different duration categories of ß-blocker use. The category reflecting no use of ß-blockers was used as the reference category for significance tests. To assess the impact of duration of misclassification, the analyses were repeated after excluding all subjects who reported ß-blocker use at baseline in order to restrict the analysis to incident users. In addition, we conducted an analysis in which we excluded all participants who did not use ß-blockers during follow-up and investigated mean BMD per cardiovascular disease risk category, to examine confounding by indication.

We calculated risks for nonvertebral fractures with a Cox proportional hazards model[21] with the exposure represented by time-dependent covariates. Use of other medication, such as thiazide and statin use, was also represented by time-varying variables. Vertebral fracture analyses were performed using logistic regression. Multiple imputation was used to impute missing information for confounding variables in the fracture analyses.[22]

Analyses were performed with SAS (Statistical Analysis System version 8, Cary, NC) and with SPSS 11.5 for Windows (SPSS Inc., Chicago, IL).

Results

In our study, we included a total of 3009 participants (1287 men and 1722 women), for whom femoral neck BMD measurements at the third visit were available. For 2713 and 2740 of these persons, also data on baseline BMD and bone structure, respectively, were available. The mean follow-up was 2787 days. During the study, 991 participants used a ß-blocker of whom 386 were past users at the time of the third visit. The median duration of ß-blocker use was 936 days. For fracture analyses, 7892 participants were alive and at risk for fracture at start of follow-up (July 1, 1991). During the study, 1218 participants had at least one nonvertebral fracture and 521 participants a frailty fracture. The first frailty fracture was in 338 cases a hip fracture, in 127 cases an upper humerus fracture, and in 56 cases a pelvis fracture. Furthermore, there were in total 315 wrist fractures and 352 hip fractures.

ß-blocker users were on average older than non-users and had a higher baseline prevalence of cardiovascular diseases (e.g. hypertension, history of myocardial infarction, diabetes mellitus) and had a higher BMD and body mass index at baseline (Table 1).

Any use of ß-blockers during follow-up was associated with a significantly higher BMD at the third examination visit; non-users had a mean BMD of 0.834 g/cm^2 (CI 95% 0.828–0.840) and ß-blocker users had a mean BMD of 0.855 g/cm^2 (0.846–0.864). In a linear regression analysis, we noticed a significant association between BMD of the femoral neck and increasing duration of ß-blocker use. Per year increase in ß-blocker use, BMD of femoral neck increased 0.005 g/cm^2 ($p < 0.001$) as estimated by a univariate linear regression analysis (Table 2). After adjustment for age, gender and duration of follow-up, the positive association remained significant. Since use of ß-blockers was associated

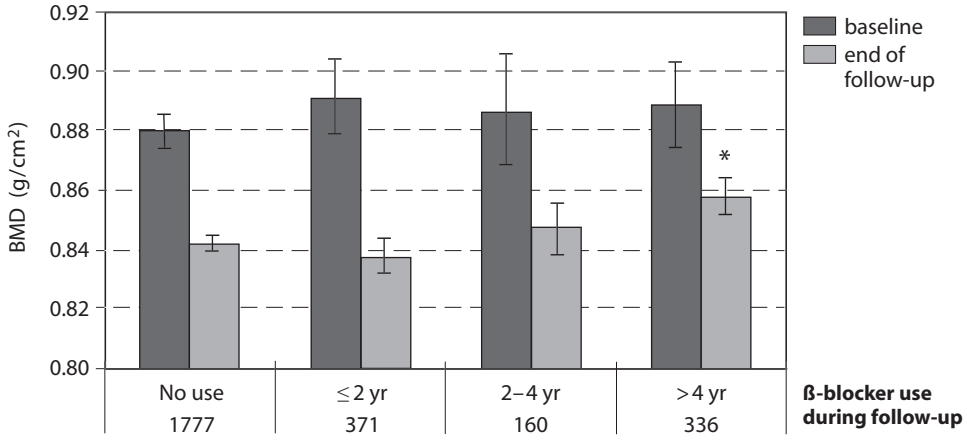
Table 1 — Baseline characteristics for ß-blocker users and non-users.

	ß-blocker users during the study	Non-users	p
Number	991 (33%)	2018 (67%)	
Number of days of follow-up	2780 (272)	2792 (277)	
Age (yr)	66.6 (6.4)	65.8 (6.8)	**
Gender (women)	564 (56.9%)	1158 (57.4%)	
Previous fracture in 5 yr before baseline	155 (16.1%)	269 (13.8%)	
Femoral neck BMD (g/cm^2)	0.90 (0.14)	0.88 (0.14)	**
Use of a walking aid	36 (3.8%)	47 (2.4%)	*
Lower limb disability	44 (4.5%)	75 (3.8%)	
Body mass index (kg/m^2)	27.0 (3.6)	26.0 (3.4)	**
MMSE	28.1 (1.5)	28.1 (1.5)	
Current smoking	167 (17.1%)	471 (23.7%)	**
Falling	10 (0.9%)	20 (0.8%)	
Diabetes mellitus	78 (7.9%)	121 (6.0%)	
History of myocardial infarction	106 (10.8%)	42 (2.1%)	**
Framingham 5-yr CVD risk	0.14 (0.09)	0.12 (0.08)	**
Hypertension	525 (54.3%)	311 (15.8%)	**
Use of a ß-blocker	416 (42.0%)	13 (0.6%)	**
Use of hormone replacement therapy	18 (1.8%)	40 (2.0%)	
Use of a serum lipid-lowering agent#	51 (5.2%)	33 (1.6%)	**
Use of diuretics#	174 (17.6%)	127 (6.3%)	**

Values are means with standard deviations or numbers with percentages. Some variables have missing values.

* = $p < 0.05$; ** = $p < 0.001$; # = data from baseline interviews, not further specified.

Figure 2 — Means of baseline femoral neck BMD (95% CI) and means of BMD at the end of the follow-up, for categories of exposure to ß-blockers.



Adjustments were made for age, gender, duration of follow-up, body mass index, hypertension, 5-yr cardiovascular disease risk, use of statins and thiazide diuretics, and (for follow-up BMD) baseline BMD.
Numbers under the bars reflect the number of subjects per category.
* $p < 0.05$ in comparisons between no use and ß-blocker use categories.

with use of statins and thiazide diuretics (odds ratio (OR) 3.6; 3.0–4.4 and OR 3.9; 3.3–4.8, respectively), and the use of statins and thiazides is also associated with an increased BMD,[23–25] we adjusted for the cumulative number of days of statin and thiazide use. Furthermore, adjustments for body mass index (for structure analyses; height and weight), hypertension, baseline BMD and 5-yr cardiovascular disease risk were made in which therewas a significant increase in BMD of 0.002 g/cm^2 per year of ß-blocker use. Additional adjustment for diabetes mellitus, smoking, cognitive impairment, lower limb disability, use of a walking aid, use of inhalation ß-mimetics, and glucocorticoids or hormone replacement therapy, did not alter the estimates. Exclusion of participants who already reported use of ß-blockers at the baseline interview (n = 429) increased the strength of the association (p = 0.001) (Table 2). After categorization of the duration of use of ß-blockers a higher femoral neck BMD was again observed with increasing duration of exposure to ß-blockers (Figure 2). Femoral neck BMD in long-term (> 4 years) ß-blocker users (BMD = 0.857 g/cm^2) was significantly higher than mean BMD of non-users (0.841 g/cm^2) and there was a significant trend of a higher mean BMD with increasing duration of ß-blocker use (p < 0.001).

Table 2 — Change in bone mineral density (g/cm^2) per year of ß-blocker use.

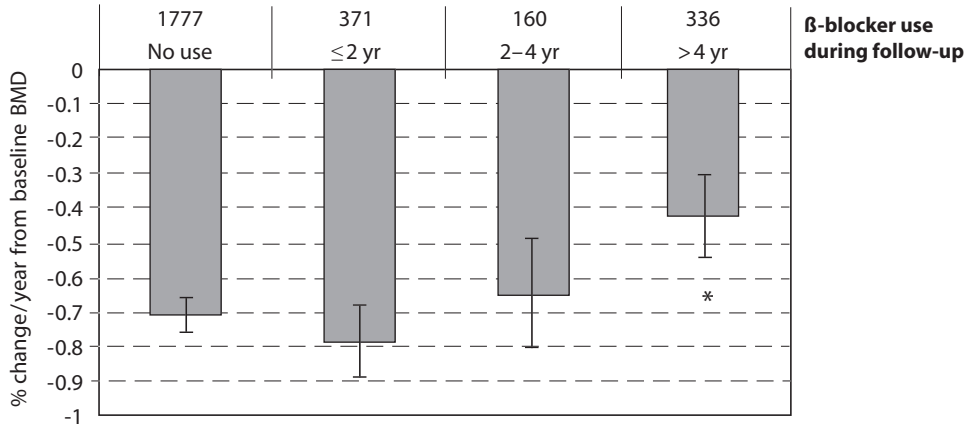
	Crude		*Model 1*		*Model 2*	
	g/cm^2 (95% CI)	p-value	g/cm^2 (95% CI)	p-value	g/cm^2 (95% CI)	p-value
All participants						
Femoral neck BMD	0.005 (0.003 ; 0.008)	< 0.001	0.006 (0.004 ; 0.008)	< 0.001	0.002 (0.001 ; 0.004)	< 0.001
Exclusion of baseline users						
Femoral neck BMD	0.006 (0.001 ; 0.011)	0.013	0.006 (0.02 ; 0.011)	0.005	0.004 (0.001 ; 0.006)	0.001

Model 1 is adjusted for age, gender and duration of follow-up.
Model 2 is adjusted for age, gender, duration of follow-up, hypertension, 5-yr cardiovascular disease risk, baseline BMD, body mass index, statin and thiazide use.

Current (0.85; 0.84–0.86) and recent ß-blocker use (0.86; 0.85–0.87) was associated with a higher BMD than no use or past use (0.84; 0.83–0.84). We examined the association between cardiovascular disease risk and BMD, after excluding all ß-blocker users to examine confounding by indication. Subjects with a baseline cardiovascular disease above the median risk did not have a higher mean BMD than subjects with a risk below the median (Data not shown).

Stratifying on gender resulted in significant positive trends for mean BMD and increasing duration of use for both men (p = 0.006) and women (p = 0.004). When we stratified on mean dose during the study period (DDD ≤ 0.6 and DDD > 0.6) and analyzed dose by means of an interaction term for dose and duration in the model, there was no statistically significant difference between high and low dose. We did not find differences in BMD for users of cardioselective (one third of all users) and non-selective ß-blockers (Data not shown). No association was found between ACE-inhibitors and BMD (Data not shown).

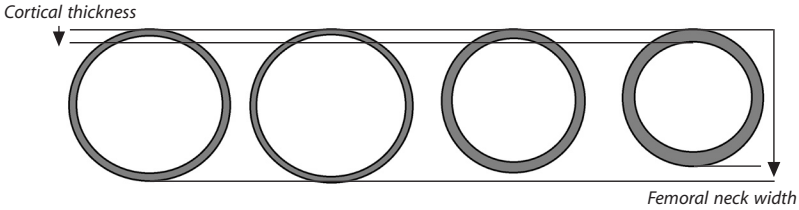
Figure 3 — Means of percentage change per year in BMD (95% CI) with duration of ß-blocker use.



