Bone Characteristics, Vitamin D and Osteoarthritis

Arjan Bergink

Bone Characteristics, Vitamin D and Osteoarthritis

Arjan Bergink

ISBN: 978-94-6421-887-9

Lay-out: Wendy Bour-van Telgen

Printing: Ipskamp Printing

Cover: Vitamin D crystals, Science Photo Library, www.sciencephoto.com

Bone Characteristics, Vitamin D and Osteoarthritis

Botkenmerken, vitamine D en artrose

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof.dr. A.L. Bredenoord

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

woensdag 16 november 2022 om 13.00 uur

door

Arjan Peter Bergink

geboren te Hengelo (Ov)

Erasmus University Rotterdam

Erasmus

Promotiecommissie

Promotoren:

Prof.dr. J.B.J. van Meurs

Prof.dr. A.G. Uitterlinden

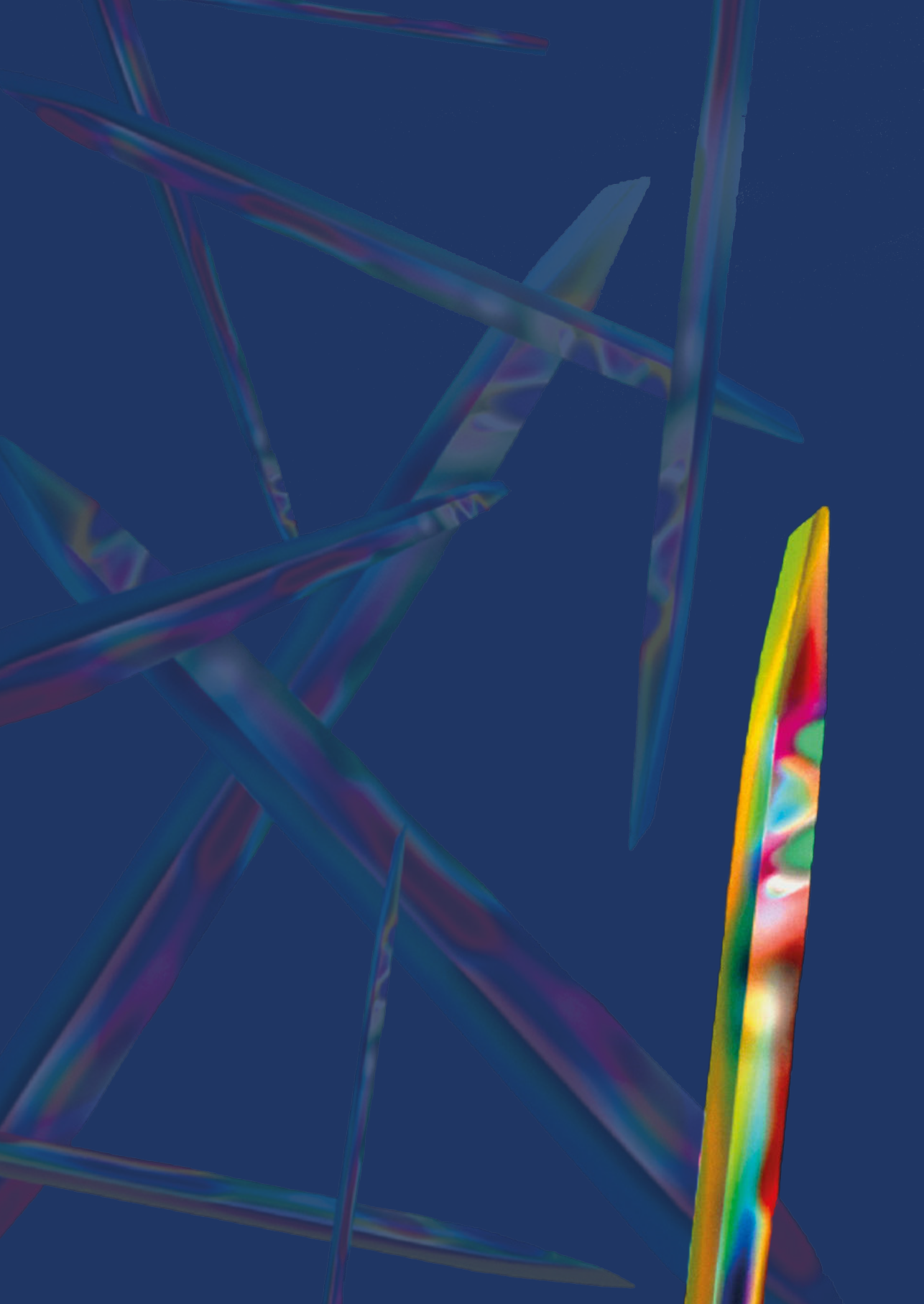
Overige leden:

Prof.dr.ir. C.P.G.M. de Groot

Prof.dr. H.B.J. Karperien

Prof.dr. F.U.S. Mattace-Raso

Content



1

GENERAL INTRODUCTION

1.1 History of osteoarthritis

The word 'osteoarthritis' is composed of the Greek words for bone (ostéon) and joint (árthron) with the extension -itis, meaning inflammation. Thus, arthritis means inflammation of the joints, and the most common form of arthritis is osteoarthritis (OA), also known as degenerative arthritis. OA is a progressive and disabling joint disease. It is affecting joint cartilage and the underlying bone leading to swelling, stiffness and pain of the joints. OA can affect the joints of both humans and animals. The oldest manifestation of this disease was found in a family of Early Cretaceous dinosaurs (Iguanodontidae), that lived between 140 and 100 million years ago[2], and other dinosaurs were diagnosed with OA as well, like Caudipteryx and Spiclypeus[2,3]. Also in ancient Egyptian mummies symptoms of OA were found[4,5], as well as in the joints of workmen in the city of Deir el-Medina (**Figure 1.1**), who constructed and decorated the pharaohs' tombs during Egypt's New Kingdom period (1550 to 1070 BCE)[6]. In Europe, signs of OA were found during the Cupper Age: Murphy et al. performed a radiologic study on Iceman Ötzi, and discovered that he suffered from spine and hip OA some 5300 years ago[7]. In contemporary humans, it most commonly affects the hands and weight bearing joints like the hips, knees, spine and feet. OA can be secondary to congenital or post-traumatic changes of the joint, pyogenic infections, joint malalignment or instability. Primary OA, occurring without obvious trauma, is usually poly-articular, and rarely occurs before the age of 40. OA of the weight bearing joints is most common in the obese individuals older than 50.

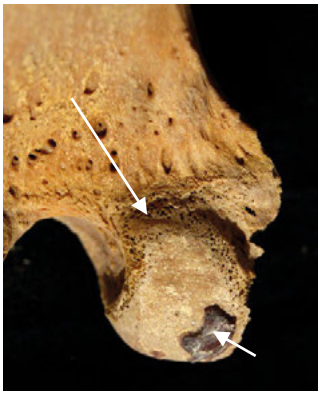


Figure 1.1 Joint of ancient Egyptian worker affected by OA[6]
(short arrow: pitting, long arrow: osteophytic lipping)

1.2 Prevalence of osteoarthritis and clinical management

At present, OA is the most common disease of the joints and a leading cause of disability among elderly in Western societies[8,9]. In 2017, it was estimated that 303 million people worldwide suffered from OA[10]. It can affect any joint, but preferentially involves the knee, hands, hip and spine. In the elderly, the knee is the most affected joint with reported prevalences ranging from 19.2% to 37.4%[11-13]. OA, with resulting pain and disability, has a considerable impact on the individual patient. The economic burden of OA on patients and society is also significant due to health care costs and loss of productivity[14].

The Netherlands Institute for Health Services Research (NIVEL) reported a prevalence in the Netherlands of OA diagnosed in 2020, at 1.557.900 subjects (64% women), most of them with knee OA (746.100), followed by hip OA (476.900)[15]. These data were collected by general practitioners based on their diagnoses (ICPC-code[16]), with no well-defined radiological criteria available. A large study that did use radiological criteria to estimate the prevalences of knee and hip OA in the Netherlands was performed by Odding et al. in 1998[17]. They drew a random sample of 1.156 men and 1.739 women from the Rotterdam Study, a large prospective cohort study of men and women aged 55 and over with the objective to investigate the incidence of, and risk factors for, chronic disabling diseases[1]. They defined radiographic OA (ROA) as a Kellgren/Lawrence score (**Table 1.1**) of 2 or more, and observed prevalent hip ROA in 14.1% of men and 15.9% of women, and 16.3% and 29.1% prevalent knee ROA, respectively.

All studies described in this thesis are performed within the Rotterdam Study, with exception of the meta-analysis (**Chapter 3.2**). In the figures below, the baseline prevalence of knee, hip and hand ROA in RS-I and RS-II (n = 7842) are presented by age groups (**Figure 1.2, 1.3 and 1.4)**. It is obvious that especially knee and hand OA is a very common condition increasing with age. Given the aging Dutch society, it is anticipated that OA will be to be the most frequent disease in the Dutch population in 2040 affecting approximately 2.3 million people. Importantly, OA and its sequela has also been identified as a risk factor for other diseases, such as cardiovascular disease, which is thought to be due to lower mobility at advanced OA disease stages. Consequently, OA has been identified as a serious disease determining morbidity and mortality in the elderly[18].

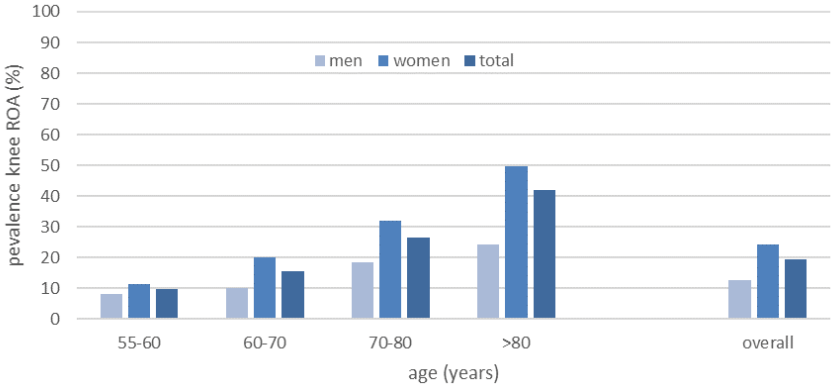


Figure 1.2 Prevalence of radiographic knee OA in the Rotterdam Study

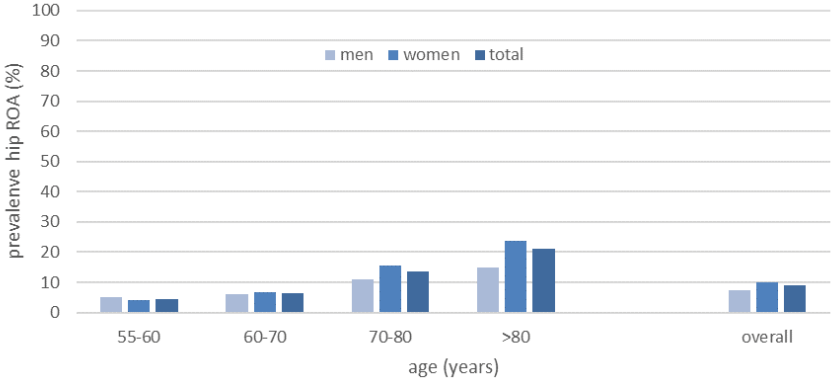


Figure 1.3 Prevalence of radiographic hip OA in the Rotterdam Study

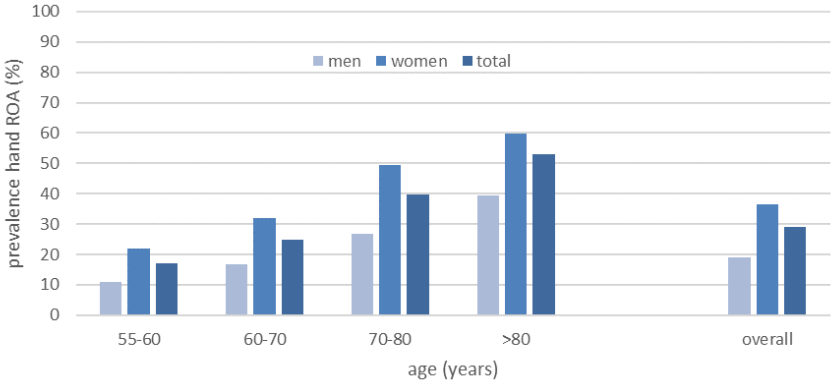


Figure 1.4 Prevalence of radiographic hand OA in the Rotterdam Study

Since there is no cure available for OA or effective preventive strategies, clinical non-surgical management of OA is almost exclusively directed at symptom reduction: treatment directed at managing pain. Non-pharmacological methods such as education and self-management, exercise, weight loss if overweight or obese, and walking aids as indicated are recommended. Pharmacologic modalities for pain reduction include acetaminophen (paracetamol) and NSAIDs (topical or oral), and intra-articular corticosteroids for hip and knee OA. If these strategies fail, joint replacement is the only available treatment option, but this often results in suboptimal resolution of the complaints as well[19-21]. Furthermore, joint replacements only last for a limited period of time[22,23], and thus are less attractive for younger patients[24]. It is therefore important to learn more about biological mechanisms underlying OA, to identify novel targets for treatment and/or prevention of this wide spread and disabling and, thus, important disease.

1.3 Classification of osteoarthritis

OA is characterized by the following changes of the synovial joint **(Figure 1.5)**:

- Clinical: tenderness, pain, stiffness, of the joint with occasional crepitus, local inflammation and effusion

- Pathological: synovial inflammation, thickening of the capsule, focal articular cartilage loss, and bone hypertrophy (subchondral sclerosis and osteophytes)

- Radiological: joint space narrowing, subchondral sclerosis, osteophytes, and occasional pseudo-cysts

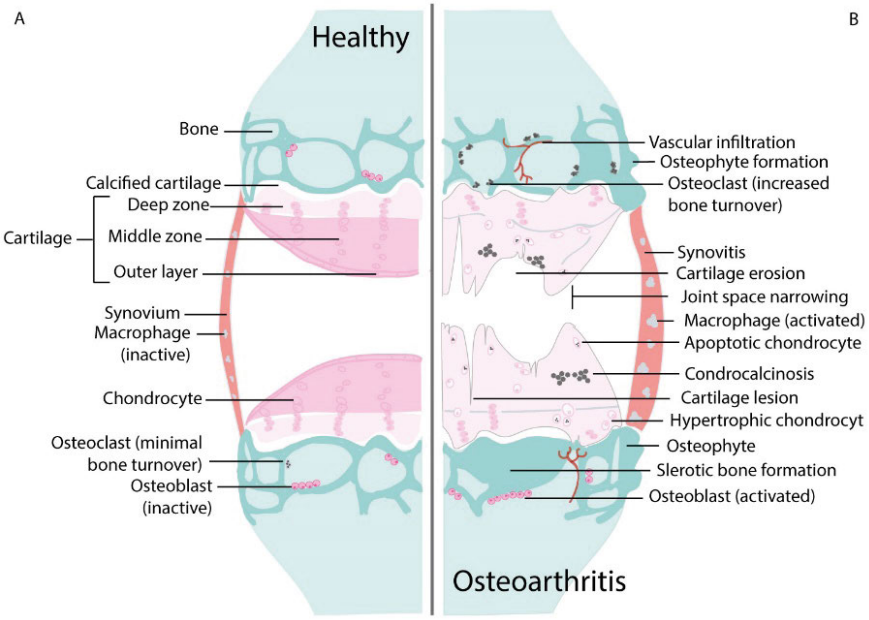
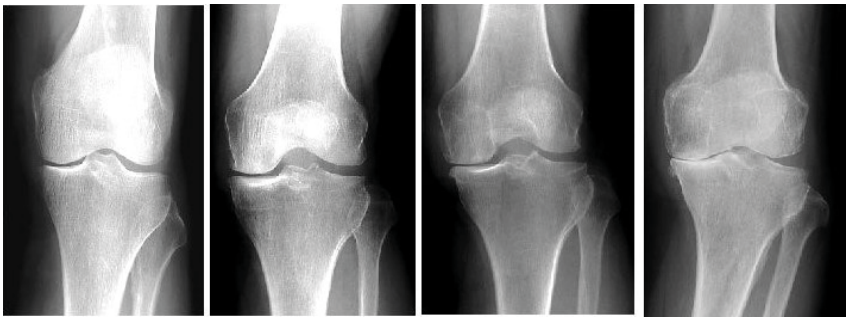


Figure 1.5 Characteristics of a normal and osteoarthritic joint (figure courtesy of Cindy Boer)

Since several phenotypes of OA exist and OA occurs at various joints with varying severity, analysis of OA across epidemiological studies is difficult. Several grading systems for pain and disability are developed[25], of which the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) score for hip and knee OA is one of the most widely used[26]. The WOMAC measures five items for pain (score range 0–20), two for stiffness (score range 0–8), and 17 for functional limitation (score range 0–68). OA can also be graded by radiological features, usually by using the Kellgren & Lawrence score[27] (**Table 1.1, Figure 1.6**), but other grading methods of radiographs, like the Alhbäck score[28], or the BLOKS and WORMS scoring systems for magnetic resonance imaging (MRI) [29-31] have been described. Interestingly, radiological abnormalities do not always correspond to clinical symptoms[17,32], and vice versa[33]. Thus, defining criteria for OA for epidemiological analyses can substantially vary between studies and is still subject of debate.

grade	description
1	doubtful narrowing of the joint space, possible osteophytic lipping
2	definite osteophytes, possible narrowing of the joint space
3	moderate multiple osteophytes, definite joint space narrowing, some sclerosis, possible deforming of bone ends
4	large osteophytes, marked joint space narrowing , severe sclerosis and definite bony end deformity

Table 1.1 Kellgren and Lawrence grading system based on radiographic findings for OA[27]



grade 1 grade 2 grade 3 grade 4

Figure 1.6 Knee radiographs with different grades of OA according to the Kellgren and Lawrence score

1.4 The etiology of osteoarthritis

'For many years confusion has existed in the minds of medical men about the significance of the articular changes called "osteo-arthritis"', T.T. Stamm wrote 8 decades ago (when mainly men were practicing medicine!) in the Lancet[34]. He wanted to end the confusion about the etiology, and thus the treatment of OA, by postulating that the disease was a direct result of pure mechanical wear, plus changes resulting from the reaction of the tissues of the joint. Other later authors preferred to make a distinction between primary and secondary OA. Primary OA, also described as idiopathic or generalized OA[35,36], was believed to be due to some intrinsic defect of cartilage, while secondary OA was thought to be the result of external factors, like congenital abnormalities, infections, or injuries of the joint[37]. However, an increasing number of investigators now believes that

the distinction between primary and secondary OA is invalid, and that OA is, in general, caused by a combination of internal and external factors[38-42].

1.4.1 The role of bone in osteoarthritis

Changes in bone occur as a result of OA, but changes in bone architecture and biology may also contribute to the development of OA. Over the years the role of bone in the etiology of OA has been increasingly subject of research. In 1972, Radin et al. postulated that repetitive impulsive loading of joints leads to trabecular microfracture[38]. This causes bone remodeling, resulting in stiffening of bone and increased stress on articular cartilage, eventually leading to cartilage breakdown and joint degeneration. The observation that patients with severe osteoporosis rarely develop OA supported their hypothesis[43]. This inverse relation between osteoporosis and OA was described also by Foss and Byers[44]. They suggested that both OA and high bone mineral density (BMD) were caused by increased physical activity (including the stress of obesity), although they also discussed the possible negative effect of increased subchondral bone density on overlying articular cartilage.

However, the afore mentioned association between high bone mineral density and OA was not only observed in weight-bearing joints: Roh and co-authors demonstrated that both cortical and trabecular bone mineral content of the distal radius and the second metacarpal bone of the hand were increased in subjects with OA of the hip, suggesting that factors other than wear, tear and local stress play a role in the pathogenesis of OA[45]. The same authors also demonstrated a significant increase in the occurrence of OA at the hands in subjects with hip OA compared to controls[46]. This finding is in line with their suggestion of one or more systemic factors causing OA.

Numerous cross-sectional studies have indicated that OA is associated with a difference in bone properties, like higher BMD[47-50] and higher bone turnover[51-57]. Associations found in cross-sectional studies, however, do not give insight in possible cause-and-effect relationships. Longitudinal studies could give more insight in the possible relation between bone characteristics and OA. Prospective studies on the relation

between aspects of bone quality and OA can be performed in two directions:

1) investigation of alterations in bone quality, for instance the incidence of fractures, in subject with and without OA at baseline, or

2) development of OA in subjects with differences in bone quality at baseline.

1.4.2 Fracture risk and osteoarthritis

Since BMD appears to be increased in subjects with OA[47,49,51,58,59], and low BMD is associated with increased fracture risk[60-62], a protective effect of OA on fracture risk might be expected. Early studies, indeed reported less fractures of the femoral neck in subjects with OA of the hip[44,63,64]. Since then, several investigators have studied the hypothesis that OA protects against fractures. Some studies supported this theory[65-67], whereas two population-based cohort studies showed no decreased fracture risk, in spite of increased BMD[68,69]. The authors of these two longitudinal? studies speculated that the absence of a protective effect of increased BMD on fracture risk might be explained by an increased fall tendency in subjects with OA. Given the inconclusive evidence on the longitudinal relationship between OA and fracture risk, more data is needed before a definitive conclusion can be drawn.

1.4.3 Bone mineral density and osteoarthritis

Another way to study the possible relation between bone characteristics and OA is to investigate whether BMD, bone turnover or fracture history influence the development of OA. Several studies investigated the relation between baseline BMD and the development of different types of OA. They indicated that high BMD at baseline is associated with increased risk of incident hand and knee OA[70]. In addition, the study of Hart and coworkers showed that women with a peripheral (non-vertebral) fracture had reduced risk of subsequently developing incident OA[71]. Finally, Bettica et al. showed that bone resorption is increased in patients with progressive knee OA, similar to that observed in patients with osteoporosis[72]. However, the only longitudinal study on the association between baseline BMD and the development of hip OA suggested that high BMD protects against the development of symptomatic radiographic hip OA[73].

1.4.4 Vitamin D and osteoarthritis

One well known factor influencing bone quality is vitamin D status. Vitamin D deficiency leads to decreased absorption of calcium and phosphate in the intestine, and diminished mineralization of the collagen matrix. Such severe deficiency causes rickets in children and osteomalacia, osteoporosis, and increased fracture risk in adults[74,75] but the influence of more subtle changes in vitamin D status is less clear.

Vitamin D3 (cholecalciferol) is taken in the diet (from fortified dairy products and fish oils) or is synthesized in the skin from 7-dehydrocholesterol by ultraviolet irradiation. The vitamin D produced by 7-dehydrocholesterol depends on the intensity of UV irradiation. In order to be biologically active and affect mineral metabolism and to have effects on numerous other diverse physiological functions including inhibition of growth of cancer cells and protection against certain immune mediated disorders, vitamin D most be converted to its active form, 1,25-dihydroxyvitamin D3. Vitamin D is transported in the blood by the vitamin D binding protein (DBP, a specific binding protein for vitamin D and its metabolites in serum) to the liver. In the liver vitamin D is hydroxylated by one or more cytochrome P450 vitamin D 25 hydroxylases (including CYP2R1, CYP2D11 and CYP2D25), resulting in the formation of 25-hydroxyvitamin D3 (25(OH)D3). 25(OH)D3, the major circulating form of vitamin D, is transported by the DBP to the kidney.

Christakos, S., Ajibade, D.V., Dhawan, P., Fechner, A.J., and Mady, L.J. 2010. Vitamin D: metabolism. *Endocrinol Metab Clin North Am* 39:243-253

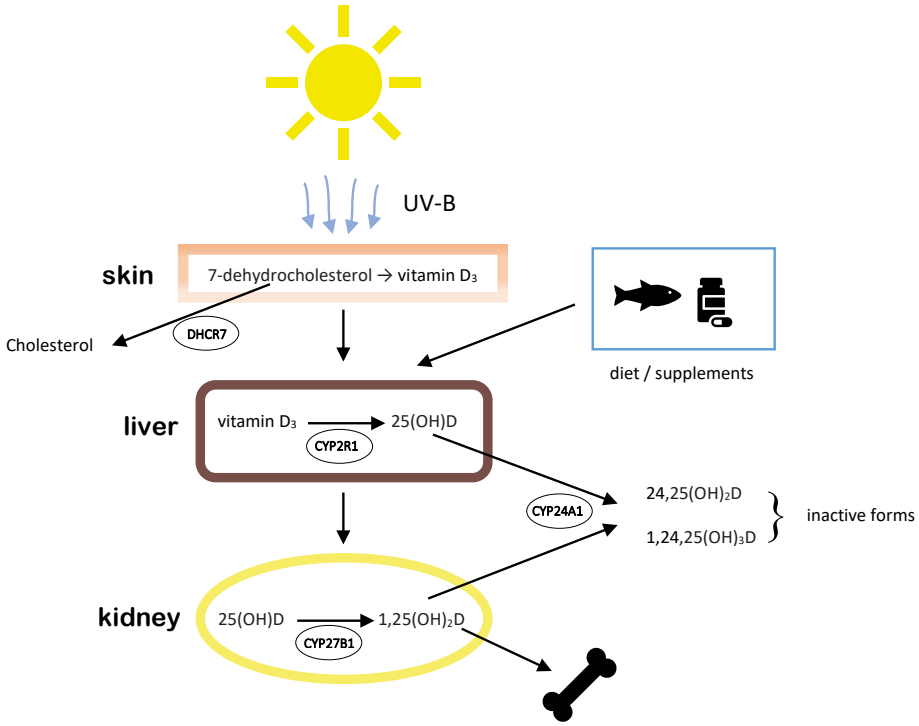


Figure 1.7 Vitamin D metabolism

Because of its influence on bone metabolism, vitamin D status might also influence the risk of developing OA. Although some cross-sectional studies found altered vitamin D serum levels in subjects with OA[76-79], other cross-sectional studies have not[80-87]. The prospective studies on the association between vitamin D levels and OA that have been performed had different endpoints (i.e., bone and/or OA phenotypes assessed in these studies) and showed conflicting results[88-91]. In their systematic review on vitamin D levels and OA, Cao and coworkers concluded that there was moderate evidence that low levels of vitamin D were associated with increased progression of radiographic knee OA, and strong evidence for an association between vitamin D and cartilage loss when combining different imaging results (radiographs and MRI) in knee OA. No evidence was found for an association of vitamin D levels and OA at other joints (i.e., hip of hand). It was

also evident that most studies were modestly sized and heterogeneity across the study with respect to different OA-definitions was high, which prevents a definite conclusion that could be drawn from this systematic review.

1.4.5 Vitamin D, genetics and osteoarthritis

As described above, past studies show no clear relation between vitamin D levels, as measured in the circulation and not at the site of the relevant joint or bone, and the development of OA. This could be due to the fact that vitamin D levels fluctuate in time, depending on variation in sun exposure, dietary intake and vitamin D supplementation (**Figure 1.7**). The vitamin D levels at time of measurement may thus not reflect the vitamin D status over a longer period of time.

A way to obtain more reliable insight of the influence of vitamin D status on the development of OA is by performing a Mendelian Randomization (MR) study. Thus, in relation to our studies, variations in genes of known function relating to OA and/or bone biology, for example genes involved in vitamin D transport, are used to examine the causal effect of a modifiable exposure (vitamin D levels) on disease (OA). Vitamin D levels change over time, and are subject to confounding factors, like outdoor activities, but genetic variations are stable over life, and therefore not influenced by confounding factors.

> Establishing causal relationships between environmental exposures and common diseases faces problems of unresolved confounding, reverse causation and selection bias that may result in spurious associations. Mendelian randomization, in which a functional genetic variant acts as a proxy for an environmental exposure, provides a means of overcoming these problems as the inheritance of genetic variants is independent of—that is randomized with respect to—the inheritance of other traits, according to Mendel's law of independent assortment. Examples drawn from exposures and outcomes as diverse as milk and osteoporosis, alcohol and coronary heart disease, sheep dip and farm workers' compensation neurosis, folate and neural tube defects are used to illustrate the applications of Mendelian randomization approaches in assessing potential environmental causes of disease. As with all genetic epidemiology studies there are problems associated with the need for large sample sizes, the non-replication of findings, and the lack of relevant functional genetic variants.

Two large meta-analyses of genome-wide association studies (GWAS) found common variants near genes involved in cholesterol synthesis (*DHCR7*), hydroxylation

(*CYP2R1*, *CYP24A1*), and vitamin D transport (*GC)* influencing vitamin D status[92,93] (**Figure 1.7)**. In an expansion of this study, two additional loci in SEC23A and in AMDHD1 were found[94]. Individuals with risk alleles at these loci had substantially elevated risk for vitamin D insufficiency. A study on the association between variants in these genes, and several forms of OA in a sufficiently sized cohort study could give more, non-biased, insight in the role of vitamin D in OA.

1.5 Study Cohort

The majority of the studies described in this thesis are performed within the Rotterdam Study (RS). The total size of the Rotterdam Study is approximately 19,000 participants and comprises four cohorts (**Figure 1.8)**. The Rotterdam Study is worldwide the largest population-based cohort with detailed longitudinal data on radiographic (and clinical) parameters of OA.

The Rotterdam Study is a prospective cohort study in the Ommoord district in the city of Rotterdam, the Netherlands on the occurrence and risk factors of chronic disease and disability 1. The recruitment of the original cohort, RS-I, started in January 1990 and consisted of 10.275 men and women aged 55 years or over. The first data collection was performed from October 1990 to July 1993. Since then, participants have been re-examined every ±5 years and the original cohort has been extended with 3 additional cohorts: RS-II; III and IV. During each visit, participants are examined in detail resulting in more than 1500 measurements per participant. In short, participants were interviewed at home and went through an extensive set of examinations, including radiographs of hands, thoraco-lumbar spine, hips and knees, bone mineral densitometry, and sample collections for genetic analyses

Hofman, A., Grobbee, D.E., de Jong, P.T., and van den Ouweland, F.A. 1991. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol 7:403-422.

Ikram, M.A., Brusselle, G.G.O., Murad, S.D., van Duijn, C.M., Franco, O.H., Goedegebure, A., Klaver, C.C.W., Nijsten, T.E.C., Peeters, R.P., Stricker, B.H., et al. 2017. The Rotterdam Study: 2018 update on objectives, design and main results. Eur J Epidemiol 32:807-850.

Radiographs of knees, hips and hands were scored at baseline (RS-I-1, between 1991 and 1993), at the second follow-up visit (RS-I-3, between 1997 and 1999) and at the third follow-up visit (RS-I-4, between 2003 and 2005) for subject who were still able to visit the research center. The mean follow-up time was approximately 8 years. Vitamin D serum levels were measured at baseline (RS-I-1)

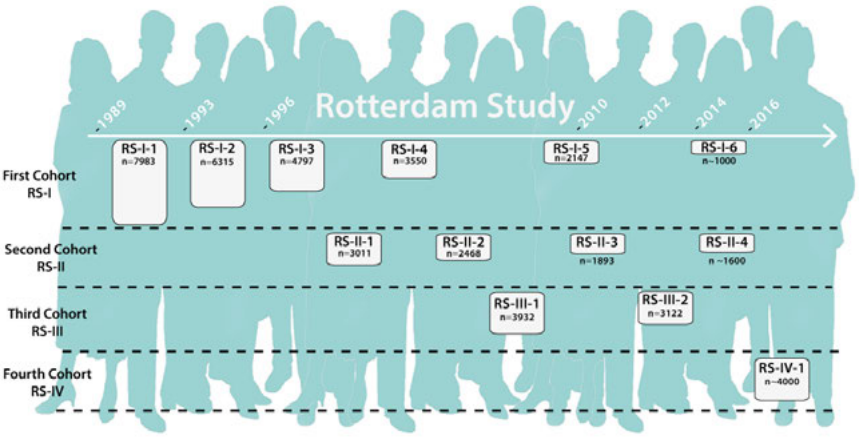


Figure 1.8 The Rotterdam Study (figure courtesy of Cindy Boer)

1.6 Study aims and outline of this thesis

OA has been considered a local degeneration of the joint for many decades. However, over time the notion arose that systemic conditions, like bone metabolism and hormone status, are of influence on the development of OA. In this thesis, therefore the possible association of several of these factors on different types of OA is examined.

In **Chapter 2.1** the results of a study on the association between baseline radiographic knee OA and incident vertebral and non-vertebral fractures are presented, and in **Chapter 2.2** the possible associations between OA and bone quality are studied longitudinally the other way around: whether baseline BMD is of influence on the incidence and progression of knee OA. **Chapter 2.3** provides an up-date and addition of this study, adding more subjects with a longer follow-up, and additionally investigating hip and hand OA.

Chapter 3.1 of this thesis describes the results of a study investigating the possible influence of vitamin D status on the development or worsening of radiological

knee OA, analyzing the data similar to the aforementioned studies. In addition, a meta-analysis on studies on vitamin D serum levels and OA is preformed (**Chapter 3.2**).

In **Chapter 4.1** the results of a Mendelian Randomization study on genetic determinants of vitamin D levels and OA risk are described, with data derived from Iceland (ref) and the UK Biobank study (ref), together comprising 23,877 knee OA cases, 17,151 hip OA cases and over 562,000 controls.

Finally, in the discussion the findings are placed in perspective, overall conclusions are drawn and future recommendations are made.

References

1. Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. European journal of epidemiology. 1991;7(4):403-422.

2. Rothschild BM, Xiaoting Z, Martin LD. Osteoarthritis in the early avian radiation: Earliest recognition of the disease in birds. Cretaceous Research. 6// 2012;35(0):178-180.

3. Mallon JC, Ott CJ, Larson PL, Iuliano EM, Evans DC. Spiclypeus shipporum gen. et sp. nov., a Boldly Audacious New Chasmosaurine Ceratopsid (Dinosauria: Ornithischia) from the Judith River Formation (Upper Cretaceous: Campanian) of Montana, USA. PloS one. 2016;11(5):e0154218.

4. Wenz W, Spranz B, Kosack W. [View of the past: Roentgenography of an Egyptian mummy]. Radiologe. Feb 1975;15(2):45-49.

5. Panzer S, Augat P, Sprenger M, et al. Correlation of atherosclerosis and osteoarthritis in ancient Egypt: A standardized evaluation of 45 whole-body CT examinations. International journal of paleopathology. Jun 2021;33:137-145.

6. Austin A. https://theconversation.com/what-tracing-a-tough-commute-in-ancient-egypt-reveals-about-osteoarthritis-85410. 2017.

7. Murphy WA, Jr., Nedden Dz D, Gostner P, Knapp R, Recheis W, Seidler H. The iceman: discovery and imaging. Radiology. Mar 2003;226(3):614-629.

8. Sacks JJ, Luo YH, Helmick CG. Prevalence of specific types of arthritis and other rheumatic conditions in the ambulatory health care system in the United States, 2001-2005. Arthritis care & research. Apr 2010;62(4):460-464.

9. Vina ER, Kwoh CK. Epidemiology of osteoarthritis: literature update. Current opinion in rheumatology. Mar 2018;30(2):160-167.

10. Disease GBD, Injury I, Prevalence C. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet. Nov 10 2018;392(10159):1789-1858.

11. Dillon CF, Rasch EK, Gu Q, Hirsch R. Prevalence of knee osteoarthritis in the United States: arthritis data from the Third National Health and Nutrition Examination Survey 1991-94. The Journal of rheumatology. Nov 2006;33(11):2271-2279.

12. Felson DT, Naimark A, Anderson J, Kazis L, Castelli W, Meenan RF. The prevalence of knee osteoarthritis in the elderly. The Framingham Osteoarthritis Study. Arthritis and rheumatism. 1987;30(8):914-918.

13. Jordan JM, Helmick CG, Renner JB, et al. Prevalence of knee symptoms and radiographic and symptomatic knee osteoarthritis in African Americans and Caucasians: the Johnston County Osteoarthritis Project. The Journal of rheumatology. Jan 2007;34(1):172-180.

14. Hunter DJ, Schofield D, Callander E. The individual and socioeconomic impact of osteoarthritis. Nature reviews. Rheumatology. Jul 2014;10(7):437-441.

15. RIVM. https://www.volksgezondheidenzorg.info/onderwerp/artrose/cijfers-context/huidige-situatie#!node-prevalentie-en-aantal-nieuwe-gevallen-van-artrose-huisartsenpraktijk. 2020.

16. International Classification of Primary Care.

17. Odding E, Valkenburg HA, Algra D, Vandenouweland FA, Grobbee DE, Hofman A. Associations of radiological osteoarthritis of the hip and knee with locomotor disability in the Rotterdam Study. Annals of the rheumatic diseases. 1998;57(4):203-208.

18. OARSI White Paper - OA as a Serious Disease. 2016; https://oarsi.org/education/oarsi-resources/oarsi-white-paper-oa-serious-disease.

19. Scott CE, Bugler KE, Clement ND, MacDonald D, Howie CR, Biant LC. Patient expectations of arthroplasty of the hip and knee. The Journal of bone and joint surgery. British volume. Jul 2012;94(7):974-981.

20. Tilbury C, Haanstra TM, Leichtenberg CS, et al. Unfulfilled Expectations After Total Hip and Knee Arthroplasty Surgery: There Is a Need for Better Preoperative Patient Information and Education. The Journal of arthroplasty. Oct 2016;31(10):2139-2145.

21. de Beer J, Petruccelli D, Adili A, Piccirillo L, Wismer D, Winemaker M. Patient perspective survey of total hip vs total knee arthroplasty surgery. The Journal of arthroplasty. Jun 2012;27(6):865-869 e861-865.

22. Evans JT, Walker RW, Evans JP, Blom AW, Sayers A, Whitehouse MR. How long does a knee replacement last? A systematic review and meta-analysis of case series and national registry reports with more than 15 years of follow-up. Lancet. Feb 16 2019;393(10172):655-663.

23. Evans JT, Evans JP, Walker RW, Blom AW, Whitehouse MR, Sayers A. How long does a hip replacement last? A systematic review and meta-analysis of case series and national registry reports with more than 15 years of follow-up. Lancet. Feb 16 2019;393(10172):647-654.

24. Wainwright C, Theis JC, Garneti N, Melloh M. Age at hip or knee joint replacement surgery predicts likelihood of revision surgery. The Journal of bone and joint surgery. British volume. Oct 2011;93(10):1411-1415.

25. Collins NJ, Misra D, Felson DT, Crossley KM, Roos EM. Measures of knee function: International Knee Documentation Committee (IKDC) Subjective Knee Evaluation Form, Knee Injury and Osteoarthritis Outcome Score (KOOS), Knee Injury and Osteoarthritis Outcome Score Physical Function Short Form (KOOS-PS), Knee Outcome Survey Activities of Daily Living Scale (KOS-ADL), Lysholm Knee Scoring Scale, Oxford Knee Score (OKS), Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), Activity Rating Scale (ARS), and Tegner Activity Score (TAS). Arthritis care & research. Nov 2011;63 Suppl 11:S208-228.

26. WOMAC. 1982; https://www.womac.com/womac/index.php.

27. Kellgren JH, Jeffrey MR, Ball J. Atlas of Standard Radiographs of Arthritis: The epidemiology of Chronic Rheumatism. Vol 2. Oxford: Blackwell Scientific Publications; 1963.

28. Ahlback S. Osteoarthrosis of the knee. A radiographic investigation. Acta Radiol Diagn (Stockh). 1968:Suppl 277:277-272.

29. Hunter DJ, Lo GH, Gale D, Grainger AJ, Guermazi A, Conaghan PG. The reliability of a new scoring system for knee osteoarthritis MRI and the validity of bone marrow lesion assessment: BLOKS (Boston Leeds Osteoarthritis Knee Score). Annals of the rheumatic diseases. Feb 2008;67(2):206-211.

30. Lynch JA, Roemer FW, Nevitt MC, et al. Comparison of BLOKS and WORMS scoring systems part I. Cross sectional comparison of methods to assess cartilage morphology, meniscal damage and bone marrow lesions on knee MRI: data from the osteoarthritis initiative. Osteoarthritis and cartilage. Nov 2010;18(11):1393-1401.

31. Felson DT, Lynch J, Guermazi A, et al. Comparison of BLOKS and WORMS scoring systems part II. Longitudinal assessment of knee MRIs for osteoarthritis and suggested approach based on their performance: data from the Osteoarthritis Initiative. Osteoarthritis and cartilage. Nov 2010;18(11):1402-1407.

32. Dahaghin S, Bierma-Zeinstra SM, Ginai AZ, Pols HA, Hazes JM, Koes BW. Prevalence and pattern of radiographic hand osteoarthritis and association with pain and disability (the Rotterdam study). Annals of the rheumatic diseases. May 2005;64(5):682-687.

33. Illingworth KD, El Bitar Y, Siewert K, Scaife SL, El-Amin S, Saleh KJ. Correlation of WOMAC and KOOS scores to tibiofemoral cartilage loss on plain radiography and 3 Tesla MRI: data from the osteoarthritis initiative. Knee surgery, sports traumatology, arthroscopy : official journal of the ESSKA. Jul 2014;22(7):1649-1658.

34. Stamm TT. Aetiology and treatment of osteo-arthritis. Lancet. 1939;2:754-756.

35. Cecil R, Archer B. Classification and treatment of chronic arthritis. Jama. 1926;87(741-6).

36. Kellgren JH, Moore R. Generalized osteoarthritis and Heberden's nodes. Br Med J. Jan 26 1952;1(4751):181-187.

37. Lee P, Rooney PJ, Sturrock RD, Kennedy AC, Dick WC. The etiology and pathogenesis of osteoarthrosis: a review. Seminars in arthritis and rheumatism. Spring 1974;3(3):189-218.

38. Radin EL, Paul IL, Rose RM. Role of mechanical factors in pathogenesis of primary osteoarthritis. Lancet. 1972;1(7749):519-522.

39. Solomon L. Patterns of osteoarthritis of the hip. The Journal of bone and joint surgery. British volume. May 1976;58(2):176-183.

40. Doherty M, Watt I, Dieppe P. Influence of primary generalised osteoarthritis on development of secondary osteoarthritis. Lancet. 1983;2(8340):8-11.

41. Palazzo C, Nguyen C, Lefevre-Colau MM, Rannou F, Poiraudeau S. Risk factors and burden of osteoarthritis. Ann Phys Rehabil Med. Jun 2016;59(3):134-138.

42. O'Neill TW, McCabe PS, McBeth J. Update on the epidemiology, risk factors and disease outcomes of osteoarthritis. Best practice & research. Clinical rheumatology. Apr 2018;32(2):312-326.

43. Smith RW, Jr., Rizek J. Epidemiologic studies of osteoporosis in Women of Puerto Rico and southeastern Michigan with special reference to age, race, national origin and to other related or associated findings. Clin Orthop Relat Res. Mar-Apr 1966;45:31-48.

44. Foss MV, Byers PD. Bone density, osteoarthrosis of the hip, and fracture of the upper end of the femur. Annals of the rheumatic diseases. 1972;31(4):259-264.

45. Roh YS, Dequeker J, Mulier JC. Bone mass in osteoarthrosis, measured in vivo by photon absorption. J Bone Joint Surg [Am]. 1974;56(3):587-591.

46. Roh YS, Dequeker J, Mulier JC. Osteoarthrosis at the hand skeleton in primary osteoarthrosis of the hip and in normal controls. Clin Orthop Relat Res. Jan-Feb 1973(90):90-94.

47. Hannan MT, Anderson JJ, Zhang Y, Levy D, Felson DT. Bone mineral density and knee osteoarthritis in elderly men and women. The Framingham Study. Arthritis and rheumatism. Dec 1993;36(12):1671-1680.

48. Hart DJ, Mootoosamy I, Doyle DV, Spector TD. The relationship between osteoarthritis and osteoporosis in the general population: the Chingford Study. Annals of the rheumatic diseases. 1994;53(3):158-162.

49. Nevitt MC, Lane NE, Scott JC, et al. Radiographic osteoarthritis of the hip and bone mineral density. The Study of Osteoporotic Fractures Research Group. Arthritis and rheumatism. 1995;38(7):907-916.

50. Lethbridge-Cejku M, Tobin JD, Scott WW, Jr., et al. Axial and hip bone mineral density and radiographic changes of osteoarthritis of the knee: data from the Baltimore Longitudinal Study of Aging. The Journal of rheumatology. 1996;23(11):1943-1947.

51. 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.

52. Peel NF, Barrington NA, Blumsohn A, Colwell A, Hannon R, Eastell R. Bone mineral density and bone turnover in spinal osteoarthrosis. Annals of the rheumatic diseases. Nov 1995;54(11):867-871.

53. Sowers M, Lachance L, Jamadar D, et al. The associations of bone mineral density and bone turnover markers with osteoarthritis of the hand and knee in pre- and perimenopausal women. Arthritis and rheumatism. 1999;42(3):483-489.

54. Stewart A, Black A, Robins SP, Reid DM. Bone density and bone turnover in patients with osteoarthritis and osteoporosis. The Journal of rheumatology. 1999;26(3):622-626.

55. El Miedany YM, Mehanna AN, El Baddini MA. Altered bone mineral metabolism in patients with osteoarthritis. Joint Bone Spine. 2000;67(6):521-527.

56. Naitou K, Kushida K, Takahashi M, Ohishi T, Inoue T. Bone mineral density and bone turnover in patients with knee osteoarthritis compared with generalized osteoarthritis. Calcified tissue international. 2000;66(5):325-329.

57. Dequeker J, Aerssens J, Luyten FP. Osteoarthritis and osteoporosis: clinical and research evidence of inverse relationship. Aging Clin Exp Res. Oct 2003;15(5):426-439.

58. Dequeker J. Inverse relationship of interface between osteoporosis and osteoarthritis. The Journal of rheumatology. 1997;24(4):795-798.

59. Zhang Y, Hannan MT, Chaisson CE, et al. Bone mineral density and risk of incident and progressive radiographic knee osteoarthritis in women: the Framingham Study. The Journal of rheumatology. Apr 2000;27(4):1032-1037.

60. Legrand E, Chappard D, Pascaretti C, et al. Bone mineral density and vertebral fractures in men. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 1999;10(4):265-270.

61. De Laet CE, van Hout BA, Burger H, Hofman A, Pols HA. Bone density and risk of hip fracture in men and women: cross sectional analysis [published erratum appears in BMJ 1997 Oct 11;315(7113):916]. Bmj. 1997;315(7102):221-225.

62. Marshall D, Johnell O, Wedel H. Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures]. Bmj. 1996;312(7041):1254-1259.

63. Pogrund H, Rutenberg M, Makin M, Robin G, Menczel J, Steinberg R. Osteoarthritis of the hip joint and osteoporosis: a radiological study in a random population sample in Jerusalem. Clin Orthop. 1982(164):130-135.

64. Byers PD, Contepomi CA, Farkas TA. A post mortem study of the hip joint. Including the prevalence of the features of the right side. Annals of the rheumatic diseases. 1970;29(1):15-31.

65. Cumming RG, Klineberg RJ. Epidemiological study of the relation between arthritis of the hip and hip fractures. Annals of the rheumatic diseases. 1993;52(10):707-710.

66. Dequeker J, Johnell O. Osteoarthritis protects against femoral neck fracture: the MEDOS study experience. Bone. 1993;14(Suppl 1):S51-56.

67. Kanis J, Johnell O, Gullberg B, et al. Risk factors for hip fracture in men from southern Europe: the MEDOS study. Mediterranean Osteoporosis Study. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 1999;9(1):45-54.

68. Jones G, Nguyen T, Sambrook PN, Lord SR, Kelly PJ, Eisman JA. Osteoarthritis, bone density, postural stability, and osteoporotic fractures: a population based study. The Journal of rheumatology. 1995;22(5):921-925.

69. Arden NK, Nevitt MC, Lane NE, et al. Osteoarthritis and risk of falls, rates of bone loss, and osteoporotic fractures. Study of Osteoporotic Fractures Research Group. Arthritis and rheumatism. Jul 1999;42(7):1378-1385.

70. Hardcastle SA, Dieppe P, Gregson CL, et al. Individuals with high bone mass have an increased prevalence of radiographic knee osteoarthritis. Bone. Feb 2015;71:171-179.

71. Hart DJ, Cronin C, Daniels M, Worthy T, Doyle DV, Spector TD. The relationship of bone density and fracture to incident and progressive radiographic osteoarthritis of the knee: the Chingford Study. Arthritis and rheumatism. 2002;46(1):92-99.

72. Bettica P, Cline G, Hart DJ, Meyer J, Spector TD. Evidence for increased bone resorption in patients with progressive knee osteoarthritis: longitudinal results from the Chingford study. Arthritis and rheumatism. Dec 2002;46(12):3178-3184.

73. Barbour KE, Murphy LB, Helmick CG, Hootman JM, Renner JB, Jordan JM. Bone Mineral Density and the Risk of Hip and Knee Osteoarthritis: The Johnston County Osteoarthritis Project. Arthritis care & research. Jan 27 2017.

74. Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. Endocrine reviews. Aug 2001;22(4):477-501.

75. Holick MF. Vitamin D deficiency. N Engl J Med. Jul 19 2007;357(3):266-281.

76. Breijawi N, Eckardt A, Pitton MB, et al. Bone mineral density and vitamin D status in female and male patients with osteoarthritis of the knee or hip. Eur Surg Res. 2009;42(1):1-10.

77. Rapin CH, Lagier R. Age-related blood changes in hip osteoarthritis patients: a possible indicator of bone quality. Annals of the rheumatic diseases. Apr 1982;41(2):215-216.

78. Ding C, Cicuttini F, Parameswaran V, Burgess J, Quinn S, Jones G. Serum levels of vitamin D, sunlight exposure, and knee cartilage loss in older adults: the Tasmanian older adult cohort study. Arthritis and rheumatism. May 2009;60(5):1381-1389.

79. Chaganti RK, Parimi N, Cawthon P, Dam TL, Nevitt MC, Lane NE. Association of 25-hydroxyvitamin D with prevalent osteoarthritis of the hip in elderly men: the osteoporotic fractures in men study. Arthritis and rheumatism. Feb 2010;62(2):511-514.

80. Bird HA, Peacock M, Storer JH, Wright V. Comparison of serum 25-OH vitamin D concentrations in rheumatoid arthritis and osteoarthrosis. Br Med J. Jun 14 1980;280(6229):1416.

81. Bird HA, Wright V, Hennes U, Theiss E. Comparison of serum 1,25-dihydroxycholecalciferol concentrations in rheumatoid arthritis and osteoarthrosis. Annals of the rheumatic diseases. Jun 1982;41(3):257-258.

82. Geusens P, Dequeker J, Verstraeten A. Age-related blood changes in hip osteoarthritis patients: a possible indicator of bone quality. Annals of the rheumatic diseases. Feb 1983;42(1):112-113.

83. Roberts NB, Holding JD, Walsh HP, et al. Serial changes in serum vitamin K1, triglyceride, cholesterol, osteocalcin and 25-hydroxyvitamin D3 in patients after hip replacement for fractured neck of femur or osteoarthritis. European journal of clinical investigation. Jan 1996;26(1):24-29.

84. Hunter DJ, Hart D, Snieder H, Bettica P, Swaminathan R, Spector TD. Evidence of altered bone turnover, vitamin D and calcium regulation with knee osteoarthritis in female twins. Rheumatology. Nov 2003;42(11):1311-1316.

85. Kalichman L, Kobyliansky E. Association between circulatory levels of vitamin D and radiographic hand osteoarthritis. Rheumatology international. Jan 2012;32(1):253-257.

86. Muraki S, Dennison E, Jameson K, et al. Association of vitamin D status with knee pain and radiographic knee osteoarthritis. Osteoarthritis and cartilage. Nov 2011;19(11):1301-1306.

87. Al-Jarallah KF, Shehab D, Al-Awadhi A, Nahar I, Haider MZ, Moussa MA. Are 25(OH)D levels related to the severity of knee osteoarthritis and function? Med Princ Pract. 2012;21(1):74-78.

88. McAlindon TE, Felson DT, Zhang Y, et al. Relation of dietary intake and serum levels of vitamin D to progression of osteoarthritis of the knee among participants in the Framingham Study. Annals of internal medicine. Sep 1 1996;125(5):353-359.

89. Lane NE, Gore LR, Cummings SR, et al. Serum vitamin D levels and incident changes of radiographic hip osteoarthritis: a longitudinal study. Study of Osteoporotic Fractures Research Group. Arthritis and rheumatism. 1999;42(5):854-860.

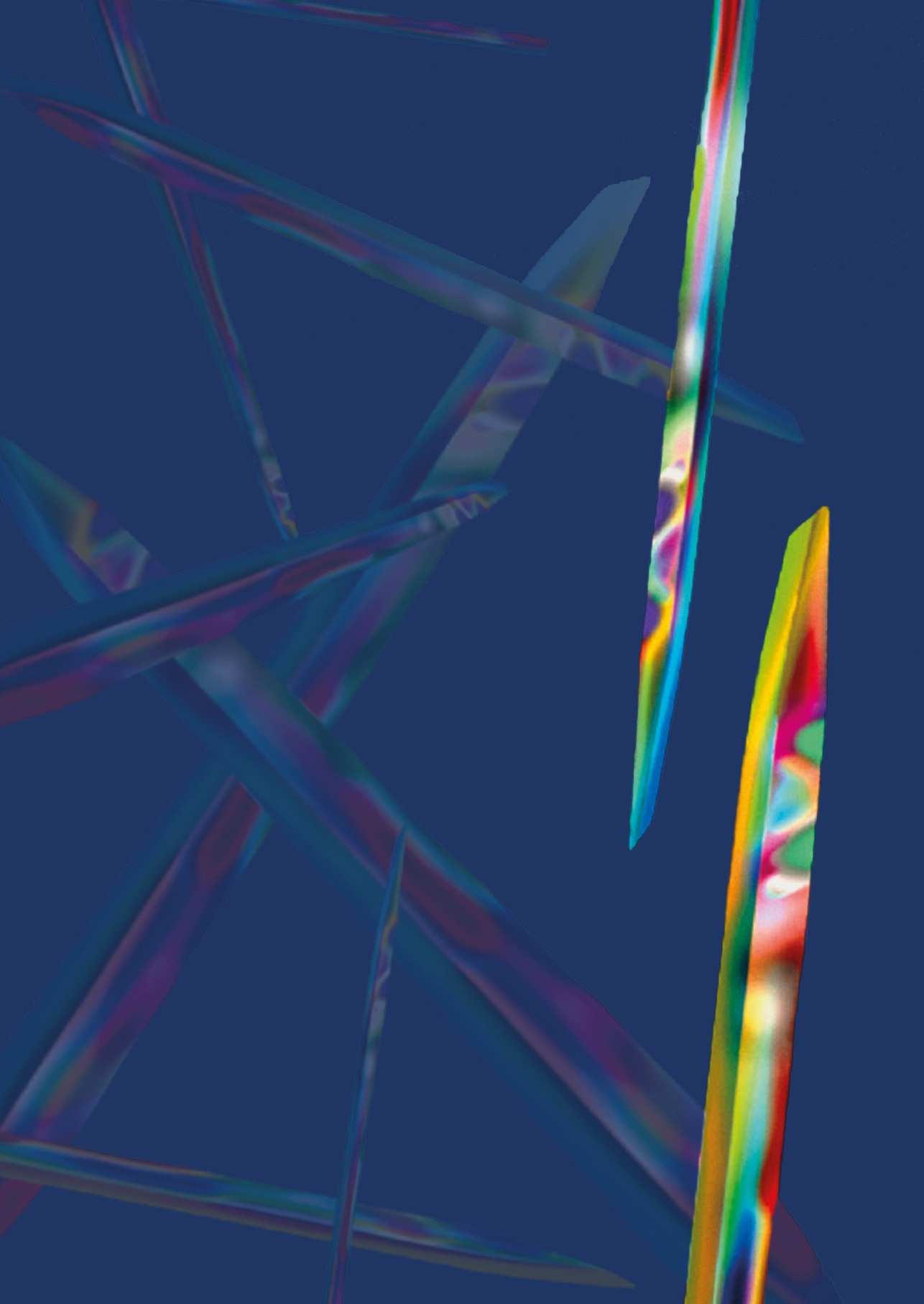
90. Felson DT, Niu J, Clancy M, et al. Low levels of vitamin D and worsening of knee osteoarthritis: results of two longitudinal studies. Arthritis and rheumatism. Jan 2007;56(1):129-136.

91. Konstari S, Paananen M, Heliovaara M, et al. Association of 25-hydroxyvitamin D with the incidence of knee and hip osteoarthritis: a 22-year follow-up study. Scandinavian journal of rheumatology. Mar 2012;41(2):124-131.

92. Ahn J, Yu K, Stolzenberg-Solomon R, et al. Genome-wide association study of circulating vitamin D levels. Human molecular genetics. Jul 1 2010;19(13):2739-2745.

93. Wang TJ, Zhang F, Richards JB, et al. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet. Jul 17 2010;376(9736):180-188.

94. Jiang X, O'Reilly PF, Aschard H, et al. Genome-wide association study in 79,366 European-ancestry individuals informs the genetic architecture of 25-hydroxyvitamin D levels. Nature communications. Jan 17 2018;9(1):260.



2

BONE CHARACTERISTICS AND OSTEOARTHRITIS

2.1 knee osteoarthritis and fracture risk

Osteoarthritis of the knee is associated with vertebral and non-vertebral fractures in the elderly: the Rotterdam Study

Arthritis Rheum 2003 Oct 15;49(5):648-57. PubMed PMID: 14558050

Bergink AP, van der Klift M, Hofman A, Verhaar JA, van Leeuwen JP, Uitterlinden AG, Pols HA

ABSTRACT

Objective. To study the association between prevalent radiographic osteoarthritis (ROA) of the knee and incident vertebral and non-vertebral fractures.

Methods. A sample of 2773 subjects was drawn from the Rotterdam Study, a prospective population-based cohort study of the elderly. Status on knee ROA was assessed at baseline using the Kellgren score. Incident non-vertebral fractures were scored for all subjects, while for 1466 subjects additional data on incident vertebral fractures were available.

Results. Although ROA cases had a higher bone mineral density (BMD), their incident fracture risk was increased as compared to non-cases. After adjustment for potential confounding factors, including parameters of postural stability, the relative risks for incident vertebral and non-vertebral fractures in the presence of knee ROA were 2.0 (95% confidence interval 1.1 to 3.4) and 1.5 (1.1 to 2.0), respectively.

Conclusions: Knee ROA is associated with an increased risk of incident vertebral and non-vertebral fractures, independent of BMD and parameters of postural stability.

INTRODUCTION

Osteoarthritis (OA) and osteoporosis are both common bone disorders in the elderly. Severe forms of these diseases rarely occur simultaneously in a single patient[1-3]. The inverse relationship between OA and osteoporosis has been described extensively in the past[4,5]; whereas osteoporosis is defined as low bone mineral density (BMD)[6], several studies indicate that OA is associated with increased BMD[5,7-10].

Low BMD has been shown to predict fracture risk in several large prospective studies[11-13]. As OA is associated with increased BMD, and high BMD is related to reduced fracture risk, a protective effect of OA on fracture risk might be expected. Early studies indeed reported less fractures of the femoral neck in subjects with osteoarthritis of the hip[1,3,14]. Since then, several investigators have studied this hypothesis, however, with conflicting results. A protective effect of osteoarthritis on hip fractures was reported by several nested cases control studies[2,15,16], whereas two population-based cohort studies on the relationship between incident fractures and OA showed no decreased fracture risk, in spite of increased BMD[17,18]. The absence of a protective effect of increased BMD on fracture risk in the latter two studies was explained by the authors as a result of an increased fall tendency in subjects with osteoarthritis.

The aim of the present study was to investigate the association between radiographic OA (ROA) and fracture risk, and the importance of BMD and postural stability in this association, using a large sample of men and women from a prospective and population-based study.

SUBJECTS AND METHODS

Subjects. The study population consisted of participants of the Rotterdam Study, a prospective cohort study of men and women aged 55 and over. The objective of the Rotterdam Study is to investigate the incidence of, and risk factors for, chronic disabling diseases. The rationale and study design have been described previously[19]. The focus is on neurogeriatric, cardiovascular, ophthalmologic and locomotor diseases, including osteoarthritis and osteoporosis. All 10275 inhabitants of Ommoord, a district in Rotterdam, the Netherlands, were invited to participate. The response rate was 78%, resulting in 7983 subjects participating in the study. Of these, 6450 subjects visited the research center for examination at baseline between 1990 and 1993. Written informed consent was obtained from each participant. The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus University Medical School.

A sample of 2773 subjects was drawn from the Rotterdam Study population. These subjects formed the non-vertebral fractures group. The selection was based on availability of data on ROA of the knees, data on possible confounding factors and follow-up information on the incidence of non-vertebral fractures[20,21]. Additionally, for 1466 subjects (the vertebral fracture group) we had data available on the incidence of thoraco-lumbar vertebral fractures.

Osteoarthritis. At baseline, between 1991 and 1993, weight-bearing anteroposterior radiographs of the knees with the patellae in central position were obtained. ROA was assessed by means of the Kellgren-Lawrence grading system in five grades (from zero to four)[22]. All radiographs were scored for osteoarthritis by two independent observers, who were blinded to all data of the participant, as described previously[20]. After each set of 150 radiographs the scores of the two readers were evaluated. Whenever the Kellgren-score differed, the two readers met to read the X-ray together to reach consensus. A subject was considered to have ROA of the knee, if the Kellgren score of one or both joints was equal to or larger than 2. The presence of osteophytes was scored on a scale of 0 to 3 at four different sites at each knee (medial / lateral distal femur

and medial / lateral proximal tibia), using a validated atlas[23]. These scores were summed and participants were regarded to have osteophytosis when being in the gender-specific highest quartile of this sumscore. Additionally, the distances between femur and tibia in the medial and lateral knee joint compartments were summed for both knees in 2279 persons (82% of the study population). Subsequently, joint space narrowing (JSN) was defined as being in the lowest gender-specific quartile of this sumscore.

Non-vertebral fractures. 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 gate keeper function. For the remaining 20% of the population, research physicians collected data from their GP's records. For hospitalized patients, discharge reports and letters from medical specialists were additionally used for verification. All fractures were coded independently by two research physicians according to the International Classification of Diseases, 10th revision (ICD-10). 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. Fractures of the skull, hand or foot were excluded, because these types of fractures are likely to be caused by trauma rather than by osteoporosis or other intrinsic factors. Validated follow-up data on all fractures and mortality was available from 1991 until the end of 1997, resulting in a mean follow-up time of 5.7 years. Only 10 subjects (0.4%) were lost to follow-up (i.e., no data on fractures or mortality available until the end of 1997) and excluded from the analyses.

Vertebral fracture assessment. Both during baseline and at the second follow-up visit, between 1997 and 1999, lateral radiographs of the thoraco-lumbar spine were taken of subjects who were able to come to the research center by a trained research assistant. At baseline, there were two research technicians available, and one of them took all the radiographs at the follow-up visit. All radiographs were taken following a standard protocol, with a distance between source and plate of 120 cm, using a Solarize FV (General Electric CGR, Milwaukee, WI). The follow-up radiographs were available for 1466 individuals

(53% of the study population), who survived an average 6.6 years after their baseline center visit and who were still able to come to our research center. These follow-up radiographs were evaluated morphometrically by Dr. McCloskey and colleagues (Sheffield, UK) by the McCloskey-Kanis method[24]. If a vertebral fracture was detected, the baseline radiograph was evaluated as well. If a fracture was already present at baseline, it was considered prevalent. 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 pathologic fractures.

Other variables. At baseline, between 1990 and 1993, an extensive baseline home interview on medical history, risk factors for chronic diseases and medication use was performed by trained interviewers. Lower limb disability was assessed using a modified version of the Stanford Health Assessment[25-27]. A lower limb disability index was obtained by calculating the mean score of answers to the following six questions: 'Are you able to stand up from a straight chair without using your arms for support?', 'Are you able to get in and out of bed?', 'Are you able to walk outdoors on flat ground?', 'Are you able to climb up five steps?', 'Are you able to bend down to pick up clothing from the floor?' and 'Are you able to get in and out of a car?'. The answers were scored as follows: 0 = yes, without difficulty, 1 = yes, with some difficulty, 2 = yes, with much difficulty, 3 = no, unable to do. In addition, subjects were asked whether they were using any form of walking aid at the moment of interview and about their fall history over the past year. We calculated a 'fall frequency index', which could range from zero to four (0 = no history of falling, 1 = less than one fall per month, 2 = more than one fall per month, but less than one per week, 3 = more than one per week, but less than daily, and 4 = daily falling). The variables lower limb disability index, use of walking aid and fall frequency index were considered to be variables of postural stability. Information on smoking status was obtained and scored as ever smoked (current and past smoking) or not.

After the home interview, subjects were invited to the research center for clinical examination. Height and weight were measured with subjects wearing indoor clothing and without shoes. Body mass index (BMI) was computed as weight in kilograms divided

by height in squared meters (kg/m^2). BMD measurements of the femoral neck and lumbar vertebrae L2 to L4 was performed by dual energy X-ray absorptiometry (DXA, Lunar DPX-L densitometer, Everberg, Belgium) as described previously[8].

Fo1low up data on postural stability, collected between 1997 and 1999, were available for the 1466 subjects, the same subjects as those with data on incident vertebral fractures.

Statistical analysis. Differences in mean age at baseline between the non-vertebral fracture group, the vertebral fracture group and their source population (the Rotterdam Study) were evaluated by means of ANOVA and the difference in gender ratio by the Chi-square test. All other differences in baseline characteristics were compared by ANCOVA testing with age and gender as covariates. BMD values were additionally adjusted for BMI. In the same way, differences between subjects with and without ROA were compared. Since BMI was significantly different between cases and non-cases, differences in BMD between ROA cases and controls were adjusted for BMI in addition to age and gender. To evaluate the association between ROA and non-vertebral fracture risk, proportional hazards regression analysis (Cox regression) was used to compute hazard ratios (HRs) with 95% confidence intervals as estimation for relative fracture risk. In contrast to the non-vertebral fractures, no information on the point of time of the occurrence of vertebral fractures was available. Therefore, logistic regression analysis was used to calculate odds ratios (ORs) with 95% confidence intervals as estimation for relative vertebral fracture risk.

First, we calculated crude hazard and odds ratios. In the next step we adjusted for age, gender, height and weight. Subsequently, we added femoral neck BMD in the regression model, and, finally, we added covariates of postural stability (lower limb disability index, use of walking aid and tendency to fall) and smoking status. Femoral neck and not lumbar spine BMD was used for adjustment, since we had no data on possible osteophytes of the lumbar spine. Such osteophytes may lead to an overestimation of the lumbar spine BMD and is, therefore, less suitable for adjustment[28,29]. Additionally, analyses stratified by gender were performed on both the vertebral and non-vertebral fracture

groups. In the limited set with complete follow-up data (being the vertebral fracture group), the hazard and odds ratios of non-vertebral respectively vertebral fracture risk were adjusted for follow-up data on postural stability.

To further investigate the role of BMD in the relation between ROA and fracture risk, a stratified analysis was performed, comparing relative risk estimates in subjects with low and high BMD. Low and high BMD was defined as being below or above the age- and gender-adjusted median of the femoral neck BMD, respectively. P-values of the differences in risks were computed using the Z-test, using the formula: $|Z| = \{\ln RE_1 - \ln RE_2\} / \{ \sqrt{} [(se_1)^2 + (se_2)^2]\}$, in which 'RE' is risk estimate, and 'se' is standard error of the regression coefficient, as computed using the regression model. We used SPSS version 10.1.0 (SPSS Inc., Chicago, USA) for all our analyses.

RESULTS

In **Table 2.1.1**, the baseline characteristics of the Rotterdam Study population, the study population with data on non-vertebral fracture incidence and the group in which vertebral fracture incidence was studied are shown. Compared to the source population of the Rotterdam Study, the non-vertebral fracture group was on average 1.6 years younger. In the vertebral fracture group, the participants were on average 4 years younger. In both groups, the variables of immobility were significantly decreased as compared to the source population: the lower limb disability index was on average a 25% lower and relatively less subjects were using a walking aid.

Table 2.1.1. Baseline characteristics of subjects in the Rotterdam Study compared to the study population (with data on ROA of the knee and non-vertebral fractures) and subgroup (with data on ROA knee and vertebral fractures).

	Rotterdam Study (n = 7983)	non-vertebral fracture group (n = 2773)	p	vertebral fracture group (n = 1466)	p
Age, years	70.6 ± 9.8	69.0 ± 7.9	< 0.01	66.4 ± 6.7	< 0.01
Female	61%	60%	0.17	59%	0.17
Height, cm	166.6 ± 7.6	166.6 ± 6.4	0.86	166.8 ± 6.4	0.65
Weight, kg	73.0 ± 13.0	73.2 ± 10.9	0.34	73.5 ± 11.0	0.12
Body mass index, kg/m^2	26.3 ± 4.5	26.4 ± 3.7	0.22	26.4 ± 3.7	0.13
BMD femoral neck, gm/cm^2	0.835 ± 0.20	0.838 ± 0.11	0.40	0.843 ± 0.11	0.14
BMD lumbar spine, gm/cm^2	1.09 ± 0.30	1.09 ± 0.21	0.74	1.09 ± 0.19	0.60
Lower limb disability index	0.44 ± 0.59	0.33 ± 0.52	< 0.01	0.32 ± 0.53	< 0.01
Fall frequency index	0.20 ± 0.48	0.20 ± 0.59	0.75	0.19 ± 0.59	0.50
Use of walking aid	11%	7%	< 0.01	7%	< 0.01
Smoking ever	64%	66%	0.03	65	0.50

Table 2.1.2 shows the baseline characteristics of the two groups, in which we studied the vertebral and non-vertebral fracture risks, stratified by ROA of the knee. In the non-vertebral fracture group, 675 subjects (24%) were diagnosed to have ROA of the knee at baseline (having a Kellgren score of two or higher), whereas in the vertebral fracture group the number of ROA cases was 320 (22%). Both groups show a similar pattern of differences in baseline characteristics in the absence and presence of ROA of the knee. In participants with ROA of the knee, the proportion of women was increased compared to subjects without ROA of the knee. Subjects with ROA of the knee were on average up to four years older. After adjustment for age and gender, they were half a centimeter taller and had a 6 kg higher weight. The age-, gender- and BMI-adjusted BMD of the femoral neck and lumbar spine was 2 to 4% higher in ROA cases in both groups. In addition, the differences in values of other factors that could confound the relationship between ROA and fracture risk are displayed. In subjects with ROA of the knee, all values of variables associated with postural instability (i.e., lower limb disability index, fall frequency index and use of walking aid) are increased. Participants with knee ROA smoked less as compared to subject without ROA of the knee.

Table 2.1.2. Characteristics of potential confounding factors in the non-vertebral fracture group (n = 2773) and vertebral fracture group
(n = 1466) by radiographic osteoarthritis of the knee.

	non-vertebral fracture group			vertebral fracture group		
	ROA absent (n = 2098)	ROA present (n = 675)	p	ROA absent (n = 1146)	ROA present (n = 320)	p
Age, years	68.0 ± 7.6	71.9 ± 8.1	< 0.01	65.9 ± 6.6	68.4 ± 6.9	< 0.01
Female	55%	73%	< 0.01	55%	72%	< 0.01
Height, cm	166.5 ± 9.1	166.9 ± 8.7	0.12	167.3 ± 9.0	167.9 ± 8.2	0.17
Weight, kg	71.9 ± 11.5	77.5 ± 12.1	< 0.01	72.8 ± 11.1	78.7 ± 12.1	< 0.01
Body mass index, kg/m^2	25.9 ± 3.5	27.8 ± 4.0	< 0.01	26.0 ± 3.4	28.0 ± 4.0	< 0.01
BMD femoral neck, gm/cm^2	0.828 ± 0.14	0.852 ± 0.14	< 0.01	0.844 ± 0.13	0.861 ± 0.14	0.03
BMD lumbar spine, gm/cm^2	1.08 ± 0.20	1.12 ± 0.21	< 0.01	1.08 ± 0.19	1.12 ± 0.20	< 0.01
Smoking ever	69%	64%	< 0.01	70%	62%	0.01
Lower limb disability index - baseline	0.27 ± 0.40	0.39 ± 0.54	< 0.01	0.18 ± 0.32	0.30 ± 0.467	< 0.01
- follow-up				0.14 ± 0.43	0.48 ± 0.59	< 0.01
Fall frequency index - baseline	0.18 ± 0.40	0.22 ± 0.48	0.05	0.15 ± 0.38	0.17 ± 0.42	0.49
- follow-up				0.22 ± 0.42	0.29 ± 0.47	0.02
Use of walking aid - baseline	5%	11%	< 0.01	2%	8%	< 0.01
- follow-up				7%	14%	< 0.01

Radiographic osteoarthritis (ROA) is defined as having a Kellgren score of two or higher. Values are means ± standard deviations (SD), or percentages.
Age and gender are crude values, with p-value estimated by ANOVA and Chi-square test. All other values are adjusted for age and gender. BMD values are additionally adjusted for BMI. Corresponding p-values are estimated by ANCOVA.

Additionally, the values for the variables as measured at the end of the follow-up period (December 1997) are shown. These variables were available for a limited number of subjects (equal to the vertebral fracture group). When comparing follow-up data to baseline data, all values have increased, especially in the subjects with ROA of the knee. Whereas no significant difference in fall frequency index between participants with and without ROA was seen at baseline, this difference was significant at follow-up.

In **Table 2.1.3**, the numbers and cumulative incidences of the different types of fractures in the absence and presence of ROA of the knee are displayed. During the mean follow-up period of 5.7 years, 196 non-vertebral fractures occurred in the total study population (total cumulative incidence: 7%). Of these fractures, the most common types were hip fractures (28% of all non-vertebral fractures) and wrist fractures (30%). In the vertebral fracture group, 77 vertebral fractures (5%) occurred during 6.6 of years of follow-up. The incidence of all types of fractures was higher in subjects with ROA of the knee than in subjects without knee ROA.

Additionally, the incidence of all fracture types in subjects diagnosed with and without osteophytosis was compared, as well as in subjects with and without joint space narrowing (JSN). For osteophytosis, but not for JSN, a similar association with incident fractures was observed as for ROA defined by Kellgren score.

Table 2.1.3. Frequencies of incident fractures by radiographic osteoarthritis (ROA) of the knee:

	ROA absent	ROA present	
fracture type	% (n / total)	% (n / total)	p
non-vertebral†	6.0% (125 / 2098)	10.5% (71 / 675)	< 0.01
- *hip*	1.4% (30 / 2098)	3.6% (24 / 675)	< 0.01
- *wrist*	1.8% (38 / 2098)	3.0% (20 / 675)	0.07
vertebral‡	4.5% (51 / 1146)	8.1% (26 / 320)	0.01

Frequencies of incident fractures by osteophytosis and joint space narrowing (JSN) of knee:

	osteophytosis			JSN		
fracture type	absent	present	p	absent	present	p
non-vertebral†	6.0%	10.2%	< 0.01	6.7%	7.2%	0.72
- *hip*	1.5%	3.3%	< 0.01	1.5%	2.2%	0.26
- *wrist*	1.8%	2.7%	0.16	1.9%	2.0%	0.85
vertebral‡	4.6%	6.6%	0.13	4.8%	6.1%	0.38

Radiographic osteoarthritis (ROA) is defined as having a Kellgren score of two or higher.
P-values are obtained by chi-square test.
† follow-up time: 5.7 ± 1.5 years
‡ follow-up time: 6.6 ± 0.4 years

The unadjusted risk estimate for a non-vertebral fracture in subjects with ROA of the knee was significantly increased as compared to subjects without ROA of the knee (**Table 2.1.4**). When adjustment was made for age and gender, the risk estimate decreased and significance was no longer reached. When height and weight were added to the regression model, the point estimate increased, and was statistically significant again. Additional adjustment for femoral neck BMD did not change the estimate. When all possible confounders were taken into account, so including use of walking aid, lower limb disability index, fall frequency index and smoking status, the risk estimate remained similar. This stepwise adjustment analysis showed similar results when we analyzed the risks for the two most frequent types of non-vertebral fractures, i.e., hip and wrist fractures, separately. The odds ratio for a vertebral fracture in the presence of ROA of the knee did not change by adjusting for potential confounding factors.

Table 2.1.4. Association between radiographic osteoarthritis of the knee and incident fracture.

fracture type	n	crude HR / OR	HR / OR*	HR / OR**	HR / OR†	HR / OR††
non-vertebral	2773	1.8 (1.4-2.5)	1.3 (0.9-1.7)	1.5 (1.1-2.1)	1.5 (1.1-2.1)	1.5 (1.1-2.0)
-hip		2.6 (1.5-4.5)	1.4 (0.8-2.5)	1.8 (1.0-3.2)	1.8 (1.0-3.4)	1.6 (0.9-2.8)
-wrist		1.7 (1.0-2.9)	1.2 (0.7-2.2)	1.5 (0.8-2.6)	1.5 (0.9-2.7)	1.5 (0.8-2.6)
vertebral	1446	1.9 (1.2-3.1)	1.6 (1.0-2.7)	1.9 (1.2-3.3)	2.0 (1.2-3.4)	2.0 (1.1-3.4)

	men		women		adjusted for follow-up data	
fracture type	n	HR / OR‡	n	HR / OR‡	n	HR / OR‡‡
non-vertebral	1120	1.3 (0.6-2.8)	1653	1.5 (1.0-2.1)	1446	1.3 (0.9-2.0)
-hip		1.7 (0.5-5.7)		1.6 (0.8-3.1)		0.6 (0.2-2.0)
-wrist		[1])		1.5 (0.8-2.7)		1.5 (0.8-3.1)
vertebral	598	0.8 (0.2-3.0)	868	2.6 (1.4-5.0)	1446	1.8 (1.0-3.2)

Associations are presented by hazard ratios (HR) for non-vertebral fractures and odds ratios (OR) for vertebral fractures,
with 95% confidence interval between parentheses:
* adjusted for age, gender; ** adjusted for age, gender, height, weight, † adjusted for age, gender, height, weight and BMD (femoral neck),
†† adjusted for age, gender, height, weight, BMD, baseline lower limb disability index, use of walking aid, fall frequency index and smoking status.
‡ adjusted for age, height, weight, BMD, baseline lower limb disability index, use of walking aid, fall frequency index and smoking status.
‡‡ adjusted for baseline age, gender, height, weight, BMD and follow-up data on lower limb disability index, use of walking aid, fall frequency index and smoking status.
[1]) No wrist fracture cases among men with ROA knee

To further examine possible confounding by gender, stratified analyses were performed. When considering fracture risk in women, where most fractures occur, significantly increased point estimates are found for both vertebral and non-vertebral fracture risk. In men, no significant increased fracture risk in the presence of knee ROA was observed.

Finally, to eliminate possible confounding effects of decreased postural stability during the follow-up period, adjustment for follow-up lower limb disability index, use of walking aid and fall frequency index was made in a limited set of subjects (i.e., the vertebral fracture group). The fracture risk in the presence of knee ROA is still increased, though significance was no longer reached for non-vertebral fracture risk.

Table 2.1.5 shows the adjusted risk estimates for subjects with knee ROA in strata of low (below the median age- and gender-adjusted BMD) and high femoral neck BMD (above the median age- and gender-adjusted BMD). The incidence of all types of fractures was highest in the low BMD group. However, the non-vertebral relative fracture risks in the presence of knee ROA in the upper BMD group are higher as compared to the risks in the lower BMD group, where the point estimates were close to unity and did not reach significance. The differences in non-vertebral risk estimates between the low and high BMD group are significant, except for the differences in hip fracture risk. There were no significant differences between the risk of a vertebral fracture in the presence of knee ROA in the low BMD group compared to the risk in the high BMD group.

Table 2.1.5. Association of radiographic osteoarthritis of the knee and incident fractures by low and high BMD.

		low BMD		high BMD		
fracture type	ROA	n / total (%)	HR / OR	n / total (%)	HR / OR	p
non-vertebral	absent	87 / 1120 (7.8)	1.1 (0.7–1.7)	38 / 978 (3.9)	2.2 (1.3–3.5)	0.04
	present	35 / 267 (13.1)		36 / 408 (8.8)		
- hip	absent	25 / 1120 (2.2)	1.2 (0.6–2.5)	5 / 978 (0.5)	3.4 (1.0–11.6)	0.17
	present	14 / 267 (5.2)		10 / 408 (2.5)		
- wrist	absent	29 / 1120 (2.6)	0.8 (0.3–1.9)	9 / 978 (0.9)	3.0 (1.2–7.4)	0.04
	present	7 / 267 (2.6)		13 / 408 (3.2)		
vertebral	absent	40 / 588 (6.8)	1.8 (0.9–3.4)	11 / 558 (2.0)	2.7 (1.0–7.4)	0.48
	present	16 / 125 (12.8)		10 / 195 (5.1)		

Low and high BMD groups are divided by the median of age- and gender-adjusted femoral neck BMD.
Associations are presented by hazard ratios (HR) for non-vertebral fractures and odds ratios (OR) for vertebral fractures,
with 95% confidence interval between parentheses. All value are adjusted for age, gender, height, weight, lower limb disability index,
use of walking aid, fall frequency index and smoking.
P-values were calculated using the Z-test.

DISCUSSION

This study shows a positive association between ROA of the knee at baseline and non-vertebral fracture risk, and is the first to report an association between knee ROA and increased vertebral fracture risk. These effects remain significant, even when potential confounding factors, i.e., age, gender, weight, height, femoral neck BMD and variables of postural stability, were taken into account. The association appears to be driven by the presence of osteophytes.

A protective effect of osteoarthritis on hip fractures was reported by several nested case control studies, which compared prevalence of self-reported OA in hip fracture cases to controls without hip fracture. In contrast, two population-based cohort

studies on the relationship between OA and incident fractures showed no decreased fracture risk[17]. Jones et al.[17] showed that the protective effect disappeared by taking into account the relationship between self-reported OA and increased tendency to fall. Arden et al.[18] studied both self-reported and radiographically confirmed OA of hip and hands and found no reduced risk of incident vertebral or non-vertebral fracture. In one retrospective study radiographic hip OA, but not knee OA, was even associated with a significant increase in the incidence of non-vertebral fractures[30]. In these three studies, the authors hypothesize that the lack of protective effect of osteoarthritis on fracture risk might be explained by an increased risk of falling due to OA. Together with differences in design and statistical power of these studies, this could explain why some studies do and others do not show a relation between OA and fracture risk.

In our study on the association of ROA of the knee and fracture risk, we attempted to account for potential confounding factors, including BMD and fall tendency. The differences in baseline characteristics we observed between subjects with and without ROA of the knee are in line with previous findings[31,32]. ROA of both knee and hip have been shown to be associated with a significantly higher femoral neck and lumbar spine BMD[5,7-9]. Subjects with ROA in our study also showed decreased postural stability, as reflected by the baseline data on fall frequency and use of a walking aid, which is in agreement with previous reports on the relationship between OA and decreased postural stability[17]. Smoking was inversely related to baseline ROA of the knee, as described previously for other study populations[31-33]. Since smoking and postural stability are also related to fracture risk, they were used to adjust the relation between ROA and incident fracture. After the adjustments for all possible confounding factors available in our study population at baseline, the odds ratio for fracture risk in the presence of knee ROA remained significantly increased. Thus, the observed relation between baseline ROA of the knee and subsequent fracture risk appears not to be explained by these variables. Another potential confounding variable is the use of hormone replacement therapy (HRT). However, of the 1266 women with data on the use of HRT in our study population, only 37 subjects (3%) used HRT. When we excluded these women from the analyses, the results remained unaltered (data not shown).

One might argue that the positive relationship between ROA and fractures could be confounded by an increased fall tendency of the subjects with ROA, and that adjusting through regression modelling cannot neutralize its strong influence on the relation. We tried to overcome this by taking several confounding factors associated with both falling and ROA into account, i.e., lower limb disability index and use of walking aid, both at baseline and at follow-up, and therefore adjusting for any potentially residual confounding effect of increased fall tendency[34]. These variables will also account for other confounding factors, like decreased muscle strength and physical activity in subjects with OA. Furthermore, the association between vertebral fracture and falling is weak; most vertebral fractures are not clinically diagnosed, and if they are, the majority followed moderate or no trauma[35]. Thus, although the association between non-vertebral fracture risk and ROA of the knee could be confounded by falling, this is not likely to be the case for vertebral fracture risk. The same applies for the potential confounding effect of physical activity on the observed association between ROA and vertebral fracture risk[36].

One of the limitations of this study is the relatively low prevalence of ROA and the low incidence of fractures in men. Separate analysis for men and women shows the association between ROA and fracture risk to be driven by the association in women. In men, no significantly increased fracture risk in the presence of knee ROA was observed, possibly due to lack of power. By adjustment for gender, we tried to overcome the potential confounding effects on fracture risk estimates. Another limitation is that follow-up data on postural stability was available for only a limited number of subjects in the non-vertebral fracture group. This reduced the power to detect a significant association between ROA and non-vertebral fracture risk when adjustment for follow-up data on postural stability was made. Furthermore, no data on physical activity or muscle strength was available. However, the potentially confounding effects of these variables are likely to be canceled out by adjusting for lower limb disability index and use of walking aid. Finally, it is likely that a health-based selection is present, since all subjects had to be able to visit the research center. In particular, this type of bias might have occurred in the group with data on vertebral fractures because in order to detect a vertebral fracture, participants had to return to the research center at the end of the follow-up period. Compared to the total

Rotterdam Study, especially the group with data on vertebral fractures was significantly younger, and the mobility (as measured by lower limb disability index and recent use of walking aid) was significantly lower compared to the source population. However, even in this younger and healthier population, with better mobility and less tendency to fall, we found a significant association of ROA and fracture risk. It seems likely, that without health-selection, a similar or even stronger association would be found.

Although the relation between ROA and fracture risk is independent of the potential confounding variables, including BMD, we did observe a significant interaction between BMD and ROA in the regression model of non-vertebral fracture risk. This means, that not only BMD and ROA are independent predictors of non-vertebral fracture risk, but also that the association of ROA and fracture risk is being modified by BMD. When the subjects were stratified by low and high BMD at baseline, we found, as expected, that the percentage of subjects (both ROA cases and non-cases) with fractures is higher in the low BMD group compared to those with high BMD. However, the relative risk of an incident non-vertebral fracture in the presence of knee ROA is larger and significantly increased in subjects with high BMD, compared to those with low BMD. Consequently, it appears that the protective effect of a high BMD on non-vertebral fracture risk is substantially diminished in subjects with ROA of the knee.

When we consider vertebral fracture risk, we also see a lower percentage of subjects with fractures in the high BMD stratum compared to the low BMD stratum. However, here no difference in relative vertebral fracture risk in the presence of ROA was observed between the low and high BMD groups. This suggests that low baseline BMD and ROA are independent predictors of vertebral fracture risk, i.e., no interaction was seen between BMD and ROA on vertebral fracture risk.

The protective effect of a high BMD on fracture risk is counteracted by the presence of ROA, especially in case of non-vertebral fractures. A potential explanation for the increased fracture risk in ROA cases is that, even though these subjects have increased BMD, their bone is of inferior quality. Indeed, several studies showed locally altered

bone properties in osteoarthritis. Kamibayashi et al. demonstrated that osteoarthritic subchondral bone of the knee had a higher bone volume fraction, but the microstructure was characterized by fewer, more widely spaced and thicker than normal trabeculae[37]. In osteoarthritic femoral heads Mansell and colleagues found an increased trabecular collagen metabolism, leading to altered bone morphology[38]. Ding and colleagues showed that the subchondral bone of the proximal tibia in early-stage OA is mechanically inferior compared to normal subchondral bone[39-41].