Adjustments were made for age, gender, duration of follow-up, body mass index, hypertension, 5-yr cardiovascular disease risk, use of statins and thiazide diuretics, and baseline BMD.
Numbers above the bars reflect the number of subjects per category.
* $p < 0.05$ in comparisons between no use and ß-blocker use categories.

ß-blocker users had significantly lower rates of bone loss, compared with non-users (Figure 3). Long-term users had a mean loss of 0.43% per year and non-users lost approximately 0.71% per year. The trend for mean rate of loss and duration of use of ß-blockers was significant ($p < 0.001$).

Figure 4 — Hip structural analysis parameters categorized by duration of ß-blocker use.



	Non-users	≤2 years	2–4 years	>4 years	
Cortical thickness (mm)	1.38	↓1.4%	0.0%	↑1.4%	*
Femoral neck width (cm)	3.14	↑0.3%	↓1.0%	↓1.7%	*
Endosteal diameter (cm)	2.87	↑0.3%	↓1.0%	↓1.6%	*
Section modulus (cm^3)	1.18	↓1.7%	↑1.7%	0.0%	
Buckling ratio	13.14	↑0.7%	↓2.1%	↓4.2%	*

The figure represents a caricature of the femoral neck cross sections (not to scale).

Adjustments were made for age, gender, duration of follow-up, height, weight, hypertension, 5-yr cardiovascular disease risk, use of statins and thiazide diuretics.
* $p < 0.05$ in comparison between non-users and ß-blocker users for > 4 years.

Hip structural analyses showed a significant 1.5% thicker cortex, a 1.7% smaller endosteal diameter and a 1.6% smaller femoral neck width after >4 years of ß-blocker use in comparison to non-users (Figure 4). The buckling ratio (reflecting bone stability) was significantly lower for long-term users (4.2%) than for non-users. There was no significant difference in femoral neck strength as represented by the section modulus.

We categorized duration of ß-blocker use in categories reflecting no use, short-term use (1 day to ≤ 2 years) and long-term use (>2 years of continuous use). ß-blocker use was not associated with risk for all nonvertebral fractures (hazard ratio (HR) 1.05; 0.84–1.30 for short-term use and HR 0.93; 0.75–1.14 for long-term use). However, frailty fracture risk was significantly decreased for long-term ß-blocker users (HR 0.67; 0.46–0.97) (Table 3). Hip fracture risk was decreased for long-term use, but this decrease did not reach statistical significance (HR 0.73; 0.47–1.14). Wrist fracture risk was, although not significantly, higher for ß-blocker users. Risk for vertebral fracture was lower, but did not reach statistical significance (OR 0.83; 0.49–1.41). Stratification on gender did not essentially affect the risk estimates. Adjusting for baseline BMD did not change the risk estimates essentially. When we analyzed cumulative dose instead of the number of days of ß-blocker use, as the exposure definition, risk estimates remained similar. Use of ACE-inhibitors was not significantly associated with risk of fracture.

Table 3 — Risk of fracture with (duration of) ß-blocker use.

	ß-blocker use	*Hazard / Odds ratio**	*Confidence intervals*
Vertebral fracture	No use	1	Reference
(166 cases)	Short-term use	1.46	0.95–2.26
	Long-term use	0.83	0.49–1.41
Nonvertebral fracture	No use	1	Reference
(1218 cases)	Short-term use	1.05	0.84–1.30
	Long-term use	0.93	0.75–1.14
Frailty fracture	No use	1	Reference
(521 cases)	Short-term use	1.26	0.91–1.74
	Long-term use	0.67	0.46–0.97
Wrist fracture	No use	1	Reference
(315 cases)	Short-term use	1.19	0.78–1.80
	Long-term use	1.27	0.87–1.84
Hip fracture	No use	1	Reference
(352 cases)	Short-term use	1.12	0.74–1.69
	Long-term use	0.76	0.49–1.18

Adjustments were made for age, gender, duraction of follow-up, body mass index, hypertension, 5-yr cardiovascular disease risk, use of statins and thiazide diuretics, falling, and previous (non)vertebral fracture.
Short-term use is ß-blocker use for ≤ 2 years.
Long-term use is ß-blocker use for > 2 years.
* Odds ratio for vertebral fracture analyses, Hazard ratio for nonvertebral fracture analyses.

Discussion

In this study we showed a significant association between long-term use of ß-blockers and a higher BMD of the femoral neck and lower rates of bone loss. The buckling ratio was lower for long-term users indicating a higher stability of the femoral neck. Risk of all non-vertebral fractures was not different for users and non-users, but risk of frailty fractures was significantly decreased for ß-blocker users.

BMD of past users was not different from that of non-users, so the effect of ß-blockers may not be permanent. On the other hand, most past users in our study did not have a long exposure to ß-blockers prior to discontinuation, so lower BMD for past users may be a reflection of short-term use. Further division of past users in duration categories of prior use was not possible due to low numbers. Both differences in rates of bone loss and mean BMD were independent of baseline BMD, as we adjusted these analyses for baseline BMD. We did not find a dose-effect relation, but the power to detect effect modification by daily dose was small due to low number of participants per duration-group.

The analyses on bone structure could provide some insight on the way ß-blockers might influence BMD. With normal ageing, thinning of the cortices takes place and because of that loss of BMD occurs. Endocortical expansion can compensate for cortical thinning due to its biomechanical effects on bone. A femoral neck with a thinner cortex but with a larger diameter can be equally strong as a smaller bone with a thicker cortex. Mechanical stimuli are believed to influence bone formation and resorption so that strains on bone remain between an upper and a lower set-point. This theory is described as "mechanostat".[26] ß-blocker users in our study had thicker cortices, smaller endosteal diameters and smaller femoral neck widths. It is possible that, like estrogens,[8] ß-blockers influenced the lower set-point in a way that more bone is conserved than mechanically required. This reduces the sensitivity to disuse of bone, i.e. the cortices do not thin over time and endocortical expansion does not occur because there is no increase in strain that needs to be compensated. However, another possibility is that the primary action of ß-blockers is inhibition of bone expansion necessitating endosteal apposition (or inhibition of endosteal resorption) to keep the strains in bone between the set-points. Inhibition of expansion is also previously shown to be associated with serum estradiol level.[27,28] Whether ß-blockers act as bone-forming agents or bone resorption inhibitors cannot be discriminated from these data. Long-term ß-blocker users also lost bone, although less than non-users. However, it may be that age-related bone loss dominates over the potential bone-forming capacities of ß-blockers and still a netto bone loss is observed. The buckling ratio, a parameter thought to predict fracture risk, was lower for ß-blocker users suggesting less cortical instability and consequent bone fragility. Section modulus is another predictor of fracture risk and gives information on the bending strength of bone, and was not different among users and non-users, probably reflecting its compensatory adaptive nature; bending strength is kept stable until a threshold is reached. Apparently, this threshold was not reached in our population.

The fact that nonvertebral fracture risk was not reduced for ß-blocker users was mainly due to a higher risk for wrist fractures for users. Risk of fracture is also influenced by exter-

nal factors, such as risk of falling. ß-blockers may increase the risk of falling due to hypotension and potential beneficial effects of the drugs on bone may be compensated by the increased risk of falling.

ß-blocker use was ascertained from pharmacy records and therefore not subject to recall bias. Participants had to collect the medications at the pharmacy in order to be classified as exposed; therefore our exposure data is potentially more accurate than self-reported ß-blocker use[29] or exposure data generated from general practitioner prescriptions.[30] These detailed pharmacy dispensing data also allowed us to study total duration of ß-blocker use and mean dose over time. To limit misclassification of duration of exposure, we excluded in a separate analysis all baseline users, who apparently used ß-blockers when the study started. This did not essentially change the results. Since there may be a link between cardiovascular disease and osteoporosis,[31,32] we adjusted for a number of cardiovascular disease risk factors, to deal with confounding. This might occur when people with cardiovascular disease are at lower risk for osteoporosis. The disease itself, but not the therapy, could then potentially be the explanation for a higher BMD and/or reduced fracture risk. In order to examine confounding by indication, we examined the mean BMD for quartiles of 5-yr cardiovascular disease risk in persons without the therapy. An increasing disease risk (indication for ß-blockers) was not associated with BMD. Furthermore, we did not observe an association between use of ACE-inhibitors, and adjustment for ß-mimetic or steroid use did not alter the estimates. This makes it unlikely that confounding by indication or contra-indication played a role.

Only one previously published study in humans examined the hypothesis that use of ß-blocking agents can influence BMD.[29] In that case-control study among 1344 women a 2.5% increase in total hip BMD and a non-significant increase in lumbar spine BMD was found for self-reported ß-blocker users. In contrast to our study, no information on duration or dose was available, nor was change in BMD or bone structure measured. Pasco et al. also found a reduced fracture risk, as did a recent study in a general practioner database.[30] Over the last few years, evidence is accumulating that bone metabolism is under ß-adrenergic control. Osteoblasts and osteoclasts were shown to have ß-adrenergic receptors,[3] that may effectuate leptin effects on bone.[1,33] Inactivation of the sympathetic nervous system impairs bone resorption by inhibiting preosteoclast differentiation.[6] Propranolol, a ß-blocker, increased bone mass in mice.[1] In contrast with the above-mentioned studies, there is also evidence for an anabolic influence of the sympathetic nervous system on bone,[34,35] so its exact role in bone metabolism needs to be examined further.

The present study supports the hypothesis that the ß-adrenergic system is involved in bone metabolism and that use of ß-blockers can prevent bone loss and lead to a lower risk of fracture. Furthermore, hip structural analysis showed that ß-blockers, like estrogens, might inhibit bone expansion and thereby increase endosteal bone apposition or inhibit endosteal bone resorption. The association between ß-blocker use and BMD and fractures, needs to be further investigated with randomized trials before we can recommend ß-blocker therapy as prevention or therapy of osteoporosis.