While all these changes were found at a local level, it might well be that ROA of the knee is associated with more systemic changes in bone, resulting in an increased fracture risk. Aspen et al. postulate that systemic factors could explain the diversity of physiological changes, like increased BMD, in generalized OA[42]. This is in line with the observation of Sowers, that women with knee OA had higher BMD and less bone turnover[43]. Furthermore, a common role of bone turnover and repair in early knee OA, defined by osteophytosis, was suggested by Hart et al.: women in the Chingford study with incident knee OA had higher baseline BMD than those without incident OA[44]. Interestingly, the women in this study with previous fractures (mainly of the wrist and the vertebrae) had less chance of developing OA. This emphasizes the importance of studying the relation between fractures and OA in follow-up studies. The bone properties associated with previous fractures and decreased incidence of OA may well be different from those associated with prevalent OA and increased incidence of fractures. Thus, when studied cross-sectionally, these two possible explanations cannot be distinguished.

That OA can be associated with changes at remote sites was also demonstrated by Gevers et al, who found altered mineralisation and biomechanical changes in the iliac crest of patients with osteoarthritis of the hand[45,46]. The result of these changes was, according to the authors, stronger but less flexible bone. Another study on iliac crest bone of subjects with OA of the hands showed increased concentrations of growth factors IGF-I, IGF-II and TGF-ß at this site[47]. The osteocalcin levels were also raised in these patients, which may reflect a generally increased biosynthetic activity of osteoblasts. These findings support our hypothesis that localized OA can be associated with generalized altered bone

quality, which could lead to the increased fracture risk we observed in our study. The fact that the association is especially observed with the presence of osteophytes strengthens our belief that an altered bone formation is responsible for both knee OA and increased fracture risk.

This systemic difference in bone characteristics between subject with and without osteoarthritis may well be related to genetic factors, as has been recently suggested by Antoniades et al.[10]. In line with these findings, Naganathan observed an increased peak bone mass in the hip in daughters of women with hand OA[48]. Furthermore, candidate genes associated with increased risk for OA, like the estrogen receptor α gene[49], the type I collagen gene[50] or the vitamin D receptor gene[51], have also been related to increased fracture risk[52-55]. This supports our hypothesis that both knee OA and fracture risk are due to a common intrinsic variation in bone structure.

In conclusion, our results show that, despite a higher BMD, subjects with ROA of the knee have an increased risk of fractures, especially of vertebral fractures. This relation appears to be driven by the osteophytosis. We postulate that decreased mechanical properties of the bone in subjects with ROA of the knee may be the underlying cause. Further investigations, like microcomputed tomography imaging of cortical and trabecular bone at peripheral sites of subjects with and without osteoarthritis[56,57] could help to elucidate these structural differences.

REFERENCES

1. Pogrund H, Rutenberg M, Makin M, Robin G, Menczel J, Steinberg R. Osteoarthritis of the hip joint and osteoporosis: a radiological study in a random population sample in Jerusalem. Clin Orthop 1982;164**:**130-5.

2. Cumming RG, Klineberg RJ. Epidemiological study of the relation between arthritis of the hip and hip fractures. Ann Rheum Dis 1993;52**:**707-10.

3. Foss MV, Byers PD. Bone density, osteoarthrosis of the hip, and fracture of the upper end of the femur. Ann Rheum Dis 1972;31**:**259-64.

4. Dequeker J. The relationship between osteoporosis and osteoarthritis. Clin Rheum Dis 1985;11**:**271-96.

5. Dequeker J. Inverse relationship of interface between osteoporosis and osteoarthritis. J Rheumatol 1997;24**:**795-8.

6. Kanis JA. Osteoporosis and osteopenia. J Bone Miner Res 1990;5**:**209-11.

7. Nevitt MC, Lane NE, Scott JC, Hochberg MC, Pressman AR, Genant HK, Cummings SR. Radiographic osteoarthritis of the hip and bone mineral density. The Study of Osteoporotic Fractures Research Group. Arthritis Rheum 1995;38**:**907-16.

8. 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**:**1-13.

9. Hannan MT, Anderson JJ, Zhang Y, Levy D, Felson DT. Bone mineral density and knee osteoarthritis in elderly men and women. The Framingham Study. Arthritis Rheum 1993;36**:**1671-80.

10. Antoniades L, MacGregor AJ, Matson M, Spector TD. A cotwin control study of the relationship between hip osteoarthritis and bone mineral density. Arthritis Rheum 2000;43**:**1450-5.

11. Legrand E, Chappard D, Pascaretti C, Duquenne M, Rondeau C, Simon Y, Rohmer V, Basle MF, Audran M. Bone mineral density and vertebral fractures in men. Osteoporos Int 1999;10**:**265-70.

12. De Laet CE, van Hout BA, Burger H, Hofman A, Pols HA. Bone density and risk of hip fracture in men and women: cross sectional analysis. Bmj 1997;315**:**221-5.

13. Marshall D, Johnell O, Wedel H. Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures [see comments]. Bmj 1996;312**:**1254-9.

14. Byers PD, Contepomi CA, Farkas TA. A post mortem study of the hip joint. Including the prevalence of the features of the right side. Ann Rheum Dis 1970;29**:**15-31.

15. Dequeker J, Johnell O. Osteoarthritis protects against femoral neck fracture: the MEDOS study experience. Bone 1993;14**:**S51-6.

16. Kanis J, Johnell O, Gullberg B, Allander E, Elffors L, Ranstam J, Dequeker J, Dilsen G, Gennari C, Vaz AL, Lyritis G, Mazzuoli G, Miravet L, Passeri M, Perez Cano R, Rapado A, Ribot C. Risk factors for hip fracture in men from southern Europe: the MEDOS study. Mediterranean Osteoporosis Study. Osteoporos Int 1999;9**:**45-54.

17. Jones G, Nguyen T, Sambrook PN, Lord SR, Kelly PJ, Eisman JA. Osteoarthritis, bone density, postural stability, and osteoporotic fractures: a population based study. J Rheumatol 1995;22**:**921-5.

18. Arden NK, Nevitt MC, Lane NE, Gore LR, Hochberg MC, Scott JC, Pressman AR, Cummings SR. Osteoarthritis and risk of falls, rates of bone loss, and osteoporotic fractures. Study of Osteoporotic Fractures Research Group. Arthritis Rheum 1999;42**:**1378-85.

19. 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**:**403-22.

20. Odding E, Valkenburg HA, Algra D, Vandenouweland FA, Grobbee DE, Hofman A. Associations of radiological osteoarthritis of the hip and knee with locomotor disability in the Rotterdam Study. Ann Rheum Dis 1998;57**:**203-8.

21. Burger H, van Daele PL, Odding E, Valkenburg HA, Hofman A, Grobbee DE, Schutte HE, Birkenhager JC, Pols HA. Association of radiographically evident osteoarthritis with higher bone mineral density and increased bone loss with age. The Rotterdam Study. Arthritis Rheum 1996;39**:**81-6.

22. Kellgren JH JM, Ball J. Atlas of Standard Radiographs of Arthritis, Volume 2: The epidemiology of Chronic Rheumatism. Oxford: Blackwell Scientific Publications; 1963.

23. Burnett S, Hart DJ, Cooper C, Spector TD. A Radiographic Atlas of Osteoarthritis. London: Springer Verlag; 1994.

24. 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**:**138-47.

25. Fries JF, Spitz PW, Young DY. The dimensions of health outcomes: the health assessment questionnaire, disability and pain scales. J Rheumatol 1982;9**:**789-93.

26. Siegert CE, Vleming LJ, Vandenbroucke JP, Cats A. Measurement of disability in Dutch rheumatoid arthritis patients. Clin Rheumatol 1984;3**:**305-9.

27. Odding E, Valkenburg HA, Algra D, Vandenouweland FA, Grobbee DE, Hofman A. Association of locomotor complaints and disability in the Rotterdam study. Ann Rheum Dis 1995;54**:**721-5.

28. Liu G, Peacock M, Eilam O, Dorulla G, Braunstein E, Johnston CC. Effect of osteoarthritis in the lumbar spine and hip on bone mineral density and diagnosis of osteoporosis in elderly men and women. Osteoporos Int 1997;7**:**564-9.

29. Rand T, Schneider B, Grampp S, Wunderbaldinger P, Migsits H, Imhof H. Influence of osteophytic size on bone mineral density measured by dual X-ray absorptiometry. Acta Radiol 1997;38**:**210-3.

30. Arden NK, Griffiths GO, Hart DJ, Doyle DV, Spector TD. The association between osteoarthritis and osteoporotic fracture: the Chingford Study. Br J Rheumatol 1996;35**:**1299-304.

31. Cooper C, Inskip H, Croft P, Campbell L, Smith G, McLaren M, Coggon D. Individual risk factors for hip osteoarthritis: obesity, hip injury, and physical activity. Am J Epidemiol 1998;147**:**516-22.

32. Felson DT, Zhang Y, Hannan MT, Naimark A, Weissman B, Aliabadi P, Levy D. Risk factors for incident radiographic knee osteoarthritis in the elderly: the Framingham Study. Arthritis Rheum 1997;40**:**728-33.

33. Felson DT, Anderson JJ, Naimark A, Hannan MT, Kannel WB, Meenan RF. Does smoking protect against osteoarthritis? Arthritis Rheum 1989;32**:**166-72.

34. Nevitt MC, Cummings SR, Kidd S, Black D. Risk factors for recurrent nonsyncopal falls. A prospective study. Jama 1989;261**:**2663-8.

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**:**221-7.

36. Gregg EW, Cauley JA, Seeley DG, Ensrud KE, Bauer DC. Physical activity and osteoporotic fracture risk in older women. Study of Osteoporotic Fractures Research Group. Ann Intern Med 1998;129**:**81-8.

37. Kamibayashi L, Wyss UP, Cooke TD, Zee B. Trabecular microstructure in the medial condyle of the proximal tibia of patients with knee osteoarthritis. Bone 1995;17**:**27-35.

38. Mansell JP, Bailey AJ. Abnormal cancellous bone collagen metabolism in osteoarthritis. J Clin Invest 1998;101**:**1596-603.

39. Ding M, Dalstra M, Linde F, Hvid I. Changes in the stiffness of the human tibial cartilage-bone complex in early-stage osteoarthrosis. Acta Orthop Scand 1998;69**:**358-62.

40. Ding M. Age variations in the properties of human tibial trabecular bone and cartilage. Acta Orthop Scand Suppl 2000;292**:**1-45.

41. Day JS, Ding M, van der Linden JC, Hvid I, Sumner DR, Weinans H. A decreased subchondral trabecular bone tissue elastic modulus is associated with pre-arthritic cartilage damage. J Orthop Res 2001;19**:**914-8.

42. Aspden RM, Scheven BA, Hutchison JD. Osteoarthritis as a systemic disorder including stromal cell differentiation and lipid metabolism. Lancet 2001;357**:**1118-20.

43. Sowers M, Lachance L, Jamadar D, Hochberg MC, Hollis B, Crutchfield M, Jannausch ML. The associations of bone mineral density and bone turnover markers with osteoarthritis of the hand and knee in pre- and perimenopausal women. Arthritis Rheum 1999;42**:**483-9.

44. Hart DJ, Cronin C, Daniels M, Worthy T, Doyle DV, Spector TD. The relationship of bone density and fracture to incident and progressive radiographic osteoarthritis of the knee: the Chingford Study. Arthritis Rheum 2002;46**:**92-9.

45. Gevers G, Dequeker J, Geusens P, Nyssen-Behets C, Dhem A. Physical and histomorphological characteristics of iliac crest bone differ according to the grade of osteoarthritis at the hand. Bone 1989;10**:**173-7.

46. Gevers G, Dequeker J, Martens M, Van Audekercke R, Nyssen-Behets C, Dhem A. Biomechanical characteristics of iliac crest bone in elderly women according to osteoarthritis grade at the hand joints. J Rheumatol 1989;16**:**660-3.

47. Dequeker J, Mohan S, Finkelman RD, Aerssens J, Baylink DJ. Generalized osteoarthritis associated with increased insulin-like growth factor types I and II and transforming growth factor beta in cortical bone from the iliac crest. Possible mechanism of increased bone density and protection against osteoporosis. Arthritis Rheum 1993;36**:**1702-8.

48. Naganathan V, Zochling J, March L, Sambrook PN. Peak bone mass is increased in the hip in daughters of women with osteoarthritis. Bone 2002;30**:**287-92.

49. Ushiyama T, Ueyama H, Inoue K, Nishioka J, Ohkubo I, Hukuda S. Estrogen receptor gene polymorphism and generalized osteoarthritis. J Rheumatol 1998;25**:**134-7.

50. Loughlin J, Sinsheimer JS, Mustafa Z, Carr AJ, Clipsham K, Bloomfield VA, Chitnavis J, Bailey A, Sykes B, Chapman K. Association analysis of the vitamin D receptor gene, the type I collagen gene COL1A1, and the estrogen receptor gene in idiopathic osteoarthritis. J Rheumatol 2000;27**:**779-84.

51. Uitterlinden AG, Burger H, Huang Q, Odding E, Duijn CM, Hofman A, Birkenhager JC, van Leeuwen JP, Pols HA. Vitamin D receptor genotype is associated with radiographic osteoarthritis at the knee. J Clin Invest 1997;100**:**259-63.

52. Houston LA, Grant SF, Reid DM, Ralston SH. Vitamin D receptor polymorphism, bone mineral density, and osteoporotic vertebral fracture: studies in a UK population. Bone 1996;18**:**249-52.

53. Keen RW, Woodford-Richens KL, Grant SF, Ralston SH, Lanchbury JS, Spector TD. Association of polymorphism at the type I collagen (COL1A1) locus with reduced bone mineral density, increased fracture risk, and increased collagen turnover. Arthritis Rheum 1999;42**:**285-90.

54. Uitterlinden AG, Weel AE, Burger H, Fang Y, van Duijn CM, Hofman A, van Leeuwen JP, Pols HA. Interaction between the vitamin D receptor gene and collagen type Ialpha1 gene in susceptibility for fracture. J Bone Miner Res 2001;16**:**379-85.

55. Langdahl BL, Lokke E, Carstens M, Stenkjaer LL, Eriksen EF. A TA repeat polymorphism in the estrogen receptor gene is associated with osteoporotic fractures but polymorphisms in the first exon and intron are not. J Bone Miner Res 2000;15**:**2222-30.

56. Ding M, Hvid I. Quantification of age-related changes in the structure model type and trabecular thickness of human tibial cancellous bone. Bone 2000;26**:**291-5.

57. Ito M, Nakamura T, Matsumoto T, Tsurusaki K, Hayashi K. Analysis of trabecular microarchitecture of human iliac bone using microcomputed tomography in patients with hip arthrosis with or without vertebral fracture. Bone 1998;23**:**163-9.

2.2 bone mineral density predicts the development of knee osteoarthritis

Bone mineral density and vertebral fracture history are associated with incident and progressive radiographic knee osteoarthritis in elderly men and women: the Rotterdam Study

Bone 2005 Oct;37(4):446-56. PubMed PMID: 16027057

Bergink AP, Uitterlinden AG, van Leeuwen JP, Hofman A, Verhaar JA, Pols HA

ABSTRACT

Objective. To study the association between baseline femoral neck and lumbar spine bone mineral density (BMD), prevalent fractures and incident and progressive radiographic osteoarthritis (ROA) of the knee in men and women.

Methods. A sample of 1403 subjects (829 women and 574 men) was drawn from the Rotterdam Study, a prospective population-based cohort study of the elderly. Incidence and progression of ROA in quartiles of femoral neck (FN) and lumbar spine (LS) BMD were determined using the Kellgren score, and separate analyses were made for men and women. Furthermore, incidence and progression of ROA were compared in subjects with and without a prevalent vertebral or non-vertebral fracture at baseline.

Results. The incidence of knee ROA of subject in the highest FN BMD (10.5%) and LS BMD (14.3%) was significantly higher than of those in the lowest quartiles (3.4% and 3.3% respectively), with corresponding adjusted odds ratios (95% confidence interval) of 2.8 (1.2 - 6.8) and 4.7 (2.1 - 10.7). The same trend was seen in the association between LS BMD and the progression of knee ROA, but no association was found between FN BMD and progression of ROA. Separate analyses for men and women both showed significant increased risks in the presence of high baseline BMD, with higher odds ratios in men than

in women, but larger confidence limits due to lower number of cases in men. Combined incidence and progression of knee ROA in subjects with a prevalent vertebral, but not with a prevalent non-vertebral fracture at baseline was 8 times lower than subject without a fracture, independent of baseline BMD.

Conclusions: High systemic BMD at baseline is associated with increased incidence and progression of knee ROA in both men and women, while a prevalent vertebral fracture has a protective effect.

Keywords: bone, density, osteoarthritis, knee, fractures

INTRODUCTION

Numerous cross-sectional studies indicated that OA is associated with increased BMD[1-5]. This led to the assumption that subjects with high BMD have increased risk of developing OA. Three prospective studies in women[6-8] and one study in a small subset of both women and men[9] indicated that high BMD at baseline is associated with increased risk of incident knee OA.

However, two of these studies found a protective effect of high BMD on progression of OA[7,8]. One study showed that women who had sustained a fracture at any site had less chance of developing knee OA, independently of their BMD at baseline[8]. This observation could indicate that, besides baseline bone mass, other aspects of bone quality could play a role in the development of OA, similar to the way prevalent vertebral fractures predict future fractures independent of BMD[10-13]. The mechanism behind this association is however still not clear, and several questions remain to be answered, for example whether the location of BMD measurement (femoral neck, lumbar spine, etc.) or fracture location is of influence on this possible association, and whether this association is present in men as well as in women.

In this study we investigate the risk of incident and progressive radiographic knee OA in both men and women with differences in baseline femoral neck, lumbar spine BMD and vertebral and non-vertebral fracture history.

MATERIALS AND METHODS

Subjects. The study population consisted of subjects of the Rotterdam Study, a prospective cohort study of men and women aged 55 and over, with the objective to investigate the incidence of, and risk factors for, chronic disabling diseases. The rationale and study design have been described previously[14]. The study population consisted of 1403 subjects (59% women), drawn from the Rotterdam Study population. The selection was based on availability of data on ROA status of the knees at baseline and on second follow-up examination, measurements of femoral neck and lumbar spine BMD, and potentially confounding factors. Differences in characteristics between the total Rotterdam Study population and our study population were described previously[15]. In brief, subjects in our study population were younger and more mobile than in the source population, the total Rotterdam Study.

Radiographic osteoarthritis. Both at baseline, between 1991 and 1993, and at the second follow-up visit, between 1997 and 1999, weight-bearing anteroposterior radiographs of the knees with the patellae in central position were obtained. Radiographic osteoarthritis (ROA) was assessed by means of the Kellgren-Lawrence grading system in five grades (from 0 to 4)[16]. Baseline Kellgren-scores were established by two independent readers as described before[17]. Both baseline and follow up radiographs were scored by a single reader (APB), who was trained by one of the aforementioned readers (EO), and who was blinded to all clinical data of the participants. Follow up radiographs were compared side by side to corresponding baseline radiograph by to identify incidence or progression of ROA. A subject was diagnosed positive with ROA of the knee, if the Kellgren score of one or both knees was two or higher at baseline. Incident ROA was diagnosed when a subject had Kellgren below 2 at either of the knees at baseline, and had a Kellgren score 2 or higher at either knee at follow-up. A subject was diagnosed with progressive ROA if the Kellgren score of either of the knees was 2 or higher, and the Kellgren score increased in that same knee during follow-up.

The presence of osteophytes was scored at baseline and at follow up by the same reader (APB) on a scale of 0 to 3 at four different sites at each knee (medial / lateral distal femur and medial / lateral proximal tibia) using a validated atlas[18] as described in our previous paper[15]. The highest score at any of the four sites of one knee determined the osteophyte grade of that knee. A subject with a grade of 2 or 3 at one knee or both was considered to have osteophytosis. Incident osteophytosis was defined as grade 0 or 1 of both knees at baseline with grade 2 or 3 of either of knees at follow-up. Progression of osteophytosis was defined as having grade 2 of either knee at baseline and grade 3 of the same knee at follow-up.

At baseline the distance between femur and tibia in the center of the medial and lateral joint compartments of the knees were measured in millimeters using a ruler[19]. When the joint space at any site was smaller than 2 standard deviations below the gender specific mean of that site the subject was defined to have joint space narrowing (JSN). This corresponded with the gender-specific lowest quartile of the combined joint spaces as described before[15]. Subsequently, the differences in joint spaces of the medial and lateral compartments of both knees, measured at baseline and measured at follow-up by the same reader (APB), were calculated. Incident JSN was defined to be present in subjects *without* JSN at baseline with a decrease in joint space at any site at follow up exceeding 2 standard deviations below the gender-specific mean difference in joint space of that site. This corresponded with a decrease of 2 millimeters at any site. Progression of JSN was defined if the difference in joint space at follow up was exceeding 2 millimeters in subjects *with* JSN at baseline.

The inter-observer variability between the two readers (APB and EO) and the intra-observer variability (APB) were low, with kappa scores ranging from 0.67 to 0.94 for cross-sectional and longitudinal measurements of joint space, osteophytosis and Kellgren score. Additionally, baseline mean Kellgren scores were calculated by adding up the 2 Kellgren scores of both knees of each subject and dividing them by two.

Vertebral fracture assessment. Both at baseline and at the second follow-up visit lateral radiographs of the thoraco-lumbar spine were taken of all subjects in our study population by a trained research assistant. All radiographs were taken following a standard protocol, with a distance between source and plate of 120 cm, using a Solarize FV (General Electric CGR). The follow-up radiographs were evaluated morphometrically by Dr. McCloskey and colleagues (Sheffield, UK) by the McCloskey-Kanis method[20]. If a vertebral fracture was detected, the baseline radiograph was evaluated as well. If a fracture was already present at baseline, it was considered prevalent. 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 a radiologist to rule out artifacts and other etiologies, such as pathological fractures.

Other variables. Between 1990 and 1993, an extensive baseline home interview on medical history and of risk factors for chronic diseases was performed by trained interviewers. Data on non-vertebral fracture history at or after age of 50 and information on smoking status at baseline was obtained. Female participants were asked for age at their last menstrual period and whether they had ever used hormone replacement therapy (HRT), with the exception of oral contraceptives. A lower limb disability was assessed using a modified version of the Stanford Health Assessment[21] as described in our previous paper[15]. After the home interview, subjects were invited to the research center for clinical examination and laboratory measurements. Height and weight were measured with subjects wearing indoor clothing and without shoes. Body mass index (BMI) was computed as weight in kilograms divided by height in squared meters (kg/m2). Bone mineral density (BMD) measurements of the femoral neck and vertebrae L2 to L4 were performed by dual energy X-ray absorptiometry (DXA, Lunar DPX-L densitometer) as described previously[5].

Statistical analysis. Differences in mean femoral neck and lumbar spine BMD in the absence and presence of baseline, incident and progressive ROA by Kellgren, osteophytosis and JSN were evaluated by means of ANOVA testing. The significance of the differences in baseline characteristics by quartiles of BMD was calculated by means of a linear regression model. P-values for differences in age, age at last menstrual period and ever HRT-use are unadjusted, and p-values for differences in height, weight and BMI were adjusted for age. All other p-values were adjusted for age and BMI. Likewise, p-values of the differences in baseline characteristics by incident and progressive ROA by Kellgren were calculated. In this case, additional adjustment for gender was made. Odds ratio's (OR), with 95% confidence intervals between parentheses, were calculated by means of logistic regression modeling, and were adjusted for baseline age, gender, body mass index (BMI), smoking status, use of walk aid, lower limb disability index and mean Kellgren score at baseline. In the separate analyses of men and women, the ORs for women were additionally adjusted for age of last menstrual period. The ORs for incidence and progression of knee ROA in the presence of a vertebral or non-vertebral fracture were additionally adjusted lumbar spine and femoral neck BMD respectively.

We used SPSS version 10.1.0 (SPSS Inc., Chicago, USA) for all our analyses.

RESULTS

In **Table 2.2.1a** the numbers of subject in our study population with and without baseline ROA defined by Kellgren as well as the numbers of subjects with absent and present osteophytosis and JSN are shown with corresponding mean femoral neck (FN) and lumbar spine (LS) BMD. At baseline, 20.5 % of all 1403 subjects had radiographic OA (women: 25.3 %, men: 13.6 %), defined by a Kellgren score of 2 and over. Osteophytosis was present in 21.8 % of the subjects (women: 26.2 %, men: 15.5 %) and 34.1 % had joint space narrowing at baseline (women: 33.8 %, men: 34.5 %).

Table 2.2.1a. Frequency of subjects in the absence and presence of baseline radiographic osteoarthritis (ROA), defined by Kellgren score, osteophytosis and joint space narrowing (JSN) of the knee with corresponding crude means of femoral neck (FN) and lumbar spine (LS) bone mineral density (BMD)

at baseline ('90-'92)									
	all			**women**			**men**		
	n (%)	FN BMD ± sd	LS BMD ± sd	n (%)	FN BMD ± sd	LS BMD ± sd	n (%)	FN BMD ± sd	LS BMD ± sd
Kellgren score									
< 2	1115	0.84 ± 0.13	1.08 ± 0.19	619	0.82 ± 0.13	1.02 ± 0.17	496	0.88 ± 0.12	1.16 ± 0.20
≥ 2	288 (20.5)	0.86* ± 0.13	1.12* ± 0.20	210 (25.3)	0.85* ± 0.13	1.08* ± 0.18	78 (13.6)	0.90 ± 0.13	1.23* ± 0.21
osteophytosis									
absent	1097	0.84 ± 0.13	1.08 ± 0.19	612	0.82 ± 0.13	1.02 ± 0.17	485	0.88 ± 0.12	1.16 ± 0.20
present	306 (21.8)	0.86* ± 0.13	1.11* ± 0.20	217 (26.2)	0.85* ± 0.13	1.07* ± 0.18	89 (15.5)	0.90· ± 0.13	1.21* ± 0.21
JSN									
absent	1139	0.85 ± 0.13	1.09 ± 0.19	624	0.82 ± 0.13	1.03 ± 0.17	515	0.88 ± 0.12	1.17 ± 0.19
present	264 (18.8)	0.84 ± 0.13	1.09 ± 0.21	205 (24.7)	0.83 ± 0.13	1.06· ± 0.18	59 (10.3)	0.88 ± 0.14	1.20 ± 0.26

*: p-value of difference in BMD < 0.05 (unadjusted)
·: p-value of difference in BMD < 0.10 (unadjusted)

Presented in **Table 2.2.1b** are the incidence and progression of ROA and its separate features after a mean follow-up-time of 6.5 years (range: 5.6 to 9.3 years) with corresponding mean FN and LS BMD. Incidence and progression of ROA according to Kellgren score, osteophytosis or JSN varied between 7 and 11% for all subjects. However, when we stratify for gender, cases were found to consist predominantly of women, resulting in low numbers of cases in men, in particular with regard to progression. Analysis of crude BMD values showed that subjects with ROA defined by Kellgren or osteophytosis at baseline had significantly increased baseline femoral neck (FN) BMD (2.4%) and lumbar spine (LS) BMD (3.7%). In separate analyses for women, we observed similar effects (3.7 % for FN BMD and 5.9 % for LS BMD), but in men with baseline ROA only the LS BMD was significantly increased (6.0%). No significant association is seen between BMD and JSN at baseline.

Table 2.2.1b. Frequency of subjects with incident and progressive radiographic osteoarthritis (ROA), defined by Kellgren score, osteophytosis and joint space narrowing (JSN) of the knee with corresponding crude means of femoral neck (FN) and lumbar spine (LS) bone mineral density (BMD)

	at follow-up ('97-'99) ‡								
		all			**women**			**men**	
	n (%)	FN BMD ± sd	LS BMD ± sd	n (%)	FN BMD ± sd	LS BMD ± sd	n (%)	FN BMD ± sd	LS BMD ± sd
incident ROA†									
absent	1041	0.84 ± 0.13	1.08 ± 0.19	563	0.81 ± 0.13	1.01 ± 0.16	478	0.88 ± 0.12	1.15 ± 0.20
present	74 (6.6)	0.89* ± 0.15	1.15* ± 0.20	56 (9.0)	0.86* ± 0.13	1.11* ± 0.18	18 (3.6)	0.99* ± 0.16	1.30* ± 0.20
progressive ROA†									
absent	263	0.87 ± 0.14	1.11 ± 0.20	187	0.85 ± 0.14	1.07 ± 0.18	76	0.90 ± 0.13	1.23 ± 0.21
present	25 (8.7)	0.85 ± 0.12	1.18· ± 0.23	23 (11.0)	0.84 ± 0.11	1.16* ± 0.22	2 (2.6)	0.98 ± 0.13	1.48· ± 0.23
incident osteophytosis									
absent	978	0.84 ± 0.13	1.08 ± 0.19	532	0.81 ± 0.13	1.01 ± 0.16	446	0.87 ± 0.12	1.15 ± 0.20
present	119 (10.8)	0.88* ± 0.14	1.13* ± 0.20	80 (13.1)	0.85* ± 0.12	1.08* ± 0.18	39 (8.0)	0.94* ± 0.14	1.25* ± 0.18
progressive osteophytosis									
absent	284	0.87 ± 0.13	1.11 ± 0.20	198	0.85 ± 0.13	1.06 ± 0.18	88	0.91 ± 0.13	1.21 ± 0.21
present	22 (7.2)	0.84 ± 0.11	1.16 ± 0.20	21 (9.7)	0.85 ± 0.10	1.15* ± 0.20	1 (1.1)	0.67·	1.30
incident JSN									
absent	1055	0.85 ± 0.13	1.09 ± 0.19	572	0.82 ± 0.13	1.03 ± 0.17	483	0.88 ± 0.12	1.16 ± 0.19
present	84 (7.4)	0.86 ± 0.13	1.12 ± 0.22	52 (8.3)	0.83 ± 0.13	1.05 ± 0.18	32 (6.2)	0.91 ± 0.11	1.24* ± 0.25
progressive JSN									
absent	235	0.84 ± 0.13	1.09 ± 0.21	180	0.82 ± 0.13	1.05 ± 0.18	55	0.88 ± 0.14	1.20 ± 0.26
present	29 (11.0)	0.85 ± 0.14	1.10 ± 0.21	25 (12.2)	0.84 ± 0.13	1.09 ± 0.20	4 (6.8)	0.90 ± 0.22	1.15 ± 0.26

ROA: radiographic osteoarthritis, as defined by a Kellgren score of ≥ 2 at one or both knees
*: p-value of difference in BMD < 0.05 (unadjusted)
·: p-value of difference in BMD < 0.10 (unadjusted)
†: incident ROA: baseline Kellgren score < 2, with follow-up Kellgren score ≥ 2, Progression ROA: baseline Kellgren score ≥ 2, increase in Kellgren score at any knee at follow-up
‡ Mean follow-up-time: 6.6 years (range: 5.0 to 9.3 years)

In subjects with incident ROA baseline FN BMD was 6.0% increased, and LS BMD 6.5%. Similar trends were seen in subjects with osteophytosis and in the separate analyses for women with incident ROA and osteophytosis. Separate analyses for men with incident ROA showed an even higher increase in FN BMD (12.5%) and LS BMD (13.0%). Subject with progression in ROA, and osteophytosis had a non-significant decrease in femoral neck BMD of 2.3 %, respectively 3.4%. However, this decrease was not observed in men, and in women with progression of ROA or osteophytosis the lumbar spine BMD was significantly increased at baseline. Except for a 6.9% increased lumbar spine BMD in men with incident JSN, no significant association between baseline BMD and incidence or progression of JSN was found, although the trend was similar compared to what we observed in subjects with incidence or progression of ROA and osteophytosis.

The characteristics of the study population (n=1403) by gender-specific quartiles of femoral neck and lumbar spine BMD are shown in **Tables 2.2.2a** and **2.2.2b**. Compared to subjects in the lowest gender-specific quartile of both femoral neck and lumbar spine BMD, subjects in the higher quartiles have significantly increased height, weight, BMI and Kellgren sum score, and have a lower prevalence of both vertebral and non-vertebral fractures at baseline.

Table 2.2.2a. Baseline characteristics study population by gender-specific quartiles of femoral neck (FN) BMD.

	all	I	II	III	IV	p-trend*
(FN) BMD (gm/cm^2, range)	0.85 (0.48-1.45)	0.70 (0.48-0.80)	0.81 (0.75-0.87)	0.88 (0.82-0.95)	1.02 (0.91-1.45)	
number	1403	365	349	342	347	
women (%)	59.1	58.9	59.3	60.2	57.9	0.87
age (yrs ± sd)	66.3 ± 6.7	67.6 ± 7.0	66.8 ± 6.7	65.8 ± 6.4	64.8 ± 6.2	< 0.01
height (cm ± sd)	167.6 ± 8.8	166.3 ± 8.8	167.1 ± 8.5	167.6 ± 8.8	169.2 ± 8.7	< 0.01
weight (kg ± sd)	74.0 ± 11.4	69.4 ± 10.6	72.7 ± 10.5	74.9 ± 10.8	79.3 ± 11.5	< 0.01
BMI (kg/m^2 ± sd)	26.4 ± 3.5	25.1 ± 3.1	26.0 ± 3.2	26.7 ± 3.6	27.7 ± 3.7	< 0.01
cigarette smoking (%)	22.6	26.6	21.5	21.6	20.5	0.11
recent use of walking aid (%)	2.6	4.4	3.2	2.0	0.9	< 0.01
lower limb disability (%)	26.6	26.9	29.8	21.6	28.0	0.36
prevalent vertebral fracture (%)	7.2	10.7	8.6	5.6	3.7	< 0.01
prevalent non-vertebral fracture (%)	20.7	24.7	23.2	18.1	16.4	0.02
Kellgren sum score	0.5 ± 0.7	0.4 ± 0.6	0.4 ± 0.7	0.5 ± 0.7	0.6 ± 0.8	0.02
Women†:						
(FN) BMD (gm/cm^2, range)	0.82 (0.48-0.74)	0.67 (0.48-0.74)	0.78 (0.75-0.81)	0.86 (0.82-0.90)	1.00 (0.91-1.45)	
number	820	212	206	205	197	
age at last menstrual period (yrs ± sd)	48.9 ± 4.9	48.4 ± 4.9	49.0 ± 5.0	48.7 ± 5.0	49.6 ± 4.8	0.04
ever HRT use (%)	21.2	17.9	19.4	22.0	25.9	0.04

values are means with standard deviations (sd) or percentages.
*p-values for trend in difference in age, age at last menstrual period and HRT use are unadjusted; for differences in height, weight and BMI are adjusted for age; all other p-values are adjusted for age and BMI.
†data on age at last menstrual period were missing for 9 women

In contrast to subjects in the highest quartiles of lumbar spine BMD (**Table 2.2.2b**), the subjects in the highest quartiles of femoral neck BMD (**Table 2.2.2a**) were younger and were more often using a walking aid compared to the subjects in the lower quartiles of femoral neck BMD. In our study population, smoking at baseline and lower limb disability were not significantly associated with differences in BMD, although subjects in the higher quartiles tended to smoke less. Women in the highest quartiles of both femoral neck and lumbar spine BMD had a later age of menopause and used more often HRT compared the women in the lowest quartiles (data on age of last menstrual period were missing for 9 women).

Table 2.2.2b. Baseline characteristics study population by gender specific quartiles of lumbar spine (LS) BMD.

	all	**I**	**II**	**III**	**IV**	**p-trend***
LS BMD (gm/cm^2, range)	1.09	0.87	1.02	1.13	1.34	
	(0.60-1.91)	(0.60-1.02)	(0.92-1.16)	(1.02-1.30)	(1.15-1.91)	
number	1403	354	340	366	343	
women (%)	59.1	58.2	58.2	59.6	60.3	0.51
age (yrs ± sd)	66.3 ± 6.7	66.8 ± 6.6	65.9 ± 6.3	65.6 ± 7.0	66.9 ± 6.8	0.97
height (cm ± sd)	167.6 ± 8.8	166.3 ± 8.9	167.5 ± 8.5	168.0 ± 8.9	168.4 ± 8.6	< 0.01
weight (kg ± sd)	74.0 ± 11.4	69.3 ± 10.8	73.4 ± 10.6	75.4 ± 11.3	78.0 ± 11.1	< 0.01
BMI (kg/m^2 ± sd)	26.4 ± 3.5	25.0 ± 3.3	26.2 ± 3.5	26.7 ± 3.4	27.5 ± 3.5	< 0.01
cigarette smoking (%)	22.6	27.7	23.5	19.7	19.5	0.09
recent use of walking aid (%)	26.6	24.6	24.1	24.3	33.5	0.18
lower limb disability (%)	2.6	2.0	2.9	3.0	2.6	0.87
prevalent vertebral fracture (%)	7.2	12.1	6.5	6.3	3.8	< 0.01
prevalent non-vertebral fracture (%)	20.7	22.9	22.6	19.7	17.5	0.10
Kellgren sum score	0.5 ± 0.7	0.4 ± 0.6	0.5 ± 0.7	0.5 ± 0.7	0.6 ± 0.8	0.03
Women†:						
LS BMD (gm/cm^2, range)	1.04	0.83	0.97	1.07	1.27	
	(0.60-1.59)	(0.60-0.91)	(0.92-1.01)	(1.02-1.14)	(1.15-1.59)	
number	820	204	196	215	205	
age at last menstrual period (yrs ± sd)	48.9 ± 4.9	48.4 ± 5.0	47.9 ± 5.4	49.5 ± 4.7	49.8 ± 4.4	< 0.01
ever HRT use (%)	21.2	18.1	16.3	19.5	30.7	< 0.01

values are means with standard deviations or percentages.
*p-values for trend in difference in age, age at last menstrual period and HRT use are unadjusted; for differences in height, weight and BMI are adjusted for age; all other p-values are adjusted for age and BMI.
†data on age at last menstrual period were missing for 9 women

In **Table 2.2.3**, the same baseline characteristics are described as in **Tables 2.2.2a** and **2.2.2b**, but now by incidence and progression of radiographic osteoarthritis by Kellgren score. Incidence of ROA is significantly associated with female gender (75.7% vs. 54.0%), increased weight (4%), BMI (8% increased) and BMD (6% increased), as well as 5.6% increased prevalence of vertebral fractures and 0.3 point increased mean Kellgren score at baseline. Similar trends are seen for progression of ROA, except for the mean femoral neck BMD: here we observed a non-significant *decrease* in subjects with progression of ROA.

Table 2.2.3. Baseline characteristics study population by incident and progression of ROA knee by Kellgren.

	baseline ROA absent			**baseline ROA present**		
	incident ROA*			progressive ROA*		
	absent	present	p-value†	absent	present	p-value†
	n = 1041	n = 74		n = 263	n = 25	
women (%)	54.0	75.7	< 0.01	71.1	92.0	0.03
age (yrs)	65.7 ± 6.5	66.5 ± 6.7	0.34	68.0 ± 7.0	69.4 ± 7.0	0.26
height (cm)	168.2 ± 8.8	165.1 ± 8.8	0.77	165.9 ± 8.2	164.0 ± 6.9	0.44
weight (kg)	73.0 ± 11.1	76.0 ± 11.3	< 0.01	76.6 ± 12.1	80.2 ± 11.6	0.02
BMI (kg/cm^2)	25.8 ± 3.2	27.9 ± 3.7	< 0.01	27.8 ± 3.9	29.9 ± 4.4	0.03
FN BMD (gm/cm^2)	0.84 ± 0.13	0.89 ± 0.15	< 0.01	0.87 ± 0.14	0.85 ± 0.12	0.55
LS BMD (gm/cm^2)	1.08 ± 0.19	1.15 ± 0.20	< 0.01	1.11 ± 0.20	1.18 ± 0.23	0.03
cigarette smoking (%)	25.1	16.2	0.53	16.0	8.0	0.80
recent use of walking aid (%)	1.7	4.1	0.23	4.9	12.0	0.23
lower limb disability (%)	22.9	33.8	0.17	36.1	60.0	0.17
prevalent vertebral fracture (%)	7.0	1.4	0.02	10.3	0.0	0.08
prevalent non-vertebral fracture (%)	19.1	20.3	0.89	27.0	20.0	0.55
Kellgren sum score	0.2 ± 0.3	0.5 ± 0.4	< 0.01	1.6 ± 0.4	1.9 ± 0.5	< 0.01
Women‡:	n = 559	n = 55		n = 183	n = 23	
age at last menstrual period	49.0 ± 5.0	50.1 ± 4.3	0.10	48.4 ± 5.0	48.0 ± 4.3	0.72
ever HRT use (%)	20.8	27.3	0.26	20.8	21.7	0.91

values are means with standard deviations and percentages
ROA: radiographic osteoarthritis, as defined by a Kellgren score of ≥ 2 at one or both knees
*incident ROA: baseline Kellgren score < 2, with follow-up Kellgren score ≥ 2, Progression ROA: baseline Kellgren score ≥ 2, increase in Kellgren score at any knee at follow-up
†p-values for difference in gender, age, age at last menstrual period and HRT use are unadjusted, p-values for difference in height, weight and body mass index (BMI) are adjusted for age and gender, all other p-values are adjusted for age, gender and BMI
‡data on age at last menstrual period were missing for 9 women

Table 2.2.4 shows percentages of incidence and progression of ROA by quartiles of femoral neck and lumbar spine BMD. The corresponding adjusted odds ratios are displayed, with the lowest BMD quartile being the reference. Subjects in the highest quartile of femoral neck BMD at baseline have nearly three times the risk of developing incident ROA at follow up compared to the subjects in the lowest femoral neck BMD quartile. Per standard deviation (sd) increase in BMD, their risk of incident ROA increases with 50%. Results for lumbar spine BMD were similar, if not stronger. Same but somewhat weaker trends were seen in the association between progression of ROA and lumbar spine BMD, but not between progression and femoral neck BMD.

Table 2.2.4. Association between gender-specific quartiles of femoral neck and lumbar spine BMD and incident and progressive radiographic OA

quartiles FN BMD (mean BMD, g/cm²)	ROA by Kellgren			quartiles LS BMD (mean BMD, g/cm²)	ROA by Kellgren		
	n	*incidence*	OR†		n	*incidence*	OR†
I (0.69)	268	3.4 %	1	**I** (0.87)	275	3.3 %	1
II (0.80)	297	6.1 %	2.1 (0.9 - 5.1)	**II** (1.01)	288	5.6 %	1.6 (0.7 - 4.0)
III (0.88)	283	6.7 %	1.6 (0.7 - 3.9)	**III** (1.13)	272	3.3 %	0.9 (0.3 - 2.5)
IV (1.01)	267	10.5 %	2.8 (1.2 - 6.8)	**IV** (1.32)	280	14.3 %	4.7 (2.1 - 10.7)
	per SD BMD		1.5 (1.1 - 1.9)		per SD BMD		1.7 (1.3 - 2.2)
	n	*progression*	OR†		n	*progression*	OR†
I (0.70)	69	8.7 %	1	**I** (0.88)	72	5.6 %	1
II (0.81)	77	7.8 %	0.7 (0.2 - 2.4)	**II** (1.04)	70	4.3 %	0.6 (0.1 - 2.9)
III (0.90)	69	10.1 %	1.2 (0.3 - 4.1)	**III** (1.17)	73	11.1 %	1.8 (0.5 - 6.7)
IV (1.04)	73	8.2 %	0.7 (0.2 - 2.9)	**IV** (1.37)	73	13.7 %	1.8 (0.5 - 6.4)
	per SD BMD		0.9 (0.5 - 1.4)		per SD BMD		1.4 (1.0 - 2.2)

In **Table 2.2.5**, the association between quartiles of femoral neck and lumbar spine BMD and combined incidence and progression of ROA stratified by gender are shown. In men more consistent and stronger effects were observed than in women. Compared to men in the lowest femoral neck and lumbar spine BMD quartiles, men in the highest quartiles have a 4, respectively 5 times increased risk of developing or worsening of ROA by Kellgren score, with ORs of 2.6 and 1.7 per SD increase in BMD respectively. The femoral neck BMD in women at baseline has no significant effect on the development of ROA in women, but women in the highest quartiles of lumbar spine BMD have a nearly 3 times increased risk of developing or worsening of ROA.