References

1. **Takeda S, Elefteriou F, Levasseur R, et al.** Leptin regulates bone formation via the sympathetic nervous system. Cell 2002; 111 (3): 305-17.
2. **Sisask G, Bjurholm A, Ahmed M, Kreicbergs A.** The development of autonomic innervation in bone and joints of the rat. Journal of the Autonomic Nervous System 1996; 59 (1-2): 27-33.
3. **Togari A.** Adrenergic regulation of bone metabolism: possible involvement of sympathetic innervation of osteoblastic and osteoclastic cells. Microsc Res Tech 2002; 58 (2): 77-84.
4. **Hohmann EL, Levine L, Tashjian AH, Jr.** Vasoactive intestinal peptide stimulates bone resorption via a cyclic adenosine 3',5'-monophosphate-dependent mechanism. Endocrinology 1983; 112 (4): 1233-9.
5. **Lerner UH, Lundberg P, Ransjo M, Persson P, Hakanson R.** Helodermin, helospectin, and PACAP stimulate cyclic AMP formation in intact bone, isolated osteoblasts, and osteoblastic cell lines. Calcified Tissue International 1994; 54 (4): 284-9.
6. **Cherruau M, Facchinetti P, Baroukh B, Saffar JL.** Chemical sympathectomy impairs bone resorption in rats: a role for the sympathetic system on bone metabolism. Bone 1999; 25 (5): 545-51.
7. **Minkowitz B, Boskey AL, Lane JM, Pearlman HS, Vigorita VJ.** Effects of propranolol on bone metabolism in the rat. J Orthop Res 1991; 9 (6): 869-75.
8. **Beck TJ, Stone KL, Oreskovic TL, et al.** Effects of current and discontinued estrogen replacement therapy on hip structural geometry: the study of osteoporotic fractures. J Bone Miner Res 2001; 16 (11): 2103-10.
9. **Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA.** Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol 1991; 7 (4): 403-22.
10. **Burger H, de Laet CE, van Daele PL, et al.** Risk factors for increased bone loss in an elderly population: the Rotterdam Study. Am J Epidemiol 1998; 147 (9): 871-9.
11. **Rivadeneira F, Houwing-Duistermaat JJ, Beck TJ, et al.** The influence of an insulin-like growth factor I gene promoter polymorphism on hip bone geometry and the risk of nonvertebral fracture in the elderly: the Rotterdam Study. J Bone Miner Res 2004; 19 (8): 1280-90.
12. **Beck TJ, Looker AC, Ruff CB, Sievanen H, Wahner HW.** Structural trends in the aging femoral neck and proximal shaft: analysis of the Third National Health and Nutrition Examination Survey dual-energy X-ray absorptiometry data. J Bone Miner Res 2000; 15 (12): 2297-304.
13. **Beck TJ, Ruff CB, Warden KE, Scott WW, Jr., Rao GU.** Predicting femoral neck strength from bone mineral data. A structural approach. Invest Radiol 1990; 25 (1): 6-18.
14. **WHO.** International Statistical Classification of Diseases and Related Health Problems, 10th revision. In. Geneva; 1992.
15. **Van der Klift M, De Laet CE, McCloskey EV, Hofman A, Pols HA.** The incidence of vertebral fractures in men and women: the Rotterdam Study. J Bone Miner Res 2002; 17 (6): 1051-6.
16. **WHO.** Anatomical Therapeutical Chemical (ATC) Classification Index. In. Oslo: World Health Organization Collaborating Centre for Drug Statistics Methodology; 1993.
17. **WHO.** Anatomical Therapeutic Classification (ATC) Index: Including Defined Daily Doses (DDD) for Plain Substances. In. Oslo; 1992.
18. **Pincus T, Summey JA, Soraci SA, Jr., Wallston KA, Hummon NP.** Assessment of patient satisfaction in activities of daily living using a modified Stanford Health Assessment Questionnaire. Arthritis Rheum 1983; 26 (11): 1346-53.
19. **Folstein MF, Folstein SE, McHugh PR.** "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975; 12 (3): 189-98.
20. **Anderson KM, Odell PM, Wilson PW, Kannel WB.** Cardiovascular disease risk profiles. Am Heart J 1991; 121 (1 Pt 2): 293-8.
21. **Cox D.** Regression models and life tables. J Royal Stat Society 1972: 187-220.
22. **Twisk J, de Vente W.** Attrition in longitudinal studies. How to deal with missing data. J Clin Epidemiol 2002; 55 (4): 329-37.
23. **Sigurdsson G, Franzson L.** Increased bone mineral density in a population-based group of 70-year-old women on thiazide diuretics, independent of parathyroid hormone levels. J Intern Med 2001; 250 (1): 51-6.

24. **Funkhouser HL, Adera T, Adler RA.** Effect of HMG-CoA reductase inhibitors (statins) on bone mineral density. J Clin Densitom 2002; 5 (2): 151-8.
25. **Montagnani A, Gonnelli S, Cepollaro C, et al.** Effect of simvastatin treatment on bone mineral density and bone turnover in hypercholesterolemic postmenopausal women: a 1-year longitudinal study. Bone 2003; 32 (4): 427-33.
26. **Frost HM.** The mechanostat: a proposed pathogenic mechanism of osteoporoses and the bone mass effects of mechanical and nonmechanical agents. Bone Miner 1987; 2 (2): 73-85.
27. **Ahlborg HG, Johnell O, Turner CH, Rannevik G, Karlsson MK.** Bone Loss and Bone Size after Menopause. N Engl J Med 2003; 349 (4): 327-34.
28. **Duan Y, Beck TJ, Wang XF, Seeman E.** Structural and biomechanical basis of sexual dimorphism in femoral neck fragility has its origins in growth and aging. J Bone Miner Res 2003; 18 (10): 1766-74.
29. **Pasco JA, Henry MJ, Sanders KM, Kotowicz MA, Seeman E, Nicholson GC.** Beta-adrenergic blockers reduce the risk of fracture partly by increasing bone mineral density: Geelong Osteoporosis Study. J Bone Miner Res 2004; 19 (1): 19-24.
30. **Schlienger RG, Kraenzlin ME, Jick SS, Meier CR.** Use of beta-blockers and risk of fractures. Jama 2004; 292 (11): 1326-32.
31. **Burnett JR, Vasikaran SD.** Cardiovascular disease and osteoporosis: is there a link between lipids and bone? Ann Clin Biochem 2002; 39 (Pt 3): 203-10.
32. **Samelson EJ, Kiel DP, Broe KE, et al.** Metacarpal cortical area and risk of coronary heart disease: the Framingham Study. Am J Epidemiol 2004; 159 (6): 589-95.
33. **Elefteriou F, Takeda S, Ebihara K, et al.** Serum leptin level is a regulator of bone mass. Proc Natl Acad Sci U S A 2004; 101 (9): 3258-63.
34. **Sandhu HS, Herskovits MS, Singh IJ.** Effect of surgical sympathectomy on bone remodeling at rat incisor and molar root sockets. Anat Rec 1987; 219 (1): 32-8.
35. **Hill EL, Turner R, Elde R.** Effects of neonatal sympathectomy and capsaicin treatment on bone remodeling in rats. Neuroscience 1991; 44 (3): 747-55

Chapter 6

General Discussion

Since the incidence of osteoporosis increases with age, and fractures – as direct consequence of osteoporosis – cause important morbidity and mortality, preventive and therapeutic strategies will become increasingly important due to ageing of the population. Cardiovascular disease incidence also increases in the ageing population and as a result cardiovascular drugs are commonly used in the elderly. If cardiovascular drugs also have effects on bone, insight into the character and quantity of these effects might play a role in cardiovascular treatment, since many elderly are at risk for both cardiovascular disease and osteoporosis. Furthermore, research on the association between the use of these drugs and bone outcomes, such as fracture risk and bone mineral density, may answer some of the questions on the potential role of these medicines in the prevention and treatment of osteoporosis.

The work presented in this thesis aims at gaining insight into the effects of cardiovascular drugs on bone in a population of elderly men and women. Three different drug groups were chosen based upon their pharmacological effect on bone: i.e. thiazide diuretics, statins and ß-blockers. The shortcomings and merits of the individual studies that were presented in this thesis have been discussed in the previous chapters. In this chapter, the main findings are summarized and discussed in the broader context of clinical practice. Finally, recommendations for future research are given.

Main findings

Thiazide diuretics

Thiazide diuretics, prescribed for treatment of hypertension, are cheap and have relatively few adverse effects.[1] Recently, a randomized trial showed that thiazide-type diuretics are more effective in preventing major forms of cardiovascular disease than angiotensin-converting enzyme (ACE) inhibitors or calcium channel blockers.[2] Thiazides are therefore excellent first choice antihypertensive agents for the general population; however, the prescription rate of thiazides has decreased over the past decade.[3] The study described in Chapter 2, together with results from other studies,[4–9] support the hypothesis that thiazide use leads to a lower risk of (hip) fracture.

The gold standard in the research on drug effects is the randomized controlled clinical trial. Risk estimates obtained in clinical trials will be unbiased by baseline prognosis and confounding by indication will be absent.[10] However, randomized trials are very expensive and pharmaceutical companies have no commercial interest in such studies because patents of thiazides have expired. Therefore, observational studies on thiazide effects on bone and fractures fill an important niche.

The risk reduction found in our study was substantial. We found an approximately 50% lower risk of hip fracture for long-term thiazide users. Analysis of the amount of overlap of thiazide use with use of the other two cardiovascular drugs which might influence fracture risk, reveals that approximately 44% and 12% of the thiazide users was concurrently using ß-blockers or statins, respectively. Although the overlap was quite substantial

and might be of influence on the risk estimate, additional analyses in which we also adjusted for use of ß-blockers and statins showed approximately the same risk estimates. We showed that the risk reducing effect of thiazides on hip fracture disappears relatively quick after discontinuation of thiazide use. Therefore, it is too early to recommend thiazides for the prevention or treatment of osteoporosis. However, when making a choice out of the available drugs to treat hypertension, thiazide diuretics should be considered, especially in persons at risk for osteoporosis.

Statins

The HMG CoA reductase inhibiting statins are prescribed to treat hypercholesterolemia. Since they were introduced on the market in the late 1980's, their prescription rate increased sharply and approximately 90% of the patients who are treated for hypercholesterolemia in the Netherlands currently uses a statin.[11] Statins inhibit an early rate-limiting step in the mevalonate pathway of the cholesterol biosynthesis.[12] Bisphosphonates, which are bone resorption inhibitors, also inhibit the mevalonate pathway a few steps more downstream.[13] Apart from potential inhibition of resorption, statins may enhance bone formation. Statins were shown to stimulate expression of bone morphogenetic protein-2 (BMP-2).[14–16] BMP-2 is a protein that is involved in osteoblast differentiation and function, and since osteoblasts are bone-forming cells, statins may also increase bone formation. Therefore an association between statins and BMD is plausible. Indeed, this association was firstly demonstrated in 1999 in bone cells.[14] We showed that users of statins have a higher mean BMD and lower rates of bone loss than persons who do not use statins and this association is duration-dependent. Long-term statin use is also associated with a lower risk of both nonvertebral and vertebral fractures.

In contrast with randomized trials, confounding by indication should always be considered in pharmaco-epidemiologic studies. We tried to investigate this phenomenon in several ways. First, we adjusted for the indication of statins, cholesterol levels and cardiovascular disease risk and compared the effects of statins with the effects of drugs prescribed for the same indication, such as nicotinic acids and fibrates. Furthermore, we investigated the association between cholesterol levels and BMD in persons without lipid-lowering drugs during follow-up. The results of all these analyses suggested that confounding by indication is very unlikely. Recently, a *healthy user effect* was proposed as an explanation for the association between statin use and osteoporosis. People who pay more attention to their health are supposed to use statins more often. In the Netherlands, however, physicians and in particular general practitioners, are encouraged to prescribe statins according to national guidelines. Although we cannot exclude that some participants did not fulfill these criteria, it is unlikely that a large number of healthy patients used statins. As a result, a substantial healthy user effect is unlikely.

The exact mechanism by which statins could act on bone is not completely understood. In our study, statin use was not associated with an individually increasing BMD during follow-up. It may be that statins have bone-forming capacities, but that age-related bone loss dominates over bone formation still resulting in a netto-effect of bone loss. Hip structural analyses comparing statin users and non-users showed thicker cortices and – not significantly – smaller endosteal diameters and wider femoral necks. The question

is whether this is due to endosteal bone apposition, less endosteal resorption, periosteal apposition, or a combination. The assessment of structural changes over time might answer this question but, unfortunately, we were not able to study this. Comparison of effects of statins on hip structural geometry with effects of bisphosphonates might also be helpful in assessing whether statins inhibit bone resorption or stimulate bone formation. Bisphosphonates are established bone resorption inhibitors and when statins have similar effects on bone structure as bisphosphonates, this would point to a bone resorption inhibition for statins as well. As far as we know, only one published report, an abstract, reported on hip structural changes in people that were treated with bisphosphonates.[17] In that study less loss of cortical thickness was observed for post-menopausal women using bisphosphonates for 3 years in comparison with women using a placebo. No data on femoral neck width were reported in that study. However, women on alendronate had practically no change in cross-sectional area over the follow-up period, but their cortices were 1.6% thinner compared to the begin of the study. This could be an indication of bone expansion, since the cross-sectional area is determined by both the diameter and the thickness of the cortex. Bone expansion is a normal compensation for thinning of cortices over time that occurs with aging; we assume therefore that non-users had increased in femoral neck width during the follow-up. The femoral neck width of statin users in our study was not significantly different from non-users and this suggests that at least some bone expansion over time occurred also in statin users. Statin users seem to have changes in structure of their hip bones that is similar with bisphosphonates, apart from an additional increase in cross-sectional area that was not observed in bisphosphonate users.

Recently, two single nucleotide polymorphisms (SNPs) in the gene encoding BMP-2 were found. The first one is the SNP in codon 37 in which transversion of thymine (leading to the amino acid serine) for guanine (leading to alanin) occurs. The second one in codon 189, is transition of thymine for adenine that leads to the amino acid arginine instead of serine. Since stimulation of expression of BMP-2 is thought to be (one of) the underlying mechanisms of action of statins on bone, we hypothesized that changes in the gene encoding BMP-2 might interact with this association. Preliminary analyses in the population of the Rotterdam Study showed no association between bone and statin use for carriers of the Arg189Ser SNP. In contrast, a significant protective association was observed for non-carriers that was stronger than the association we found in the whole cohort. This indicates effect modification. At the moment, little has been published on these polymorphisms and therefore we do not know whether their allele frequency differs between races or ethnicities. Since a minority (one third) was carrier in our population, it could be that other populations have fewer carriers and would benefit less from statin use. This could explain why some studies did not observe an association between statin use and bone.

All studies on statins and bone in this thesis suggest a beneficial effect. Causality, however, cannot be proven in observational studies. Randomized trials could provide more evidence, but must be properly conducted and include people at risk for osteoporosis and fractures. The re-analyses of two clinical trials designed to investigate the relation between statins and cardiovascular disease did not show significant associations between

statin use and fracture risk.[18,19] However, less than 20% of the participants were women and the participants were relatively young. Fracture incidence was therefore low and also the power to detect possible associations. Together with meta-analyses of observational studies that had reliable data on statin exposure and confounders, randomized clinical trials investigating statin effects on bone should be carried out to add more evidence for a causal relation between statins and osteoporosis. At the moment, we therefore cannot recommend statins for the indication of osteoporosis until such trials have been performed. Since the currently available statins are all designed to act in the liver, and not on bone, newer statins should be developed with a higher affinity for bone. Transdermal application of statins, instead of ingesting pills, might bypass the liver and lead to higher concentrations in bone. In that way, statins would probably have stronger effects on bone and might even be used to treat osteoporosis. However, when statins would only be beneficial to bone in non-carriers of the previously mentioned polymorphism, a minority of the population would profit from statin use.

Apolipoprotein E polymorphism

A recently discovered pathway involved in bone metabolism is that of apolipoprotein E (ApoE). ApoE is a protein of which three genetically determined isoforms exist, the so-called E*2, E*3 and E*4 isoform. It is assumed that, among others, ApoE mediates vitamin K transport which in turn influences bone turnover.[20,21] In some studies the hypothesis was brought forward that low density lipoprotein (LDL) levels are increased in subjects with a polymorphism in exon 4 of the gene encoding ApoE, namely the E*4 allele, and that accumulation of oxidized lipids in the subendothelial space of bone may lead to inhibition of osteoblast differentiation.[22] At variance with some other studies,[23–28] we did not observe an association between the ApoE polymorphism and osteoporosis. There are other polymorphisms in the gene encoding ApoE that we did not investigate. These may affect the transcription rate and absolute levels of ApoE. Meta-analyses of large datasets are needed to further investigate all polymorphisms in the ApoE gene.

The study described in this thesis was the largest population-based study on the association between the ApoE polymorphism and osteoporosis carried out so far. Although we did not observe an association, we do not exclude the possibility that for certain subgroups this polymorphism might be associated with osteoporosis. Recently, we observed a significant association between the use of statins and presence of the E*4 allele; carriers were more often using statins (16%) than non-carriers (13%, $p = 0.01$). This is biologically plausible, since subjects carrying the E*4 allele have higher LDL levels which can be treated with statins. Statins are often used in combination with other cardiovascular drugs and consequently we found that 37% of the carriers used a ß-blocker, compared with 35% of non-carriers ($p = 0.06$). In contrast, non-carriers used more (29%) thiazides than carriers (26%, $p = 0.04$). Consequently, a potentially detrimental effect of the E*4 allele could be masked by more frequent use among carriers of cardiovascular medicines which are beneficial to bone. Additional analyses in which we adjusted for baseline use of serum lipid-lowering drugs, diuretics and ß-blockers, showed a borderline significant association between the presence of the E*4 allele and BMD (BMD of non-carriers: 0.888 (95% CI 0.882–0.894), BMD of carriers: 0.877 (95% CI 0.868–0.887), p-value = 0.06). However, rates

of bone loss were similar for carriers and non-carriers. Finally, fracture risk appears to be similar between carriers and non-carriers after adjustments for drug use (hazard ratio 1.05; 95% CI 0.91–1.22). Therefore it seems reasonable to assume that major confounding by use of these drugs is absent in our population. Nevertheless, we recommend adjusting for cardiovascular drug use in future observational research regarding effects of ApoE*4 allele.

ß-blockers

The most widely used cardiovascular drugs in the Rotterdam Study were ß-blockers. The effects of ß-blockers on bone are supposed to result from blocking of the leptin signaling pathway and, by that, preventing the negative effects of leptin on bone.[29] We showed that use of ß-blockers is associated with a higher mean BMD and lower rates of bone loss. A duration-dependent relation was observed, i.e. the association became stronger with a longer duration of ß-blocker use resulting in a 2% higher BMD and a 40% lower rate of loss for persons who used ß-blockers for more than 4 years. The risk of a vertebral fracture was lower among ß-blocker users, but was not statistically significant. There was a significant association between a lower risk of fractures that typically occur at older age (upper humerus, hip and pelvis fractures) and use of ß-blockers. However, we did not find an association with risk of all nonvertebral fractures. We hypothesized that this may be due to an increased risk of falling that may compensate beneficial effects of the medicine in younger people.

Hip structural analyses showed differences in cortical thickness, endosteal diameter and femoral neck width between users and non-users. Thicker cortices and smaller endosteal diameters could indicate either less bone resorption or endosteal apposition. The effects of ß-blockers and statins on bone structure appear not to be completely similar. ß-blocker users had a significantly smaller femoral neck width in contrast with statin users. Since statin users had thicker cortices (and still bone expansion) it is not likely that cortices of ß-blocker users were so thick that strains, induced by mechanical stimuli, did not reach the threshold for stimulation of bone formation. A potential explanation for the observed structure in ß-blocker users is therefore inhibition of bone expansion with compensatory inhibition of endosteal resorption or stimulation of endosteal apposition. Inhibition of bone expansion is previously associated with serum estradiol levels.[30,31] It might be that ß-blockers have a similar effect on bone structure as estrogen.

Because we did not find an association between occurrence of all fractures and the use of ß-blockers, it is questionable whether ß-blockers can be used as anti-osteoporotic agents. Although we observed a significantly higher BMD and lower rates of bone loss, fracture incidence is the most important clinical outcome. Patients do not notice low BMD or high rates of bone loss, but they do suffer whenever a fracture occurs. A previous analysis in the Rotterdam Study pointed out that BMD is a predictor of fracture risk, but does not explain fracture incidence entirely.[32] Experience learns that denser bones are not necessarily stronger bones, as 30 years of research on fluoride for treatment of osteoporosis has shown.[33] The goal in osteoporosis treatment should always be to lower fracture incidence. At this moment, statins seem to be better candidates in the future treatment of osteoporosis than ß-blockers.

The relevance of the study on ß-blocker use is the additional proof of a ß-adrenergic influence on bone. Selective blocking of adrenergic receptors on osteoblasts may have stronger effects on bone than the currently used ß-blockers have. Although ß-blockers may not be first-rate anti-osteoporotic drugs, a better understanding of bone metabolism is a prerequisite for developing new medicines against osteoporosis.

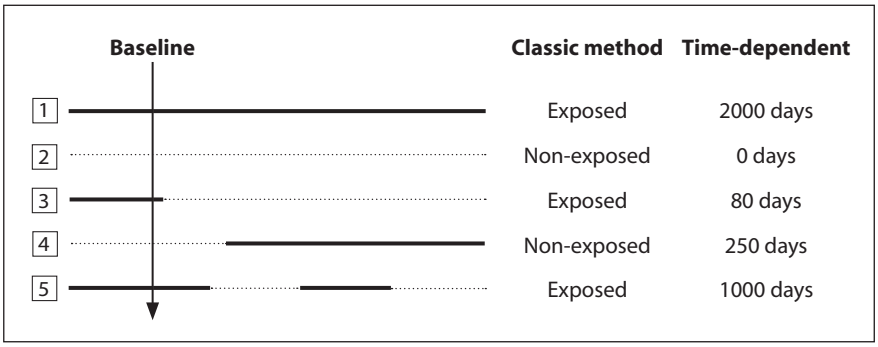
Methodological considerations

The methodological considerations of the presented studies have been discussed in the individual chapters. In the current paragraph, we review an important epidemiological issue: confounding by indication. Furthermore we will discuss time-dependent exposure variables and how we dealt with missing data for confounding variables.

Confounding by indication is a term used when a variable is a risk factor for a disease among non-exposed persons and is also associated with the exposure of interest, without being an intermediate step in the causal pathway between the exposure and the disease.[34] Until recently cardiovascular disease and osteoporosis were seen as two separate disease entities that both increase in prevalence with aging. However, it seems that there are similar pathophysiological mechanisms underlying both diseases. Examples of factors that increase the risk of osteoporosis and cardiovascular diseases are advanced age, menopause, dyslipidemia, hyperhomocystinemia, hypertension, and diabetes. Nitric oxide (NO), in addition to its known atheroprotective effects, appears to also play a role in osteoblast function and bone turnover.[35] Consequently, when studying the association between the use of cardiovascular drugs and (consequences) of osteoporosis, one must be aware of the fact that people that use cardiovascular drugs may have a different a priori risk of osteoporosis compared to non-users. To control for potential confounding by indication, we adjusted for the 5-year risk of cardiovascular disease as computed with the Framingham algorithm.[36] Furthermore, we adjusted for the indication of the therapy, i.e. serum cholesterol levels in the analyses on statin use and hypertension in the analyses on ß-blocker use and we adjusted for drugs that are prescribed for the same indication, respectively other lipid-lowering drugs and ACE-inhibitors. Finally, adjustment for use of ß-mimetics, which are drugs that were contra-indicated in persons using ß-blockers, did not alter the risk estimates. Residual confounding cannot be excluded. As described above, the factors that are of influence on both risk of cardiovascular disease and osteoporosis affect the incidence of these diseases in the same direction. Therefore, it is very unlikely that residual confounding can explain the results from the individual studies, because, when presence of cardiovascular disease would be of influence on the risk estimates, it would increase the risk of fracture or decrease the mean BMD. Since we found the opposite, residual confounding by indication would only attenuate the associations we described in this thesis.