Table 2.2.5. Association between quartiles of femoral neck and lumbar spine BMD and combined incidence & progression of ROA stratified by gender.

gender	quartiles FN BMD (mean BMD, g/cm^2)	n	ROA by Kellgren		quartiles LS BMD (mean BMD, g/cm^2)	n	ROA by Kellgren	
			incidence & progression	OR†			incidence & progression	OR†
men	**I** (0.73)	150	2.0 %	1	**I** (0.93)	148	1.4 %	1
	II (0.84)	142	1.4 %	0.7 (0.1 - 4.4)	**II** (1.09)	142	1.4 %	0.9 (0.1 - 6.7)
	III (0.91)	136	2.9 %	1.7 (0.4 - 8.1)	**III** (1.23)	148	2.7 %	1.6 (0.3 - 9.1)
	IV (1.05)	146	7.5 %	4.3 (1.1 - 17.5)	**IV** (1.44)	136	8.8 %	5.0 (1.1 - 23.4)
		per SD BMD		2.6 (1.6 - 4.2)		per SD BMD		1.7 (1.1 - 2.7)
women‡	**I** (0.67)	212	6.6 %	1	**I** (0.83)	204	5.4 %	1
	II (0.78)	206	9.2 %	1.3 (0.6 - 2.6)	**II** (0.97)	196	7.7 %	1.2 (0.5 - 2.8)
	III (0.86)	205	9.8 %	1.2 (0.5 - 2.5)	**III** (1.07)	215	6.5 %	0.9 (0.4 - 2.2)
	IV (1.00)	197	12.7 %	1.2 (0.6 - 2.9)	**IV** (1.27)	205	18.5 %	2.6 (1.2 - 5.4)
		per SD BMD		1.0 (0.8 - 1.3)		per SD BMD		1.4 (1.1 - 1.8)

† Odds ratio's (OR), with 95% confidence intervals between parentheses, and p-values for trend are adjusted for baseline age, gender, body mass index (BMI), smoking status, use of walk aid lower limb disability index and ROA sum score at baseline. In women, the odds ratios and p-values were additionally adjusted for age at last menstrual period and HRT use.

‡ Data on age of last menstrual period and HRT use were missing for 9 women

Table 2.2.6 shows the percentage of all subjects with either incident or progressive ROA in the absence and presence of prevalent vertebral or non-vertebral fracture at baseline with the corresponding odds ratios. Subjects with a prevalent vertebral fracture before baseline measurements have a significantly reduced risk of developing or progression of ROA of the knee. Inversely, subjects without vertebral fracture have an 8 times increased risk (95%C.I. 1.1-60.9) of incident or progressive ROA defined by Kellgren compared to those with a history of vertebral fracture. Whether subjects have a history of non-vertebral fracture has no influence on their risk of developing ROA or worsening of ROA. When we additionally adjusted for age at menopause and HRT use, the risk of incidence or progression of ROA in women in the absence or presence of prevalent fractures showed similar risk estimates (data not shown).

Table 2.2.6. The association between prevalent vertebral and non-vertebral fracture and incidence & progression of ROA

	prevalent vertebral fracture				**prevalent non-vertebral fracture**			
	absent		present		absent		present	
	%	(cases/total)	%	(cases/total)	%	(cases/total)	%	(cases/total)
ROA by Kellgren								
incidence	7.0 %	(73/1041)	1.4 %	(1/74)	6.5 %	(59/901)	7.0 %	(15/214)
progression	9.6 %	(25/267)	0 %	(0/27)	9.4 %	(20/212)	6.6 %	(5/76)
incidence & progression	7.5 %	(98/1302)	1.0 %	(1/101)	7.1 %	(79/1113)	6.9 %	(20/290)
OR[†]	0.12 (0.02 – 0.91)				0.92 (0.54 – 1.56)			

[†] Odds ratio's (OR), with 95% confidence intervals between parentheses, are adjusted for baseline age, gender, body mass index (BMI), bone mineral density (BMD) (for vertebral fractures: lumbar spine BMD and for non-vertebral fractures: femoral neck BMD), smoking status, lower limb disability, use of walk aid and baseline ROA sum score.

DISCUSSION

In this study we investigated the predictive value of baseline femoral neck, lumbar spine BMD and prevalent vertebral and non-vertebral fractures on the incidence and progression of knee ROA in men and women. Our first crude analyses showed results similar to those of past studies in the Chingford and the Framingham cohorts[7,8]: subjects with incident ROA, in particular osteophytosis, have increased baseline femoral neck BMD, and those with progression tend to have decreased FN BMD. However, when we consider men and women with progression of ROA separately, the decrease in femoral neck BMD is no longer seen. Furthermore, when adjustment for age, gender, BMI, and mobility (use of walk aid and lower limb disability) was made we still observed a strong association between high femoral neck BMD and incident knee OA, but, in contrast to the aforementioned studies, not between low femoral neck BMD and progression.

Besides baseline femoral neck BMD, we studied the effect of differences in lumbar spine on knee ROA at follow up. The association of incidence and progression of knee OA by Kellgren score we found with high baseline lumbar spine BMD was more consistent than with femoral neck BMD, both in women and in men. When we compared the crude baseline BMD of subjects with and without incidence and progression of the two separate features of knee OA, osteophytosis and JSN, we found significant associations for osteophytosis similar to the association with OA by Kellgren, but not for JSN (although the trend was the same). This could well be explained by the fact that the Kellgren score, in particular the lower scores, depends largely on the presence of osteophytes, while JSN mainly plays a role in the higher Kellgren scores[16]. Since analyzing the association between crude BMD and incident and progressive osteophytosis and JSN separately gave us no additional information compared to analysis of OA by Kellgren alone, we restricted our further analyses associations of BMD with knee ROA according to Kellgren score.

One way of establishing the quality of bone is to measure the BMD at several sites: when BMD is low the bone quality is decreased and this results in increased fracture risk[22]. However, BMD is only one of several measurements that express bone quality, besides

for example ultrasound measurement[23-26] and modulus of elasticity[27,28]. In a previous paper we reported that, in spite of higher BMD, subjects with baseline knee ROA have increased fracture risk[15], so knee ROA may be associated with altered quality of bone as well. Though some studies have suggested that local changes associated with high BMD at a specific site may cause osteoarthritic changes at the same site[29-31], we hypothesized that differences in femoral neck and lumbar spine BMD may be a measurement for altered systemic bone quality, resulting in the development of OA at other sites than those of BMD measurement. The suggestion that OA is related to a generalized condition of bone is supported by several other studies[5,6,32-35].

In order to get more insight in this presumed relation between bone quality and the development of OA, we should not merely investigate baseline BMD, but other aspects of altered bone strength as well, like prevalent fractures. In this paper we distinguished subjects with prevalent vertebral and non-vertebral fractures and compared their risk of developing incident and progressive knee OA with subjects without fracture history. Our findings were in line with those of Hart et al[8]. However, they found a significant relation with peripheral fractures, whereas we found that a history of vertebral fractures was significantly associated with reduced risk of developing knee ROA. In a cross-sectional examination of the same study population, Arden and colleagues found prevalent hip OA to be associated with increased osteoporotic fracture risk, despite having increased BMD[36]. However, subjects with spine OA had reduced fracture risk, and since no relation between fracture risk and hand or knee OA was seen, the authors suggested that the increased fracture risk in subjects with hip OA might be due to postural instability.

There are some limitations of this study. Although our subjects were drawn from a population-based study, the Rotterdam Study, they were selected based on the availability of follow-up radiographs. This selection introduces a health-selection-bias, since these subjects survived the follow-up period, and were healthy enough to visit the research center. However, since the incidence and progression of ROA in these healthier subjects will be lower than in a population based sample, we can assume that if this health-selection would have influenced the associations we found, it would underestimate the

true effect. Another limitation is the relatively low prevalence, incidence and progression of ROA men. Since the association between baseline BMD and incidence was in the same direction as for progression of ROA, we combined incidence and progression in our sub-analysis for men and women. For similar reasons of low power, we combined incidence and progression in our analysis of the association between prevalent fractures and the development of ROA. Finally, no data on OA of the spine were available in our study. Thus, the apparently more robust association between high lumbar spine BMD and the development of knee ROA may by biased by the presence of spinal OA[37-39]. On the other hand, the influence of osteophytes at hip on femoral neck BMD measurements is not clear. In order to interfere with the BMD measurements, which are taken at the smallest section of the femoral neck, the osteophytes should have been exceptionally large. If present at all, these kinds of osteophytes will be very rare, and not likely to bias the association we found. Furthermore, the strength of the relation between OA of the knees and osteophytosis of either spine or hip is disputable. For example, the increased risk of knee OA for women with high BMD Hart et al found in the Chingford study didn't alter by adjusting for spinal osteophytosis[2]. We know that clustering between joint sites in osteoarthritis (OA) is more common than would be expected simply from the rising prevalence of the disorder with age[40]. However, in our study the crude Spearman correlation coefficient between maximum Kellgren scores at either knee and maximum Kellgren scores at either hip was 0.12 ($N = 1377$, $p < 0.01$). No data on osteophytes of the hip were available, but given the weak correlation between Kellgren scores of hip and knee, we can assume that the association between osteophytes at the hip and those at the knee is at least as weak.

Although the results we found in this study are overall in line with those of the few prospective studies previously published on the relation between BMD and the development of OA, some differences need attention.

In their study on 473 women in the Framingham cohort, Zang et al. suggest that high baseline femoral neck BMD might be associated with an increased risk of incident knee OA, especially osteophyte development. However, they found a protective effect of high femoral neck BMD on progression of OA[7]. In our study, we also observe lower crude

mean femoral neck BMD in women with progressive ROA (**Table 2.2.1a**). However, 12% of the subjects with progressive ROA are using a walking aid compare to 5% of subjects without progressive ROA, and their lower limb disability index is two times increased (**Table 2.2.3**). Thus, the lower BMD in subject likely to develop progressive ROA may by associated with lower mobility, or higher morbidity. When adjusted for mobility, we do not observe a protective effect of high femoral neck BMD on progressive ROA (**Table 2.2.4**). In addition, although the research-question is the same, their study design differs from ours. They performed their analyses comparing knees, not subjects, to increase the power of their study. Furthermore, the moment of measurement of BMD in the Framingham study was halfway the knee ROA follow-up period. Thus, at the moment of determination of BMD, the ROA status was unclear, and therefore it was not possible to determine whether incidence or progression occurs after the BMD measurement.

One longitudinal study of 830 British women examined, besides baseline BMD, the relationship of fractures and the incidence and progression of knee OA[8]. Again, high baseline BMD was found to be associated with increased incidence of OA, but had a protective effect on progressive OA. Additionally, the authors state that women with a peripheral fracture had reduced risk of subsequently developing incident OA. However, since the development of OA occurred somewhere between baseline and follow-up, i.e., the same period in which the fractures were sustained, cause and effect could not be distinguished.

Another prospective study on bone mass, bone loss and incident OA was performed by Sowers et al. Their study showed that of 482 women 9 subjects with incident knee OA had higher baseline BMD[6]. However, their study population was much younger than ours, and as a consequence the incidence of knee OA was low (1.9%).

Most previous studies were performed in women. Only one study included men in their investigation, as described in the paper of Hochberg et al. In a subset of 119 men and 76 women they found that the odds of developing knee OA were 1.64 (95% CI, 1.03 to 2.61) for each standard deviation increase in spinal BMD[9], similar to the estimates we

derived. However, no separate analysis for men and women was made.

In this large, population-based study, we investigated the possible relation between several indicators of bone strength (baseline femoral neck and lumbar spine BMD and history of vertebral and non-vertebral fracture before baseline) and subsequent incidence or progression of knee ROA to get more insight in the mechanism behind the development of this disorder. Women, but men as well, with high baseline BMD have increased risk of developing knee ROA. This effect seems to be driven by osteophytosis, and seems to be stronger for lumbar spine BMD than for femoral neck BMD. Similarly, the protective effect of a prevalent fracture was only present if the fracture was located at the spine. This could suggest that the association we found is mediated by influences on trabecular, rather than cancellous, bone. Both vertebral bone mass and OA are associated with estrogen status[41-44]. To account for a possible bias due to differences in estrogen exposure, we adjusted for age at menopause and hormone replacement therapy in our separate analysis for women. However, this did not alter the risk estimates. Still, the association between baseline BMD and development of OA may be mediated by the estrogen pathway. Polymorphisms in the estrogen receptor alpha (ERα) gene are found to be associated with both vertebral fracture and bone loss[45,46] and with OA[47,48]. However, other candidate genes, like the VDR receptor gene are associated with both bone mass and OA as well[49-53], and thus several other pathways may play a modulating role.

We showed that baseline BMD, especially at the lumbar spine, predicts the development of knee ROA, and that the presence of a vertebral fracture protects against this development, independently of BMD. This supports the hypothesis that differences in BMD, fracture risk and subsequent risk of knee OA are due to a common intrinsic variation in bone structure and metabolism, rather than a result of local mechanical conditions. Further investigations are needed to clarify the mechanism behind this association.

REFERENCES

1. Hannan MT, Anderson JJ, Zhang Y, Levy D, Felson DT. Bone mineral density and knee osteoarthritis in elderly men and women. The Framingham Study. Arthritis Rheum 1993; 36:1671-80.

2. Hart DJ, Mootoosamy I, Doyle DV, Spector TD. The relationship between osteoarthritis and osteoporosis in the general population: the Chingford Study. Ann Rheum Dis 1994; 53:158-62.

3. Nevitt MC, Lane NE, Scott JC, et al. Radiographic osteoarthritis of the hip and bone mineral density. The Study of Osteoporotic Fractures Research Group. Arthritis Rheum 1995; 38:907-16.

4. Lethbridge-Cejku M, Tobin JD, Scott WW, Jr., et al. Axial and hip bone mineral density and radiographic changes of osteoarthritis of the knee: data from the Baltimore Longitudinal Study of Aging. J Rheumatol 1996; 23:1943-7.

5. Burger H, van Daele PL, Odding E, et al. Association of radiographically evident osteoarthritis with higher bone mineral density and increased bone loss with age. The Rotterdam Study. Arthritis Rheum 1996; 39:81-6.

6. Sowers M, Lachance L, Jamadar D, et al. The associations of bone mineral density and bone turnover markers with osteoarthritis of the hand and knee in pre- and perimenopausal women. Arthritis Rheum 1999; 42:483-9.

7. Zhang Y, Hannan MT, Chaisson CE, et al. Bone mineral density and risk of incident and progressive radiographic knee osteoarthritis in women: the Framingham Study. J Rheumatol 2000; 27:1032-7.

8. Hart DJ, Cronin C, Daniels M, Worthy T, Doyle DV, Spector TD. The relationship of bone density and fracture to incident and progressive radiographic osteoarthritis of the knee: the Chingford Study. Arthritis Rheum 2002; 46:92-9.

9. Hochberg MC, Lethbridge-Cejku M, Tobin JD. Bone mineral density and osteoarthritis: data from the Baltimore Longitudinal Study of Aging. Osteoarthritis Cartilage 2004; 12 Suppl A:S45-8.

10. Ross PD, Genant HK, Davis JW, Miller PD, Wasnich RD. Predicting vertebral fracture incidence from prevalent fractures and bone density among non-black, osteoporotic women. Osteoporos Int 1993; 3:120-6.

11. Burger H, van Daele PL, Algra D, et al. Vertebral deformities as predictors of non-vertebral fractures. Bmj 1994; 309:991-2.

12. Nevitt MC, Ross PD, Palermo L, Musliner T, Genant HK, Thompson DE. Association of prevalent vertebral fractures, bone density, and alendronate treatment with incident vertebral fractures: effect of number and spinal location of fractures. The Fracture Intervention Trial Research Group. Bone 1999; 25:613-9.

13. Black DM, Arden NK, Palermo L, Pearson J, Cummings SR. Prevalent vertebral deformities predict hip fractures and new vertebral deformities but not wrist fractures. Study of Osteoporotic Fractures Research Group. J Bone Miner Res 1999; 14:821-8.

14. 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; 7:403-22.

15. Bergink AP, van der Klift M, Hofman A, et al. Osteoarthritis of the knee is associated with vertebral and nonvertebral fractures in the elderly: the Rotterdam Study. Arthritis Rheum 2003; 49:648-57.

16. Kellgren JH, Jeffrey MR, Ball J. Atlas of Standard Radiographs of Arthritis: The epidemiology of Chronic Rheumatism. Vol. 2. Oxford: Blackwell Scientific Publications, 1963.

17. Odding E, Valkenburg HA, Algra D, Vandenouweland FA, Grobbee DE, Hofman A. Associations of radiological osteoarthritis of the hip and knee with locomotor disability in the Rotterdam Study. Ann Rheum Dis 1998; 57:203-8.

18. Burnett, S., Hart, D. J., Cooper, C., and Spector, T. D. A Radiographic Atlas of Osteoarthritis. London: Springer Verlag; 1994.

19. Altman, R. D., Fries, J. F., Bloch, D. A., Carstens, J., Cooke, T. D., Genant, H., Gofton, P., Groth, H., McShane, D. J., Murphy, W. A., and et al. Radiographic assessment of progression in osteoarthritis. Arthritis Rheum 30:1214-25.; 1987.

20. McCloskey EV, Spector TD, Eyres KS, et al. The assessment of vertebral deformity: a method for use in population studies and clinical trials. Osteoporos Int 1993; 3:138-47.

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; 26:1346-53.

22. Meltzer M, Lessig HJ, Siegel JA. Bone mineral density and fracture in postmenopausal women. Calcif Tissue Int 1989; 45:142-5.

23. van Daele PL, Burger H, Algra D, et al. Age-associated changes in ultrasound measurements of the calcaneus in men and women: the Rotterdam Study. J Bone Miner Res 1994; 9:1751-7.

24. Schott AM, Weill-Engerer S, Hans D, Duboeuf F, Delmas PD, Meunier PJ. Ultrasound discriminates patients with hip fracture equally well as dual energy X-ray absorptiometry and independently of bone mineral density. J Bone Miner Res 1995; 10:243-9.

25. Gregg EW, Kriska AM, Salamone LM, et al. The epidemiology of quantitative ultrasound: a review of the relationships with bone mass, osteoporosis and fracture risk. Osteoporos Int 1997; 7:89-99.

26. Kann P, Bergink AP, Fang Y, et al. The collagen Ia1 SP1 polymorphism is associated with differences in ultrasound transmission velocity in the calcaneus in postmenopausal women. Calcif Tissue Int 2002; 70:450-6.

27. Greenfield MA, Craven JD, Wishko DS, Huddleston AL, Friedman R, Stern R. The modulus of elasticity of human cortical bone: an in vivo measurement and its clinical implications. Radiology 1975; 115:163-6.

28. Kann P, Bergink AP, Beyer J. Determination of the modulus of elasticity of bone material by an acoustical approach at the forearm distinguishes women with and without vertebral fractures independent of bone mineral density. Exp Clin Endocrinol Diabetes 2002; 110:161-5.

29. Kamibayashi L, Wyss UP, Cooke TD, Zee B. Trabecular microstructure in the medial condyle of the proximal tibia of patients with knee osteoarthritis. Bone 1995;17**:**27-35.

30. Ding M, Dalstra M, Linde F, Hvid I. Changes in the stiffness of the human tibial cartilage-bone complex in early-stage osteoarthrosis. Acta Orthop Scand 1998;69**:**358-62.

31. Day JS, Ding M, van der Linden JC, Hvid I, Sumner DR, Weinans H. A decreased subchondral trabecular bone tissue elastic modulus is associated with pre-arthritic cartilage damage. J Orthop Res 2001;19**:**914-8.

32. Gevers G, Dequeker J, Martens M, Van Audekercke R, Nyssen-Behets C, Dhem A. Biomechanical characteristics of iliac crest bone in elderly women according to osteoarthritis grade at the hand joints. J Rheumatol 1989; 16:660-3.

33. Schnitzler CM, Mesquita JM, Wane L. Bone histomorphometry of the iliac crest, and spinal fracture prevalence in atrophic and hypertrophic osteoarthritis of the hip. Osteoporos Int 1992; 2:186-94.

34. Dequeker J, Mohan S, Finkelman RD, Aerssens J, Baylink DJ. Generalized osteoarthritis associated with increased insulin-like growth factor types I and II and transforming growth factor beta in

cortical bone from the iliac crest. Possible mechanism of increased bone density and protection against osteoporosis. Arthritis Rheum 1993; 36:1702-8.

35. Sowers M. Epidemiology of risk factors for osteoarthritis: systemic factors. Curr Opin Rheumatol 2001; 13:447-51.

36. Arden, N. K., Griffiths, G. O., Hart, D. J., Doyle, D. V., and Spector, T. D. The association between osteoarthritis and osteoporotic fracture: the Chingford Study. Br J Rheumatol 35:1299-304.; 1996.

37. Slosman DO, Rizzoli R, Donath A, Bonjour JP. Vertebral bone mineral density measured laterally by dual-energy X-ray absorptiometry. Osteoporos Int 1990; 1:23-9.

38. Masud T, Langley S, Wiltshire P, Doyle DV, Spector TD. Effect of spinal osteophytosis on bone mineral density measurements in vertebral osteoporosis. Bmj 1993; 307:172-3.

39. Rand, T., Schneider, B., Grampp, S., Wunderbaldinger, P., Migsits, H., and Imhof, H. Influence of osteophytic size on bone mineral density measured by dual X-ray absorptiometry. Acta Radiol 38:210-3.; 1997.

40. Cooper, C., Egger, P., Coggon, D., Hart, D. J., Masud, T., Cicuttini, F., Doyle, D. V., and Spector, T. D. Generalized osteoarthritis in women: pattern of joint involvement and approaches to definition for epidemiological studies. J Rheumatol 23:1938-42.; 1996.

41. Moore M, Bracker M, Sartoris D, Saltman P, Strause L. Long-term estrogen replacement therapy in postmenopausal women sustains vertebral bone mineral density. J Bone Miner Res 1990; 5:659-64.

42. Felson DT, Nevitt MC. The effects of estrogen on osteoarthritis. Curr Opin Rheumatol 1998; 10:269-72.

43. Oliveria SA, Felson DT, Klein RA, Reed JI, Walker AM. Estrogen replacement therapy and the development of osteoarthritis. Epidemiology 1996; 7:415-9.

44. Tsai CL, Liu TK. Osteoarthritis in women: its relationship to estrogen and current trends. Life Sci 1992; 50:1737-44.

45. van Meurs JB, Schuit SC, Weel AE, et al. Association of 5' estrogen receptor alpha gene polymorphisms with bone mineral density, vertebral bone area and fracture risk. Hum Mol Genet 2003; 12:1745-54.

46. Kobayashi N, Fujino T, Shirogane T, et al. Estrogen receptor alpha polymorphism as a genetic marker for bone loss, vertebral fractures and susceptibility to estrogen. Maturitas 2002; 41:193-201.

47. Ushiyama T, Ueyama H, Inoue K, Nishioka J, Ohkubo I, Hukuda S. Estrogen receptor gene polymorphism and generalized osteoarthritis. J Rheumatol 1998; 25:134-7.

48. Bergink AP, van Meurs JB, Loughlin J, et al. Estrogen receptor alpha gene haplotype is associated with radiographic osteoarthritis of the knee in elderly men and women. Arthritis Rheum 2003; 48:1913-22.

49. Colin EM, Uitterlinden AG, Meurs JB, et al. Interaction between vitamin D receptor genotype and estrogen receptor alpha genotype influences vertebral fracture risk. J Clin Endocrinol Metab 2003; 88:3777-84.

50. Uitterlinden AG, Burger H, Huang Q, et al. Vitamin D receptor genotype is associated with radiographic osteoarthritis at the knee. J Clin Invest 1997; 100:259-63.

51. Uitterlinden AG, Burger H, Huang Q, et al. Relation of alleles of the collagen type Ialpha1 gene to bone density and the risk of osteoporotic fractures in postmenopausal women. N Engl J Med 1998; 338:1016-21.

52. Uitterlinden AG, Burger H, van Duijn CM, et al. Adjacent genes, for COL2A1 and the vitamin D receptor, are associated with separate features of radiographic osteoarthritis of the knee. Arthritis Rheum 2000; 43:1456-64.

53. Loughlin J, Sinsheimer JS, Mustafa Z, et al. Association analysis of the vitamin D receptor gene, the type I collagen gene COL1A1, and the estrogen receptor gene in idiopathic osteoarthritis. J Rheumatol 2000; 27:779-84.

2.3 bone mineral density and the development of knee, hip and hand osteoarthritis

Are Bone Mineral Density and Fractures Related to the Incidence and Progression of Radiographic Osteoarthritis of the Knee, Hip, and Hand in Elderly Men and Women? The Rotterdam Study

Arthritis Rheumatol 2019 Mar;71(3):361-9. PubMed PMID: 30264891

Bergink AP, Rivadeneira F, Bierma-Zeinstra SM, Zillikens MC, Ikram MA, Uitterlinden AG, and van Meurs JB

ABSTRACT

Objective. To examine the longitudinal relationship between bone mineral density (BMD) and the incidence and progression of knee, hip and hand osteoarthritis, and the relationship between prevalent vertebral and non-vertebral fractures and the incidence and progression of osteoarthritis in elderly men and women in the Rotterdam Study.

Methods. Age- and sex-specific quartiles of baseline femoral neck BMD (FN-BMD) were constructed for a total number of 4,154 subjects. Radiographs were scored for incidence and progression of knee and hip osteoarthritis, and for incidence of hand osteoarthritis. Prevalent vertebral fractures were scored using the McCloskey/Kanis method, and prevalent non-vertebral fractures were reported by baseline interview.

Results. Subjects in the highest quartile of FN-BMD had an increased risk of incident knee radiographic osteoarthritis (ROA) (OR 1.58; 95%CI: 1.14 to 2.18), and an increased risk of incident hip ROA (OR 1.57; 95%CI: 1.06 to 2.32) compared to the lowest quartile. No significant relationship was found between high FN-BMD and progression

of knee or hip ROA, or the incidence of hand ROA. Prevalent vertebral and non-vertebral fractures were not related to the incidence or progression of knee or hip ROA. Vertebral fractures were however associated with incident hand ROA (OR 1.74; 95%CI: 1.02 to 2.98).

Conclusion. Results from the present study confirm earlier studies and thus provide strong evidence that high FN-BMD is a prognostic risk factor for the development of knee and hip ROA. Vertebral fractures were found to be a risk factor for incident hand ROA.

INTRODUCTION

The inverse relation between osteoporosis (OP) and osteoarthritis (OA) has been described extensively in the past decades[1, 2]. However, whether high bone mineral density (BMD) is the cause or consequence of OA is unclear. A number of prospective studies[3-7] indicated that high BMD at baseline is associated with increased risk of incident knee OA. Two of these studies found a protective effect of high BMD on progression of knee OA[4, 5], while Nevitt and al. found that progression was not significantly related to BMD[7].

However, almost no data is available on the relationship between BMD and incidence or progression of hip and hand OA. A previous review concluded that the relationship between OA and OP is elusive and that especially longitudinal studies show no clear relation between OA and OP[8].

We have previously studied the association between baseline BMD, incident and progressive radiographic OA of the knee and prevalent vertebral and non-vertebral fractures in 1,403 men and women[9]. We found that high baseline BMD was associated with increased incidence of knee OA, and that subjects with a prevalent vertebral fracture had less risk of incident or progressive knee OA. Since our publication, few longitudinal studies have been performed to further examine the influence of BMD on the development of OA. A recent review summarized all available published data on the relationship between BMD and longitudinal OA, adding up to a total of 4,942 individuals (including 1,403

subjects of our own Rotterdam Study), showing increased risk for the development of knee OA in subjects with high baseline BMD[10].

Regarding the development of hip OA, even less longitudinal studies are available. Hochberg mentioned a dose–response relationship between the quartile of baseline BMD and the incidence of radiographic hip OA in the SOF study[11], while Barbour et al. recently found no association between high BMD and radiographic hip OA in the Johnston County Osteoarthritis Project[12].

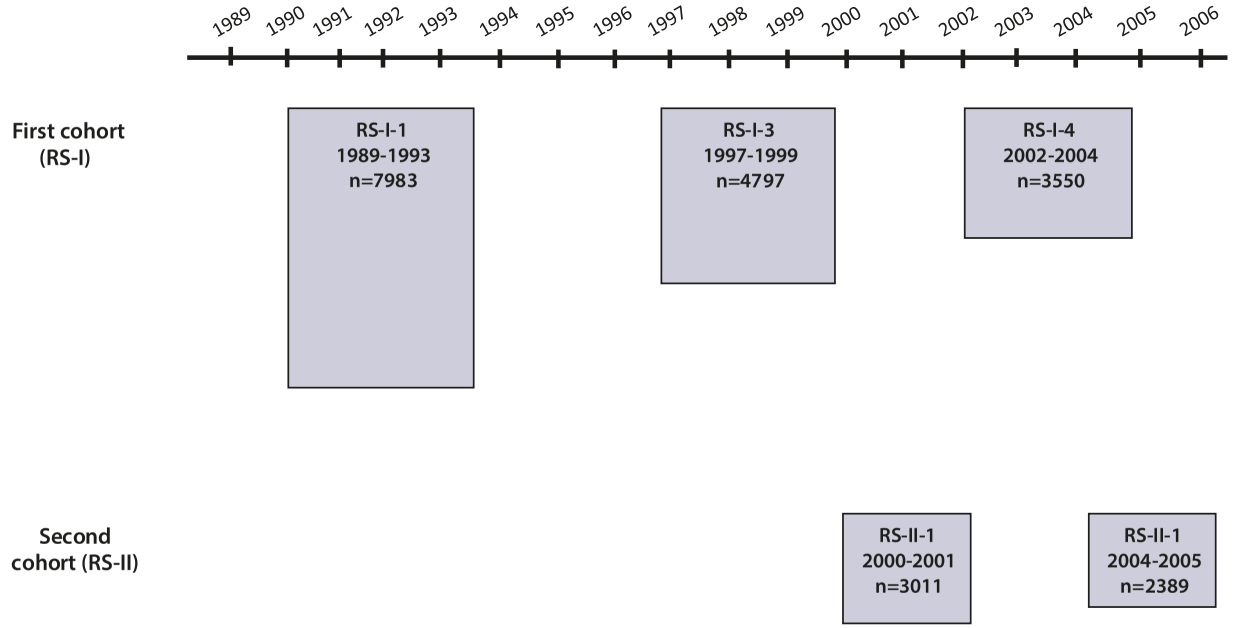
To our knowledge, only one longitudinal study on baseline BMD and incident hand OA was performed, showing no association[3]. Hand OA is especially interesting in this respect, since development of OA in the hands, especially of DIP and DIP-joints, is thought to be due to more systemic influences, like adiposity[13], sex hormone levels[14] and genetic influences[15] rather than local (mechanical) loading.

In the current study, we aimed to study the association between baseline BMD and the development of hip and hand OA, and between prevalent vertebral and non-vertebral fractures and the risk of OA. In addition, we wanted to verify the positive association between high BMD and the development of knee radiographic OA (ROA) we found previously in the Rotterdam Study in an expanded study population (total sample size is 4,154) drawn from the Rotterdam Study-I and II, with extended (now 8.4 years) follow up time.

MATERIALS AND METHODS

Subjects. The study population consisted of subjects of the Rotterdam Study-I (RS-I) and Rotterdam Study-II (RS-II). The rationale and study design have been described previously[16, 17]. The study population consisted of 3,005 subjects (55.8% women), drawn from RS-I, and 1,149 subjects (54.4% women) drawn from RS-II (**Figure 2.3.1**: cohorts in the total Rotterdam Study). The selection was based on the availability of radiographs of the knees, hips and hands at baseline and follow-up examinations, and data on BMD, prevalent vertebral and non-vertebral fractures and potentially confounding factors at baseline.

Figure 2.3.1. Cohorts in the Rotterdam Study



RS: Rotterdam Study

Radiographic osteoarthritis. Radiographs of knees, hips and hands were taken at three visits for RS-I: RS-I-1 (baseline measurement, between 1998 and 1993), RS-I-3 (between 1997 and 1999, mean follow up time 6.5 years) and RS-I-4 (between 2002 and 2004, mean follow up time 11.9 years, **Figure 2.3.1**).

Table 2.3.1. Baseline characteristics study populations by age- and sex-specific quartiles of FN-BMD: RS-I & RS-II

			RS-I			
	All	**Quartile 1**	**Quartile 2**	**Quartile 3**	**Quartile 4**	**p-trend***
FN-BMD (gm/cm^2, range)	0.89 (0.49-1.49)	0.74 (0.49-0.88)	0.84 (0.70-0.96)	0.92 (0.76-1.05)	1.05 (0.83-1.49)	
number	3005	755	757	752	741	
women (%)	55.8	55.6	55.9	55.6	56.3	
age (yrs ± sd)	65.3 ± 6.5	65.4 ± 6.6	65.3 ± 6.4	65.3 ± 6.6	65.2 ± 6.5	
height (cm ± sd)	168.4 ± 9.1	167.4 ± 9.5	167.9 ± 9.3	168.7 ± 8.8	169.6 ± 8.9	1.0×10^{-6}
weight (kg ± sd)	74.5 ± 11.5	69.4 ± 11.0	73.5 ± 10.8	76.5 ± 10.7	78.8 ± 11.5	1.2×10^{-64}
BMI (kg/m^2 ± sd)	26.3 ± 3.5	24.7 ± 3.1	26.1 ± 3.4	26.9 ± 3.3	27.4 ± 3.6	3.0×10^{-57}
			RS-II			
	All	**Quartile 1**	**Quartile 2**	**Quartile 3**	**Quartile 4**	**p-trend***
FN-BMD (gm/cm^2, range)	0.93 (0.48-1.46)	0.78 (0.48-0.93)	0.89 (0.67-1.00)	0.97 (0.77-1.09)	1.10 (0.85-1.46)	
number	1149	287	290	290	282	
women (%)	54.4	54.4	54.5	54.5	54.3	
age (yrs ± sd)	63.1 ± 6.4	63.3 ± 6.6	63.1 ± 6.4	62.8 ± 6.4	62.8 ± 6.4	
height (cm ± sd)	168.8 ± 8.9	167.8 ± 9.2	168.9 ± 8.7	169.0 ± 9.1	169.8 ± 8.6	0.9×10^{-2}
weight (kg ± sd)	77.1 ± 13.1	71.4 ± 12.4	76.0 ± 12.4	78.9 ± 12.5	82.2 ± 12.8	4.3×10^{-25}
BMI (kg/m^2 ± sd)	27.0 ± 3.9	25.3 ± 3.3	26.6 ± 3.5	27.6 ± 3.9	28.5 ± 4.0	2.7×10^{-26}

values are means with standard deviations (sd) or percentages, *unadjusted p-values
FN-BMD: femoral neck bone mineral density, BMI: body mass index, RS: Rotterdam Study

For RS-II, radiographs were taken at two visits: RS-II-1 (baseline, between 2000 and 2001) and at RS-II-2 (between 2004 and 2005, mean follow-up time 4.1). The overall mean follow-up time was 8.4 years (standard deviation (SD) 2.2, range 3.7 to 13.6) for subjects in RS-I and for RS-II 4.1 years (SD 0.6, range 1.1 to 5.8). Prevalence, incidence and progression of ROA were scored by the Kellgren/Lawrence (K/L) grading system[18] as

described previously[9, 19, 20]. In short, prevalence of ROA of the knee or hip was defined as a K/L score of ≥2 at baseline at one or both knees/hips. Prevalence of hand ROA was defined as presence of a K/L score ≥2 in 2 out of 3 hand joint groups for one or both hands. Incident ROA was defined when a subject had no prevalent ROA (a K/L score <2) of both knees, both hips or both hands at baseline, and a K/L score of ≥2 at follow-up (RS-I-3, RS-I-4 or RS-II-2) of one or both knees, one or both hips or one or both hands, respectively. Progressive ROA was defined as an increase of K/L score in subjects with prevalent ROA of that same joint. Thus, the analysis is person-based: incident OA can only occur in subjects without prevalent ROA at both the left and right joint, progression of ROA can only occur in subjects with prevalent ROA at the left, right or both joints. A third measure was calculated: incidence or progression of OA. This was defined by having either incident ROA or showing progression of ROA. This measure was constructed for comparison with our previous study[9], and for reasons of power when stratifying for study population. Subjects that received total joint replacement during follow up were excluded from the analysis of that joint group. The radiographs in RS-I and RS-II were obtained using the same protocol, and were scored by PhD-students trained by a radiologist. The interobserver agreement was 0.71 for RS-I and 0.68 for RS-II[20]. Data on incidence of hand OA was available for 2,118 subjects.

Fracture assessment. Prevalent non-vertebral fractures: Between 1989 and 1993, an extensive baseline home interview on medical history and of risk factors for chronic diseases was performed by trained interviewers. Data on non-vertebral fracture history at or after age of 50 was obtained as described previously[9, 16]. Vertebral fractures: Both at baseline (between 1989 and 1993) and at the second follow-up visit (between 1997 and 1999), radiographs of the thoracolumbar spine were available for 2,920 individuals from RS-I. The thoracolumbar spine radiographs of the follow-up visit were scored for the presence of vertebral fracture using the McCloskey/Kanis method, as described previously[21]. If vertebral fractures were detected, the baseline radiograph was also evaluated. If the vertebral fracture was already present at baseline, it was considered to be a prevalent fracture. If it was not present at baseline, the fracture was defined as incident.

Bone mineral density. BMD measurements of the femoral neck were performed at baseline using dual energy X-ray absorptiometry (DXA, Lunar DPX-L densitometer). Standard positioning was used with anterior-posterior scans of the right proximal femur unless there was a history of hip fracture or prosthesis implantation. In the latter case, the left side was scanned[9, 22].

Other variables. Data on age, sex, height, weight, body mass index (BMI) and other potentially confounding (use of a walking aid, lower limb disability and smoking) variables were obtained as described previously[9, 16].

Statistical analysis. Age- and sex-specific quartiles of femoral neck BMD (FN-BMD) for RS-I and RS-II were created by forming quartiles of FN-BMD by age groups per 5 years for men and women separately. The significance of the differences in height, weight and BMI by these quartiles was calculated by means of a linear regression model. Odds ratios (ORs) with 95% confidence intervals (95%CI) for the association between FN-BMD quartiles and ROA were calculated by means of logistic regression modeling, and were adjusted for baseline age, sex, BMI, study population, follow up time and corresponding K/L sum score at baseline. This sum score was calculated by adding up the K/L scores (0-4) of both knees, both hips or both hand joints, thus creating sum scores for each separate joint group. This was done in order to adjust for the potential confounding effect of mild OA at baseline (a K/L score 1 of one or both joints) on the incidence of ROA, and of the severity of OA at baseline for progression of ROA. FN-BMD per SD was calculated stratified by sex and study population, and ORs for the association between SD increase in FN-BMD and ROA were calculated by means of logistic regression modeling as well, and adjusted for baseline age, sex, BMI, study population, follow up time and corresponding K/L sum score at baseline. ORs for the association between the incidence or progression of knee, hip and hand ROA and prevalent vertebral and non-vertebral fractures in RS-I were calculated by means of logistic regression modeling, and were adjusted for baseline age, sex, body mass index, femoral neck bone mineral density, use of walking aid, lower limb disability, fall tendency and corresponding K/L sum score at baseline. We used IBM© SPSS version 22 for all our analyses.

RESULTS

In **Table 2.3.1** the baseline characteristics of the RS-I and RS-II study populations by age- and sex-specific quartiles of FN-BMD are shown. The mean height, weight and BMI were significantly higher with increasing FN-BMD. Subjects in RS-II were on average 2 years younger compared to those in RS-I, and had significantly increased weight and BMI. RS-I included 389 incident and 236 progressive knee ROA cases, 221 incident and 116 progressive hip ROA cases, and 320 incident hand ROA cases. In RS-II, 65 incident and 51 progressive knee ROA cases were present, 32 incident and 21 progressive hip ROA cases, and 96 incident hand ROA cases.

Bone mineral density

Table 2.3.2 shows the association between age- and sex-adjusted quartiles of FN-BMD and the incidence of knee and hip ROA, the progression of knee and hip ROA, and the combined measure for incidence or progression. An increase of incidence in knee ROA was seen with high FN-BMD: subjects in the highest FN-BMD quartile had a 58% increased risk of incident knee ROA compared to subjects in the lowest quartile (OR 1.58; 95%CI: 1.14 to 2.18). The effect for progression of knee OA was non-significant (OR 1.07; 95%CI: 0.64 to 1.78), the risk for incident knee ROA or progression of knee ROA was 42% increased (OR 1.42; 95%CI: 1.09 to 1.86). The risk for incident knee ROA increased 15% with each SD increase in FN-BMD (OR 1.15; 95%CI: 1.04 to 1.28). No significant progression of ROA was seen per SD increase in FN-BMD (OR 0.89; 95%CI: 0.77 to 1.03).

Table 2.3.2. Association between age- and sex-adjusted quartiles of FN-BMD and incident and progressive knee and hip ROA

			Knee ROA, RS-I & -II			
quartiles FN-BMD	Incidence cases/total (%)	OR*	Progression cases/total (%)	OR*	Incidence or progression cases/total (%)	OR*
Quartile 1	90/933 (9.6 %)	1 (reference)	42/109 (38.5 %)	1 (reference)	132/1042 (12.7 %)	1 (reference)
Quartile 2	112/902 (12.4 %)	1.29 (0.93 - 1.78)	70/145 (48.3 %)	1.39 (0.82 - 2.38)	182/1047 (17.4 %)	1.34 (1.02 - 1.75)
Quartile 3	116/868 (13.4 %)	1.28 (0.92 - 1.78)	78/174 (44.8 %)	1.09 (0.65 - 1.84)	194/1042 (18.6 %)	1.23 (0.94 - 1.61)
Quartile 4	136/811 (16.8 %)	1.58 (1.14 - 2.18)	97/212 (45.8 %)	1.07 (0.64 - 1.78)	233/1023 (22.8 %)	1.42 (1.09 – 1.86)
	per increase sd FN-BMD	1.15 (1.04 - 1.28)	per increase sd FN-BMD	0.89 (0.77 - 1.03)	per increase sd FN-BMD	1.06 (0.97 - 1.15)
			Hip ROA, RS-I & -II			
quartiles FN-BMD	Incidence cases/total (%)	OR†	Progression cases/total (%)	OR†	Incidence or progression cases/total (%)	OR†
Quartile 1	49/992 (4.9 %)	1 (reference)	21/50 (42.0 %)	1 (reference)	70/1042 (6.7 %)	1 (reference)
Quartile 2	59/991 (6.0 %)	1.01 (0.68 - 1.52)	30/56 (53.6 %)	1.71 (0.74 - 3.96)	89/1047 (8.5 %)	1.17 (0.81 - 1.68)
Quartile 3	59/986 (6.0 %)	1.04 (0.69 - 1.56)	30/56 (53.6 %)	1.61 (0.70 - 3.75)	89/1042 (8.5 %)	1.17 (0.81 - 1.70)
Quartile 4	86/930 (9.2 %)	1.57 (1.06 - 2.32)	56/93 (60.2 %)	2.17 (0.99 - 4.79)	142/1023 (13.9 %)	1.82 (1.28 - 2.59)
	per increase sd FN-BMD	1.07 (0.95 - 1.22)	per increase sd FN-BMD	1.32 (1.04 - 1.66)	per increase sd FN-BMD	1.16 (1.05 - 1.29)

*Adjusted odds ratio's (OR), with 95% confidence intervals between parentheses, are adjusted for age, sex, body mass index, study population, follow up time and knee ROA sum score at baseline

† Adjusted odds ratio's (OR), with 95% confidence intervals between parentheses, are adjusted for age, sex, body mass index, study population, follow up time and hip ROA sum score at baseline

FN-BMD: femoral neck bone mineral density, ROA: radiographic osteoarthritis, RS: Rotterdam Study, sd: standard deviation

Subjects in the highest FN-BMD quartile had a 57% higher risk for incident hip ROA compared to the lowest quartile (OR 1.57; 95%CI: 1.06 to 2.32). The higher risk for progression of radiographic hip OA was not significant (OR 2.17; 95%CI: 0.99 to 4.79), while an 82% increased risk of incidence or progression of hip ROA (OR 1.82; 95%CI: 1.28 to 2.59) was observed. The increased risk for incidence or progression of hip ROA per SD FN-BMD was 16% (OR 1.16; 95%CI: 1.05 to 1.29). In contrast to radiographic knee OA, the latter association was driven by the progression of radiographic hip OA: the increased risk of progression per SD FN-BMD increase was 32% (OR1.32; 95%CI: 1.04 to 1.66). The increased risk of incident hip ROA per SD FN-BMD was not significant (OR 1.07; 95%CI: 0.95 to 1.22).