The second methodological issue to be discussed is the use of time-dependent variables for representation of the exposure. Previous studies investigating the use of cardiovascular drugs and BMD or incidence of fractures often extrapolated baseline data on exposure over the total study period. The risk of biased estimates resulting from extrapolating base-

Figure 1 — Exposure definitions.



The classic method of extrapolating baseline data over the study period versus representation of the exposure with time-dependent variables. The solid lines represent exposed time and the dotted lines represent non-exposed time. For persons [1] and [2] there is no bias introduced by extrapolating the baseline exposure data. However, person [3] is considered exposed when in fact he discontinued medication use soon after baseline. The opposite applies to person [4]. Person [5] discontinues and starts again during the study period.

line data can be deduced from Figure 1. Since exposure to medication is time-dependent, which means that people start and discontinue drug use over time, an accurate estimation of risk of an event with drug use must take this time-dependency into account. Apart from creating more precise exposure definitions, time-dependent analyses also allow for investigating timing of drug use in relation to the event. Sensitivity analyses can compare exposure defined as the summed number of days of several periods of drug use (Figure 1; person 5 : 1000 days) with exposure defined as only the last period of use before the event (Figure 1; person 5 : 250 days).

Time-dependent analyses are more laborious and more demanding of statistical software, but the resulting decrease in misclassification makes that this probably will be the standard in future observational research on drug effects, provided that the data allow for such analyses.

Most epidemiologic studies encounter missing data for several covariates. This can be caused by logistic problems, such as a machine that failed on the day that a participant visited the research center, or by refusal of the participant to have a specific parameter measured. The first case is an example of randomly missing data, i.e. the measurement is missing independently of the outcome that should have been measured. The second case is an example of not randomly missing data; the fact that the information is missing depends on the true, but unobserved, value. An illustration of this is the fact that persons who drink too much alcohol more often refuse to answer the question regarding their average daily alcohol consumption than persons who abstain from alcohol. Software packages typically used for analyzing data, delete any case with a missing covariate to perform a complete case analysis. The deletion of cases reduces power, and creates the potential for bias in the resulting estimates. Furthermore, external validity can be compromised because part of the study population is deleted.[37,38]

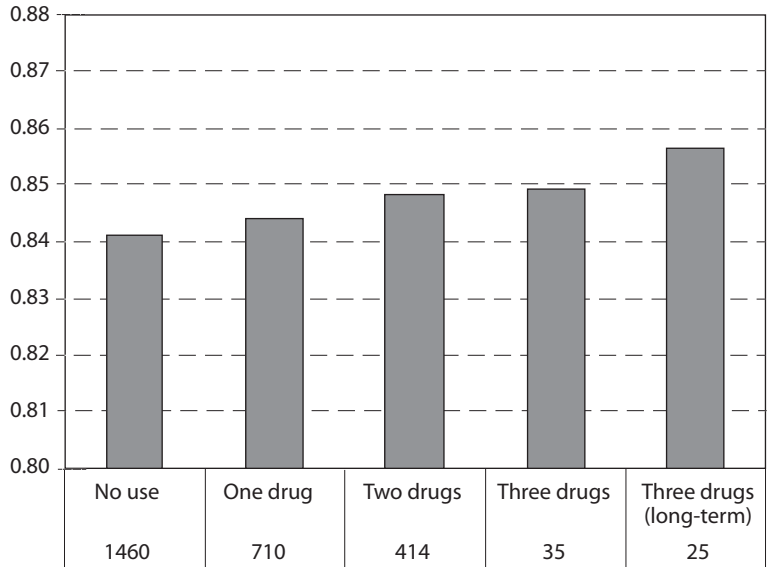
There are several ways to deal with missing values; firstly, to accept that participants are deleted from the analysis, that is conducting a so-called complete case analysis. As mentioned above, this may lead to biased results. Secondly, adjusting for missing values by categorizing a variable and also creating a dummy variable for the missing data. Thirdly, imputation of the missing value by either single imputation (replacing all missing values by the mean or median group value) or by multiple imputation. Previous studies have shown that single imputation can be fairly reliable.[39] However, other researchers stated that both single and multiple imputation gave reliable results, but only when there was less than 10% of the data missing. When 10% to 60% of the participants had missing data on a variable, multiple imputation should be the method of choice.[40] Multiple imputation replaces each missing value with a set of plausible estimates that represent the uncertainty about the right value to impute.[37,38] This is only possible under three assumptions; data are randomly missing, but the missing values are dependent on values of other, actually measured, variables. Every variable may have missing values and the data must be from a continuous multivariate distribution. With a Markov Chain Monte Carlo model, a distribution of possible estimates for the missing values is created, from which samples can be drawn. Subsequently, several complete datasets are created from the original one, with the missing values imputed separately for each dataset. We created for the studies in this thesis 5 datasets by multiple imputation. The sets were analyzed separately and a covariance matrix was created from which results of the 5 datasets were combined into one risk estimate. In this way we could include all participants in the fracture analyses. We did not perform multiple imputation to prevent deletion of participants for analyses on BMD, hip bone structure and rates of bone loss, but in future this will probably become the standard way in analyzing epidemiological data as accessible, user-friendly computer programs are becoming available to perform multiple imputation.

Future research

Combined effects of cardiovascular drugs

In this thesis, we described the associations between use of either thiazides, statins or ß-blockers, and BMD, hip bone structure and fractures. A very important question remains whether combination of these drugs leads to addition or even multiplication of the separate effects. For associations between drug use and BMD, bone structure and rates of bone loss, few people used the three drugs at the same time, and power to detect interaction of use of these drugs was therefore low. The number of people with data available on BMD measurements who used all three drugs together was 72 and for 60 of them also data on potential confounders were present. Of these 60 participants, only 25 had used all these medicines for more than one year before the BMD measurement of the third examination round (between 1997 and 1999). Figure 2 shows the mean BMD at the third examination round for groups with no use of thiazides, ß-blockers or statins and for combinations of these drugs. Due to low power, there were no significant differences between the groups. However, the trend test was significant ($p = 0.02$). These findings suggest that

Figure 2 — Mean BMD of participants at the third examination round by use of thiazides, ß-blockers and statins.



The bars represent the mean BMD of the specific group and the numbers under the bars reflect the number of participants per group.
The mean BMD is adjusted for age, gender, duration of follow-up, hypertension, body mass index, 5-yr cardiovascular risk and baseline BMD.

combined use of these drugs may have a stronger effect on bone than use of only one or two of these medicines. These results should be considered with care, because only few people used all three drugs and we did not determine whether they used the drugs consecutively or concurrently.

The analyses to determine the risk of fracture with the combined use of these medicines are quite complicated due to the fact that exposure to these drugs was expressed with time-dependent variables reflecting current use, and very few people used the three drugs simultaneously. This might be studied, however, in future research.

Other drugs with potential effects on bone

In this thesis we examined the association between use of three cardiovascular drugs and osteoporosis. There is another type of drug that is often prescribed to patients with cardiovascular disease, namely oral anticoagulants. In the Netherlands, oral anticoagulants are available as coumarins. These drugs are indicated for the prevention and treatment of arterial and venous thromboembolic diseases. The coumarins antagonize vitamin K through inhibition of the enzyme, vitamin K epoxide reductase. Vitamin K functions as a cofactor in the posttranslational y-carboxylation of glutamic acid and, as a result of oral anticoagulant therapy, there is production of nonfunctional, undercarboxylated proteins, including osteocalcin and matrix Gla protein.[41] An increased concentration of

inactivated osteocalcin has been associated with a decrease in bone mineral density[42] and a heightened risk of rib and vertebral fracture.[43,44] In contrast with the drugs described in this thesis, use of coumarins is hypothesized to lower BMD and increase fracture risk. Combined use of either thiazides, ß-blockers or statins with coumarins can neutralize the beneficial effects of the previous drugs and further research on an association between oral anticoagulants and bone is therefore required.

As stated above, nitric oxide (NO) appears to play a role in osteoblast function and bone turnover.[45] Isosorbide mononitrate and isosorbide dinitrate are used in the treatment of angina pectoris. Since these agents act as a NO donor, they might prevent both bone loss and stimulate bone resorption. A recently published trial that included 144 women randomized to either 5 mg or 20 mg isosorbide mononitrate or to placebo found a significant decrease in urine N-telopeptide, a marker of bone resorption, and a significantly increased serum bone-specific alkaline phosphatase.[46] It would be interesting to examine the association between use of nitrates and fracture incidence and BMD.

Besides cardiovascular drugs, there are many types of medicines that also have unintentional effects on bone, such as glucocorticoids and antidepressive agents.

Soon after introduction of glucocorticoids as treatment for many inflammatory diseases, it was clear that they could induce osteoporosis.[47] However, there is still controversy whether low-dose regimens also induce osteoporosis and a higher fracture risk.[48] Conflicting results also have been presented regarding the effects of inhaled corticosteroids on BMD and fracture risk.[49,50] Furthermore, polymorphisms of the glucocorticoid receptor may modulate the effect of the therapy. In the Rotterdam Study we have precise data on glucocorticoid use and further research investigating dose-dependency of the oral glucocorticoid effect on bone, the effect of inhaled glucocorticoids and interaction with glucocorticoid receptor polymorphisms can contribute to clinical practice in prescribing these agents.

Use of selective serotonin-reuptake inhibitors (SSRI's) blocks the serotonin transporter and relieves the symptoms of depression by potentiating serotonergic activity. Recently, functional serotonergic pathways in bone have been proposed.[51,52] Preliminary clinical studies showed detrimental effects of SSRI's; SSRI's were shown to decrease BMD in men and increase bone loss in women.[53,54] It might be interesting to investigate the association between SSRI use and osteoporosis in both men and women from the Rotterdam Study.

We made the assumption that use of the cardiovascular drugs of interest influenced bone cells, but we did not investigate the concentration of these medicines in bone. Although some mechanisms of action of the drugs on bone are hypothesized, the precise biological explanation for a potential causal relation was often absent. Epidemiology can be considered as a starting point in the research of causal relations and we therefore also advocate further research on effects of these drugs in animal and bone cell models.

References

1. **Savage PJ, Pressel SL, Curb JD, et al.** Influence of long-term, low-dose, diuretic-based, antihypertensive therapy on glucose, lipid, uric acid, and potassium levels in older men and women with isolated systolic hypertension: The Systolic Hypertension in the Elderly Program. SHEP Cooperative Research Group. Arch Intern Med 1998; 158 (7): 741-51.
2. Major outcomes in high-risk hypertensive patients randomized to angiotensin-converting enzyme inhibitor or calcium channel blocker vs diuretic: The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). Jama 2002; 288 (23): 2981-97.
3. **Onder G, Gambassi G, Landi F, et al.** Trends in antihypertensive drugs in the elderly: the decline of thiazides. J Hum Hypertens 2001; 15 (5): 291-7.
4. **Cauley JA, Cummings SR, Seeley DG, et al.** Effects of thiazide diuretic therapy on bone mass, fractures, and falls. The Study of Osteoporotic Fractures Research Group. Ann Intern Med 1993; 118 (9): 666-73.
5. **Ray WA, Griffin MR, Downey W, Melton LJ.** Long-term use of thiazide diuretics and risk of hip fracture. Lancet 1989; 1 (8640): 687-90.
6. **Felson DT, Sloutskis D, Anderson JJ, Anthony JM, Kiel DP.** Thiazide diuretics and the risk of hip fracture. Results from the Framingham Study. JAMA 1991; 265 (3): 370-3.
7. **Herings RM, Stricker BH, de Boer A, Bakker A, Sturmans F, Stergachis A.** Current use of thiazide diuretics and prevention of femur fractures. J Clin Epidemiol 1996; 49 (1): 115-9.
8. **Rashiq S, Logan RF.** Role of drugs in fractures of the femoral neck. Br Med J (Clin Res Ed) 1986; 292 (6524): 861-3.
9. **LaCroix AZ, Wienpahl J, White LR, et al.** Thiazide diuretic agents and the incidence of hip fracture. N Engl J Med 1990; 322 (5): 286-90.
10. **Grobbee DE, Hoes AW.** Confounding and indication for treatment in evaluation of drug treatment for hypertension. Bmj 1997; 315 (7116): 1151-4.
11. **Mantel-Teeuwisse AK, Klungel OH, Verschuren WM, Porsius AJ, de Boer A.** Time trends in lipid lowering drug use in The Netherlands. Has the backlog of candidates for treatment been eliminated? Br J Clin Pharmacol 2002; 53 (4): 379-85.
12. **Tobert JA.** New developments in lipid-lowering therapy: the role of inhibitors of hydroxymethylglutaryl-coenzyme A reductase. Circulation 1987; 76 (3): 534-8.
13. **van Beek E, Lowik C, van der Pluijm G, Papapoulos S.** The role of geranylgeranylation in bone resorption and its suppression by bisphosphonates in fetal bone explants in vitro: A clue to the mechanism of action of nitrogen-containing bisphosphonates. J Bone Miner Res 1999; 14 (5): 722-9.
14. **Mundy G, Garrett R, Harris S, et al.** Stimulation of bone formation in vitro and in rodents by statins. Science 1999; 286 (5446): 1946-9.
15. **Ohnaka K, Shimoda S, Nawata H, et al.** Pitavastatin enhanced BMP-2 and osteocalcin expression by inhibition of Rho-associated kinase in human osteoblasts. Biochem Biophys Res Commun 2001; 287 (2): 337-42.
16. **Sugiyama M, Kodama T, Konishi K, Abe K, Asami S, Oikawa S.** Compactin and simvastatin, but not pravastatin, induce bone morphogenetic protein-2 in human osteosarcoma cells. Biochem Biophys Res Commun 2000; 271 (3): 688-92.
17. **Greenspan SL, Bhattacharya R, Beck TJ, Resnick NM, Parker RA.** The effect of hormone replacement therapy, alendronate, or the combination of both on hip structural geometry: a 3-year, randomized, double-blind, placebo-controlled, NIH-funded clinical trial. J Bone Miner Res 2003; 18 (Suppl 2): S92 (Abstract F356).
18. **Pedersen TR, Kjekshus J.** Statin drugs and the risk of fracture. 4S Study Group. JAMA 2000; 284 (15): 1921-2.
19. **Reid IR, Hague W, Emberson J, et al.** Effect of pravastatin on frequency of fracture in the LIPID study: secondary analysis of a randomised controlled trial. Long-term Intervention with Pravastatin in Ischaemic Disease. Lancet 2001; 357 (9255): 509-12.
20. **Knapen MH, Hamulyak K, Vermeer C.** The effect of vitamin K supplementation on circulating osteocalcin (bone Gla protein) and urinary calcium excretion. Ann Intern Med 1989; 111 (12): 1001-5.
21. **Kohlmeier M, Salomon A, Saupe J, Shearer MJ.** Transport of vitamin K to bone in humans. J Nutr 1996; 126 (4 Suppl): 1192S-6S.

22. **Parhami F, Garfinkel A, Demer LL.** Role of Lipids in Osteoporosis. Arterioscler Thromb Vasc Biol 2000; 20 (11): 2346-8.
23. **Salamone LM, Cauley JA, Zmuda J, et al.** Apolipoprotein E gene polymorphism and bone loss: estrogen status modifies the influence of apolipoprotein E on bone loss. J Bone Miner Res 2000; 15 (2): 308-14.
24. **Shiraki M, Shiraki Y, Aoki C, et al.** Association of bone mineral density with apolipoprotein E phenotype. J Bone Miner Res 1997; 12 (9): 1438-45.
25. **Cauley JA, Zmuda JM, Yaffe K, et al.** Apolipoprotein E polymorphism: A new genetic marker of hip fracture risk –The Study of Osteoporotic Fractures. J Bone Miner Res 1999; 14 (7): 1175-81.
26. **Pluijm SM, Dik MG, Jonker C, Deeg DJ, Kamp GJ, Lips P.** Effects of Gender and Age on the Association of Apolipoprotein E epsilon4 with Bone Mineral Density, Bone Turnover and the Risk of Fractures in Older People. Osteoporos Int 2002; 13 (9): 701-9.
27. **Johnston JM, Cauley JA, Ganguli M.** APOE 4 and hip fracture risk in a community-based study of older adults. J Am Geriatr Soc 1999; 47 (11): 1342-5.
28. **Kohlmeier M, Saupe J, Schaefer K, Asmus G.** Bone fracture history and prospective bone fracture risk of hemodialysis patients are related to apolipoprotein E genotype. Calcif Tissue Int 1998; 62 (3): 278-81.
29. **Takeda S, Elefteriou F, Levasseur R, et al.** Leptin regulates bone formation via the sympathetic nervous system. Cell 2002; 111 (3): 305-17.
30. **Ahlborg HG, Johnell O, Turner CH, Rannevik G, Karlsson MK.** Bone Loss and Bone Size after Menopause. N Engl J Med 2003; 349 (4): 327-34.
31. **Seeman E.** Periosteal bone formation--a neglected determinant of bone strength. N Engl J Med 2003; 349 (4): 320-3.
32. **Schuit SC, van der Klift M, Weel AE, et al.** Fracture incidence and association with bone mineral density in elderly men and women: the Rotterdam Study. Bone 2004; 34 (1): 195-202.
33. **Dequeker J, Declerck K.** Fluor in the treatment of osteoporosis. An overview of thirty years clinical research. Schweiz Med Wochenschr 1993; 123 (47): 2228-34.
34. **Salas M, Hofman A, Stricker BH.** Confounding by indication: an example of variation in the use of epidemiologic terminology. Am J Epidemiol 1999; 149 (11): 981-3.
35. **McFarlane SI, Muniyappa R, Shin JJ, Bahtiyar G, Sowers JR.** Osteoporosis and cardiovascular disease: brittle bones and boned arteries, is there a link? Endocrine 2004; 23 (1): 1-10.
36. **Anderson KM, Odell PM, Wilson PW, Kannel WB.** Cardiovascular disease risk profiles. Am Heart J 1991; 121 (1 Pt 2): 293-8.
37. **Rubin DB, Schenker N.** Multiple imputation in health-care databases: an overview and some applications. Stat Med 1991; 10 (4): 585-98.
38. **Schafer JL, Graham JW.** Missing data: our view of the state of the art. Psychol Methods 2002; 7 (2): 147-77.
39. **Arnold AM, Kronmal RA.** Multiple imputation of baseline data in the cardiovascular health study. Am J Epidemiol 2003; 157 (1): 74-84.
40. **Barzi F, Woodward M.** Imputations of missing values in practice: results from imputations of serum cholesterol in 28 cohort studies. Am J Epidemiol 2004; 160 (1): 34-45.
41. **Hauschka PV, Lian JB, Cole DE, Gundberg CM.** Osteocalcin and matrix Gla protein: vitamin K-dependent proteins in bone. Physiol Rev 1989; 69 (3): 990-1047.
42. **Sato Y, Honda Y, Kunoh H, Oizumi K.** Long-term oral anticoagulation reduces bone mass in patients with previous hemispheric infarction and nonrheumatic atrial fibrillation. Stroke 1997; 28 (12): 2390-4.
43. **Caraballo PJ, Heit JA, Atkinson EJ, et al.** Long-term use of oral anticoagulants and the risk of fracture. Arch Intern Med 1999; 159 (15): 1750-6.
44. **Vergnaud P, Garnero P, Meunier PJ, Breart G, Kamihagi K, Delmas PD.** Undercarboxylated osteocalc in measured with a specific immunoassay predicts hip fracture in elderly women: the EPIDOS Study. J Clin Endocrinol Metab 1997; 82 (3): 719-24.
45. **Evans DM, Ralston SH.** Nitric oxide and bone. J Bone Miner Res 1996; 11 (3): 300-5.
46. **Jamal SA, Cummings SR, Hawker GA.** Isosorbide mononitrate increases bone formation and decreases bone resorption in postmenopausal women: a randomized trial. J Bone Miner Res 2004; 19 (9): 1512-7.

47. **Curtis PH, Clark WS, Herndon CH.** Vertebral fractures resulting from prolonged cortisone and corticotropin therapy. J Am Med Assoc 1954; 145: 467.
48. **Reid IR, Heap SW.** Determinants of vertebral mineral density in patients receiving long-term glucocorticoid therapy. Arch Intern Med 1990; 150 (12): 2545-8.
49. **van Staa TP, Leufkens HG, Cooper C.** Use of inhaled corticosteroids and risk of fractures. J Bone Miner Res 2001; 16 (3): 581-8.
50. **Herrala J, Puolijoki H, Impivaara O, Liippo K, Tala E, Nieminen MM.** Bone mineral density in asthmatic women on high-dose inhaled beclomethasone dipropionate. Bone 1994; 15 (6): 621-3.
51. **Westbroek I, van der Plas A, de Rooij KE, Klein-Nulend J, Nijweide PJ.** Expression of serotonin receptors in bone. J Biol Chem 2001; 276 (31): 28961-8.
52. **Battaglino R, Fu J, Spate U, et al.** Serotonin regulates osteoclast differentiation through its transporter. J Bone Miner Res 2004; 19 (9): 1420-31.
53. **Diem S, Blackwell T, Yaffe K, Bauer DC, Ensrud K.** Use of selective serotonin receptor inhibitors increases the rate of hip bone loss. J Bone Miner Res 2004; 19 (Suppl 1): S90 (Abstract).
54. **Haney EM, Chan BKS, Lambert L, et al.** SSRI use is associated with lower BMD among men. J Bone Miner Res 2004; 19 (Suppl 1): S7 (Abstract).