No significant association between high FN-BMD and the incidence of radiographic hand OA was found, as is shown in **Table 2.3.3**. Additional adjustment for other potentially confounding variables (use of a walking aid, lower limb disability and smoking) available for subjects in RS-I, did not change the risk estimates for knee, hip or hand ROA (data not shown).

Table 2.3.3. Association between age- and sex-adjusted quartiles of FN-BMD and incident hand ROA

	Hand ROA, RS-I & -II	
quartiles	Incidence	OR*
FN-BMD	cases/total (%)	
Quartile 1	103/565 (18.2 %)	1 (reference)
Quartile 2	98/532 (18.4 %)	1.01 (0.73 - 1.40)
Quartile 3	112/520 (21.5 %)	1.22 (0.88 - 1.69)
Quartile 4	103/501 (20.6 %)	1.03 (0.74 - 1.44)
	per increase sd FN-BMD	1.01 (0.90 - 1.13)

*Adjusted odds ratio's (OR), with 95% confidence intervals between parentheses, are adjusted for age, sex, body mass index, study population, follow up time and hand ROA sum score at baseline
FN-BMD: femoral neck bone mineral density, ROA: radiographic osteoarthritis, RS: Rotterdam Study, sd: standard deviation

In **Supplementary Table 2.3.1** the incidence and progression of ROA by quartiles of FN-BMD for RS-I and RS-II separately are shown. The incidence or progression of knee and hip ROA for subjects in RS-II was approximately half compared to the incidence or progression for subjects in RS-I in each quartile. The incidence or progression of knee and hip ROA increased per quartile in both RS-I and RS-II. This resulted in a 36% increased risk for incident or progressive knee ROA for subjects in the highest quartile compared those in the lowest quartiles in RS-I (OR 1.36; 95%CI: 1.01 to 1.84), and an 84% increased risk for hip ROA (OR 1.84; 95%CI: 1.26 to 2.70). In RS-II, the increased risks in the highest quartiles failed to reach significance. However, for the incidence or progression of knee ROA a 30% increased risk per SD FN-BMD was observed in RS-II.

Supplementary Table 2.3.1. Association between age- and sex-adjusted quartiles of FN- BMD and incident and progressive knee and hip ROA: RS-I & RS-II separately

	Knee ROA, RS-I		**Knee ROA, RS-II**	
quartiles FN-BMD	Incidence or progression cases/total (%)	OR*	Incidence or progression cases/total (%)	OR*
Quartile 1	112/755 (14.8 %)	1 (reference)	20/287 (7.0 %)	1 (reference)
Quartile 2	159/757 (21.0 %)	1.41 (1.05 - 1.90)	23/290 (7.9 %)	1.03 (0.51 - 2.05)
Quartile 3	167/752 (22.2 %)	1.30 (0.96 - 1.75)	27/290 (9.3 %)	0.97 (0.49 - 1.91)
Quartile 4	187/741 (25.2 %)	1.36 (1.01 - 1.84)	46/282 (16.3 %)	1.67 (0.88 - 3.19)
	per increase sd FN-BMD	1.00 (0.90 - 1.10)	per increase sd FN-BMD	1.30 (1.08 - 1.58)
	Hip ROA, RS-I		**Hip ROA, RS-II**	
quartiles FN-BMD	Incidence or progression cases/total (%)	OR†	Incidence or progression cases/total (%)	OR†
Quartile 1	59/755 (7.8 %)	1 (reference)	11/287 (3.8 %)	1 (reference)
Quartile 2	75/757 (9.9 %)	1.14 (0.77 - 1.70)	14/290 (4.8 %)	1.38 (0.54 - 3.54)
Quartile 3	78/752 (10.4 %)	1.13 (0.76 - 1.70)	11/290 (3.8 %)	1.52 (0.58 - 4.00)

	Hip ROA, RS-I		Hip ROA, RS-II	
Quartile 4	125/741 (16.9 %)	1.84 (1.26 - 2.70)	17/282 (6.0 %)	1.83 (0.72 - 4.64)
	per increase sd FN-BMD	1.14 (1.01 - 1.29)	per increase sd FN-BMD	1.18 (0.91 - 1.54)

*Adjusted odds ratio's (OR), with 95% confidence intervals between parentheses, are adjusted for age, sex, body mass index, study population, follow up time and knee ROA sum score at baseline
† Adjusted odds ratio's (OR), with 95% confidence intervals between parentheses, are adjusted for age, sex, body mass index, study population, follow up time and hip ROA sum score at baseline
FN-BMD: femoral neck bone mineral density, ROA: radiographic osteoarthritis, RS: Rotterdam Study, sd: standard deviation

In RS-I, the percentage of subjects with incident hand OA tended to increase in the higher BMD quartiles (**Supplementary Table 2.3.2**), with a significantly increased risk of 52% for subject in the third quartile (OR 1.52; 95%CI: 1.03 to 2.23), but no significant association was seen was seen for the highest quartile (OR1.18; 95%CI: 0.79 to 1.76). In RS-II, no significant association between FN-BMD and incident hand ROA was found.

Supplementary Table 2.3.2. Association between age- and sex-adjusted quartiles of FN-BMD and incident hand ROA: RS-I & RS-II separately

	Hand ROA, RS-I		Hand ROA, RS-II	
quartiles	Incidence	OR*	Incidence	OR*
FN-BMD	cases/total (%)		cases/total (%)	
Quartile 1	74/342 (21.6 %)	1	29/223 (13.0 %)	1
Quartile 2	75/322 (23.3 %)	1.12 (0.76 - 1.65)	23/210 (11.0 %)	0.79 (0.43 - 1.46)
Quartile 3	90/307 (29.3 %)	1.52 (1.03 - 2.23)	22/213 (10.3 %)	0.65 (0.34 - 1.23)
Quartile 4	81/310 (26.1 %)	1.18 (0.79 - 1.76)	22/191 (11.5 %)	0.70 (0.37 - 1.34)
	per increase sd FN-BMD	1.05 (0.91 - 1.21)	per increase sd FN-BMD	0.87 (0.71 - 1.08)

*Adjusted odds ratio's (OR), with 95% confidence intervals between parentheses, are adjusted for age, sex, body mass index, study population, follow up time and hand ROA sum score at baseline
FN-BMD: femoral neck bone mineral density, ROA: radiographic osteoarthritis, RS: Rotterdam Study, sd: standard deviation

Prevalent fractures

Table 2.3.4 shows the association between prevalent vertebral and non-vertebral fractures and the combined measure of incidence or progression of radiographic knee and hip OA, and the incidence of radiographic hand OA. No significant associations were found between fractures and radiographic knee and hip OA, and with non-vertebral fractures and the incidence of hand ROA. Subjects with a vertebral fracture at baseline, however, had a 74% increased risk of incident hand ROA (OR 1.74; 95%CI: 1.02 to 2.98) after adjustments for possible confounding factors.

Table 2.3.4. Knee, hip and hand ROA by prevalent vertebral and non-vertebral fractures, RS-I

	Knee ROA		**Hip ROA**		**Hand ROA**	
prevalent fracture type	Incidence or progression cases/total (%)	OR*	Incidence or progression cases/total (%)	OR*	Incidence cases/total (%)	OR*
vertebral fracture						
absent	541/2597 (20.8 %)	1 (reference)	306/2710 (11.3 %)	1 (reference)	304/1229 (24.7 %)	1 (reference)
present	38/211 (18.0 %)	0.72 (0.48 - 1.09)	25/210 (11.9 %)	0.96 (0.57 - 1.61)	24/74 (32.4 %)	1.74 (1.02 - 2.98)
non-vertebral fracture						
absent	516/2596 (19.9 %)	1 (reference)	290/2686 (10.8 %)	1 (reference)	310/1228 (25.2 %)	1 (reference)
present	148/579 (25.1 %)	1.21 (0.96 - 1.53)	84/596 (14.1 %)	1.25 (0.92 - 1.68)	55/250 (22.0 %)	0.77 (0.54 - 1.10)

OR*: Odds ratio, with 95% confidence intervals between parentheses, adjusted for age, sex, body mass index, femoral neck bone mineral density, use of walking aid, lower limb disability, fall tendency and corresponding ROA sum score at baseline
ROA: radiographic osteoarthritis, RS: Rotterdam Study

DISCUSSION

Results of this study present strong evidence that high BMD is a significant risk factor for the development of subsequent knee and hip OA. High baseline FN-BMD is significantly related to the incidence of radiographic knee and hip OA, but not to the incidence of radiographic hand OA. Furthermore, high baseline FN-BMD is not associated with the progression of radiographic knee OA. The association between high FN-BMD and the progression of radiographic hip OA is not significant, but there is a significant increase in risk of progressive hip OA per SD increase of FN-BMD. Prevalent vertebral fractures are associated with the incidence of radiographic hand osteoarthritis, but not with any of the measures in knee and hip ROA. Non-vertebral fractures are not associated with incidence or progression of knee, hip or hand OA.

Bone mineral density

Results on the relationship between BMD and the incidence on knee ROA confirm earlier studies[3-7], and strengthens the evidence that high FN-BMD is an important risk factor for developing subsequent knee ROA. In contrast to knee OA, almost no previous data was available on the association between high BMD and incident hip OA. The current study provides evidence that high BMD is also a significant risk factor for incident radiographic hip ROA. This finding confirms the results mentioned by Hochberg et al.[11] They concluded that a greater BMD increases the risk that an elderly white woman will develop radiographic hip OA when the diagnosis of OA is based upon osteophytosis, but not when the diagnosis is based upon the development of JSN alone. However, no numbers, percentages or ORs were presented in this paper. The conflicting results reported by Barbour et al.[12] on the other hand, might be explained by the limited number of subjects in their study. In our study, we observed that in the smaller sub group, RS-II, the association between the highest quartile of FN-BMD and incidence or progression of knee and hip ROA failed to reach significance, probably due to lack of power (**Supplementary Table 2.3.1)**. The increase in risk of incident or progressive knee ROA per SD increase of FN-BMD however was significant in RS-II. This could be due to the increased power by analyzing per SD increase in BMD, instead of per quartile.

No association of high FN-BMD and progression of knee ROA was observed in this study. This is consistent with previous findings[7] and might be due to collider bias[23] or lack of power, since the number of cases with progressive knee OA is low.

Table 2.3.5. Characteristics past study versus present study

	Past study*	**Present study**	
	RS-I	RS-I	RS-II
Number of patients	1,403	3,005	1,149
Follow-up time (yrs)	6.5	8.4	4.1
Incident knee ROA	74	389	65
Progressive knee ROA	25	236	51

ROA: radiographic osteoarthritis
RS: Rotterdam Study
* Bergink AP, Uitterlinden AG, Van Leeuwen JP, Hofman A, Verhaar JA, and Pols HA. Bone mineral density and vertebral fracture history are associated with incident and progressive radiographic knee osteoarthritis in elderly men and women: the Rotterdam Study. Bone 2005;37:446-56.

Comparing the results on knee ROA of the present study with results reported by Bergink et al. in 2005 (**Table 2.3.5**), it can be concluded that both studies found that high BMD at baseline is associated with increased risk of incident knee OA during follow-up. The present study, thus, confirms previous results and provides stronger evidence. Nevertheless, the increased risk of incident knee ROA per SD increase of FN-BMD in 2005 was 50% (OR 1.5; 95%CI: 1.1 to 1.9), while in the present study the increased risk is lower, 15% (OR 1.15; 95%CI: 1.04 to 1.28). Looking at the results from RS-I and RS-II separately, it can be concluded that the increased risk per SD increase of FN-BMD is higher in RS-II than in RS-I (OR 1.30; 95%CI: 1.08 to 1.58 and OR 1.00; 95%CI: 0.90 to 1.10, respectively, **Supplementary Table 2.3.1)**. A possible explanation for the higher OR in RS-II, which is more similar to the OR in 2005, is that the mean age of the RS-II study population in the present study, like the mean age of the subjects in RS-I in 2005, is relatively low, and that the risk attenuates with extended follow-up time and aging of participants. In younger patients the development of subsequent ROA might be more likely to be caused by systemic effects associated with high BMD, and to a lesser extent by environmental influences.

The present study is the first prospective study to provide consistent evidence for the relationship between high baseline BMD and incident hip OA. The risk for incident hip ROA and knee ROA is similar. But other than for knee ROA, a significant increased risk was found for progression of hip ROA per SD FN-BMD. This is also translated into the higher increased risk for incidence or progression in hip ROA, compared to knee ROA. However, the risk for progression of hip ROA alone was not significantly increased for subject in the highest FN-BMD quartile.

Following the fact that contrary to knee and hip ROA, no associations were found between BMD and the incidence of hand ROA, it seems that the positive association between BMD and ROA is strongest in weight-bearing joints. This might be due to local mechanical influences. Repetitive forces on subchondral bone with altered bone characteristics, like increased stiffness of cortical bone, might lead to increased deterioration of overlying cartilage. Consequently, this is more pronounced in weight-bearing joints.

Osteophytes around the femoral head may influence femoral neck BMD and will result in higher BMD measured at the femoral neck. They can, thus confound the association between BMD and hip OA. However, previous studies with data from the Rotterdam Study showed that hypertrophic hip OA was associated with elevated BMD also measured at remote sites, like the skull[24]. Discrimination between atrophic and hypertrophic ROA seems important since atrophic OA is associated with deceased BMD, and hypertrophic OA with increased BMD[24]. In the present study radiographic OA was classified using the K/L score, in which the formation of osteophytes, besides joint space narrowing, determines the severity of OA. Consequently, this study focused on the incidence and progression of hypertrophic, rather than atrophic AO. Since atrophic AO is considered to be a different disease type than hypertrophic OA[25, 26], and associated with different risk factors[24, 27], the present results can't be generalized to all types of OA, especially atrophic OA.

Prevalent fractures

In our previous research a significant protective effect of prevalent vertebral fractures on the risk of incidence and progression of knee ROA was reported. These results were not confirmed by the present study. Although a decreased risk of incident and progressive knee OA was observed, the association was not significant (OR 0.72; 95%CI: 0.48 to 1.09). The present study, however, provides evidence for an increased risk of hand ROA in subjects with prevalent vertebral fractures (OR 1.74; 95%CI: 1.02 to 2.98). It is likely that a common risk factor influences both the incidence of OA in non-weight bearing joints and vertebral fracture risk. Stronger correlations between genetic factors and OA are seen in hand OA compared to knee or hip OA[15, 28]. Likewise recent studies show that vertebral fracture risk is influenced by genetic factors[29, 30]. It is plausible that a common (heritable) bone characteristic affects both vertebral fracture risk and the incidence of hand OA. Another possible explanation for this observation might be that subjects with a prevalent vertebral fracture are more dependent on the use of a walking aid, leading to an increased risk of hand OA[31].

Methodological considerations

In the present study, the incidence and progression of ROA were combined in a composite measure 'incidence or progression' to be able to compare our results to our past study[9] and to decrease multiple testing when analyzing RS-I and RS-II separately, and when analyzing the association between prevalent fractures and ROA. In addition, we feel this is a valid approach considering that the development and worsening of OA is a continuous process and the distinction between incident and progressive OA remains artificial. We constructed quartiles of FN-BMD to compare subjects with low BMD to those with high BMD, and analyzed per increase SD FN-BMD to evaluate the effect of BMD change on incidence or progression of OA in the total study population, thus increasing power. To avoid multiple testing and to avoid the known profound confounding effect of lumbar spine osteophytes on BMD measurements, analyses were done with FN-BMD, and not with lumbar spine BMD.

Participants were selected based on the availability of follow-up radiographs. This selection introduces a possible selection bias, since these subjects survived the follow-up period, and were healthy enough to visit the research center. Furthermore, since selection of the study population was based on both the availability of data on exposure (BMD) and outcome (incidence or progression of OA), collider bias may be introduced[23]. A possible association between baseline BMD and especially progressive knee or hip OA could be obscured by this type of bias, since subjects with progressive OA are less likely to return to the research center for follow examinations.

In order to adjust for the potential confounding effect of mild OA at baseline (K/L score 1 at one or both joints) on the incidence of ROA, and of the severity of OA at baseline for progression of ROA, we adjusted for site-specific K/L sum score. However, if mild OA at baseline does not confound the possible association between baseline BMD and incidence or progressive OA, but is independently associated with exposure (BMD) and outcome (incidence or progressive OA), adjusting for it could lead to collider bias[23, 32]. Therefore we performed additional analyses without adjustment for baseline site-specific K/L sumscore. The odds ratios without adjustment for the baseline sumscore were marginally higher than with adjustment for the baseline K/L sumscore, which argues against collider bias.

Finally, we excluded subjects with joint replacements in our analyses as they are not merely an expression of worsening of OA, but also –and perhaps mainly- of pain experience and burden to daily living. This could weaken an association between baseline BMD and radiographic OA. In our study population RS1, 21 subjects received a total knee replacement (TKR) and 46 subjects received a total hip replacement (THR) during a mean follow up time of 8.4 years. Including subjects with TKRs and THPs in the analyses resulted in slightly increased ORs for incident knee and hip OA of subjects in the highest quartiles of baseline BMD compared to those in the lowest BMD quartiles (data not shown).

In conclusion, the present large longitudinal study confirms earlier studies and thus provides strong evidence that high FN-BMD is a prognostic risk factor for the

development of subsequent radiographic knee and hip OA. No evidence was provided for high FN-BMD as a prognostic risk factor for progression of radiographic knee or hip, or for the incidence of radiographic hand OA. However, a significant higher risk of progression of hip ROA was found for each SD increase in FN-BMD. The protective effect of vertebral fractures for the incidence or progression of radiographic knee OA could not be confirmed by the present study, but vertebral fractures were found to be a risk factor for the incidence of hand OA.

REFERENCES

1. Stewart A, and Black AJ. Bone mineral density in osteoarthritis, *Current opinion in rheumatology* 2000;12:464-467.

2. Dequeker J, Aerssens J, and Luyten FP. Osteoarthritis and osteoporosis: clinical and research evidence of inverse relationship, *Aging clinical and experimental research* 2003;15:426-439.

3. Sowers M, Lachance L, Jamadar D, et al. The associations of bone mineral density and bone turnover markers with osteoarthritis of the hand and knee in pre- and perimenopausal women, *Arthritis and rheumatism* 1999;42:483-489.

4. Zhang Y, Hannan MT, Chaisson CE, et al. Bone mineral density and risk of incident and progressive radiographic knee osteoarthritis in women: the Framingham Study, *The Journal of rheumatology* 2000;27:1032-1037.

5. Hart DJ, Cronin C, Daniels M, et al. The relationship of bone density and fracture to incident and progressive radiographic osteoarthritis of the knee: the Chingford Study, *Arthritis and rheumatism* 2002;46:92-99.

6. Hochberg MC, Lethbridge-Cejku M, and Tobin JD. Bone mineral density and osteoarthritis: data from the Baltimore Longitudinal Study of Aging, *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society* 2004;12 Suppl A:S45-48.

7. Nevitt MC, Zhang Y, Javaid MK, et al. High systemic bone mineral density increases the risk of incident knee OA and joint space narrowing, but not radiographic progression of existing knee OA: the MOST study, *Annals of the rheumatic diseases* 2010;69:163-168.

8. Im GI, and Kim MK. The relationship between osteoarthritis and osteoporosis, *Journal of bone and mineral metabolism* 2014;32:101-109.

9. Bergink AP, Uitterlinden AG, Van Leeuwen JP, et al. Bone mineral density and vertebral fracture history are associated with incident and progressive radiographic knee osteoarthritis in elderly men and women: the Rotterdam Study, *Bone* 2005;37:446-456.

10. Hardcastle SA, Dieppe P, Gregson CL, et al. Osteoarthritis and bone mineral density: are strong bones bad for joints?, *BoneKEy reports* 2015;4:624.

11. Hochberg MC. Do risk factors for incident hip osteoarthritis (OA) differ from those for progression of hip OA?, *The Journal of rheumatology. Supplement* 2004;70:6-9.

12. Barbour KE, Murphy LB, Helmick CG, et al. Bone Mineral Density and the Risk of Hip and Knee Osteoarthritis: The Johnston County Osteoarthritis Project, *Arthritis care & research* 2017.

13. Visser AW, Ioan-Facsinay A, de Mutsert R, et al. Adiposity and hand osteoarthritis: the Netherlands Epidemiology of Obesity study, *Arthritis research & therapy* 2014;16:R19.

14. Sowers MF, Hochberg M, Crabbe JP, et al. Association of bone mineral density and sex hormone levels with osteoarthritis of the hand and knee in premenopausal women, *American journal of epidemiology* 1996;143:38-47.

15. Doherty M. Genetics of hand osteoarthritis, *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society* 2000;8:S8-10.

16. Hofman A, Grobbee DE, de Jong PT, et al. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study, *European journal of epidemiology* 1991;7:403-422.

17. Hofman A, Darwish Murad S, van Duijn CM, et al. The Rotterdam Study: 2014 objectives and design update, *European journal of epidemiology* 2013;28:889-926.

18. Kellgren JH, Jeffrey MR, and Ball J (1963) *Atlas of Standard Radiographs of Arthritis: The epidemiology of Chronic Rheumatism*, Vol. 2, Blackwell Scientific Publications, Oxford.

19. Odding E, Valkenburg HA, Algra D, et al. Associations of radiological osteoarthritis of the hip and knee with locomotor disability in the Rotterdam Study, *Annals of the rheumatic diseases* 1998;57:203-208.

20. Kerkhof HJ, Bierma-Zeinstra SM, Arden NK, et al. Prediction model for knee osteoarthritis incidence, including clinical, genetic and biochemical risk factors, *Annals of the rheumatic diseases* 2014;73:2116-2121.

21. McCloskey EV, Spector TD, Eyres KS, et al. The assessment of vertebral deformity: a method for use in population studies and clinical trials, *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA* 1993;3:138-147.

22. Burger H, van Daele PL, Odding E, et al. Association of radiographically evident osteoarthritis with higher bone mineral density and increased bone loss with age. The Rotterdam Study, *Arthritis and rheumatism* 1996;39:81-86.

23. Munafo MR, Tilling K, Taylor AE, et al. Collider scope: when selection bias can substantially influence observed associations, *International journal of epidemiology* 2018;47:226-235.

24. Castano-Betancourt MC, Rivadeneira F, Bierma-Zeinstra S, et al. Bone parameters across different types of hip osteoarthritis and their relationship to osteoporotic fracture risk, *Arthritis and rheumatism* 2013;65:693-700.

25. Panoutsopoulou K, Thiagarajah S, Zengini E, et al. Radiographic endophenotyping in hip osteoarthritis improves the precision of genetic association analysis, *Annals of the rheumatic diseases* 2017;76:1199-1206.

26. Conrozier T, Ferrand F, Poole AR, et al. Differences in biomarkers of type II collagen in atrophic and hypertrophic osteoarthritis of the hip: implications for the differing pathobiologies, *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society* 2007;15:462-467.

27. Conrozier T, Merle-Vincent F, Mathieu P, et al. Epidemiological, clinical, biological and radiological differences between atrophic and hypertrophic patterns of hip osteoarthritis: a case-control study, *Clinical and experimental rheumatology* 2004;22:403-408.

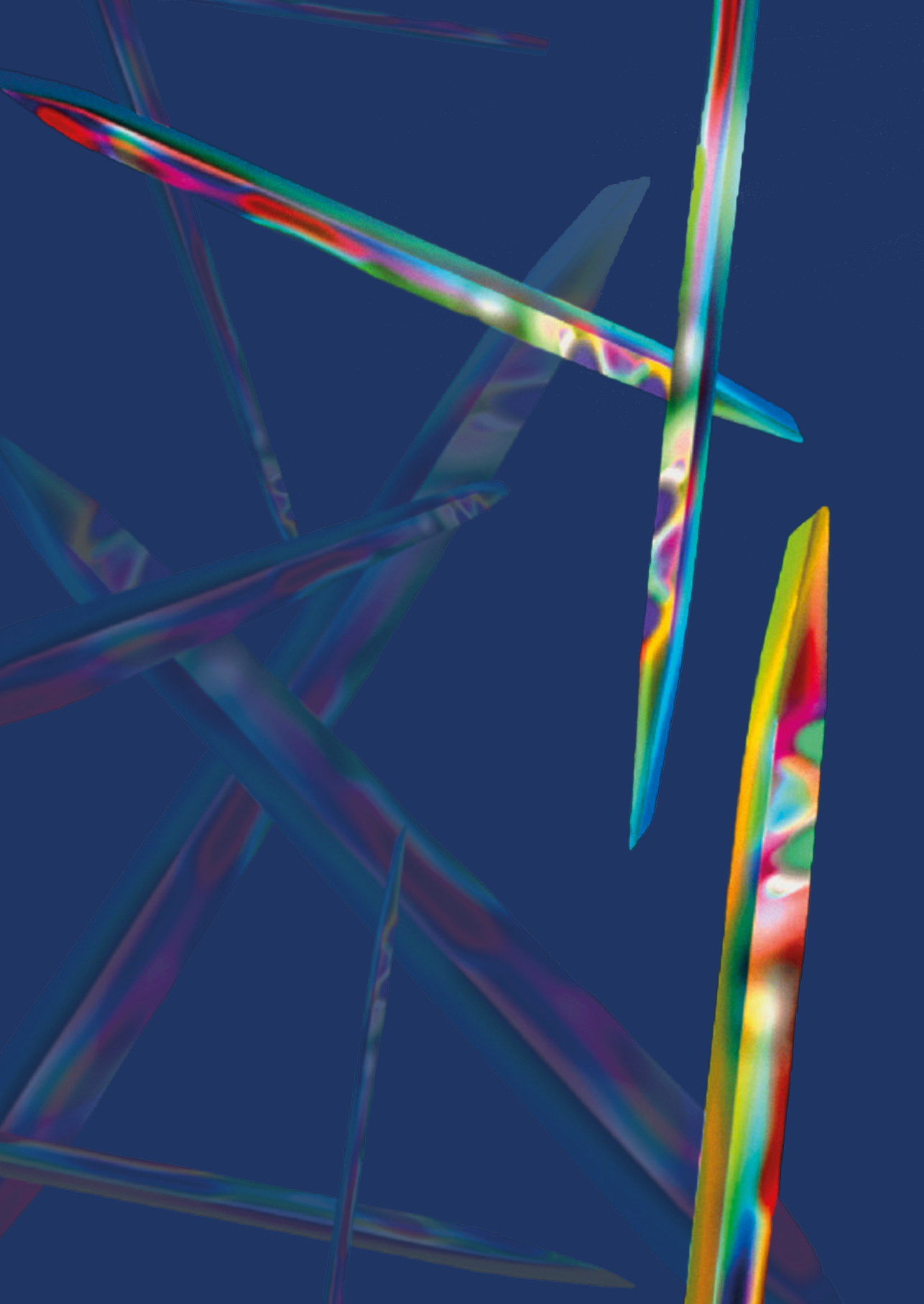
28. MacGregor AJ, Li Q, Spector TD, et al. The genetic influence on radiographic osteoarthritis is site specific at the hand, hip and knee, *Rheumatology* 2009;48:277-280.

29. Nielson CM, Liu CT, Smith AV, et al. Novel Genetic Variants Associated With Increased Vertebral Volumetric BMD, Reduced Vertebral Fracture Risk, and Increased Expression of SLC1A3 and EPHB2, *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research* 2016;31:2085-2097.

30. Liu CT, Karasik D, Zhou Y, et al. Heritability of prevalent vertebral fracture and volumetric bone mineral density and geometry at the lumbar spine in three generations of the Framingham study, *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research* 2012;27:954-958.

31. Werner RA, Waring W, and Maynard F. Osteoarthritis of the hand and wrist in the post poliomyelitis population, *Archives of physical medicine and rehabilitation* 1992;73:1069-1072.

32. Cole SR, Platt RW, Schisterman EF, et al. Illustrating bias due to conditioning on a collider, *International journal of epidemiology* 2010;39:417-420.



3

VITAMIN D AND OSTEOARTHRITIS

3.1 dietary vitamin D intake, serum vitamin D levels and knee osteoarthritis

Vitamin D status, bone mineral density, and the development of radiographic osteoarthritis of the knee: The Rotterdam Study

J Clin Rheumatol 2009 Aug;15(5):230-7. PubMed PMID: 19654490

Bergink AP, Uitterlinden AG, van Leeuwen JP, Buurman CJ, Hofman A, Verhaar JA, Pols HA

ABSTRACT

Objective. To study the association between baseline vitamin D status, bone mineral density (BMD) and the development of radiographic osteoarthritis (ROA) of the knee in a large population-based cohort of men and women.

Methods. A sample of 1248 subjects (728 women and 520 men) was drawn from the Rotterdam Study, a prospective population-based cohort study of the elderly. At baseline, vitamin D dietary intake was determined, and BMD and 25-hydroxy vitamin D (25(OH)D) serum levels were measured. After a mean follow-up time of 6.5 years incidence and progression of knee ROA of was assessed.

Results. The mean vitamin D intake in our study population was 64 IU/day and the mean 25(OH)D level 66 nmol/l. Vitamin D levels were associated with baseline BMD, particularly in subjects with baseline knee ROA. Progressive ROA occurred in 5.1% of the participants in the highest tertile of vitamin D intake against 12.6% in the lowest tertile, resulting in an adjusted odds ratio of 7.7 (95% C.I. 1.3 to 43.5). Both intake and levels of 25(OH)D were not significantly related to incident ROA. However, we found a significant interaction between vitamin D intake and BMD in the association with incident knee ROA (p=0.03): in subjects with low lumbar spine BMD at baseline we observe an increasing incidence of knee ROA with decreasing vitamin D intake and serum levels.

Conclusions. Low dietary vitamin D intake increases the risk of progression of knee ROA. Particularly in subjects with low baseline BMD, vitamin D status seems to influence the incidence and progression of knee ROA. Thus, improving the vitamin D status in elderly could protect against the development and worsening of knee OA, especially in those with low BMD.

INTRODUCTION

Local changes in subchondral bone play an important role in the development of osteoarthritis (OA)[1, 2], while altered bone characteristics at remote sites are found in subjects with OA[3, 4]. Epidemiological studies have shown that several aspects of bone health, like bone mineral density (BMD), bone turnover and fracture risk, are associated with OA[5-7]. Since vitamin D influences bone quality[8], it is hypothesized that vitamin D status has an effect on the risk of the development or progression of OA. Bischoff-Ferrari and colleagues found in their cross-sectional study of 228 subjects with primary radiographic knee OA a high prevalence of sub-optimal vitamin D levels with a positive association between vitamin D status and femoral neck (FN) BMD[9]. They suggested that correction of sub-optimal vitamin D levels could help reducing fracture risk as well as progression of OA. To our knowledge, two prospective epidemiological studies have found an association between dietary intake and serum levels of vitamin D and the development of radiographic OA. The first study was performed by McAlindon and colleagues[10], who studied 556 subjects (37% men) over a period of 8.5 years, with 162 subjects having prevalent knee osteoarthritis. In their analysis of separate knees, they showed a 3- to 4-fold increased risk of progression of OA in the middle and lower tertiles compared to the higher tertiles of dietary intake of vitamin D as well as baseline serum 25-hydroxy vitamin D levels. The other study investigating vitamin D levels and OA was performed by Lane and colleagues[11]. Their study of 237 women over a follow-up period of 8 years showed a 3-fold increased risk of incident joint space narrowing (JSN) at the hips of women in the middle and lower tertiles of serum 25-hydroxy vitamin D levels at baseline. In both studies, adjustment for baseline BMD did not change the risk estimates. In contrast, a recent study of Felson and colleagues suggests that vitamin D status is unrelated to the risk of joint space or cartilage loss in knee OA[12].

In our study we investigated 1248 men and women during 6.5 years for the possible influence of vitamin D status on the development or worsening of radiological knee OA, and whether BMD plays a role in the association. We analyzed our data similar to the aforementioned studies in order to be able to compare our results with their findings.

MATERIALS AND METHODS

Subjects. The study population consisted of subjects of the Rotterdam Study, a prospective cohort study of men and women aged 55 and over, with the objective to investigate the incidence of, and risk factors for, chronic disabling diseases. The rationale and study design have been described previously[13]. The study population consisted of 1248 subjects (58% women), drawn from the Rotterdam Study population. The selection was based on the availability of radiographs of the knees at baseline and second follow-up examinations and data on potentially confounding factors at baseline. Differences in characteristics between the complete Rotterdam Study and our study population were described previously[14]. Participants had to be able to come to our research center to have their radiographs taken. Thus, subjects in our study population were more mobile and on average 4 years younger than in the source population, the total Rotterdam Study.

Radiographic osteoarthritis. Both at baseline, between 1991 and 1993, and at the second follow-up visit, between 1997 and 1999, weight-bearing anteroposterior radiographs of the knees in fully extended position, with the patellae in central position, were obtained. The mean follow-up-time was 6.5 years (range: 5.8 to 9.3 years). Prevalence, incidence and progression of ROA by the Kellgren-Lawrence grading system[15], and of osteophytosis and JSN were scored as described previously[7]. In short, baseline scores were established by two independent readers[16]. Follow up radiographs were compared side by side to corresponding baseline radiograph by to identify incidence or progression of ROA. ROA of the knee was defined as a Kellgren score of 2 or more at baseline at one or both knees. Incident ROA was diagnosed when a subject had a Kellgren score below 2 at both knees at baseline, and had a Kellgren score of 2 or higher at either knee at follow-up. A subject was diagnosed with progressive ROA if the Kellgren score of either of the knees

was 2 or higher at baseline, and the Kellgren score increased in that same knee during follow-up.

Vitamin D intake and serum levels assessment. At baseline, dietary vitamin D intake and total energy intake (caloric intake) was assessed using a semiquantitative food frequency questionnaire[17]. Baseline 25-hydroxy vitamin D (25(OH)D) serum levels were measured using radioimmunoassays (IDS Ltd, Boldon, UK, www.idsltd.com). The sensitivity of the test is 3 nmol/l, the range 4 to 400 nmol/l, the intra-assay accuracy is <8%, the inter-assay accuracy <12%.

Other variables. Between 1990 and 1993, an extensive baseline home interview on medical history and of risk factors for chronic diseases was performed by trained interviewers. Information on smoking status was obtained and subjects were asked about their fall frequency over the past year. If the fall frequency was not zero, the participant was said to have a positive fall tendency. Health status was obtained by scoring the answer to the question 'How is your general health compared to members of your age group?' with -1 ('worse'), 0 ('same') or 1 ('better'). A disability index was assessed using a modified version of the Stanford Health Assessment Questionnaire, which provides information over eight categories of activities of daily living as described previously[18, 19]. After the home interview, subjects were invited to the research center for clinical examination and laboratory measurements. Height and weight were measured with subjects wearing indoor clothing and without shoes. Body mass index (BMI) was computed as weight in kilograms divided by height in squared meters (kg/m^2). BMD measurements of the FN and lumbar vertebrae L2 to L4 were performed by dual energy X-ray absorptiometry (DXA, Lunar DPX-L densitometer) as described previously[5]. At the second follow-up visit, between 1997 and 1999, all measurements were repeated.

Statistical analysis. The significance of the association between FN BMD and 25(OH)D serum levels in the absence and presence of baseline knee ROA was determined by linear regression modeling, adjusted for age, sex, BMI, smoking status, health status, disability index, fall tendency and baseline joint space narrowing. We used cutoff levels

as described by Bischoff-Ferrari et al[9] to classify vitamin D status, who considered subjects with 25(OH) vitamin D levels below 40 nmol/l vitamin D deficient, levels between 40 and 80 nmol/l hypovitaminosis D, and above 80 nmol/l vitamin D replete. Odds ratios (ORs) with 95% confidence intervals (C.I.) for incident and progressive knee ROA by tertiles of vitamin D were calculated by means of logistic regression modeling, and adjusted for the confounding factors described above. The analyses with vitamin D intake were additionally adjusted for caloric intake as advocated in previous papers[10, 20], and the analyses with vitamin D serum levels were additionally adjusted for time of season to adjust for sun exposure. Crude and adjusted p-values for trend were calculated with logistic regression modeling as well, with vitamin D tertiles put in the model as continuous instead of categorical variable. In addition, odds ratios of incident ROA by high (> 1.1 g/cm^2) and low (< 1.1 g/cm^2) lumbar spine (LS) BMD in different tertiles of vitamin D were calculated, and adjusted for the confounding factors as described above as well. The p-value for interaction was calculated by logistic regression modeling, with as dependent variable incident knee ROA, and as covariates LS BMD, vitamin D status, the product of LS BMD and vitamin D status, and confounding factors.

We used SPSS version 12.0.1 (SPSS Inc., Chicago, USA) for all our analyses.

RESULTS

Vitamin D status. Figure 3.1.1a depicts the distribution of dietary vitamin D intake in our study population. The mean and median vitamin D intake in our study population were 64.1 and 53.1 IU per day: of all subjects 2.1% had a daily intake of more than 200 IU, and only 2 subjects exceeded an intake of 400 IU per day. In **Figure 3.1.1b** the distribution of 25(OH)D serum levels is shown. The mean and median 25(OH)D serum levels are 66 and 62 nmol/l, respectively, with a range of 15 to 188 nmol/l.

Figure 3.1.1a. Distribution of dietary vitamin D intake

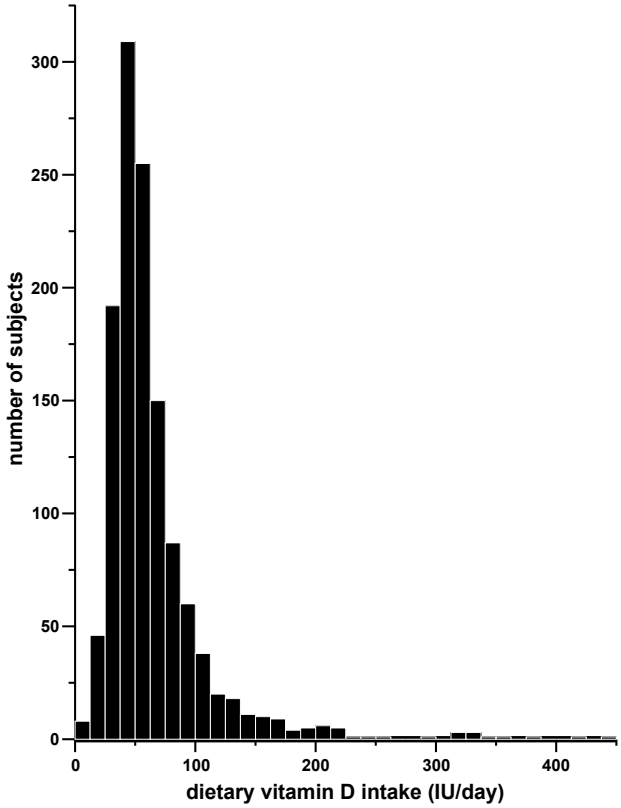
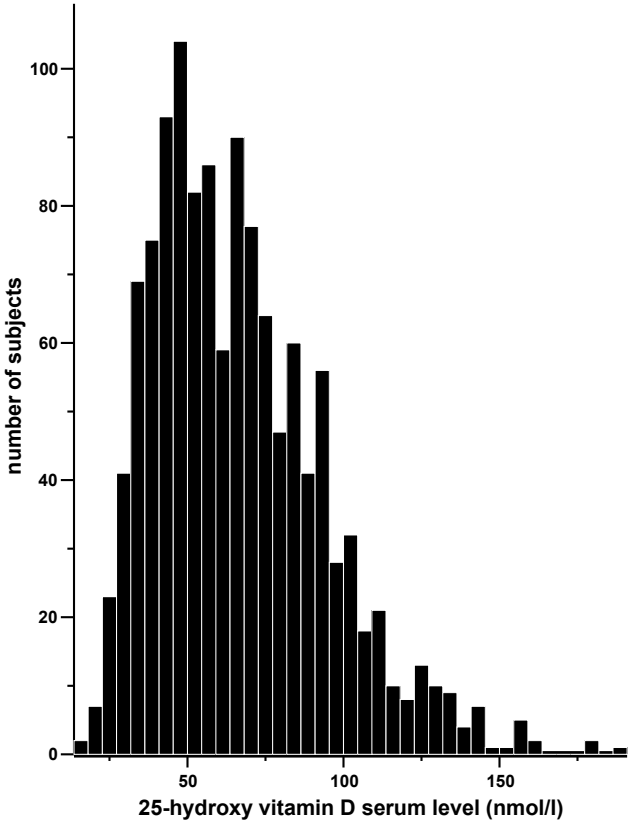
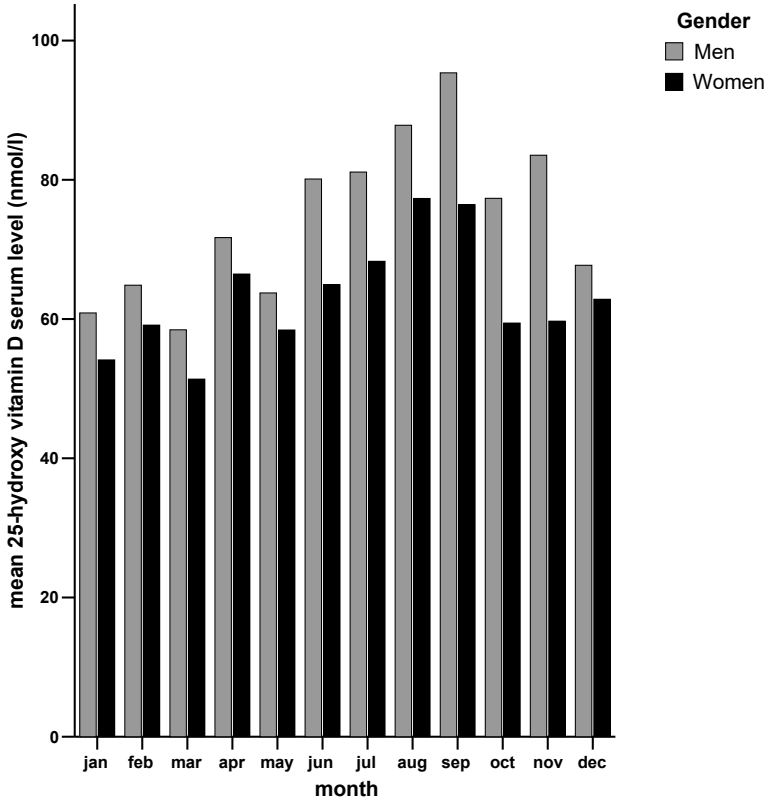


Figure 3.1.1b. Distribution of 25(OH)D serum levels



In **Figure 3.1.2**, we can observe that mean 25-hydroxy serum levels varied with the month in which blood was drawn from our participants. High levels were found in samples drawn in August and September, low levels from December until March. Overall, men had 17% higher serum levels than women ($p<0.01$).

Figure 3.1.2. Mean 25(OH)D serum levels in men and women by month of baseline examination



Vitamin D and knee ROA. Table 3.1.1 shows the baseline characteristics of our study population by tertiles of dietary vitamin D intake and by tertiles of 25(OH)D serum level. The highest dietary vitamin D intake tertile consisted of 1.3 years younger subjects and 25% less women compared to the lowest tertile. Furthermore, these subjects considered themselves healthier, tended to fall less and had 6% higher 25(OH) vitamin D serum levels. Other baseline characteristics did not differ significantly with dietary vitamin D intake.

With increasing 25(OH)D serum levels, the percentage of women decreased with 20%, subjects were up to 2 years younger, and their BMI 2% lower. FN and LS BMD increased with 6% and 4%, respectively, and the dietary vitamin D intake was 10 IU per day higher in the upper tertiles compared to the lower tertile. Furthermore, these subjects had a lower disability index, tended to fall less, and had less joint space narrowing at baseline.

Table 3.1.1. Baseline characteristics of the study population by quartiles of vitamin D intake and serum levels

		tertiles of dietary vitamin D intake (IU/day, mean & range)				**tertiles of 25(OH) vitamin D serum levels** (nmol/l, mean & range)			
	total	**I**	**II**	**III**	p- trend	**I**	**II**	**III**	p-trend
		34 (5 - 44)	53 (44 - 64)	105 (64 - 438)		39 (15 - 50)	62 (51 - 74)	98 (75 - 188)	
number	1248	416	416	416		414	423	411	
caloric intake (Kcal/day)	1980 ± 498	1709 ± 377	1974 ± 397	2257 ± 525	< 0.01	1897 ± 474	1973 ± 485	2070 ± 502	< 0.01
vitamin D intake (IU/day)	64 ± 44.5					57 ± 37	68 ± 45	67 ± 50	< 0.01
25(OH) vitamin D (nmol/l)	66 ± 27	64 ± 28	67 ± 28	68 ± 27	0.03				
women (%)	58.3	70.2	59.6	45.2	< 0.01	67.9	59.6	47.4	< 0.01
age (yrs)	66.2 ± 6.7	66.8 ± 6.9	66.3 ± 6.6	65.5 ± 6.6	< 0.01	68.7 ± 6.8	65.8 ± 6.6	64.1 ± 5.8	< 0.01
BMI (kg/m^2)	26.3 ± 3.5	26.3 ± 3.6	26.5 ± 3.7	26.2 ± 3.3	0.92	26.6 ± 4.0	26.5 ± 3.4	26.0 ± 3.1	< 0.01
FN BMD (g/cm^2)	0.85 ± 0.13	0.85 ± 0.14	0.85 ± 0.13	0.85 ± 0.13	0.23	0.82 ± 0.13	0.85 ± 0.13	0.87 ± 0.13	< 0.01
LS BMD (g/cm^2)	1.09 ± 0.19	1.09 ± 0.20	1.09 ± 0.19	1.11 ± 0.19	0.20	1.08 ± 0.20	1.09 ± 0.19	1.12 ± 0.20	< 0.01
health status	0.46 ± 0.61	0.37 ± 0.65	0.49 ± 0.58	0.52 ± 0.60	< 0.01	0.43 ± 0.62	0.46 ± 0.63	0.48 ± 0.58	0.32
cigarette smoking (%)	22.2	20.9	19.7	26.0	0.08	22.5	22.9	21.2	0.66
disability index	0.22 ± 0.32	0.25 ± 0.37	0.20 ± 0.27	0.21 ± 0.32	0.09	0.28 ± 0.38	0.21 ± 0.30	0.16 ± 0.27	< 0.01
positive fall tendency (%)	14.5	19.0	13.2	11.3	< 0.01	17.1	14.9	11.4	0.02
ROA knee by Kellgren (%)	20.0	20.9	20.4	18.8	0.44	22.2	18.7	19.2	0.28
osteophytosis (%)	21.6	21.4	22.6	20.9	0.87	23.4	21.0	20.4	0.30
joint space narrowing (%)	18.3	20.2	19.0	15.6	0.09	23.7	17.7	13.4	< 0.01

values are crude means with standard deviations (range) or percentages
BMI: body mass index, FN BMD: femoral neck bone mineral density, LS BMD: lumbar spine bone mineral density

In **Table 3.1.2** incidence and progression of knee ROA by tertiles of vitamin D intake and serum levels with corresponding odds ratios are presented. Overall, 6.5 % of the subjects had incident ROA, and 9.2 % had progressive ROA. With decreasing dietary intake of vitamin D, the percentage of subjects with progressive knee ROA increased: 5.1% of the participants in the highest tertile of dietary vitamin D intake tertile had progressive ROA, against 12.6% in the lowest tertile. The corresponding crude odds ratio of 2.7 did not reach significance. However, after adjustment for all confounding factors, the odds ratio was significantly increased to 7.7. The adjustment for caloric intake was responsible for reaching a point estimate of 3.7, while additional adjustment for BMI raised the odds ratio to 4.8, adding age and sex raised it further to in 5.7, and further adding baseline JSN raised the OR to 6.6 (data not shown). Without adjustment for caloric intake, no combination of adjustments resulted in a significantly increased odds ratio.

Table 3.1.2. Incidence / progression of knee ROA by tertiles of vitamin D intake and serum levels with corresponding odds ratios

tertiles of dietary vitamin D intake (mean & rang, IU/day)	**incident ROA** n / total (%)	crude OR	adjusted OR†	**progressive ROA** n / total (%)	crude OR	adjusted OR†
34 (5 – 44)	21 / 329 (6.4%)	1.2 (0.6 - 2.3)	1.0 (0.5 - 2.2)	11 / 87 (12.6%)	2.7 (0.8 - 8.8)	7.7 (1.3 - 43.5)
53 (44 – 64)	26 / 331 (7.9%)	1.5 (0.8 - 2.8)	1.4 (0.7 - 2.7)	8 / 85 (9.4%)	1.9 (0.6 - 6.7)	2.5 (0.6 - 11.2)
105 (64 – 438)	18 / 338 (5.3%)	1	1	4 / 78 (5.1%)	1	1
		p-trend = 0.57	p-trend = 0.98†		p-trend = 0.10	p-trend = 0.02
tertiles of 25(OH) vitamin D serum levels (mean & rang, nmol/l)	**incident ROA** n / total (%)	crude OR	adjusted OR‡	**progressive ROA** n / total (%)	crude OR	adjusted OR‡
39 (15 - 50)	22 / 322 (6.8%)	1.2 (0.6 - 2.3)	0.9 (0.4 - 1.8)	15 / 92 (16.3%)	2.9 (1.0 - 8.3)	2.1 (0.6 - 7.4)
62 (51 - 74)	24 / 344 (7.0%)	1.2 (0.7 - 2.3)	1.0 (0.5 - 1.9)	3 / 79 (3.8%)	0.6 (0.1 - 2.5)	0.6 (0.1 - 3.0)
98 (75 - 188)	19 / 332 (5.7%)	1	1	5 / 79 (6.3%)	1	1
		p-trend = 0.56	p-trend = 0.66‡		p-trend = 0.02	p-trend = 0.17‡

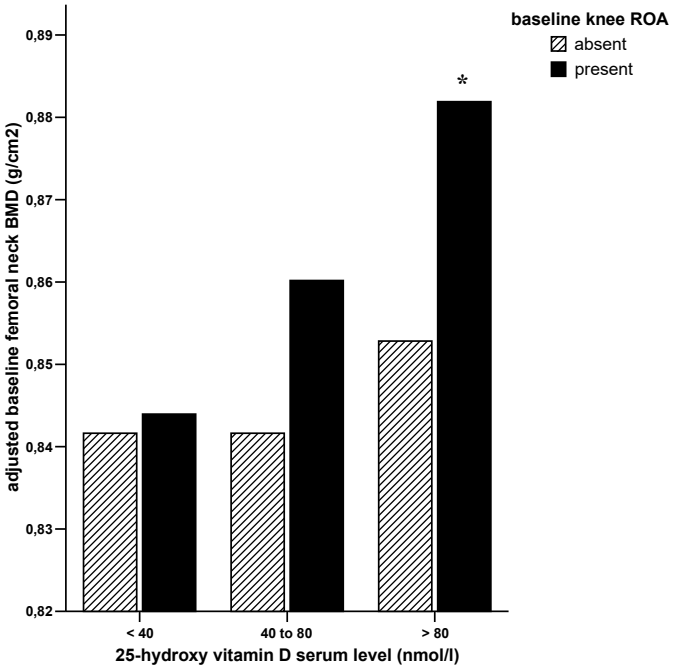
p-trend = p-value for trend
OR = odds ratio with 95% confidence limits between parentheses
† adjusted for baseline age, sex, body mass index, femoral neck bone mineral density, health status, smoking, disability index, fall tendency, baseline joint space narrowing and caloric intake
‡ adjusted for baseline age, sex, body mass index, femoral neck bone mineral density, health status, smoking, disability index, fall tendency, baseline joint space narrowing and time of the season

Similar trends were observed analyzing baseline vitamin D serum levels and ROA at follow-up. The percentage of subjects with progressive ROA in the lowest tertile of 25(OH)D serum levels was almost 3 times higher than of subjects in the highest tertile (16.3% versus 6.3%). However, after adjustment for any of the confounding variables significance was lost. Additional analysis for incidence and progression of the separate features of knee ROA (osteophytosis and JSN) showed similar trends (data not shown).

When we performed a gender-specific separate analysis on the 728 women in our study population, 11.7 % had *incident* JSN in the lowest 25(OH)D level tertile (15 to 46 nmol/l), compared to 5.7% in the highest tertile (70 to 178 nmol/l). After adjustment for confounding factors, including age, BMI and FN BMD, the odds ratio of incident JSN of the knee was 2.1 (95%CI: 0.9 to 4.7, data not shown).

Interaction with BMD. Figure 3.1.3 shows the adjusted baseline mean FN BMD by categories of 25(OH)D serum levels in subjects with and without baseline knee ROA. In subjects without knee ROA no significant differences in BMD between the vitamin D categories were seen, while in participants with knee ROA the mean adjusted FN BMD increased from 0.84 to 0.88 g/cm^2 with increasing 25(OH)D serum levels (p-value for trend = 0.03). There was significant interaction between baseline OA and 25(OH)D serum levels on FN BMD (p-value for interaction = 0.03). Similar differences in adjusted LS BMD were observed between subjects with baseline knee ROA in different categories of 25(OH)D serum levels, ranging from 1.09 g/cm^2 in the lowest category of 25(OH)D to 1.15 g/cm^2 in the highest (data not shown). However, no significant interaction between baseline OA and vitamin D serum levels on LS BMD was observed.

Figure 3.1.3. Adjusted† baseline femoral neck BMD by categories of 25(OH)D serum levels in subjects with and without knee ROA



† adjusted for age, sex, BMI, smoking status, health status, disability index, fall tendency and baseline joint space narrowing

The incidence and progression of knee ROA by tertiles of vitamin D intake and serum levels, stratified by the corresponding median of baseline LS BMD, is presented in **Table 3.1.3**. In subjects with low LS BMD at baseline we observe an increasing incidence of knee ROA with decreasing vitamin D intake and serum levels. In contrast, in subjects with high BMD, the incidence of knee ROA is higher, but does not differ between tertiles of vitamin D intake and serum levels. There is a significant interaction between vitamin D intake and baseline LS BMD in the association with incident knee ROA (p=0.03). This interaction is not seen in the association between vitamin D intake or levels, BMD and progressive knee ROA. When we combine incidence and progressive ROA, the influence of baseline BMD is reflected in the higher adjusted odds ratios in subjects with low BMD. Similar percentages with slightly lower odds ratios were observed when the same analyses were performed in subjects with high and low FN BMD (data not shown).

Table 3.1.3. Incidence / progression of knee ROA by tertiles of vitamin D intake and serum levels, stratified by the corresponding median of baseline lumbar spine BMD

	median baseline lumbar spine BMD		median baseline lumbar spine BMD		median baseline lumbar spine BMD			
	low (0.62-1.15 g/cm²)	**high** (1.02-1.91 g/cm²)	**low** (0.62-1.22 g/cm²)	**high** (1.06-1.91 g/cm²)	**low** (0.63-1.16 g/cm²)		**high** (1.03-1.91 g/cm²)	
tertiles of dietary vitamin D intake (mean & rang, IU/day)	incident ROA % (n/total)		progressive ROA % (n/total)		incident or progressive ROA % (n/total)	adjusted OR†	incident or progressive ROA % (n/total)	adjusted OR†
34 (5 – 44)	6.1% (10/165)	6.7% (11/164)	7.7% (3/39)	16.7% (8/48)	5.7% (12/209)	2.6 (0.7-9.8)	9.7% (20/207)	1.1 (0.5-2.5)
53 (44 – 64)	5.2% (9/174)	10.8% (17/157)	8.3% (4/48)	10.8% (4/37)	5.6% (12/216)	2.2 (0.6-7.4)	11.0% (22/200)	1.3 (0.7-2.7)
105 (64 – 438)	1.8% (3/168)	8.8% (15/170)	2.5% (1/40)	7.9% (3/38)	1.9% (4/211)	1	8.8% (18/205)	1
	interaction: p = 0.03†		interaction: p = 0.83†		interaction: p = 0.09†			
tertiles of 25(OH) vitamin D serum levels (mean & rang, nmol/l)	incident ROA % (n/total)		progressive ROA % (n/total)		incident or progressive ROA % (n/total)	adjusted OR ‡	incident or progressive ROA % (n/total)	adjusted OR‡
39 (15 - 50)	6.6% (11/166)	7.1% (11/156)	12.5% (4/40)	20.5% (9/44)	7.4% (16/215)	2.2 (0.7-6.9)	10.6% (21/199)	0.8 (0.4-1.6)
62 (51 - 74)	3.9% (7/179)	10.3% (17/165)	0% (0/40)	7.7% (3/39)	3.2% (7/221)	1.2 (0.3-3.9)	9.9% (20/202)	0.9 (0.4-1.8)
98 (75 - 188)	2.5% (4/162)	8.8% (15/170)	5.1% (2/39)	7.5% (3/40)	2.5% (5/200)	1	9.0% (19/211)	1
	interaction: p = 0.09‡		interaction: p = 0.81‡		interaction: p = 0.17‡			

OR = odds ratio with 95% confidence limits between parentheses
† adjusted for baseline age, sex, body mass index, health status, smoking, disability index, fall tendency, baseline joint space narrowing and caloric intake
‡ adjusted for baseline age, sex, body mass index, health status, smoking, disability index, fall tendency, baseline joint space narrowing and time of the season

DISCUSSION

The relation between BMD, vitamin D status and OA is unclear, and studies on the relationship between vitamin D and osteoarthritis have all used different determinants and endpoints[9-11, 21, 22]. In this prospective study we wanted to test the hypothesis that low baseline vitamin D intake and serum levels increase the risk of development or worsening of radiographic knee OA in a large population-based cohort. Furthermore, we wanted to examine the role of BMD in this possible association.

Only 2 subjects in our entire study population of 1248 subjects had a dietary intake of more than 400 IU of vitamin D per day, which is the recommended daily allowance for persons between the age of 51 and 70 years[23]. Nevertheless, the margins of the serum level tertiles in our study were similar to those found in the Framingham study and in the Study of Osteoporotic Fractures[10, 11]. To compare our findings with past results, we analyzed the possible association between baseline vitamin D status and the development and progression of knee ROA at follow-up in the same way McAlindon et al. did for their subjects in the Framingham study[10]. That is, we compared incidence and progression of ROA between tertiles of intake and serum levels of vitamin D, and we adjusted for the same confounding factors. We also found low vitamin D intake to be associated with progression of knee ROA. However, the mean dietary vitamin D intake in the Framingham study population was considerably higher compared to our study population (321 vs. 64 IU/day): 97% of our subjects had an intake between the limits of the lowest tertile (3 to 170 IU/day) in the Framingham study. This could indicate that there is no threshold for dietary vitamin D intake to prevent progression of knee ROA. Mean serum levels of 25(OH) D in our study and in the Framingham study were nearly the same (66 and 74 nmol/l, respectively), resulting in equivalent limits for the tertiles. Although 25(OH)D values from different laboratories can not be compared unless cross-calibration has been performed[24], similar associations were found in both the Framingham Study and Rotterdam Study: the percentage of subjects with progressive ROA in the lowest tertile of 25(OH)D serum levels was almost 3 times higher than of subjects in the highest tertile in both studies. However, in our study the significance of this association was lost after adjustment for confounding

factors. The covariates that were independently responsible for the loss of significance were disability, sex, BMI, and age, in order of strength of influence. This suggests the presence of confounding by lifestyle and health status.

Apart from differences between study populations in the mean vitamin D intake, our study and the study of McAlindon et al. differ in several other aspects. First, McAlindon and colleagues used numbers of knees in their analyses, presumably to increase power, and adjusted for correlation between fellow knees by using the generalized estimating equation approach of Liang and Zeger[25], while we used subjects with or without incident or progressive knee ROA in our analyses. Furthermore, dietary vitamin D data and vitamin D serum levels in the Framingham study were not collected at baseline (between 1983 and 1985) but during follow-up (between 1987 and 1989), i.e., approximately halfway the follow-up time of the study (between 1992 and 1993). This could obscure the association between exposure and outcome. Finally, the Framingham study population was more than twice as small as our study population, the subjects were slightly older (mean age 70.3 years versus 66.2 years), and had lower FN BMD (0.77 vs. 0.85 g/cm^2, although this last difference might be due to differences in densitometers used). On the other hand, our study populations were similar with regard to percentage women (63% vs. 58%), BMI (26.1 vs. 26.3) and prevalent knee OA (2.9 % vs. 2.0%).

The study of Lane and colleagues had different endpoints (hip OA) than ours, but their results were in accordance with our findings[11]. In this relatively small study (n = 237), consisting of women only, separate joints rather than subjects were compared, like in the study of McAlindon, and most likely to increase power. Women in the middle and lowest tertiles of 25(OH)D serum level had a more than 3 times increased risk to develop hip JSN. This is in line with the results of our separate analysis of the women in our study: the odds ratio for incident knee ROA for women in the lowest 25(OH)D level tertile compared to women in the highest tertile was 2.1 (95%CI: 0.9 to 4.7).

As is observed in other studies[26-28], vitamin D status was positively associated with mean BMD in our study population. Since our previous study has shown that high BMD is

associated with increased risk of incident knee ROA[7], we expected adjustment for BMD to influence the possible association between vitamin D status and the development of OA. However, like in the studies of McAlindon et al. and Lane et al., adjustment for BMD did not substantially change any risk estimate. Thus, BMD does not seem to be directly involved in the mechanism by which low vitamin D status might increase the risk of development or worsening of OA. Yet, we show here that baseline OA status does affect the association between 25(OH)D levels and BMD at baseline. The same finding was previously described by Bischoff-Ferrari et al., who observed a significant positive association between serum 25(OH)D and BMD in individuals with primary knee OA[9]. Since these persons had high prevalence of low 25(OH)D status, the authors suggested vitamin D supplementation to enhance BMD in individuals with OA. However, since these observations were cross-sectional, little insight is gained in the causal relation between these three factors. To further investigate the relation between BMD, vitamin D status and the development of OA, we assessed the incidence and progression of knee ROA by baseline dietary vitamin D intake and serum levels stratified by high and low baseline BMD. We found that the association between low vitamin D status and the incidence of knee ROA was limited to subjects with low baseline LS BMD (i.e., below the median). In subjects with high baseline BMD, incident and progressive knee ROA was more frequently observed than in subjects with low baseline BMD. This association between high BMD and the development of OA was reported previously in our and other study populations[7, 29]. However, the interaction with vitamin D status and BMD in their influence on the development of ROA we found in this study has not been described before to our knowledge.

The underlying mechanism of the way vitamin D status might influence the development or progression of OA is unclear. BMD seems to have no direct, but rather a modifying role in the association. We hypothesize that vitamin D status influences bone quality, and that this bone characteristic is not reflected by BMD measurement. This is supported by the observation of Ding and colleagues, who found that BMD did not reflect mechanical properties of subchondral bone in early-stage osteoarthritis[30]. On this local level, mechanical properties like stiffness or elastic modulus are associated with early degenerative changes[2, 31]. Vitamin D status affects these mechanical properties[32],

and could therefore influence the susceptibility for OA. Another possible explanation of the underlying mechanism could be the association of low serum 25(OH)D levels with increased serum parathyroid hormone (PTH), causing an increase of bone turnover[33, 34]. Increased bone turnover has been associated with OA[21, 35-37]. Finally, several studies suggest a direct effect of vitamin D metabolites on articular chondrocytes[38-40]. In this way, vitamin D status could directly influence the condition of articular cartilage, and therefore the susceptibility for OA.

There are some limitations of this study. Although our subjects were drawn from a population-based study, the Rotterdam Study, they were selected based on the availability of follow-up radiographs. This selection introduced a health-selection-bias, since these subjects survived the follow-up period, and were fit enough to visit the research center. This resulted in 4 years younger and less disabled subjects than in our source population[14]. However, since the incidence and progression of ROA in these healthier subjects will be lower than in a population based sample, we can assume that if this health-selection would have influenced the associations we found, it would underestimate the true effect. Another bias could have been introduced by the limited information on several confounding factors. Though food frequency questionnaires have been shown to give a reliable representation of actual intake of most foods[41, 42], supplementary vitamin D intake was not taken into account. However, vitamin D supplement use in the Netherlands is limited. Furthermore, no data on muscle strength and the extent the participants were exposed to sunlight were available. Since low vitamin D status is associated with unhealthy lifestyle factors, like higher BMI, lower BMD, higher disability and more falls (possibly caused by decreased muscle strength) as we found in our study, the risk of confounding is high. Although we attempted to adjust for these confounding factors as thoroughly as possible, it is possible that the subjects with low vitamin D status are less healthy subjects who are more susceptible for OA. The observation that only after adjustment for caloric intake the association between dietary vitamin D intake and progressive knee ROA reaches significance might also reflect this relation, since caloric intake was inversely related to baseline knee and progressive knee ROA in our study, even after adjustment for age, sex and BMI (data not shown).

In this study, we confirmed the observation that low dietary vitamin D intake increases the risk of progression of knee ROA. Low vitamin D serum levels appeared to be associated with increased risk of progressive ROA, but after adjustment for confounding factors, no significant relation was found. In subjects with low baseline BMD, vitamin D status seemed to influence the incidence and progression of knee ROA. Thus, improving the vitamin D status in elderly could protect against the development and worsening of knee OA, especially in those with low BMD.

REFERENCES

1. Mansell, J.P., Tarlton, J.F., and Bailey, A.J. (1997). Biochemical evidence for altered subchondral bone collagen metabolism in osteoarthritis of the hip. Br J Rheumatol 36, 16-19.

2. Ding, M., Dalstra, M., Linde, F., and Hvid, I. (1998). Changes in the stiffness of the human tibial cartilage-bone complex in early-stage osteoarthrosis. Acta Orthop Scand 69, 358-362.

3. Aspden, R.M., Scheven, B.A., and Hutchison, J.D. (2001). Osteoarthritis as a systemic disorder including stromal cell differentiation and lipid metabolism. Lancet 357, 1118-1120.

4. Dequeker, J., Mohan, S., Finkelman, R.D., Aerssens, J., and Baylink, D.J. (1993). Generalized osteoarthritis associated with increased insulin-like growth factor types I and II and transforming growth factor beta in cortical bone from the iliac crest. Possible mechanism of increased bone density and protection against osteoporosis. Arthritis and rheumatism 36, 1702-1708.

5. Burger, H., van Daele, P.L., Odding, E., Valkenburg, H.A., Hofman, A., Grobbee, D.E., Schutte, H.E., Birkenhager, J.C., and Pols, H.A. (1996). Association of radiographically evident osteoarthritis with higher bone mineral density and increased bone loss with age. The Rotterdam Study. Arthritis and rheumatism 39, 81-86.

6. Arden, N.K., Nevitt, M.C., Lane, N.E., Gore, L.R., Hochberg, M.C., Scott, J.C., Pressman, A.R., and Cummings, S.R. (1999). Osteoarthritis and risk of falls, rates of bone loss, and osteoporotic fractures. Study of Osteoporotic Fractures Research Group. Arthritis and rheumatism 42, 1378-1385.

7. Bergink, A.P., Uitterlinden, A.G., Van Leeuwen, J.P., Hofman, A., Verhaar, J.A., and Pols, H.A. (2005). Bone mineral density and vertebral fracture history are associated with incident and progressive radiographic knee osteoarthritis in elderly men and women: the Rotterdam Study. Bone 37, 446-456.

8. Lips, P. (2001). Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. Endocrine reviews 22, 477-501.

9. Bischoff-Ferrari, H.A., Zhang, Y., Kiel, D.P., and Felson, D.T. (2005). Positive association between serum 25-hydroxyvitamin D level and bone density in osteoarthritis. Arthritis and rheumatism 53, 821-826.

10. McAlindon, T.E., Felson, D.T., Zhang, Y., Hannan, M.T., Aliabadi, P., Weissman, B., Rush, D., Wilson, P.W., and Jacques, P. (1996). Relation of dietary intake and serum levels of vitamin D to progression of osteoarthritis of the knee among participants in the Framingham Study. Annals of internal medicine 125, 353-359.

11. Lane, N.E., Gore, L.R., Cummings, S.R., Hochberg, M.C., Scott, J.C., Williams, E.N., and Nevitt, M.C. (1999). Serum vitamin D levels and incident changes of radiographic hip osteoarthritis: a longitudinal study. Study of Osteoporotic Fractures Research Group. Arthritis and rheumatism 42, 854-860.

12. Felson, D.T., Niu, J., Clancy, M., Aliabadi, P., Sack, B., Guermazi, A., Hunter, D.J., Amin, S., Rogers, G., and Booth, S.L. (2007). Low levels of vitamin D and worsening of knee osteoarthritis: results of two longitudinal studies. Arthritis and rheumatism 56, 129-136.

13. Hofman, A., Grobbee, D.E., de Jong, P.T., and van den Ouweland, F.A. (1991). Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol 7, 403-422.

14. Bergink, A.P., van der Klift, M., Hofman, A., Verhaar, J.A., van Leeuwen, J.P., Uitterlinden, A.G., and Pols, H.A. (2003). Osteoarthritis of the knee is associated with vertebral and nonvertebral fractures in the elderly: the Rotterdam Study. Arthritis and rheumatism 49, 648-657.

15. Kellgren, J.H., Jeffrey, M.R., and Ball, J. (1963). Atlas of Standard Radiographs of Arthritis: The epidemiology of Chronic Rheumatism.(Oxford: Blackwell Scientific Publications).

16. Odding, E., Valkenburg, H.A., Algra, D., Vandenouweland, F.A., Grobbee, D.E., and Hofman, A. (1998). Associations of radiological osteoarthritis of the hip and knee with locomotor disability in the Rotterdam Study. Annals of the rheumatic diseases 57, 203-208.

17. Klipstein-Grobusch, K., den Breeijen, J.H., Goldbohm, R.A., Geleijnse, J.M., Hofman, A., Grobbee, D.E., and Witteman, J.C. (1998). Dietary assessment in the elderly: validation of a semiquantitative food frequency questionnaire. Eur J Clin Nutr 52, 588-596.

18. Pincus, T., Summey, J.A., Soraci, S.A., Jr., Wallston, K.A., and Hummon, N.P. (1983). Assessment of patient satisfaction in activities of daily living using a modified Stanford Health Assessment Questionnaire. Arthritis and rheumatism 26, 1346-1353.

19. van Daele, P.L., Burger, H., Algra, D., Hofman, A., Grobbee, D.E., Birkenhager, J.C., and Pols, H.A. (1994). Age-associated changes in ultrasound measurements of the calcaneus in men and women: the Rotterdam Study. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 9, 1751-1757.

20. Willett, W.C., Howe, G.R., and Kushi, L.H. (1997). Adjustment for total energy intake in epidemiologic studies. Am J Clin Nutr 65, 1220S-1228S; discussion 1229S-1231S.

21. Hunter, D.J., Hart, D., Snieder, H., Bettica, P., Swaminathan, R., and Spector, T.D. (2003). Evidence of altered bone turnover, vitamin D and calcium regulation with knee osteoarthritis in female twins. Rheumatology 42, 1311-1316.

22. Glowacki, J., Hurwitz, S., Thornhill, T.S., Kelly, M., and LeBoff, M.S. (2003). Osteoporosis and vitamin-D deficiency among postmenopausal women with osteoarthritis undergoing total hip arthroplasty. J Bone Joint Surg Am 85-A, 2371-2377.

23. Institute of Medicine, F.a.N.B. (1999). Dietary Reference Intakes: Calcium, Phosphorus, Magnesium, Vitamin D and Fluoride.(Washington, DC: National Academy Press).

24. Lips, P., Chapuy, M.C., Dawson-Hughes, B., Pols, H.A., and Holick, M.F. (1999). An international comparison of serum 25-hydroxyvitamin D measurements. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 9, 394-397.

25. Liang, K.Y., and Zeger, S.L. (1986). Longitudinal data analysis using generalized linear models. Biometrika 73, 13-22.

26. Bhattoa, H.P., Bettembuk, P., Ganacharya, S., and Balogh, A. (2004). Prevalence and seasonal variation of hypovitaminosis D and its relationship to bone metabolism in community dwelling postmenopausal Hungarian women. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 15, 447-451.

27. Baeksgaard, L., Andersen, K.P., and Hyldstrup, L. (1998). Calcium and vitamin D supplementation increases spinal BMD in healthy, postmenopausal women. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 8, 255-260.

28. Bischoff-Ferrari, H.A., Dietrich, T., Orav, E.J., and Dawson-Hughes, B. (2004). Positive association between 25-hydroxy vitamin D levels and bone mineral density: a population-based study of younger and older adults. Am J Med 116, 634-639.

29. Zhang, Y., Hannan, M.T., Chaisson, C.E., McAlindon, T.E., Evans, S.R., Aliabadi, P., Levy, D., and Felson, D.T. (2000). Bone mineral density and risk of incident and progressive radiographic knee osteoarthritis in women: the Framingham Study. The Journal of rheumatology 27, 1032-1037.

30. Ding, M., Danielsen, C.C., and Hvid, I. (2001). Bone density does not reflect mechanical properties in early-stage arthrosis. Acta Orthop Scand 72, 181-185.

31. Day, J.S., Ding, M., van der Linden, J.C., Hvid, I., Sumner, D.R., and Weinans, H. (2001). A decreased subchondral trabecular bone tissue elastic modulus is associated with pre-arthritic cartilage damage. Journal of orthopaedic research : official publication of the Orthopaedic Research Society 19, 914-918.

32. Aerssens, J., van Audekercke, R., Talalaj, M., Geusens, P., Bramm, E., and Dequeker, J. (1996). Effect of 1alpha-vitamin D3 and estrogen therapy on cortical bone mechanical properties in the ovariectomized rat model. Endocrinology 137, 1358-1364.

33. Parisien, M., Silverberg, S.J., Shane, E., de la Cruz, L., Lindsay, R., Bilezikian, J.P., and Dempster, D.W. (1990). The histomorphometry of bone in primary hyperparathyroidism: preservation of cancellous bone structure. The Journal of clinical endocrinology and metabolism 70, 930-938.

34. van Doorn, L., Lips, P., Netelenbos, J.C., and Hackeng, W.H. (1993). Bone histomorphometry and serum concentrations of intact parathyroid hormone (PTH(1-84)) in patients with primary hyperparathyroidism. Bone Miner 23, 233-242.

35. Ooms, M.E., Lips, P., Roos, J.C., van der Vijgh, W.J., Popp-Snijders, C., Bezemer, P.D., and Bouter, L.M. (1995). Vitamin D status and sex hormone binding globulin: determinants of bone turnover and bone mineral density in elderly women. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 10, 1177-1184.

36. Stewart, A., Black, A., Robins, S.P., and Reid, D.M. (1999). Bone density and bone turnover in patients with osteoarthritis and osteoporosis. The Journal of rheumatology 26, 622-626.

37. Naitou, K., Kushida, K., Takahashi, M., Ohishi, T., and Inoue, T. (2000). Bone mineral density and bone turnover in patients with knee osteoarthritis compared with generalized osteoarthritis. Calcified tissue international 66, 325-329.

38. Garabedian, M., Du Bois, M.B., Corvol, M.T., Pezant, E., and Balsan, S. (1978). Vitamin D and cartilage. I. In vitro metabolism of 25-hydroxycholecalciferol by cartilage. Endocrinology 102, 1262-1268.

39. Harmand, M.F., Thomasset, M., Rouais, F., and Ducassou, D. (1984). In vitro stimulation of articular chondrocyte differentiated function by 1,25-dihydroxycholecalciferol or 24R,25-dihydroxy-cholecalciferol. Journal of cellular physiology 119, 359-365.

40. Tetlow, L.C., and Woolley, D.E. (2001). Expression of vitamin D receptors and matrix metalloproteinases in osteoarthritic cartilage and human articular chondrocytes in vitro. Osteoarthritis and cartilage 9, 423-431.

41. Malekshah, A.F., Kimiagar, M., Saadatian-Elahi, M., Pourshams, A., Nouraie, M., Goglani, G., Hoshiarrad, A., Sadatsafavi, M., Golestan, B., Yoonesi, A., et al. (2006). Validity and reliability of a new food frequency questionnaire compared to 24 h recalls and biochemical measurements: pilot phase of Golestan cohort study of esophageal cancer. Eur J Clin Nutr 60, 971-977.

42. Johnson, B., Hackett, A., Roundfield, M., and Coufopoulos, A. (2001). An investigation of the validity and reliability of a food intake questionnaire. J Hum Nutr Diet 14, 457-465.

3.2 meta-analysis of vitamin D serum levels and the development of osteoarthritis of knee, hip and hand

25-Hydroxyvitamin D and osteoarthritis: A meta-analysis including new data

Semin Arthritis Rheum. 2016 Apr;45(5):539-46. PubMed PMID: 26522138

Bergink AP, Zillikens MC, van Leeuwen JP, Hofman A, Uitterlinden AG, van Meurs JB

ABSTRACT

Objectives: To study the relationship between 25-hydroxy (OH) vitamin D serum levels and osteoarthritis (OA) of the knee, hip and hand in a meta-analysis, with up-dated and expanded results of our previous study.

Methods: Pubmed was searched from February 1975 to December 2014 for articles assessing the relationship between vitamin D levels and OA. In our meta-analysis, 6 cross-sectional and 6 longitudinal studies were included. The number of subjects in these studies ranged from 99 to 1,248 subjects. The latter 1,248 subjects (58% women) were drawn from the Rotterdam Study, a prospective population-based cohort study of the elderly. At baseline, 25(OH) vitamin D serum levels were measured and prevalent OA of knees, hips and hands was scored by the Kellgren-Lawrence grading system. After a mean follow-up time was 8.4 years, incidence and progression of OA were assessed.

Results: No clear association between vitamin serum levels and prevalent, incident or progressive knee, hip or hand OA was observed. The quality of most studies was low, and the results conflicting. Meta-analysis of three cross-sectional studies on vitamin D levels and knee joint space narrowing (JSN) showed an increased risk of prevalent JSN with decreasing vitamin D levels (OR 1.52, 95% CI 1.15 to 2.01). The association observed in the meta-analysis of three studies on low vitamin D levels and incident and progressive

knee OA was not significant (OR 1.37, 95% CI 0.97 to 1.92), however when considering solely progressive knee OA, the risk was significantly increased (OR 2.40, 95% CI 1.22 to 4.72).

Conclusions: Epidemiological studies do not provide evidence of an independent association between 25(OH) vitamin D serum levels with hip or hand OA. When analyzing subgroups of knee OA, significant associations of low vitamin D levels with prevalent knee JSN and with progressive knee OA were observed. Overall, the results of this study do not support the advice to supplement vitamin D to prevent the onset or worsening of osteoarthritis, except perhaps for progressive knee OA.

INTRODUCTION

Vitamin D plays an important role in bone metabolism; low vitamin D status is associated with decreased bone mineral density and impaired bone remodelling[1, 2]. Epidemiological studies have shown that several aspects of bone health, like increased bone mineral density (BMD), bone turnover and fracture risk, are associated with the development of osteoarthritis (OA)[3-6]. Thus, via its influence on bone, vitamin D might have an indirect effect on the development of OA. Furthermore, in vitro studies showed vitamin D metabolism in articular cartilage[7, 8]. This could implicate a direct effect of vitamin D on OA susceptibility. Finally, low vitamin D status is associated with muscle weakness[9-11], while decreased quadriceps muscle strength is related to the development of OA[12-15].

Several studies have investigated the association between 25(OH) vitamin D serum levels and OA. Some cross-sectional studies showed significant lower 25(OH) vitamin D serum levels in subjects with OA[16-19], while other cross-sectional studies found no association[5, 20-24]. Two prospective epidemiological studies have found an association between serum low levels of vitamin D and the development of radiographic OA[25, 26]. In contrast, three other longitudinal studies found no association between vitamin D levels and OA[27-29].

Thus, up till now there is still debate whether vitamin D plays a role in the development of OA. Therefore, we performed a meta-analysis of available data in recent literature on 25(OH) vitamin D serum levels and radiographic OA (ROA). In this meta-analysis we included data of our study on the association between vitamin D levels and knee OA published in 2009[29], with up-dated data on incident and progressive knee OA, and additional data on prevalent and incident ROA of the hip and hand in the Rotterdam Study-I.

MATERIALS AND METHODS

Association analyses of the Rotterdam Study-I

Subjects. The study population consisted of subjects of the Rotterdam Study-I (RS-I), a prospective cohort study of men and women aged 55 and over, with the objective to investigate the incidence of, and risk factors for, chronic disabling diseases. The rationale and study design have been described previously[30-34] (www.erasmus-epidemiology.nl/rotterdamstudy). The study population consisted of 1,248 subjects (58% women), drawn from RS-I. The selection was based on the availability of radiographs of the knees at baseline and follow-up, and data on potentially confounding factors at baseline. The baseline characteristics of our study population are described in **Supplementary Table S3.2.1.**

Supplementary Table S3.2.1. Baseline characteristics study population Rotterdam Study

		tertiles of 25(OH) vitamin D serum levels			
	total	**I**	**II**	**III**	**p- trend**
number	1248	414	423	411	
25(OH) vitamin D (nmol/l)	66 ± 27	39 (15 - 50)	62 (51 - 74)	98 (75 - 188)	
women (%)	58.3	67.8	60.0	47.4	< 0.01
age (yrs)	66.2 ± 6.7	68.7 ± 6.8	65.8 ± 6.6	64.1 ± 5.8	< 0.01*
BMI (kg/m^2)	26.3 ± 3.5	26.6 ± 4.0	26.5 ± 3.4	26.0 ± 3.1	0.12†
fn BMD (g/cm^2)	0.85 ± 0.13	0.82 ± 0.13	0.85 ± 0.13	0.87 ± 0.13	0.01‡
health status	0.46 ± 0.61	0.44 ± 0.63	0.47 ± 0.63	0.49 ± 0.58	0.01§
disability index	0.22 ± 0.32	0.28 ± 0.38	0.21 ± 0.30	0.16 ± 0.27	< 0.01§
positive fall tendency (%)	14.5	17.1	14.9	11.4	0.21§
smoking (%)	22.4	22.5	23.4	21.2	< 0.01§

values are crude means with standard deviations (range) or percentages
BMI: body mass index, fn BMD: femoral neck bone mineral density, ls BMD: lumbar spine bone mineral density
* adjusted for sex,
† adjusted for sex & age
‡ additionally adjusted for sex, age & body mass index
§ additionally adjusted for sex, age, body mass index & femoral neck bone mineral density

Radiographic osteoarthritis. Radiographs of knees, hips and hands were scored at baseline (RS-I-1, between 1991 and 1993), at the second follow-up visit (RS-I-3, between 1997 and 1999) and at the third follow-up visit (RS-I-4, between 2003 and 2005) for subject who were still able to visit the research center. The mean follow-up time was 8.4 years (range 3.7 to 13.6). Prevalence, incidence and progression of ROA were scored by the Kellgren/Lawrence (K/L) grading system[35], and of joint space narrowing (JSN) were scored as described previously[6, 36]. In short, prevalent ROA of the knee or hip was defined as a Kellgren score of ≥2 at baseline at one or both knees/hips. Prevalent hand ROA was defined as presence of a K/L score ≥2 in 2 out of 3 hand joint groups of each or both hands. Incident ROA was diagnosed when a subject had a K/L score <2 at baseline, and had a K/L score of ≥2 at follow-up. Progressive ROA was defined as increase of K/L score in subjects with baseline ROA. No data on progressive hand OA were available. JSN was defined as a joint space of 2 millimeters (mm) below the median joint space in mm at any site. Incident JSN was diagnosed in subjects without JSN at baseline, and with JSN at

follow up. Progressive JSN was diagnosed in subjects with JSN at baseline, and a decrease of joint space of 1 mm at any site.

Other variables. Baseline 25(OH) vitamin D serum levels were measured using radioimmunoassays (IDS Ltd, Boldon, UK, www.idsltd.com). The sensitivity of the test is 3 nmol/l, the range 4 to 400 nmol/l, the intra-assay accuracy is <8%, the inter-assay accuracy <12%. Data on confounding variables age, gender, body mass index (BMI), femoral neck bone mineral density (FNBMD), health status, disability index, fall tendency, cigarette smoking and time of season were obtained as described previously[29, 30].

Statistical analysis. Gender-specific tertiles of 25(OH) vitamin D serum levels were created, odds ratios (ORs) with 95% confidence intervals (CI) for prevalent ROA by tertiles of vitamin D and per standard deviation (SD) of 25(OH) vitamin D serum levels were calculated by means of logistic regression modeling, and adjusted according to two models: model 1 with adjustments for age, gender, and BMI, and model 2 with adjustments for age, gender, BMI, FNBMD, disability index, fall tendency and for time of season. The analyses of incident and progressive ROA by serum vitamin D tertiles were additionally adjusted for baseline ROA sum score. We used SPSS version 19.0.0 (SPSS Inc., Chicago, USA) for these analyses.

Literature Study

Identification of studies. Relevant articles were identified by a systematic search using the database of Pubmed with [vitamin D OR 25-hydroxyvitamin OR 25-OH-D] AND [osteoarthritis OR OA] AND [serum OR level OR levels] as keywords. We tried to expand the number of studies in our meta-analysis by using alterative search terms like 'concentration' and 'plasma', but adding these terms did not lead to the inclusion of other applicable studies. Subsequently, the search was extended by screening of the reference lists of the included studies for review. The following inclusion criteria applied for this review: a) listed in Pubmed, b) publication in the English language, c) study in humans, d) the article represents original data, e) the disease of interest is OA, f) subject without OA are compared with subjects with prevalent OA or incident OA, or subject

with OA are compared with subjects with progressive OA, g) the study reports on vitamin D measurements in serum, h) the study investigates the relationship between OA and 25(OH) vitamin D serum levels presenting odds ratios (ORs), or difference in median or mean, i) the full-text article was available. In total 130 studies, published between February 1975 and December 2014, were identified in the database, of which 111 studies were excluded on the basis of the title and/or abstract. Of the 19 studies retrieved for full examination, 7 studies were excluded because they did not fulfil the inclusion criteria. Finally, 12 studies were included in the meta-analysis. In **Figure 3.2.1**, the flowchart of the selection process is depicted.