Chapter 7

Summary
Samenvatting

Osteoporosis is a disease that is characterized by low bone mineral density (BMD) and microarchitectural deterioration of bone tissue. The most important clinical outcome of osteoporosis is a fracture. Fractures cause important morbidity and mortality and preventive and therapeutic strategies will become increasingly important due to aging of the population. Cardiovascular disease incidence also increases in the aging population and as a result cardiovascular drugs are commonly used in elderly. Cardiovascular drugs were previously hypothesized to have unintentional effects on bone and the objective of this thesis was to investigate the character and quantity of these unintentional effects. Since many elderly are at risk for both cardiovascular disease and osteoporosis, side effects of drugs might play a role in the decision which drug to prescribe for cardiovascular diseases. Furthermore, research on the association between the use of these drugs and bone outcomes, such as fracture risk and bone mineral density, may answer some of the questions on the potential role of these medicines in the prevention and treatment of osteoporosis. All the studies presented in this thesis were based on data of the Rotterdam Study. This is a large prospective population-based cohort study among persons aged 55 years or older who were living in a suburb of Rotterdam in the Netherlands. This study included at baseline in 1990 7983 men and women.

Chapter 2 describes the association between risk of hip fracture and use of thiazide diuretics. Thiazides are known to decrease renal calcium excretion and, more than 20 years ago, the first report on effects of thiazides on BMD was published. In the years thereafter also studies on the association between thiazides and fracture incidence appeared. Except for an occasional increased risk, almost all studies found a protective effect of thiazides. However, due to more or less severe limitations to the previous studies on the domain of exposure data and/or data on confounding variables, it was still not known how long thiazides have to be used in order to have a beneficial effect. Furthermore, it was not clear how long the effect persists after discontinuation of use. In our study we showed that thiazides had to be taken continuously for at least 1 year to find a 54% decreased hip fracture risk. This risk reduction quickly disappears after discontinuation; after 4 months the risk was shown to be equal to non-users.

In ***Chapter 3***, studies of the association between use of statins, which are cholesterol-lowering drugs, and bone are described. Statins are inhibitors of a rate-limiting enzyme in the mevalonate pathway of the cholesterol synthesis. This mevalonate pathway is also involved in bone metabolism. In vitro, statins have been shown to stimulate bone formation, but observational studies of BMD and fracture risk among statin users gave contradictory results. ***Chapter 3.1*** presents the association between statin use and BMD and bone structural geometry of the femoral neck. We show that there is a duration-dependent relationship between use of statins and a higher BMD. When participants had taken a statin for more than 4 years, the BMD of that group was significantly higher than mean BMD of non-users. Not only BMD, but also the structural geometry of bone is a

predictor of fracture risk. We found that long-term statin users had significantly thicker cortices of the femoral neck. A predictor of fracture risk derived from the structure of the hip, the buckling ratio, was lower for statin users, suggesting a lower fracture risk. ***Chapter 3.2*** focuses on the association between risk of vertebral fracture, BMD of the lumber vertebrae and use of statins. Vertebral fractures are the most common and typical fractures in osteoporosis patients and are associated with increased morbidity and mortality. Most vertebral fractures are not symptomatic and are, in contrast to other fractures, not related to falls. Because comparisons of multiple radiographs, taken at different points in time are essential to study the incidence of vertebral fractures, this type of fracture is not well studied. The adjusted relative risk for incident vertebral fracture in users of statins (compared to non-users) was 0.58 (95% confidence interval 0.34–0.99). The relative risk decreased upon higher cumulative use to 0.52 (0.28–0.97) for use for more than 365 days during the study period. Statin use was not significantly associated with lumbar spine BMD. The association between risk of all nonvertebral fractures and statin use is described in ***Chapter 3.3***. Again, a beneficial effect of statins has been reported. Adjusted risk for nonvertebral fracture was lower for current statin users compared to non-users (HR 0.81; 95% confidence interval 0.61–1.07). When statins were continuously used for at least 2 years the risk of nonvertebral fracture was significantly reduced to 0.57 (0.37–0.90).

Twin and family studies showed that bone density and bone turnover are affected by genetic factors. It was estimated that 50–80% of the variability in BMD is explained by genetic factors. A recently emerged pathway involved in bone metabolism is that of apolipoprotein E (ApoE). ApoE is a protein of which three isoforms exist, the E*2, E*3 and E*4 isoforms. It is thought that ApoE mediates vitamin K transport, which in turn influences bone turnover. Another hypothesis is that low density lipoprotein (LDL) levels in subjects with the E*4 allele are increased and that accumulation of oxidized lipids in subendothelial space of bone may lead to inhibition of osteoblast differentiation. ***Chapter 4*** describes the study of the association between a polymorphism in the ApoE gene and BMD, change in BMD and risk of a fracture. Our data from this, to our knowledge, largest study performed on the association between ApoE and osteoporosis, do not support the hypothesis that the ApoE*4 risk allele is associated with BMD, increased bone loss, or an increased risk of osteoporotic fracture.

There is accumulating evidence that the sympathetic nervous system is involved in regulation of bone metabolism. Adrenergic receptors have been found on osteoblasts and osteoclasts and animal studies showed sympathetic innervation of bone with neuropeptides that modulated bone resorption. It was hypothesized that leptin regulates bone formation via the sympathetic pathway. Propranolol, a ß-blocking agent, increased bone formation in mice. ***Chapter 5*** focuses on the association between use of ß-blockers, BMD and structural geometry of the femoral neck and fracture risk. Long-term users (>4 years) of ß-blockers had a significantly higher femoral neck BMD (0.858 g/cm^2; CI 95% 0.851–0.864) compared with non-users (0.841; 0.839–0.844). The increase in BMD was reflected in a thicker cortex. Furthermore, ß-blocker users had smaller femoral neck widths than

non-users. It is not clear whether these differences are due to inhibition of bone resorption or stimulation of bone formation. The risk of a fracture in general was not different between users and non-users. However, the risk for a fracture that typically occurs at older age (frailty fracture; upper arm, hip or pelvis) was significantly decreased for persons who had continuously used a ß-blocker for more than 2 years (Hazard ratio 0.67; 0.46–0.97). The risk of an incident vertebral fracture was lower (0.83) but not significantly decreased for long-term ß-blockers.

In the general discussion (***Chapter 6***), the main findings are brought together and placed in the context of current scientific knowledge and clinical practice. Some methodological issues are discussed and recommendations for future research are made.

Osteoporose is een ziekte, die wordt gekenmerkt door een lage minerale dichtheid van het bot (BMD) en door een verslechtering van de micro-architectuur van botweefsel. Het meest kenmerkende gevolg van osteoporose is het ontstaan van fracturen. Fracturen leiden tot een hoge morbiditeit en mortaliteit. Preventieve en therapeutische maatregelen lijken steeds belangrijker te worden, gezien de veroudering van de bevolking.

Elk geneesmiddel heeft bijwerkingen en veel geneesmiddelen hebben ook bijwerkingen op botweefsel. Het meest bekende voorbeeld hiervan vormt het gebruik van glucocorticosteroïden met zijn nadelige effecten. Medicatiegebruik kan echter ook positieve bijwerkingen hebben op bot, die zichtbaar worden door een lagere incidentie van fracturen en een hogere botdichtheid. Door de vergrijzing stijgt de incidentie van hart- en vaatziekten en neemt het gebruik van cardiovasculaire medicatie toe. Bijwerkingen op bot van deze groep geneesmiddelen zou dus van grote betekenis kunnen zijn voor ontstaan van osteoporose. Er is een ruime keus op het gebied van cardiovasculaire medicatie en meerdere combinaties van geneesmiddelen zijn mogelijk. Indien nu zou blijken dat sommige middelen tevens een gunstige invloed op bot hebben, is een betere afweging mogelijk van de in te stellen behandeling bij cardiovasculaire ziekten. Zo kan men bij een postmenopausale vrouw met hypertensie en een polsfractuur in de voorgeschiedenis kiezen om een middel te geven dat niet alleen de hoge bloeddruk aanpakt maar tevens een gunstige invloed op haar botweefsel zal hebben.

Thiazide diuretica, voorgeschreven ter behandeling van hypertensie, betreffen een groep oudere cardiovasculaire geneesmiddelen met potentieel gunstige bijwerkingen. Maar ook gebruik van statinen en ß-blockers zou kunnen leiden tot een betere botkwaliteit en minder fracturen. Thiaziden remmen de natriumreabsorptie in de nier, en daardoor ook de calciumexcretie. Dit leidt tot een gunstigere calciumbalans. Statinen remmen een enzym, HMG-CoA reductase, dat actief is in de mevalonaatroute van het cholesterol metabolisme. Door remming van dit enzym worden lagere cholesterolspiegels in het bloed bereikt. Maar omdat de mevalonaatroute ook belangrijk is voor het botmetabolisme, zou gebruik van statinen ook een effect op bot kunnen hebben. ß-blockers tenslotte, remmen het ß-adrenerge systeem en hebben daardoor gunstige effecten op het hart. Onlangs ontdekte men dat botweefsel vezels van het sympathische zenuwstelsel bevat en dat er op osteoblasten en osteoclasten ß-adrenerge receptoren zijn aangetroffen. De invloed van leptine op bot wordt uitgeoefend via het sympathische zenuwstelsel en ß-blockers kunnen zo de (ongunstige) invloed van leptine op bot verminderen.

Hoofdstuk 1 beschrijft de achtergronden en het doel van het onderzoek, beschreven in dit proefschrift. Wij trachtten de effecten van gebruik van thiazide diuretica, statinen en ß-blockers op bot te karakteriseren en kwantificeren. Tevens keken we naar de associatie tussen bot enerzijds en een polymorfisme van het apolipoproteïne E gen anderzijds.

Alle studies beschreven in dit proefschrift zijn uitgevoerd met gegevens van de Erasmus Gezondheid en Ouderen (ERGO) studie. Dit is een prospectieve cohortstudie, die is opgezet om het optreden van ziektes – en de daarop van invloed zijnde determinanten – te onderzoeken in een oudere populatie. De studie omvat 3105 mannen en 4878 vrouwen uit een wijk in Rotterdam (Ommoord), die bij het begin van de studie tenminste 55 jaar oud waren. In 1990 werd gestart met het interviewen van alle deelnemers en daarna werden zij in het studiecentrum onderzocht. Zo werden gegevens verkregen over medische voorgeschiedenis, leefwijzen en dieetgewoonten. Voorts werden lichamelijk onderzoek, bloedonderzoek en neuropsychologisch onderzoek verricht. Ook de minerale dichtheid van het bot (BMD) werd gemeten. Er werden röntgenfoto's gemaakt, onder andere van de wervelkolom en deze foto's werden beoordeeld op de aanwezigheid van wervelfracturen. Het interview en het onderzoek op het studiecentrum werden herhaald in 1993 en 1995. Het ontstaan van nieuwe wervelfracturen en verandering in BMD kon dus worden gemeten door vergelijking van de meetresultaten aan het begin en het eind van de studie. Tussentijds werden alle belangrijke veranderingen in de gezondheid van de deelnemers gerapporteerd door de huisartsen. Tevens werden alle fracturen en overlijdensgevallen gemeld.
Voor alle deelnemers aan de ERGO studie was informatie beschikbaar betreffende hun geneesmiddelgebruik op basis van apotheekgegevens. Deze informatie was beschikbaar vanaf 1 januari 1991. Zo konden we voor elke dag van de studie berekenen welke medicijnen de deelnemers op dat moment gebruikten. Ook de dosis en de duur van het voorafgaande gebruik waren bekend.