Methodological quality assessment. All studies were scored for the following items: 1) information on recruitment of cases, 2) information on recruitment of controls, 3) size of the study, 4) longitudinal or cross-sectional data, 5) information for all subjects on age, gender and BMI, 6) a description of 25-hydroxy (OH) vitamin D measurement, 7) OA defined according to ACR criteria, a total joint replacement due to primary OA, the Kellgren/Lawrence (K/L) score[37] or using a validated atlas, 8) clearly description of statistical methods, 9) adjustments fully made for age, gender and BMI in the analyses, 10) results presented as either geometric mean, quartiles, tertiles or median with p-values or ORs with 95% confidence limits.

Meta-analysis. The program 'Comprehensive Meta-analysis' by Biostat (www.meta-analysis.com) was used. In order If heterogeneity existed ($I^2 > 25$) a random-effects model (DerSimonian and Laird [38]) was used for the analysis, otherwise a fixed effects model (inverse variance method) was applied.

RESULTS

Updated results from the Rotterdam Study

Table 3.2.1 shows the results of our analyses in the Rotterdam Study-I of vitamin D levels by prevalent, incident and progressive ROA of knee, hip and hand. The analyses are an up-date of, and addition to, the analyses presented in our previous paper[29].

Table 3.2.1. Radiographic osteoarthritis by 25(OH) vitamin D serum levels in the Rotterdam Study

tertiles 25(OH)D (nmol/l)	**Knee ROA (Kellgren)**					
	prevalent cases/total (%)	OR†	**incident** cases/total (%)	OR‡	**progressive** cases/total (%)	OR‡
III (75-188)	68/411 (16.5)	1	38/343 (11.1)	1	18/66 (27.3)	1
II (51-74)	74/421 (17.6)	0.81 (0.55-1.19)	45/347 (13.0)	1.02 (0.62-1.69)	20/68 (29.4)	1.37 (0.59-3.20)
I (15-50)	89/414 (21.5)	0.76 (0.50-1.14)	51/325 (15.7)	1.24 (0.73-2.11)	35/82 (35.4)	2.11 (0.87-5.08)
per SD increase vit 25(OH)D		1.20 (1.02-1.42)		0.95 (0.76-1.19)		0.76 (0.53-1.08)
tertiles 25(OH)D (nmol/l)	**Knee JSN**					
	prevalent cases/total (%)	OR†	**incident** cases/total (%)	OR‡	**progressive** cases/total (%)	OR‡
III (75-188)	55/411 (13.4)	1	18/356 (5.1)	1	8/55 (14.5)	1
II (51-74)	75/423 (17.7)	1.17 (0.79-1.73)	25/348 (7.2)	1.38 (0.71-2.69)	5/75 (6.7)	0.39 (0.11-1.37)
I (15-50)	98/414 (23.7)	1.39 (0.93-2.07)	31/316 (9.8)	1.83 (0.92-3.66)	14/98 (14.3)	0.59 (0.20-1.77)
per SD increase vit 25(OH)D		0.91 (0.77-1.08)		0.74 (0.54-1.00)		1.11 (0.71-1.73)

tertiles 25(OH)D (nmol/l)	**Hip ROA (Kellgren)**					
	prevalent cases/total (%)	OR†	**incident** cases/total (%)	OR‡	**progressive** cases/total (%)	OR‡
III (75-188)	35/408 (8.6)	1	26/373 (7.0)	1	21/33 (63.6)	1
II (51-74)	37/420 (8.8)	0.85 (0.51-1.41)	34/383 (8.9)	1.14 (0.65-1.99)	15/30 (50.0)	0.65 (0.19-2.24)
I (15-50)	40/411 (9.7)	0.75 (0.43-1.29)	35/366 (9.6)	1.19 (0.66-2.16)	19/30 (63.3)	0.68 (0.14-3.32)
per SD increase vit 25(OH)D		1.19 (0.97-1.48)		0.91 (0.71-1.16)		1.44 (0.83-2.49)
tertiles 25(OH)D (nmol/l)	**Hip JSN**					
	prevalent cases/total (%)	OR†	**incident** cases/total (%)	OR‡	**progressive** cases/total (%)	OR‡
III (75-188)	53/398 (13.3)	1	20/341 (5.9)	1	7/44 (15.9)	1
II (51-74)	68/410 (16.6)	1.25 (0.84-1.87)	30/339 (8.8)	1.20 (0.64-2.22)	5/64 (7.8)	0.27 (0.07-1.06)
I (15-50)	79/395 (20.0)	1.46 (0.97-2.22)	29/307 (9.4)	1.14 (0.59-2.21)	8/68 (11.8)	0.37 (0.10-1.44)
per SD increase vit 25(OH)D		0.88 (0.74-1.05)		0.93 (0.70-1.23)		1.57 (0.89-2.75)
tertiles 25(OH)D (nmol/l)	**Hand ROA (Kellgren)**					
	prevalent cases/total (%)	OR†	**incident** cases/total (%)	OR‡		
III (75-188)	69/391 (17.6)	1	55/220 (25.5)	1		
II (51-74)	108/395 (27.3)	1.40 (0.98-2.01)	53/197 (26.9)	1.08 (0.65-1.79)		
I (15-50)	123/388 (31.7)	1.25 (0.85-1.83)	35/160 (21.9)	0.76 (0.43-1.36)		
per SD increase vit 25(OH)D		0.90 (0.77-1.06)		0.98 (0.78-1.22)		

†OR, with 95% CI between parentheses, are adjusted for baseline age, gender, body mass index, femoral neck bone mineral density, fall tendency, disability index, health status, cigarette smoking and time of season

‡OR, with 95% CI between parentheses, are adjusted for baseline age, gender, body mass index, femoral neck bone mineral density, fall tendency, disability index, health status, cigarette smoking, time of season ROA sum score at baseline

The percentages of subjects with prevalent, incident and progressive knee ROA and JSN increases with decreasing vitamin D levels. However, after adjustment for confounding factors, only a positive association between 25(OH) vitamin D serum levels and prevalent ROA is seen (OR 1.20, 95% CI 1.02 to 1.42), while increasing vitamin D levels show a borderline protective effect on incident knee JSN (p=0.05). Prevalent, incident or progressive hip ROA and JSN show no significant association with vitamin D levels after adjustment in our study population. No association between 25(OH) vitamin D serum levels and prevalent or incident hand ROA is observed.

Table 3.2.2. Results of the methodological quality assessment of the articles included in this review

Studies	size of the study (>100 cases and controls)	longitudinal	definition of OA according to standard criteria*	information on age, gender and BMI available	complete adjustment for age, gender and weight/BMI	description vitamin 25(OH)D measurement	statistical methods	results as geometric mean (p-value), quartiles/ tertiles/ median or differences in SD (odds ratio)
McAlindon et al., 1996	+	+/-	+	+	+	+	+	+
Lane et al, 1999	+	+	+	+	+	+	+	+
Hunter et al, 2003	+	-	+	+	+	+	+	-
Felson et al, 2007	+	+	+	+	+	+	+	+
Ding et al, 2009	+	-	+	+	+	+	+	-
Bergink et al, 2009, up-dated in 2013	+	+	+	+	+	+	+	+
Chaganti, 2010	+	-	+	+	-	+	+	+
Heidari, 2010	+	-	+	-	-	+	+	+

Studies	size of the study (>100 cases and controls)	longitudinal	definition of OA according to standard criteria*	information on age, gender and BMI available	complete adjustment for age, gender and weight/BMI	description vitamin 25(OH)D measurement	statistical methods	results as geometric mean (p-value), quartiles/ tertiles/ median or differences in SD (odds ratio)
Al-Jarallah, 2012	-	-	+	+	+	+	+	+
Kalichman, 2012	+	-	+	+	+	+	+	+
Konstari, 2012	+	+	-	+	+	+	+	+
Zhang, 2014	+	+/-	+	+	-	-	+	+

* Standard criteria are: OA defined by ACR criteria, K/L score, score based on validated atlas or total joint replacement. + implies that complete information is available, - implies that information is not completely available or the feature was not applied (i.e. for complete adjustments for age/gender/BMI this was either not applied or information was incomplete in the paper). +/-: OA data were obtained longitudinally, but serum vitamin D levels were determined halfway the follow up period.

Results of the meta-analysis

Methodological quality assessment: In **Table 3.2.2,** the aspects of quality of the studies as mentioned in the methods section are

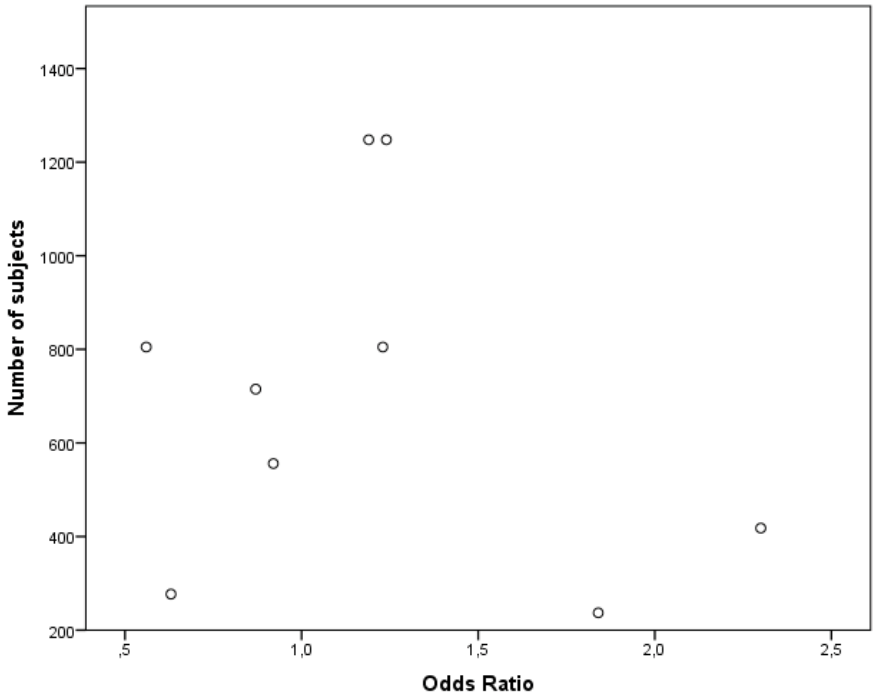
given. In summary, most studies selected subjects and controls from large cohort studies, with populations described in previous publications. The lowest number of participants was 99[39]. Six studies presented cross-sectional data[5, 18, 19, 24, 39, 40]. The other six studies were longitudinal[25-29, 41], although 25(OH) vitamin D serum levels in the Framingham study and the Osteoarthritis Initiative (OAI) Study were not collected at baseline but approximately halfway the follow-up time of the study: In the Framingham Study, baseline photographs were taken between 1983 and 1985, and follow up radiographs between 1992 and 1993, while the blood samples were collected between 1987 and 1989[25], and in the OAI Study, the change in radiographic JSN score was measured between the 24- and 48-month OAI visit, while 25(OH) vitamin D serum levels were measured at 30- or 36-month OAI visit[41]. All authors, with exception of Konstari et al., used the OA definition according to international standards or determined OA using a validated atlas. Except for Chaganti et al., all studies provided information on age, gender as well as BMI, and made adjustments for these confounders in the analyses. More information on the adjustments made in the different studies is provided in **Supplementary Table S3.2.2.** An accurate description of vitamin D serum level measurements and statistical methods was provided in all studies, with exception of Zhang and co-workers. All but two studies[5, 40] displayed their results as geometric mean (p-value) or quartiles/tertiles/median (odds ratio). There was no indication of publication bias in the studies we included in our meta-analysis: the funnel plot of included studies shows a nearly equal distribution of studies around the neutral odds ratio 1.0 (**Supplementary Figure S3.2.1**).

Supplementary Table S3.2.2. Adjustments in analyses of studies in meta-analysis

Studies	age	gender	weight/ BMI	BMD	baseline OA	activity/ disablity	health status	time of season	vitamin D supplement use
McAlindon et al., 1996	+	+	+	-	-	+	+	-	-
Lane et al, 1999	+	n/a	+	+	-	+	+	-	+
Hunter et al, 2003	n/a	n/a	+	-	n/a	-	-	-	-
Felson et al, 2007	+	+	+	-	+	-	-	-	-
Ding et al, 2009	+	+	+	-	n/a	-	+	-	-
Bergink et al, 2009, up-dated in 2013	+	+	+	+	+	+	+	+	-
Chaganti, 2010	+	n/a	-	-	n/a	+	+	+	-
Heidari, 2010	+	+	-	-	n/a	-	-	-	-
Al-Jarallah, 2012	+	+	+	-	n/a	-	-	-	-
Kalichman, 2012	+	+	+	-	n/a	-	-	-	-
Konstari, 2012	+	+	+	-	-	+	+	+	-
Zhang, 2014	+	-	-	-	-	-	-	-	+

+: data provided
-: data not provided
n/a: not appropriate

Supplementary Figure S3.2.1. Funnel plot of included studies



Results for the association between 25(OH) vitamin D serum levels and OA: in total 12 studies assessing the relationship between 25(OH) vitamin D serum levels and prevalent, incident or progressive of OA, were included in this meta-analysis.

The characteristics, endpoints and results of the studies on the prevalence of OA are shown in **Table 3.2.3**, and the characteristics of the studies on incidence and/or progression of OA in **Table 3.2.4.** Combining all cross-sectional studies, including our own novel data on prevalent OA, a total of 4,277 subjects was analysed, with a 25(OH) vitamin D levels ranging from 29 to 86 nmol/l. Two cross-sectional studies were population-based[5, 29], and provided information on prevalence of knee ROA. Bergink et al. found 18.5% ROA prevalence (231 cases), the study of Hunter et al., described 474 cases (29%) with prevalent osteophytosis. Although the total study-population mentioned in the paper of Hunter was 1,644, only 458 subjects (229 twin pairs discordant for OA) were used to calculate the odds ratio of ROA per standardized unit difference of serum vitamin D.

Table 3.2.3. Results of the included cross-sectional studies on 25(OH) vitamin D serum levels and OA

reference	total study population	mean 25(OH)D serum levels	endpoint	cases		OR (95%CI)
Hunter et al, 2003	458 (100% ♀)	86 nmol/l	knee OPH	?	OR per SD (33 nmol/l)	1.21 (0.78-1.85)
Ding et al, 2009	880 (50% ♀)	53 nmol/l	knee OPH	?	≥ 50 vs < 50 nmol/l	1.58 (0.69-3.59)
			knee JSN	?	≥ 50 vs < 50 nmol/l	1.68 (1.13-2.50)
Chaganti et al, 2010	1,104 (100% ♂)	65 nmol/l	hip ROA	?	tertile 3 vs 2 (≥ 75 vs 37.5 to 75 nmol/l)	2.01 (1.10–3.67)
					tertile 3 vs 1 (≥ 75 vs < 37.5 nmol/l)	1.99 (0.83–4.74)
Heidari et al, 2010	298 (♀:♂=?)	91 nmol/l	knee OA	148	≥ 50 vs < 50 nmol/l	1.62 (0.97-2.60)
Al-Jarallah et al , 2012	99 (91% ♀)	29 nmol/l	knee OPH	55	≥ 50 vs < 50 nmol/l	1.28 (0.17-9.95)
			knee JSN	87	≥ 50 vs < 50 nmol/l	2.68 (0.23-31.63)
Kalichman et al, 2012	190 (52% ♀)	60 nmol/l	hand OA	111	OR per SD (16 nmol/l)	0.94 (0.86-1.01)

OR: Odds Ratio, SD: standard deviation,
OPH: osteophytosis, JSN: joint space narrowing,
♀: female, ♂: male
?: number of cases nor specified

Table 3.2.4. Results of the included longitudinal studies on 25(OH) vitamin D serum levels and OA

reference	total study population	Mean 25(OH)D serum levels	follow up time	endpoint	cases	analyses	OR (95%CI)
McAlindon et al., 1996	556 (63% ♀)	74 nmol/l	8.5 years?	incident knee ROA[1]	75	tertile 3 vs 1 (>90 vs <60 nmol/l)	0.92 (0.45-1.87)
				progressive knee ROA[1]	62	tertile 3 vs 1 (>90 vs <60 nmol/l)	2.89 (1.01-8.25)
				progressive knee OPH	?	tertile 3 vs 1 (>90 vs <60 nmol/l)	3.1 (1.3-7.5)
				progressive knee JSN	?	tertile 3 vs 1 (>90 vs <60 nmol/l)	2.3 (0.9-5.5)
Lane et al, 1999	237 (100% ♀)	66 nmol/l	8 years	incident hip ROA[2]	24	tertile 3 vs 1 (>75 vs <55 nmol/l)	1.84 (0.52-6.54)
				incident hip JSN	50	tertile 3 vs 1 (>75 vs <55 nmol/l)	3.34 (1.13-9.86)
Felson et al, 2007	715 Framingham (53% ♀)	49 nmol/l	9.5 years	incident knee JSN	203	tertile 3 vs 1 (> 57.5 vs < 42.5 nmol/l)	0.87 (0.51-1.47)
						≥ 50 vs < 50 nmol/l	0.83 (0.54-1.27)
	277 BOKS (41% ♀)	51 nmol/l	2.5 years?	incident knee JSN	91	tertile 3 vs 1 (> 57.5 vs < 42.5 nmol/l)	0.56 (0.28-1.12)
						≥ 50 vs < 50 nmol/l	0.63 (0.35-1.14)
			2.5 years?	incident knee cartilage loss[3]	116?	≥ 50 vs < 50 nmol/l	0.74 (0.50-1.09)
						OR per SD	1.01 (0.99-0.03)
Konstari et al, 2012	805 (55.3%♀)	46 nmol/l (?)		incident knee OA[4]	40	quartile 4 vs 1 (> 142.5 vs < 82.5 nmol/l)	1.23 (0.49-3.13)
				incident hip OA[4]	94	quartile 4 vs 1 (> 142.5 vs < 82.5 nmol/l)	0.56 (0.09-3.45)

reference	total study population	Mean 25(OH)D serum levels	follow up time	endpoint	cases	analyses	OR (95%CI)
Zhang, 2014	418 (47.1%♀)	65.5 nmol/l	2 years	incident & progressive knee JSN	66	≥ 37.5 vs < 37.5 nmol/l	2.3 (1.1-4.5)
	271			progressive knee JSN	53	≥ 37.5 vs < 37.5 nmol/l	3.2 (1.5-6.8)
Bergink, 2009, up-dated in 2013	1,248 (58% ♀)	66 nmol/l	8.4 years	prevalent, incident and progressive ROA & JSN of knee, hip and hand	20-231	tertile 3 vs 1 & OR per SD	Table 4-6

ROA= radiographic osteoarthritis, JSN = joint space narrowing
?: number of cases nor specified
[1] according to Kellgren-Lawrence score, [2] according to IRF-score, [3] assessed by MRI, [4] clinical score

The total number of subject in all longitudinal studies was 4,256, with study populations varying between 237 to 1,248 subjects, 25(OH) vitamin D levels from 46 to 74 nmol/l, and the follow-up time from 2 to 9.5 years. Due to differences in follow-up time and definitions in end-point, a wide range in incidence and progression of was observed.

The studies in our meta-analysis used different methods to explore the association between vitamin D levels and OA risk. Most studies divided their subjects in tertiles[25-27, 29] or quartiles[28] of vitamin D levels, other studies used cut-off values of vitamin D levels (<37.5 nmol/l[41], < 50 nmol/l[19, 27, 39, 40] or < 75 nmol/l[18]), or calculated odds ratios per standard deviation of mean vitamin D levels[5, 24, 27].

Figures 3.2.3 to 3.2.4 show the results of our meta-analysis. In **Figure 3.2.2a** the odds ratios with confidence limits for the studies with data on prevalent knee ROA or osteophytosis are displayed. In the study of Heidari, a borderline significant positive association between low vitamin D levels and prevalent ROA is observed, while the associations found in the other studies are non-significant. Overall, no significant association was observed between low vitamin D levels and prevalent ROA. Data on prevalent knee joint space narrowing was available in the studies of Ding, Al-Jarallah and Bergink (**Figure 3.2.2b**). When combined, they show a significantly increased risk of prevalent JSN in the presence of low vitamin D with an odds ratio of 1.58 (CI 1.20 to 2.08, p=0.001). In **Figure 3.2.3a**, all studies with data on incident or progressive knee ROA are displayed. We combined incident and progressive ROA in our analysis, because they are mutually exclusive: subject with incident ROA have no knee OA at baseline, and subjects with progressive ROA have prevalent knee OA at baseline. The meta-analysis shows a non-significant trend towards a positive association between low vitamin D and ROA is. When analysing solely studies on progressive ROA with complete adjustments, a significant 2.4-fold increased risk of progression of ROA is observed in subjects in the lower tertile of vitamin D levels (15-50 nmol/l) compared to those in the upper tertile (75-188 nmol/l, data shown in **Supplementary Figure S3.2.2**). In **Figure 3.2.3b** we can observe that meta-analysis of all studies on incident and progressive knee JSN shows no association with vitamin D levels. Meta-analyses of studies on incident hip ROA and JSN show also no association with vitamin D levels (**Figures 3.2.4a and 3.2.4b**).

Figure 3.2.2a. Meta-analysis of studies on prevalent knee ROA/osteophytosis

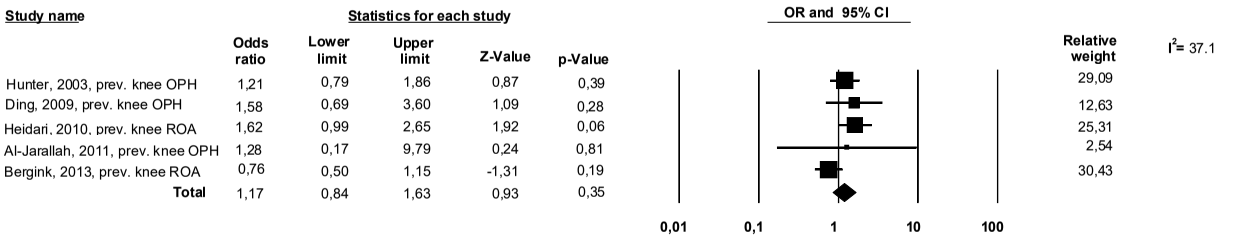


Figure 3.2.2b. Meta-analysis of studies on prevalent knee JSN

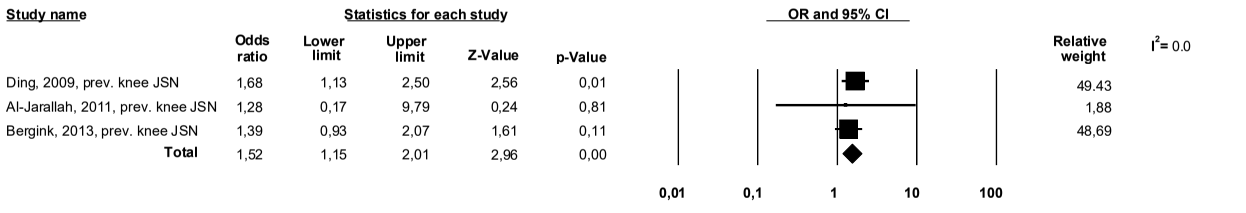


Figure 3.2.3a. Meta-analysis of studies on incident and progressive knee ROA/osteophytosis

Study name	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value	Relative weight
McAlindon, 1996, inc. knee ROA	0,92	0,45	1,88	-0,23	0,82	22,89
McAlindon, 1996, progr. knee ROA	2,89	1,01	8,26	1,98	0,05	10,53
Konstari, 2012, inc. knee, clinical	1,23	0,49	3,11	0,44	0,66	13,51
Bergink, 2013, inc. knee ROA	1,24	0,71	2,15	0,76	0,44	38,15
Bergink, 2013, progr. knee ROA	2,11	0,87	5,10	1,66	0,10	14,92
Total	1,37	0,97	1,92	1,81	0,07	

Statistics for each study | OR and 95% CI | I^2= 5.6

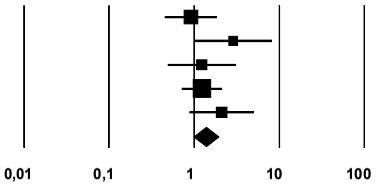


Figure 3.2.3b. Meta-analysis of studies on incident and progressive knee JSN

Study name	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value	Relative weight
McAlindon,1996, prog. knee JSN	2,30	0,93	5,69	1,80	0,07	14,96
Felson,2007, Fram, inc.&prog. knee JSN	0,87	0,51	1,48	-0,52	0,61	19,88
Felson,2007, BOKS, inc.&prog. knee JSN	0,56	0,28	1,12	-1,64	0,10	17,72
Zhang,2014, prog. knee JSN	3,20	1,50	6,81	3,02	0,00	16,89
Bergink,2013, inc. knee JSN	1,83	0,92	3,65	1,72	0,09	17,75
Bergink,2013, prog. knee JSN	0,59	0,20	1,76	-0,95	0,34	12,80
Total	1,26	0,71	2,23	0,79	0,43	

Statistics for each study | OR and 95% CI | I^2= 71.6

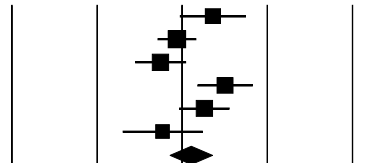


Figure 3.2.3a. Meta-analysis of studies on incident and progressive knee ROA/osteophytosis

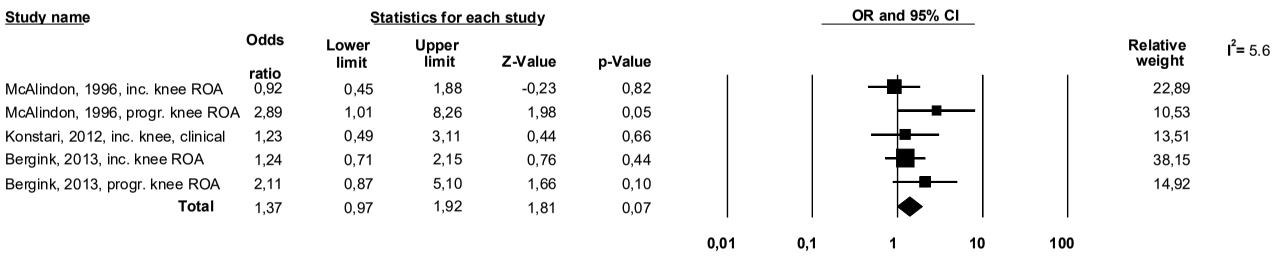
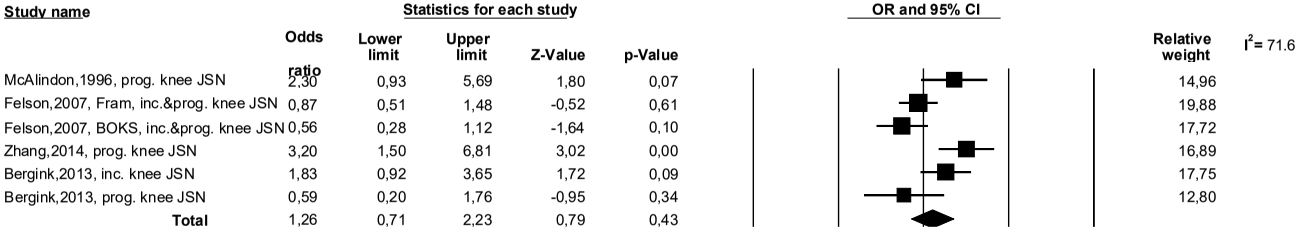


Figure 3.2.3b. Meta-analysis of studies on incident and progressive knee JSN



DISCUSSION

This meta-analysis of cross-sectional and longitudinal studies does not provide a clear answer to the question whether vitamin D levels and osteoarthritis are associated. Low vitamin D levels are not significantly associated with prevalent ROA or incident and progressive ROA combined.

In our study of 1,248 subjects from the Rotterdam Study-I, overall increasing percentages of subjects with prevalent, incident and progressive knee, hip and hand OA with decreasing vitamin D levels are seen. This association is largely driven the relation between age and vitamin D: older subjects have lower vitamin D levels and have increased prevalence and incidence of OA. After adjustment for age and other confounding factors, the association between vitamin D levels and OA is no longer seen, except for a positive association between 25(OH) vitamin D serum levels per standard deviation and prevalent knee ROA (**Table 3.2.1**). Environmental factors could possibly explain this unexpected positive association: subjects that perform hard labor by working outside may be more prone to have developed knee OA in the past.

When we consider prevalent knee JSN, an increased risk is observed in subjects with low vitamin D levels combining the studies of Ding, Al-Jarallah and Bergink. This finding is only partially confirmed in longitudinal studies: increased risk of development or worsening of knee JSN is seen in the studies of McAlindon and Zhang, but contradicted by those of Felson and Bergink. Furthermore, there is no clinical evidence of a protective effect of vitamin D on JSN. In a recently performed randomized controlled trial, McAlindon et al found no effect of vitamin D supplementation on cartilage volume loss in patients with symptomatic osteoarthritis[42].

In the meta-analyses of longitudinal studies, a significant association is only observed when analysing studies with progressive knee ROA as endpoint. This is in line with the conclusions of Cao et al, who stated in their systematic review that there was moderate evidence to suggest that low levels of vitamin D levels were positively

associated with progression of knee ROA[43]. In this review, they also stated that there was strong evidence for the association between cartilage loss with incident/progressive knee JSN/cartilage loss. However, one of the studies they refer to for this strong evidence has hip JSN as endpoint (Lane et al, 1999[26]), while they neglect the results of the longitudinal study of Felson et al., who found no association with knee cartilage loss[27].

There are several explanations for the observed significant associations between vitamin D levels and prevalent JSN in our meta-analysis. The observation could be due to multiple testing: the more endpoints are investigated (ROA and JSN, both cross-sectional and longitudinal), the more likely a significant association is found. In table 4, 14 different tests are presented. If these were independent test, the chance of finding one or more significant differences in 14 tests would be 51%, and the 'significance-cut-off' should be lowered from 0.05 to 0.0036. However, these tests are not independent: prevalent OA is associated with incident OA, and knee OA is associated with hand OA. Another explanation is insufficient adjustment for confounding factors. Low vitamin D is associated with lifestyle factors like wearing a veil, low education, high parity and low physical activity[44, 45]. These factors can confound the association between vitamin D and ROA. However, in all studies that found a significant association, adjustments were made for these lifestyle factors. A third possible explanation is that the observed associations represent a true effect. In that case, the development of joint space narrowing is actually influenced by 25(OH) vitamin D serum levels. This could be directly via the effect of vitamin D metabolites on articular chondrocytes as suggested by several studies[7, 8, 46], or indirectly via altered mechanical proprieties of the subchondral bone[47], as we hypothesized in our previous paper[29].

With regard to the supposed association with progressive knee ROA, the question is why this association is found with progressive, and not with incident knee ROA. Subjects with incident knee OA do not have OA at baseline, in contrast to subject with progressive OA, and are therefore younger, less likely to be female and have lower BMI. So, differences in characteristics within the study populations, and not in endpoints, could cause the difference in outcomes per study, despite adjustments for confounding factors.

Not only within studies, but also between studies the characteristics of the study groups differed. Considering the longitudinal studies, baseline characteristics of McAlindon's original Framingham Study differed considerably from the Framingham Offspring Study: the original Framingham Study consisted of 63% women, the mean age was 70.3 years, the mean BMI 26.1 kg/m^2 and the baseline vitamin D level was 74 nmol/l, while in the Framingham Offspring study and the Boston Osteoarthritis of the Knee Study (BOKS) [27], the percentages female were 53.1 and 41.4%, mean age 53.1 and 66.2 years respectively, the BMI 27.4 and 31.2 kg/m^2. This could explain the different, almost opposite, results Felson et al. found in their study compared to previous studies on the association between vitamin D levels and ROA. It is conceivable that younger people are more resistant to the adverse effects of low vitamin D levels. Furthermore, since the mean baseline vitamin D levels in the study of Felson is low, the subjects in their highest tertile (above 23 ng/ml = 57.5 nmol/l) are just above the threshold for vitamin D deficiency[48, 49], thus comparing very low with low vitamin D levels, and not with normal levels. Baseline characteristics of the participants of Lane and Bergink are more similar to the original Framingham study. This possibly explains the similarity in the outcomes.

To draw conclusions from a meta-analysis, the included studies should preferably be large, population-based and longitudinal, since cross-sectional studies are very prone to be biased by confounding factors. Only 6 studies were longitudinal, and the population sizes were small, resulting in low numbers of cases (incident and progressive AO), thus low power. For example, for hip JSN, when we compare two tertiles (lowest and highest) with each 500 subjects, we had 80% power to detect an increase of incidence from 6% to 11% (i.e., an increase of 83%). This means that we can only exclude effects of vitamin D on osteoarthritis which are larger than 83% difference, smaller effects cannot be excluded. Larger sample sizes are needed to perform sub-analyses of different OA endpoints and subgroups of subjects.

In conclusion, this meta-analysis does not demonstrate an association between vitamin D levels and hip or hand OA. Subgroup analyses show significant associations of low vitamin D levels with prevalent knee JSN and progressive knee OA: this could be due

to multiple testing, but also due to the fact that specific associations only occur in specific subgroups. Overall, the results of this study do not support the advice to supplement vitamin D to prevent the onset or worsening of osteoarthritis, except perhaps for progressive knee OA.

REFERENCES

1. Lips, P. (2001). Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. Endocrine reviews 22, 477-501.

2. Christodoulou, S., Goula, T., Ververidis, A., and Drosos, G. (2013). Vitamin D and bone disease. BioMed research international 2013, 396541.

3. Burger, H., van Daele, P.L., Odding, E., Valkenburg, H.A., Hofman, A., Grobbee, D.E., Schutte, H.E., Birkenhager, J.C., and Pols, H.A. (1996). Association of radiographically evident osteoarthritis with higher bone mineral density and increased bone loss with age. The Rotterdam Study. Arthritis and rheumatism 39, 81-86.

4. Arden, N.K., Nevitt, M.C., Lane, N.E., Gore, L.R., Hochberg, M.C., Scott, J.C., Pressman, A.R., and Cummings, S.R. (1999). Osteoarthritis and risk of falls, rates of bone loss, and osteoporotic fractures. Study of Osteoporotic Fractures Research Group. Arthritis and rheumatism 42, 1378-1385.

5. Hunter, D.J., Hart, D., Snieder, H., Bettica, P., Swaminathan, R., and Spector, T.D. (2003). Evidence of altered bone turnover, vitamin D and calcium regulation with knee osteoarthritis in female twins. Rheumatology 42, 1311-1316.

6. Bergink, A.P., Uitterlinden, A.G., Van Leeuwen, J.P., Hofman, A., Verhaar, J.A., and Pols, H.A. (2005). Bone mineral density and vertebral fracture history are associated with incident and progressive radiographic knee osteoarthritis in elderly men and women: the Rotterdam Study. Bone 37, 446-456.

7. Garabedian, M., Du Bois, M.B., Corvol, M.T., Pezant, E., and Balsan, S. (1978). Vitamin D and cartilage. I. In vitro metabolism of 25-hydroxycholecalciferol by cartilage. Endocrinology 102, 1262-1268.

8. Tetlow, L.C., and Woolley, D.E. (2001). Expression of vitamin D receptors and matrix metalloproteinases in osteoarthritic cartilage and human articular chondrocytes in vitro. Osteoarthritis and cartilage 9, 423-431.

9. Bischoff, H.A., Stahelin, H.B., Urscheler, N., Ehrsam, R., Vonthein, R., Perrig-Chiello, P., Tyndall, A., and Theiler, R. (1999). Muscle strength in the elderly: its relation to vitamin D metabolites. Arch Phys Med Rehabil 80, 54-58.

10. Dretakis, O.E., Tsatsanis, C., Fyrgadis, A., Drakopoulos, C.G., Steriopoulos, K., and Margioris, A.N. (2010). Correlation between serum 25-hydroxyvitamin D levels and quadriceps muscle strength in elderly cretans. J Int Med Res 38, 1824-1834.

11. Visser, M., Deeg, D.J., and Lips, P. (2003). Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): the Longitudinal Aging Study Amsterdam. The Journal of clinical endocrinology and metabolism 88, 5766-5772.

12. Hart, L.E. (2004). Quadriceps strength as a predictor of knee osteoarthritis progression. Clin J Sport Med 14, 104-105.

13. Ikeda, S., Tsumura, H., and Torisu, T. (2005). Age-related quadriceps-dominant muscle atrophy and incident radiographic knee osteoarthritis. Journal of orthopaedic science : official journal of the Japanese Orthopaedic Association 10, 121-126.

14. Segal, N.A., and Glass, N.A. (2011). Is quadriceps muscle weakness a risk factor for incident or progressive knee osteoarthritis? Phys Sportsmed 39, 44-50.

15. Bennell, K.L., Wrigley, T.V., Hunt, M.A., Lim, B.W., and Hinman, R.S. (2013). Update on the role of muscle in the genesis and management of knee osteoarthritis. Rheum Dis Clin North Am 39, 145-176.

16. Breijawi, N., Eckardt, A., Pitton, M.B., Hoelzl, A.J., Giesa, M., von Stechow, D., Haid, F., and Drees, P. (2009). Bone mineral density and vitamin D status in female and male patients with osteoarthritis of the knee or hip. Eur Surg Res 42, 1-10.

17. Rapin, C.H., and Lagier, R. (1982). Age-related blood changes in hip osteoarthritis patients: a possible indicator of bone quality. Annals of the rheumatic diseases 41, 215-216.

18. Chaganti, R.K., Parimi, N., Cawthon, P., Dam, T.L., Nevitt, M.C., and Lane, N.E. (2010). Association of 25-hydroxyvitamin D with prevalent osteoarthritis of the hip in elderly men: the osteoporotic fractures in men study. Arthritis and rheumatism 62, 511-514.

19. Heidari, B., Heidari, P., and Hajian-Tilaki, K. (2011). Association between serum vitamin D deficiency and knee osteoarthritis. International orthopaedics 35, 1627-1631.

20. Bird, H.A., Peacock, M., Storer, J.H., and Wright, V. (1980). Comparison of serum 25-OH vitamin D concentrations in rheumatoid arthritis and osteoarthrosis. Br Med J 280, 1416.

21. Bird, H.A., Wright, V., Hennes, U., and Theiss, E. (1982). Comparison of serum 1,25-dihydroxycholecalciferol concentrations in rheumatoid arthritis and osteoarthrosis. Annals of the rheumatic diseases 41, 257-258.

22. Geusens, P., Dequeker, J., and Verstraeten, A. (1983). Age-related blood changes in hip osteoarthritis patients: a possible indicator of bone quality. Annals of the rheumatic diseases 42, 112-113.

23. Roberts, N.B., Holding, J.D., Walsh, H.P., Klenerman, L., Helliwell, T., King, D., and Shearer, M. (1996). Serial changes in serum vitamin K1, triglyceride, cholesterol, osteocalcin and 25-hydroxyvitamin D3 in patients after hip replacement for fractured neck of femur or osteoarthritis. European journal of clinical investigation 26, 24-29.

24. Kalichman, L., and Kobyliansky, E. (2012). Association between circulatory levels of vitamin D and radiographic hand osteoarthritis. Rheumatology international 32, 253-257.

25. McAlindon, T.E., Felson, D.T., Zhang, Y., Hannan, M.T., Aliabadi, P., Weissman, B., Rush, D., Wilson, P.W., and Jacques, P. (1996). Relation of dietary intake and serum levels of vitamin D to progression of osteoarthritis of the knee among participants in the Framingham Study. Annals of internal medicine 125, 353-359.

26. Lane, N.E., Gore, L.R., Cummings, S.R., Hochberg, M.C., Scott, J.C., Williams, E.N., and Nevitt, M.C. (1999). Serum vitamin D levels and incident changes of radiographic hip osteoarthritis: a longitudinal study. Study of Osteoporotic Fractures Research Group. Arthritis and rheumatism 42, 854-860.

27. Felson, D.T., Niu, J., Clancy, M., Aliabadi, P., Sack, B., Guermazi, A., Hunter, D.J., Amin, S., Rogers, G., and Booth, S.L. (2007). Low levels of vitamin D and worsening of knee osteoarthritis: results of two longitudinal studies. Arthritis and rheumatism 56, 129-136.

28. Konstari, S., Paananen, M., Heliovaara, M., Knekt, P., Marniemi, J., Impivaara, O., Arokoski, J., and Karppinen, J. (2012). Association of 25-hydroxyvitamin D with the incidence of knee and hip osteoarthritis: a 22-year follow-up study. Scandinavian journal of rheumatology 41, 124-131.

29. Bergink, A.P., Uitterlinden, A.G., Van Leeuwen, J.P., Buurman, C.J., Hofman, A., Verhaar, J.A., and Pols, H.A. (2009). Vitamin D status, bone mineral density, and the development of radiographic osteoarthritis of the knee: The Rotterdam Study. J Clin Rheumatol 15, 230-237.

30. Hofman, A., Grobbee, D.E., de Jong, P.T., and van den Ouweland, F.A. (1991). Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol 7, 403-422.

31. Hofman, A., Breteler, M.M., van Duijn, C.M., Krestin, G.P., Pols, H.A., Stricker, B.H., Tiemeier, H., Uitterlinden, A.G., Vingerling, J.R., and Witteman, J.C. (2007). The Rotterdam Study: objectives and design update. Eur J Epidemiol 22, 819-829.

32. Hofman, A., Breteler, M.M., van Duijn, C.M., Janssen, H.L., Krestin, G.P., Kuipers, E.J., Stricker, B.H., Tiemeier, H., Uitterlinden, A.G., Vingerling, J.R., et al. (2009). The Rotterdam Study: 2010 objectives and design update. Eur J Epidemiol 24, 553-572.

33. Hofman, A., van Duijn, C.M., Franco, O.H., Ikram, M.A., Janssen, H.L., Klaver, C.C., Kuipers, E.J., Nijsten, T.E., Stricker, B.H., Tiemeier, H., et al. (2011). The Rotterdam Study: 2012 objectives and design update. Eur J Epidemiol 26, 657-686.

34. Hofman, A., Darwish Murad, S., van Duijn, C.M., Franco, O.H., Goedegebure, A., Ikram, M.A., Klaver, C.C., Nijsten, T.E., Peeters, R.P., Stricker, B.H., et al. (2013). The Rotterdam Study: 2014 objectives and design update. Eur J Epidemiol 28, 889-926.

35. Kellgren, J.H., Jeffrey, M.R., and Ball, J. (1963). Atlas of Standard Radiographs of Arthritis: The epidemiology of Chronic Rheumatism.(Oxford: Blackwell Scientific Publications).

36. Kerkhof, H.J., Bierma-Zeinstra, S.M., Castano-Betancourt, M.C., de Maat, M.P., Hofman, A., Pols, H.A., Rivadeneira, F., Witteman, J.C., Uitterlinden, A.G., and van Meurs, J.B. (2010). Serum C reactive protein levels and genetic variation in the CRP gene are not associated with the prevalence, incidence or progression of osteoarthritis independent of body mass index. Annals of the rheumatic diseases 69, 1976-1982.

37. Kellgren, J.H., and Lawrence, J.S. (1957). Radiological assessment of osteo-arthrosis. Annals of the rheumatic diseases 16, 494-502.

38. DerSimonian, R., and Laird, N. (1986). Meta-analysis in clinical trials. Control Clin Trials 7, 177-188.

39. Al-Jarallah, K.F., Shehab, D., Al-Awadhi, A., Nahar, I., Haider, M.Z., and Moussa, M.A. (2012). Are 25(OH)D levels related to the severity of knee osteoarthritis and function? Med Princ Pract 21, 74-78.