Hoofdstuk 2 beschrijft de associatie tussen het risico op heupfracturen en het gebruik van thiazide diuretica. Van thiaziden is bekend dat zij de excretie van calcium in de nieren verhinderen. Reeds 20 jaar geleden verscheen het eerste onderzoek waarin werd aangetoond dat thiaziden een effect kunnen hebben op BMD. In de jaren daarna werden studies gepubliceerd die een verlaagd risico op fracturen lieten zien bij thiazidegebruik, maar er waren ook studies waarin geen verandering werd aangetoond in het risico op fracturen bij gebruik van deze plaspillen. De voorgaande onderzoeken vermeldden echter geen minimale duur van gebruik die nodig was voor het effect zou optreden, omdat het niet mogelijk was dit te onderzoeken door gebrek aan bepaalde gegevens. Ook was niet duidelijk hoe lang na staken van deze diuretica de beschermende werking aanhield.
In onze studie toonden wij aan dat na een jaar van continu thiazidegebruik het risico op een heupfractuur 54% lager was dan bij niet-gebruikers. Na staken van thiaziden verdween deze risicoreductie echter weer. Vier maanden na staken konden wij geen significant verschil meer aantonen ten opzichte van niet-gebruikers.

In ***Hoofdstuk 3*** beschrijven we een aantal studies naar de associatie tussen het gebruik van cholesterolverlagende statinen enerzijds, en fracturen en botweefsel anderzijds. Statinen remmen het enzym HMG CoA reductase dat een belangrijke stap in de mevalonaat route van de cholesterolsynthese katalyseert. Deze mevalonaatroute is ook betrokken bij het botmetabolisme. Van statinen is bekend dat zij, bij laboratorium onderzoek op bot-

cellen, botvorming kunnen stimuleren. Echter, observationele studies naar effecten op BMD en fractuur risico onder mensen lieten controversiële resultaten zien.
Hoofdstuk 3.1 beschrijft de associatie tussen gebruik van statinen en BMD en structurele geometrie van de heup. We laten zien dat er een gebruiksduur-afhankelijke relatie bestaat tussen statinen en BMD. Hoe langer mensen statinen slikken, hoe hoger de BMD wordt. Na 4 jaar is de BMD van statinegebruikers significant hoger dan die van niet-gebruikers. Omdat niet alleen de BMD van belang is voor het risico op fracturen, maar ook de afmetingen van het bot, keken we ook naar de relatie tussen statinegebruik en structurele geometrie van de heup. We zagen dat langdurig statinegebruik geassocieerd was met een dikkere cortex.
Hoofdstuk 3.2 richt zich op de vraag of statinegebruik geassocieerd is met het risico op wervelfracturen en de BMD van de lumbale wervelkolom. Wervelfracturen zijn de meest voorkomende en meest typische fracturen bij patiënten met osteoporose. De meeste wervelfracturen zijn echter asymptomatisch en zijn, in tegenstelling tot andere fracturen, niet gerelateerd aan vallen. Vanwege het feit dat de meeste asymptomatisch zijn is het bestuderen van wervelfracturen niet eenvoudig. Om een compleet beeld te krijgen is het nodig dat er met enige tussenpoos röntgenfoto's worden gemaakt bij een individu, zodat deze foto's met elkaar kunnen worden vergeleken om te beoordelen of er (nieuwe) wervelfracturen zijn ontstaan. In de ERGO studie werden deze foto's bij 3469 mensen gemaakt aan het begin van de studie en tijdens de onderzoeksronde van 1997. Het – voor confounders gecorrigeerde – risico op een wervelfractuur onder statinegebruikers was 0.58 (95% betrouwbaarheidsinterval (CI 95) 0.34 – 0.99). We vonden een duur-afhankelijke relatie. Bij statinegebruik van meer dan een jaar was dit risico bijna 50 % lager dan het risico bij niet-gebruikers (CI 95 0.28 – 0.97). We zagen echter geen significante associatie tussen statinegebruik en de BMD van de wervelkolom.
Het risico op niet-vertebrale fracturen onder statinegebruikers wordt beschreven in ***Hoofdstuk 3.3***. Opnieuw zagen we een positieve bijwerking van statinen op bot. Het (gecorrigeerde) risico op een niet-vertebrale fractuur was lager voor statinegebruikers dan voor niet-gebruikers (HR 0.81, CI 95 0.61–1.07). Wanneer statinen gedurende tenminste 2 jaar continu werden gebruikt, zagen we dat het risico op een niet-vertebrale fractuur significant lager was (HR 0.57, CI 95 0.37 – 0.90).

Studies bij tweelingen en overige verwanten laten zien dat botdichtheid en bot turnover door genetische factoren beïnvloed worden. Ongeveer 50 – 80 % van de variabiliteit in BMD kan worden verklaard door deze factoren. Recent werd ontdekt dat het apolipoproteïne E (ApoeE) een belangrijke component vormt van het botmetabolisme. ApoE is een eiwit waarvan drie isovormen bekend zijn: de E*2, E*3 en E*4 isovorm. We denken dat ApoE het vitamine K transport beïnvloed, en vitamine K op zijn beurt is van invloed op de bot turnover. Een andere hypothese is dat *low density lipoprotein* (LDL) spiegels zijn verhoogd onder personen met het E*4 allel en dat daardoor geoxideerde vetten in de subendotheliale ruimte van bot kunnen accumuleren. Dit kan weer leiden tot verhindering van osteoblast differentiatie. ***Hoofdstuk 4*** beschrijft de studie naar de associatie tussen dit polymorfisme van het ApoE gen en BMD, verandering in BMD over de tijd en het risico op een fractuur. Deze studie is tot op heden, voorzover ons bekend, de grootste studie

naar de associatie tussen dit gen en osteoporose. Wij konden echter niet de hypothese bevestigen dat dit gen geassocieerd is met BMD, met verandering in BMD of met het fractuurrisico.
In de laatste jaren wordt het steeds duidelijker dat het sympathische zenuwstelsel betrokken is bij de regulatie van het bot metabolisme. Adrenerge receptoren zijn gevonden op osteoblasten en osteoclasten en dier-experimenteel onderzoek liet zien dat bot geïnnerveerd is met sympathische zenuwvezels en dat neuropeptiden botmetabolisme kunnen beïnvloeden. De hypothese rees dat leptine via het sympathische zenuwstelsel botvorming reguleert. Toediening van propranolol, een ß-blocker, aan muizen leidde tot verhoogde botvorming.

Hoofdstuk 5 richt zich op de associatie tussen het gebruik van ß-blockers, fracturen, BMD en structurele geometrie van de heup. Langdurig gebruik van ß-blockers (>4 jaar) was geassocieerd met een significant hogere BMD (0.858 g/cm^2; CI 95 0.851–0.864) van de heup vergeleken met niet-gebruikers (HR 0.841; CI 95 0.839–0.844). De toename in BMD werd weerspiegeld in een dikkere cortex. ß-blocker gebruikers hadden een kleinere diameter van de heup. Het is niet duidelijk of deze verschillen ontstaan zijn door inhibitie van botresorptie of door stimulatie van botvorming. Het risico op een fractuur was niet verschillend voor gebruikers van ß-blockers en niet-gebruikers. Alleen het risico op een van de 3 fracturen die vooral bij ouderen voorkomen (bovenarm, heup en bekken) was significant lager voor personen die meer dan 2 jaar continu een ß-blocker hadden gebruikt (Hazard ratio 0.67; 0.46–0.97). Het risico op een wervelfractuur was lager dan bij niet-gebruikers (HR 0.83) maar niet significant lager.

In de algemene discussie (***Hoofdstuk 6***) worden de belangrijkste bevindingen uit dit proefschrift besproken in de context van de huidige wetenschappelijke inzichten en de klinische praktijk. Tevens worden er enkele methodologische zaken besproken en worden er aanbevelingen gedaan voor de toekomst.

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Mariëtte

* George Wald (Nobelprijs-rede)

List of publications

Schoofs MW, van der Klift M, Hofman A, de Laet CE, Herings RM, Stijnen T, Pols HA, Stricker BH. Thiazide diuretics and the risk for hip fracture. Ann Intern Med. 2003 Sep 16; 139 (6); 476-82.

Schoofs MW, Sturkenboom MC, van der Klift M, Hofman A, Pols HA, Stricker BH. HMG-CoA reductase inhibitors and the risk of vertebral fracture. J Bone Miner Res. 2004 Sep; 19 (9) 1525-30.

Schoofs MW, van der Klift M, Hofman A, van Duijn CM, Stricker BH, Pols HA, Uitterlinden AG. ApoE gene polymorphisms, BMD, and fracture risk in elderly men and women: the Rotterdam study. J Bone Miner Res. 2004 Sep; 19 (9): 1490-6.

Schoofs MW, Maartense E, Eulderink F, Vreede RW. Cryptosporidiosis leading to an unsuspected diagnosis of AIDS. Neth J Med. 2004 Jun; 62 (6): 198-200.

Schoofs MW, Rivadeneira F, Sturkenboom MC, van Leeuwen JP, Beck TJ, Hofman A, Stricker BH, Pols HA. Epidemiological support for involvement of the sympathetic nervous system on bone. Submitted.

Schoofs MW, Sturkenboom MC, Rivadeneira F, Beck TJ, Hofman A, Pols HA, Stricker BH. Long-term use of statins is associated with bone mineral density. Submitted.

Schoofs MW, Sturkenboom MC, Hofman A, Pols HA, Stricker BH. Long-term statin use is associated with a reduced incidence of nonvertebral fractures. Submitted.

About the author

Mariëtte Schoofs was born on May 17th, 1974 in Veldhoven, The Netherlands. In June 1992, she graduated from the "van Maerlantlyceum" in Eindhoven (Gymnasium ß) and in September of the same year she started with the study "biomedical sciences" in Leiden. After obtaining her propaedeuse in 1993, she continued her education with the study medicine in Leiden. During this study she did a research project on antimicrobial characteristics of a synthetic peptide at the Department of Infectious Diseases of the Leiden University Medical Center (LUMC, Dr. P. Nibbering). She obtained her medical degree in November 1999. Subsequently she worked 6 months as a research physician at the Department of General Practice of the LUMC and from May 2000 until February 2001, she worked as a resident in internal medicine at the "Reinier de Graaf Gasthuis" in Delft (Dr. E. Maartense).
In March 2001 she started the research described in this thesis at the Pharmacoepidemiology Unit (Prof. dr. B. H. Ch. Stricker) of the Department of Epidemiology & Biostatistics (Prof. dr. A. Hofman) and the Department of Internal Medicine (Prof. dr. H. A. P. Pols) of the Erasmus MC in Rotterdam. During this period she obtained a MSc degree in Clinical Epidemiology from the Netherlands Institute for Health Sciences in Rotterdam.
In May 2002 she received a "Young Investigator Award" from the International Osteoporosis Foundation and in May 2003 and June 2005 she received the same award from the European Calcified Tissue Society.
In January 2005 she started her residency in internal medicine at the "Reinier de Graaf Gasthuis" in Delft under the supervision of Dr. E. Maartense.
Mariëtte is married to Yde de Jong and they have a daughter, Edith.


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