40. Ding, C., Cicuttini, F., Parameswaran, V., Burgess, J., Quinn, S., and Jones, G. (2009). Serum levels of vitamin D, sunlight exposure, and knee cartilage loss in older adults: the Tasmanian older adult cohort study. Arthritis and rheumatism 60, 1381-1389.

41. Zhang, F.F., Driban, J.B., Lo, G.H., Price, L.L., Booth, S., Eaton, C.B., Lu, B., Nevitt, M., Jackson, B., Garganta, C., et al. (2014). Vitamin D deficiency is associated with progression of knee osteoarthritis. J Nutr 144, 2002-2008.

42. McAlindon, T., LaValley, M., Schneider, E., Nuite, M., Lee, J.Y., Price, L.L., Lo, G., and Dawson-Hughes, B. (2013). Effect of vitamin D supplementation on progression of knee pain and cartilage

volume loss in patients with symptomatic osteoarthritis: a randomized controlled trial. Jama 309, 155-162.

43. Cao, Y., Winzenberg, T., Nguo, K., Lin, J., Jones, G., and Ding, C. (2013). Association between serum levels of 25-hydroxyvitamin D and osteoarthritis: a systematic review. Rheumatology 52, 1323-1334.

44. Gannage-Yared, M.H., Chemali, R., Yaacoub, N., and Halaby, G. (2000). Hypovitaminosis D in a sunny country: relation to lifestyle and bone markers. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 15, 1856-1862.

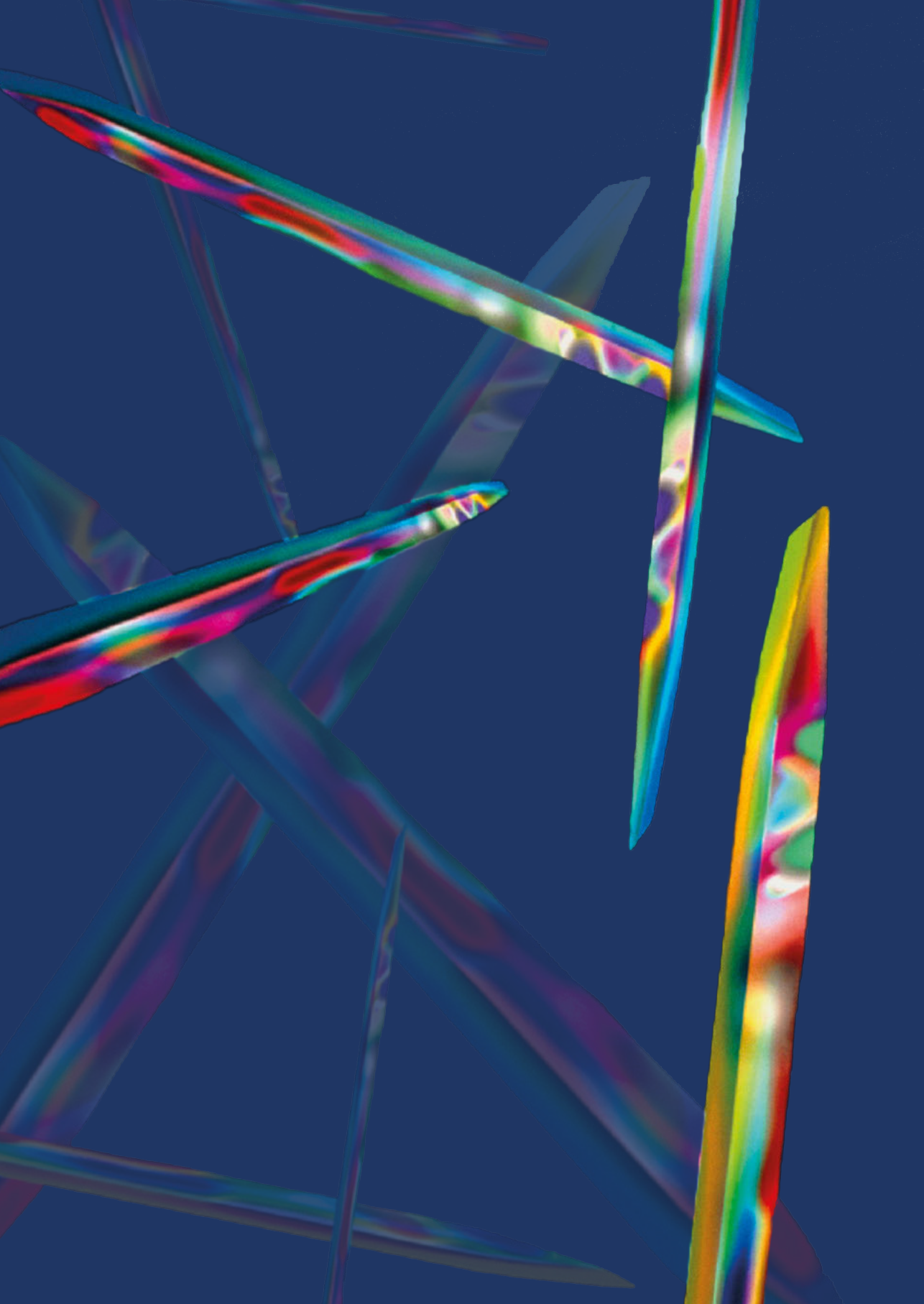
45. Guzel, R., Kozanoglu, E., Guler-Uysal, F., Soyupak, S., and Sarpel, T. (2001). Vitamin D status and bone mineral density of veiled and unveiled Turkish women. J Womens Health Gend Based Med 10, 765-770.

46. Harmand, M.F., Thomasset, M., Rouais, F., and Ducassou, D. (1984). In vitro stimulation of articular chondrocyte differentiated function by 1,25-dihydroxycholecalciferol or 24R,25-dihydroxycholecalciferol. Journal of cellular physiology 119, 359-365.

47. Aerssens, J., van Audekercke, R., Talalaj, M., Geusens, P., Bramm, E., and Dequeker, J. (1996). Effect of 1alpha-vitamin D3 and estrogen therapy on cortical bone mechanical properties in the ovariectomized rat model. Endocrinology 137, 1358-1364.

48. Holick, M.F. (2007). Vitamin D deficiency. N Engl J Med 357, 266-281.

49. Bouillon, R., Norman, A.W., and Lips, P. (2007). Vitamin D deficiency. N Engl J Med 357, 1980-1981; author reply 1981-1982.



4

GENETICS, VITAMIN D AND OSTEOARTHRITIS

4.1 genetic variants associated with vitamin D levels and osteoarthritis

Mendelian randomization study on vitamin D levels and osteoarthritis risk: a concise report

Rheumatology. 2021 Jul 1;60(7):3409-12. PubMed PMID: 33463692

Bergink AP, Trajanoska K, Uitterlinden AG, van Meurs JB

ABSTRACT

Objective. The role of vitamin D in osteoarthritis (OA) is unclear and previous epidemiological studies gave inconsistent results. We conducted a two sample Mendelian randomization (MR) study to investigate the causal relationship between genetically determined serum vitamin D levels and hip/knee OA.

Methods. Six single nucleotide polymorphisms (SNPs) associated with vitamin D levels in the SUNLIGHT Consortium were selected as instrumental variables. Summary statistics of the SNPs effects on osteoarthritis (OA) were derived from the Iceland and UK Biobank, comprising 23,877 knee OA cases, 17,151 hip OA cases and over 562,000 controls. The control samples match the osteoarthritis cases in age, sex, and county of origin.

Results. The MR analyses showed no causal association between genetically determined vitamin D levels and knee OA (OR 1.03, 95% CI 0.84 to 1.26) or hip OA (OR 1.06, 95% CI 0.83 to 1.35).

Conclusion. Genetic variations associated with low vitamin D serum levels are not associated with increased risk of OA of hip or knee in community–dwelling older adults, suggesting that vitamin D levels are not causally linked to osteoarthritis. It is therefore unlikely that vitamin D supplementation protects against hip or knee OA.

INTRODUCTION

Vitamin D status is of influence on bone metabolism; while severe deficiency causes rickets in children and osteomalacia in adults, more mild deficiencies lead to a decrease in bone mineral density (BMD) and increased fracture risk[1]. Since osteoarthritis (OA) is associated with bone characteristics like increased BMD[2] and fracture risk[3], it is hypothesized that vitamin D status influences the development of OA. However, studies on the association between vitamin D levels and OA show inconsistent results[4].

One possible explanation for these conflicting results is that the vitamin D status of the subjects measured in the circulation at one time point does not reflect the actual vitamin D status over a longer period of time. The vitamin D levels at time of blood withdrawal may be influenced by factors such as sun exposure, dietary intake and vitamin D supplementation, which cannot be completely corrected for by adjustment or matching cases and controls. Another problem in studies on the association between vitamin D levels and OA is the possible confounding effect of physical activity level: lower activity could lead to less sun exposure, resulting in lower vitamin D levels.

A way to obtain more reliable estimates of the vitamin D status over a longer period of time without confounding is by considering genetic variants that determine vitamin D levels. A meta-analyses of genome-wide association study (GWAS) in the SUNLIGHT consortium found common variants near genes involved in cholesterol synthesis (*DHCR7*), hydroxylation (*CYP2R1, CYP24A1*), and vitamin D transport (*GC*) influencing vitamin D status[5]. In an expansion of this study, two additional loci in SEC23A and in AMDHD1 were found[6]. Individuals with risk alleles at these loci had substantially elevated risk for vitamin D insufficiency.

We conducted a two sample Mendelian randomization (MR) study to examine whether there is a causal relationship between genetically increased vitamin D levels and hip/knee OA. MR is a study design in which genetic variants are used as proxy for modifiable exposure to test the unconfounded effect of the exposure on a specific outcome.

METHODS

Six single nucleotide polymorphisms (SNPs) associated with 25-hydroxyvitamin D (25OHD) level were identified from the expanded SUNLIGHT GWAS meta-analysis[6] (79,366 discovery sample and a 40,562 replication sample replicates): rs3755967 (at GC), rs12785878 (at NADSYN1/DHCR7), rs10741657 (at CYP2R1), rs17216707 (at CYP24A1), rs10745742 (at AMDHD1) and rs8018720 (at SEC23A). Next, we conducted an MR study to describe the effect of genetically lowered 25OHD on the odds of hip and knee OA in the Iceland and the UK Biobank meta-analysis[7]. The Icelandic study included 5,714 individuals with hip osteoarthritis and 4,672 individuals with knee osteoarthritis, as well as more than 172,000 controls. The UK Biobank study contained 11,437 individuals with hip osteoarthritis, 19,205 with knee osteoarthritis, and more than 389,000 controls, all of white British ancestry, selected from the UK Biobank resource of 500,000 British volunteers[7].

In our MR analysis, we assessed the effects of the SNPs upon risk of hip and knee OA, weighting the effect of each SNP by its effect upon vitamin D. Then the individual estimates of the effect were then pooled using statistically efficient estimators formally analogous to those of inverse-variance-weighted (IVW) meta-analysis[8]. In addition, we used weighted median (WM), a MR method which generates consistent effect estimates as long as >50% of the SNPs are valid instrumental variables[9]. The R package *MendelianRandomization*[10] was used to generate the IVW and WM MR effect estimates.

RESULTS

In **Table 4.1.1,** the association between the six SNPs associated with vitamin D levels and hip and knee OA are shown. In the Iceland population, two SNPs reached nominal significance for one of the studied OA phenotypes (rs12785878 for hip OA, and rs10745742 for knee OA). In the UK biobank population, rs17216707 was significantly associated with knee OA. The combined analysis showed only a nominal significant association for rs17216707, where the C-allele (associated with a lower Vitamin D level) was associated with a decreased risk for knee OA.

Table 4.1.1. Vitamin D SNPs & hip/knee osteoarthritis

					Iceland		UK Biobank		Iceland and UK Biobank combined			
Phenotype	SNP	Chromosome	Effect allele	Other allele	OR	P-value	OR	P-value	OR	lower 95%CI	upper 95%CI	P-value
Hip OA	rs3755967	4	T	C	1,027	0,316	1,016	0,283	1,019	0,993	1,045	0,154
	rs12785878	11	G	T	1,055	0,035	0,984	0,345	1,005	0,978	1,033	0,703
	rs10741657	11	A	G	1,009	0,721	0,982	0,201	0,989	1,013	0,966	0,352
	rs17216707	20	C	T	0,970	0,334	1,028	0,120	1,014	0,984	1,045	0,372
	rs10745742	12	T	C	1,002	0,935	0,984	0,249	0,988	1,012	0,965	0,335
	rs8018720	14	G	C	0,982	0,605	1,012	0,492	1,006	0,975	1,038	0,708
Knee OA	rs3755967	4	T	C	1,025	0,384	1,015	0,190	1,017	0,995	1,038	0,124
	rs12785878	11	G	T	1,053	0,052	1,008	0,534	1,017	0,994	1,040	0,159
	rs10741657	11	A	G	1,022	0,401	0,995	0,639	1,000	1,019	0,979	0,915
	rs17216707	20	C	T	0,994	0,850	0,953	0,001	0,959	0,983	0,935	0,001
	rs10745742	12	T	C	0,937	0,015	0,988	0,281	0,981	1,000	0,962	0,055
	rs8018720	14	G	C	1,004	0,918	1,002	0,910	1,002	0,976	1,028	0,887

SNP: single nucleotide polymorphism, OR: odds ratio, OA: osteoarthritis

Table 4.1.2 shows the results of the MR analysis between vitamin D levels and knee and hip OA. No causal effect of the genetic risk variants on knee or hip OA were found: for knee OA the odds ratio (OR) was 1.03 with 95% confidence interval (CI) 0.84 to 1.26 (inverse variance weighted (IVW) method) and for hip OA the OR was 1.06, with 95% CI 0.83 to 1.35. With the weight median method similar results were found.

Table 4.1.2. Results MR-analyses

Trait	Method	Fixed Model				
		β	SE	OR* (95% CI)	P-value	P-heterogeneity
knee OA	IVW	0.032	0.103	1.03 (0.84 - 1.26)	0.76	0.63
	weighted median	0.077	0.112	1.08 (0.87 – 1.34)	0.49	
hip OA	IVW	0.059	0.124	1.06 (0.83 - 1.35)	0.63	0.98
	weighted median	0.085	0.136	1.09 (0.83 – 1.42)	0.53	

*per one unit increase in vitamin D (nmol/l)
IVW: inverse variance weighted

DISCUSSION

Past observational studies on the association between vitamin D serum levels and OA led to conflicting results. These inconsistencies may be explained by confounding factors associated with low vitamin D levels. Especially in cross-sectional studies a possible association can be influenced by the fact that subjects with OA of the lower limbs are less mobile, and are, thus, less exposed to sunlight and so will have lower vitamin D serum levels. In longitudinal studies low vitamin D levels at baseline could also indicate low activity level, which could be related to worsening of OA; Zhang et al. demonstrated that exercise may decrease cytokine and cytokine-related protein levels in the synovial fluid and inhibit inflammatory factor-mediated cartilage degradation in knee OA patients[11]. To eliminate the influence of lifestyle factors associated with low vitamin D levels, we conducted a MR study on vitamin D levels in relation to OA.

Our MR analyses, with six SNPs associated with vitamin D levels as instrumental variables, and comprising 23,877 knee OA cases, 17,151 hip OA cases and over 562,000 controls, showed no causal association between genetically determined vitamin D levels and knee or hip OA. This is in line with most RCT conducted to study the effect of vitamin D on the development of OA, finding no evidence for benefits of supplementation[12].

There are some limitations to our study. The osteoarthritis case definition originated from electronic health records and clinical evaluations, and was not defined by both symptoms and radiographs, using the Kellgren and Lawrence grade to define radiographic OA, as recommend for population cohort studies[13]. We can therefore not rule out that specific radiologic features of OA, like prevalent joint space narrowing of the knee or progressive knee OA, are associated with low vitamin D levels, as shown in past studies[4]. Furthermore, the combined effect on the vitamin D levels of the 6 SNPs we used, could be too little to detect and association with knee or hip OA. Another limitation is that our MR study was based on only 6 SNPs as identified in earlier GWAS and with 7.5% explained variance[6], while the latest GWAS on 25OH vitamin D levels has identified 143 independent SNPs explaining 13% of the variance[14]. Currently, we can therefore not

exclude that associations might have been missed because of the low explained variance, and do not expect this to change when using the MR instrument based on 143 SNPs. Finally, in our MR analyses we assume that there is a linear exposure-risk association, thus non-linear relations, like threshold-effect, or U-shaped relations, will not be detected.

The strength of this study is that by studying genetically predicted vitamin D levels instead of a one-time measurement of circulating vitamin D levels, a possible association with OA is not confounded by life style factors such as activity level, vitamin D intake or supplementation. We conclude that the common genetic variants associated with low serum vitamin D levels are not associated with increased risk of hip or knee OA. This implicates that the supplementation of vitamin D does not protect against hip or knee OA.

REFERENCES

1. Lips, P. (2001). Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. Endocrine reviews 22, 477-501.
2. Bergink, A.P., Rivadeneira, F., Bierma-Zeinstra, S.M., Zillikens, M.C., Ikram, M.A., Uitterlinden, A.G., and van Meurs, J.B.J. (2019). Are Bone Mineral Density and Fractures Related to the Incidence and Progression of Radiographic Osteoarthritis of the Knee, Hip, and Hand in Elderly Men and Women? The Rotterdam Study. Arthritis & rheumatology 71, 361-369.
3. Bergink, A.P., van der Klift, M., Hofman, A., Verhaar, J.A., van Leeuwen, J.P., Uitterlinden, A.G., and Pols, H.A. (2003). Osteoarthritis of the knee is associated with vertebral and nonvertebral fractures in the elderly: the Rotterdam Study. Arthritis and rheumatism 49, 648-657.
4. Bergink, A.P., Zillikens, M.C., Van Leeuwen, J.P., Hofman, A., Uitterlinden, A.G., and van Meurs, J.B. (2016). 25-Hydroxyvitamin D and osteoarthritis: A meta-analysis including new data. Semin Arthritis Rheum 45, 539-546.
5. Wang, T.J., Zhang, F., Richards, J.B., Kestenbaum, B., van Meurs, J.B., Berry, D., Kiel, D.P., Streeten, E.A., Ohlsson, C., Koller, D.L., et al. (2010). Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet 376, 180-188.
6. Jiang, X., O'Reilly, P.F., Aschard, H., Hsu, Y.H., Richards, J.B., Dupuis, J., Ingelsson, E., Karasik, D., Pilz, S., Berry, D., et al. (2018). Genome-wide association study in 79,366 European-ancestry individuals informs the genetic architecture of 25-hydroxyvitamin D levels. Nature communications 9, 260.
7. Styrkarsdottir, U., Lund, S.H., Thorleifsson, G., Zink, F., Stefansson, O.A., Sigurdsson, J.K., Juliusson, K., Bjarnadottir, K., Sigurbjornsdottir, S., Jonsson, S., et al. (2018). Meta-analysis of Icelandic and UK data sets identifies missense variants in SMO, IL11, COL11A1 and 13 more new loci associated with osteoarthritis. Nature genetics 50, 1681-1687.
8. Burgess, S., Butterworth, A., and Thompson, S.G. (2013). Mendelian randomization analysis with multiple genetic variants using summarized data. Genetic epidemiology 37, 658-665.
9. Bowden, J., Davey Smith, G., Haycock, P.C., and Burgess, S. (2016). Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. Genetic epidemiology 40, 304-314.

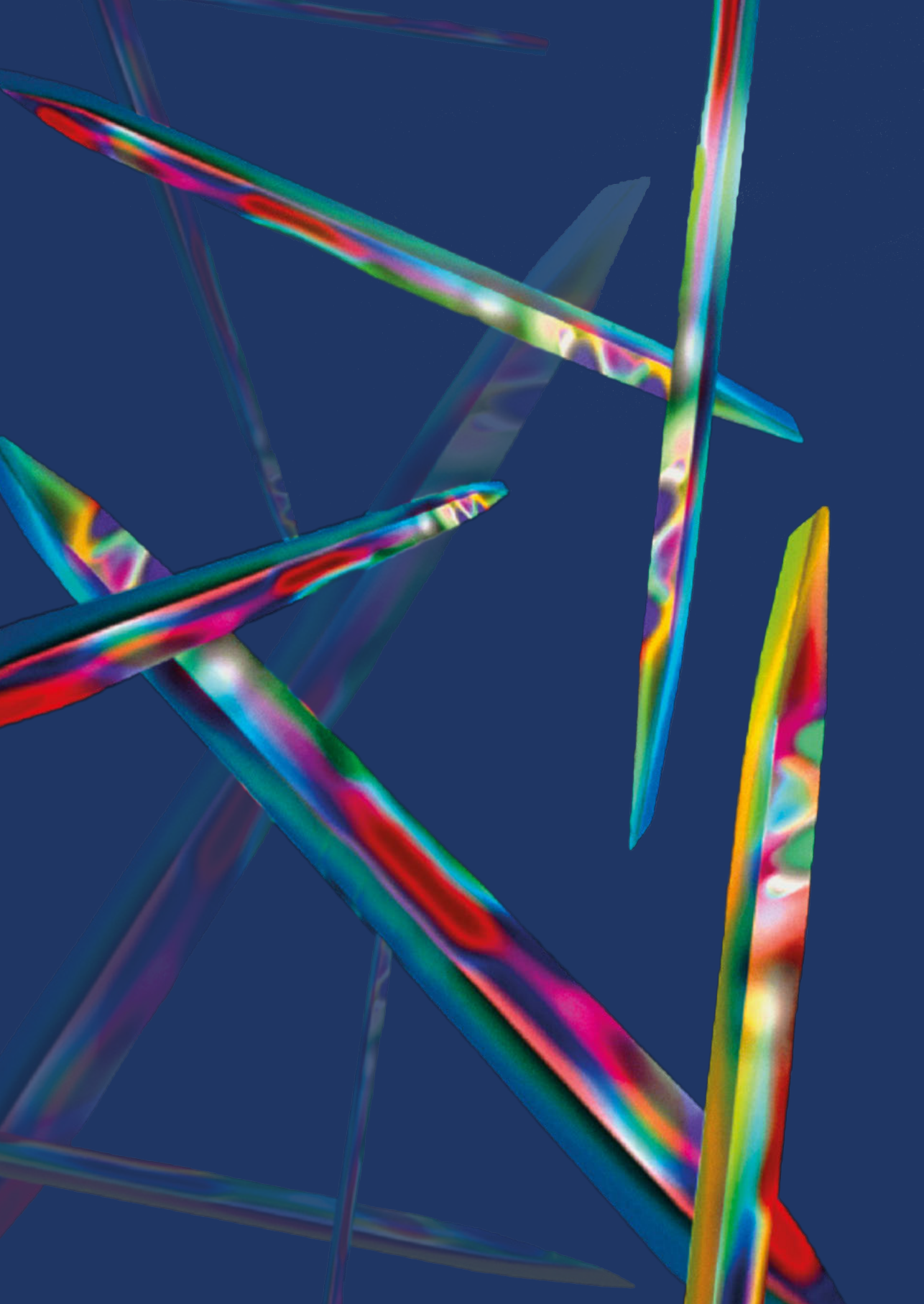
10. Yavorska, O.O., and Burgess, S. (2017). MendelianRandomization: an R package for performing Mendelian randomization analyses using summarized data. International journal of epidemiology 46, 1734-1739.

11. Zhang, S.L., Liu, H.Q., Xu, X.Z., Zhi, J., Geng, J.J., and Chen, J. (2012). Effects of exercise therapy on knee joint function and synovial fluid cytokine levels in patients with knee osteoarthritis. Mol Med Report.

12. Hussain, S., Singh, A., Akhtar, M., and Najmi, A.K. (2017). Vitamin D supplementation for the management of knee osteoarthritis: a systematic review of randomized controlled trials. Rheumatology international 37, 1489-1498.

13. Leyland, K.M., Gates, L.S., Nevitt, M., Felson, D., Bierma-Zeinstra, S.M., Conaghan, P.G., Engebretsen, L., Hochberg, M., Hunter, D.J., Jones, G., et al. (2018). Harmonising measures of knee and hip osteoarthritis in population-based cohort studies: an international study. Osteoarthritis and cartilage 26, 872-879.

14. Revez, J.A., Lin, T., Qiao, Z., Xue, A., Holtz, Y., Zhu, Z., Zeng, J., Wang, H., Sidorenko, J., Kemper, K.E., et al. (2020). Genome-wide association study identifies 143 loci associated with 25 hydroxyvitamin D concentration. Nature communications 11, 1647.



5

GENERAL DISCUSSION

'It is with some trepidation that I offer for your consideration the subject of osteo-arthritis, and especially the particular form of osteo-arthritis, which most frequently attacks the vertebrae, the sacro-iliac joints and the hips. It is a subject, however, of vast interest to the general practitioner, although it receives but scant attention from him. I repeat that it is, or rather should be of vast interest, for it is so common an ailment that it occurs almost daily in the practice of each one.' In 1909 Hunkin started his declamation before the San Francisco Medical Society with these sentences[1].

More than a century and numerous studies later, the mechanism behind the development of osteoarthritis (OA) is still not unravelled. It is known that OA is more than mechanical wear of the joint, but investigators are still in search which local and systemic factors are of influence on its development.

5.1 Risk factors for osteoarthritis

Osteoarthritis is -in general- a disease of the elderly. As people age, the prevalence of OA in nearly all joints increases[2; 3]. For example, in the main study population of this thesis, the Rotterdam Study, the prevalence of knee OA (Kellgren score of 2 or more) ranged from 11% in subjects aged 55-60, to 56% for those between age 85 and 90 (**Figure 5.1.1**).

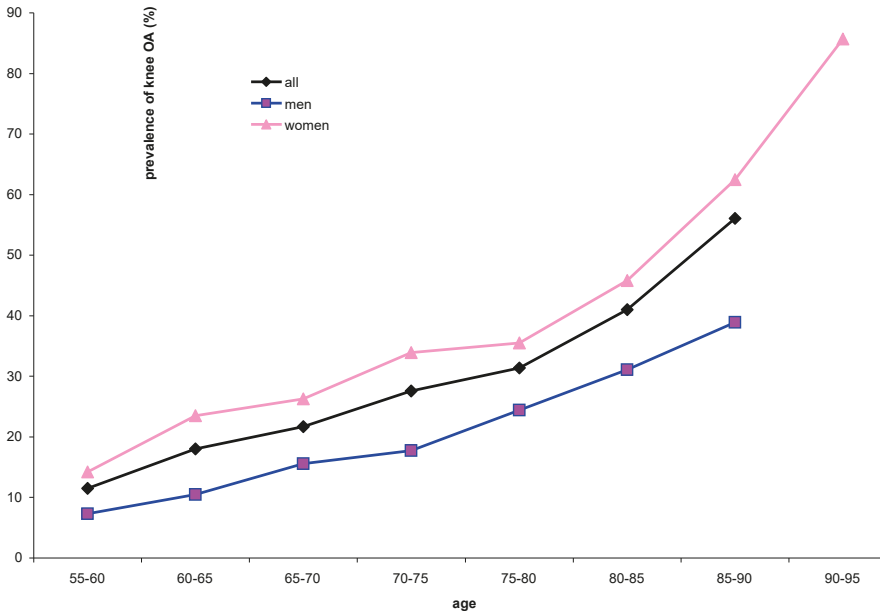


Figure 5.1.1. Distribution of prevalent knee OA among different age-groups and by gender in the Rotterdam Study at baseline (1990 -1993)

Because of the strong relation with age, OA was historically regarded as a pure degenerative disease influenced by wear and tear[4]. Typically, the joints that are used the most also suffer from OA most frequently. For example, the ankles, feet and knees of soccer players, the shoulders and elbows of pneumatic-drill operators, the hips of farmers, the patellofemoral knee joints of cyclists and the hands of boxers are most affected by osteoarthritis[5].

However, as more insight is gained in the mechanisms causing OA, the condition is now no longer considered an inevitable consequence of growing old[6]. Several systemic factors, like obesity, bone mineral density (BMD), and muscle strength, have been associated with the development of OA[7; 8]. Rather than directly causing OA, these factors contribute to the development of OA by making the joint more susceptible to the effects of external risk factors that include wear and tear, nutrition and injury (**Figure 5.1.2**). These

systemic factors are to a greater or lesser extent genetically determined, and can alter during the life course of the patient[9].

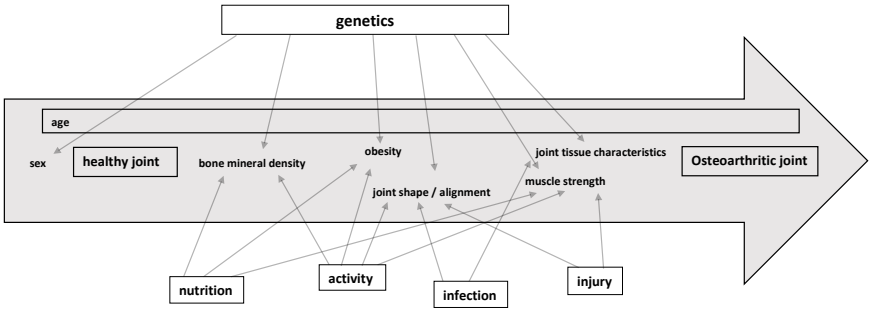


Figure 5.1.2: Life course factors influencing the development of OA

In this thesis, several suggested risk factors for OA were investigated for their role in the development of OA, in particular those related to bone health, while also making use of increased insights in genetic factors for OA. Therefore, inter-individual differences in fracture risk, bone mineral density, vitamin D status and genetic predisposition were studied in subjects to examine their influence on baseline OA, and on incidence and progression of OA in several joints. The studies were mainly performed in the Rotterdam Study, that has documented OA (by radiographs) and a wealth of related characteristics since the start, but also in other cohorts. The Rotterdam Study is a prospective cohort study on risk factors and determinants of chronic diseases in middle-aged and elderly persons, that started in 1990. It therefore consists of relatively healthy participants and has long follow-up times and such a large longitudinal study design with many other characteristics measured in the study participants, preceding development of disease, offers several advantages in understanding cause and effect.

5.2 BMD, fracture risk and osteoarthritis

Bone Mineral Density

BMD in relation to OA has been under much investigation over decades. In the early seventies, Foss and Byers were the first to observe increased metacarpal bone density in subjects with hip OA[10]. Since then, numerous (cross-sectional) studies have confirmed this observation, showing that OA of the hands, lumbar spine, hips and knees is associated with higher BMD[11-16]. This led to the assumption that subjects with high BMD have increased risk of developing OA. Several prospective studies, almost exclusively among women, showed that women with high BMD at baseline had indeed increased risk of incident knee OA[17-19]. In the prospective study presented in this thesis and which was among 2,745 men and women of the Rotterdam Study, these findings were confirmed, and a 5 times higher risk for men in the upper BMD quartile was demonstrated as well **(Chapter 2.2)**. Later, Nevitt et al. also studied men and demonstrated a 3-fold increased risk of incident OA in the upper baseline BMD quartile[20], confirming higher point estimates for men than women. In **Chapter 2.3**, the analyses of the association between BMD, fracture risk and OA were repeated in a larger and extended study population (4,154 subjects), and apart from knee OA, also hip and hand OA were analysed. Overall, these analyses confirmed the earlier found association between high BMD and increased risk of development of knee OA, albeit with more accurate point estimates that were somewhat lower (i.e., OR 1.6 (with 95% CI 1.1 to 2.2) instead of OR of 2.8 (with 95% CI 1.2 to 6.8)). The point estimates were similar for men and women, in contrast to the smaller study presented in Chapter 2.2, were the men in the upper quartile of BMD had a two times increased risk compared to women. Furthermore, this large prospective study is the first to provide consistent evidence for the relationship between high baseline BMD and OA at another joint, i.e., incident hip OA. This relationships was driven by the association between BMD and OA in women (data not shown). Indeed, a recent study confirmed a higher risk for hip OA in high bone mass individuals (n = 136, 72% women)[21]. No associations were found between BMD and the incident hand OA.

A possible explanation for the observed relation between high BMD and the development of knee and hip OA is a diminished ability to restore micro injuries of the weight-bearing joint in subjects with high BMD. The subchondral bone in these subjects, as the rest of their bone, is denser, and has less shock-absorbing capacity, leading to cartilage damage. It is also conceivable that the high BMD does not reflect bone of 'higher quality'. As was observed, the 'high BMD subjects' with knee OA have increased fracture risk (**Chapter 2.1**). Although the density of this bone is higher, it could have mechanically inferior characteristics. As Ding et al. demonstrated in early knee OA, there is increased trabecular thickness and density but relatively decreased connectivity, leading to reduced mechanical properties of subchondral bone [22; 23], making the overlying cartilage more susceptible to damage. This could also explain the fact that no clear association between high BMD and hand OA was found, since the hand is a non-weight-bearing joint.

Another possible pathway connecting high BMD with increased OA-risk is the endocrine pathway. It is suggested that generalised osteoarthritis is a hormonally mediated disease, and it could be that women with relative high endogenous estrogen concentrations, especially around the menopause, are predisposed to generalised OA[24]. This hypothesis could also explain the increased BMD in women developing OA, since estrogens protects against bone loss[25]. Such a mechanism could also be applicable to men, since age-related bone loss is related to estrogen deficiency in men as well[26]. This also might explain the inverse relation between prevalent vertebral fracture and the development of knee OA (**Chapter 2.2)**: low estrogen status predisposes to increased fracture risk and protects against OA. However, in a systematic review, de Klerk et al. found no clear association between female hormonal aspects and osteoarthritis of the hand, hip or knee [27]. Since then, numerous studies on the possible association between estrogen and OA did not lead to a conclusive answer[28-30]. The lack of association might be explained by the heterogeneity of the studied subjects. For example, past studies on estrogen status and osteoarthritis have not discriminated between subgroups based on bone health. Future studies should be performed, measuring estrogen levels in specific subgroups, for instance in subjects with and without vertebral fractures, assessing the development of OA over time. Alternatively, a Mendelian randomization (MR) study could be performed

to asses a possible causal relationship between estrogen levels and OA. MR uses genetic variation as a natural experiment to investigate the causal relations between potentially modifiable risk factors and health outcomes in observational data[31]. In this case, genetic determinants of circulating estrogen levels could be used to asses a causal relation with different types of OA[32]. In addition, other aspects of hormonal exposure, such as age at menarche and age at natural menopause are also under genetic control and, thus, it would be interesting to explore these in an MR study in relation to OA [33].

Finally, although many potential factors were included in the used risk models, the possibility cannot be ruled out that confounding factors are (partly) responsible for the associations observed. The association between high BMD and OA may -for instance- be biased by a history of hard labour, resulting in strong bones, less bone loss, and early degenerative joint disease. And although adjusted for parameters of postural stability (lower limb disability, fall frequency and use of walking aid), the association between fracture history and decreased risk of OA may still be explained by a common lifestyle factor: subjects who suffered a fracture are likely to be more cautious, and put less demand on their joints, thus decreasing the risk of OA.

Fractures

High BMD protects against fractures[34]. However, although BMD was higher in OA cases as observed in the studies in this thesis, this increased BMD did not protect against fractures (**Chapter 2.1.)** There are several possible explanations for the observation of increased fracture risk among OA cases. First, it could be that the higher BMD in subjects with OA represents bone of less quality, and thus does not protect against fractures. The second possible explanation could be a higher fall frequency in OA patients. Several studies found that subjects with OA had an increased fall tendency, suggesting that increased falls might be causing the higher fracture rate in OA patients[35; 36]. Although adjustment was made for several types of postural stability, this factor could still explain the association observed between knee OA and increased fracture risk.

Inversely, a prevalent vertebral fracture was observed to protect against the development of knee OA independent of BMD **(Chapter 2.2)**. This again suggests that another indicator of bone strength or quality than BMD is involved in the association between fracture history and OA. However, this observed protective effect of fracture on knee OA failed to reach significance in the extended analyses with longer follow-up (**Chapter 2.3**). It is possible that the relationship between vertebral fracture and knee OA development is only present if both events are occurring relatively close together. In contrast, subjects with a prevalent vertebral fracture had an increased risk for hand OA. Since the hand joints are not weight-bearing, this might lead to a different possible association between bone characteristics and OA, compared to the knee and hip joints.

In conclusion, these results suggest a common bone characteristic apart from BMD seems to be involved in development OA. In the next paragraph a possible candidate is examined.

5.3 Vitamin D and OA

Vitamin D plays an important role in bone metabolism; low vitamin D status is associated with decreased bone mineral density and impaired bone remodeling[37; 38].

The consequences of vitamin D deficiency, defined as a serum 25-hydroxyvitamin D (25OHD) concentration of less than 50 nmol/l, are secondary hyperparathyroidism and bone loss, leading to osteoporosis and fractures, mineralization defects, which may lead to osteomalacia in the long term[39; 40]. If altered bone characteristics play a role in the development of OA, vitamin D status could be of influence through its effect on bone. Yet, cross-sectional studies have found conflicting evidence for an association of vitamin D levels and OA[41-43]. However, cross-sectional studies are highly prone to bias: participants suffering from OA are, for example, less mobile and therefore less likely to go out in the sunlight. Thus, to investigate a possible association between low vitamin D status and the development of OA, prospective studies are more reliable while in addition meta-analyses can provide better estimates of true effects across many studies and assess heterogeneity.

In 1996, McAlindon et al. were the first to conduct a prospective study on the association between dietary vitamin D and circulating vitamin D levels, measured as 25OHD. In their analysis of 556 men and women in the Framingham study, a 3- to 4-fold increased risk of progression of knee OA was observed in subjects in the lowest and middle tertile of dietary intake of vitamin D (3 to 347 IU/d) compared to the highest tertile (386 to 1612 IU/d), and a 3-fold increased risk of progression of knee OA in men and women in the lowest and middle tertile of baseline serum 25OHD (12.5 to 82.5 nmol/l) compared to the highest tertile (90 to 197.5 nmol/l)[44]. Since then, several longitudinal studies on the association between vitamin D levels and different OA types were conducted, and again with conflicting results[45-48]. In an effort to verify the results of McAlindon et al., a study on vitamin D status and incidence and progression of nee OA among 1248 men and woman within the Rotterdam Study was conducted (**Chapter 3.1)**. A significant association was observed between low dietary vitamin D intake (less than 44 IU/d) and progressive knee OA, but no association between 25OHD levels (as defined by tertiles) and incident or progressive knee OA was seen. Indeed, an updated and extended analysis of 25OHD serum levels in relation to knee, hip and hand OA, with a mean follow up times of 8.4 years, and now taken together with a meta-analysis combining all published studies, showed very little -if any- evidence that vitamin D levels are associated with incidence and/or progression of OA (**Chapter 3.2**).

The most likely explanation for the lack of association is -of course- that there truly is no relation between OA and low vitamin D status. Yet, it could also be due to the heterogeneity of the included studies: most studies were small, and had many different end-points. A difference in the endpoints used in a study, for example OA of weight- versus non-weight-bearing joints, symptomatic versus radiographic OA, could weaken the measurement of a possible real association. Another reason for the lack of association could be that a single measurement of serum 25OHD levels does not give an accurate representation of someone's vitamin D status over a longer period of time. To eliminate the influence of possible confounders, an MR study was performed to studied genetic variations predisposing subjects for low vitamin D levels throughout their life, in relation to OA. Six single nucleotide polymorphisms (SNPs), which were found to be associated

at genome-wide significance level with circulating serum 25OHD levels in the SUNLIGHT Consortium, were selected as instrumental variables[49]. These genetic markers are thought to be a reflection of a lifetime vitamin D status and allow for the assessment of a possible causal relation with osteoarthritis. The MR study was well-powered and included 23,877 knee OA cases, 17,151 hip OA cases and over 562,000 controls, derived from the Iceland and UK Biobank[50], as described in **Chapter 4.1**. Importantly, the MR analyses showed no causal association between genetically determined vitamin D levels and knee or hip OA. This suggests that lifetime decreased vitamin D levels do not increase the risk of OA. These results are in line with a recent large genetic study that also found no causal role for vitamin D on fracture risk[51] and many other traits[52]. Conversely, it was found in that study that many diseases and risk factors have an (in)direct causal effect on vitamin D levels. This strongly suggests that observed variations in 25OHD level are much more likely the result of a disease rather than the cause of it. This suggestion, that vitamin D is a simple bystander instead of a contributing factor to several diseases, is made by an increasing number of investigators[53-55], and the results of this thesis support that notion as well.

Thus, in conclusion, after analysing the existing large amounts of data in the meta-analysis on 25OHD levels and OA, and analysing data on genetic variants associated with low 25OHD and OA, no consistent association was observed between low 25OHD and the occurrence of OA. These results also support the findings of several clinical intervention trials, showing no effect of supplementation with vitamin D on the progression of knee osteoarthritis[56; 57]. Yet, a meta-analysis of randomized controlled trials showed that vitamin D supplementation was effective in improving the pain and function in patients with knee OA[58], possibly by improving upper leg strength[59-62].Thus, any potential beneficial effect of vitamin D supplementation is possibly not by a direct protective effect on OA, but on decreasing the impact of OA, for example by improving muscle strength. Therefore, additional studies are needed to examine this potential causal effect of vitamin D on muscle strength, and how this may influence the possible relation of vitamin D with effects of OA rather than the origin of OA.

5.4 Overall conclusion and future research

Methodological considerations

In this thesis, the relationship between different aspects of bone quality was investigated, such as BMD, fracture risk and vitamin D status, and OA. High BMD was found to be associated with an increased risk of development of knee OA and hip OA. In contrast to what could be expected, increased fracture risk, which is associated with low BMD, did not protect against the development of OA. And *vice versa*, OA did not protect against incident fractures, but was associated with increased fracture risk. So, it seems that some other systemic factor related to bone quality influences the development of OA. The investigations in this thesis, including a Mendelian Randomization study, of a potential candidate factor reflecting bone quality, i.e., 25OHD as a reflection of overall vitamin D status, showed no consistent association with the development of OA. The most likely reason for not finding an association is that there is truly no association between vitamin D status and OA. However, it could also be that the subjects and the investigated endpoints are too heterogeneous, thus obscuring the effects that might be found in specific subgroups. Yet, the evidence collected so far, including studies in this thesis, points in the direction of there being no relationship between vitamin D and the origin of OA.

OA is now considered a heterogeneous disease in which several subtypes of OA can be defined[63; 64]. Patients with OA can be divided into subgroups, or phenotypes, on the basis of many different dimensions of the disease, such as phenotypes based on aetiological grounds, structural features or symptomatic presentation[65]. Such different phenotypes might be relevant to consider also when examining bone-related factors.

Weight bearing

A relevant subgroup differentiation to examine the etiological role of risk factors in OA is comparing OA in weight-bearing joints versus non-weight-bearing joints. Weight-bearing joints experience repetitive forces, and this could diminish the ability to restore micro injuries of these joints, especially in subjects with high BMD. The subchondral bone in these subjects, as the rest of their bone, is denser, and has less shock-absorbing

capacity, leading to cartilage damage. Since the association between BMD and the development of OA is observed in the weight-bearing joints (knees and hips), but not in the hands (**Chapter 2.3**), this implies that loading forces play a role in the association. This observation is supported by several studies which suggest that mechanical loading plays an import role in the development of OA of weight-bearing joints, whereas systemic factors are associated with OA of non-weight-bearing joints[66; 67].

Osteophytes versus JSN

Another important distinction that should be made when studying OA is between OA characterized by osteophytes versus OA with predominant joint space narrowing (JSN), described as hypertrophic, or bone-forming, OA versus atrophic OA[68; 69]. The widely used Kellgren-Lawrence scale combines these features of OA, but they appear to represent different disease types, both epidemiological, clinical, as well as biological[68-71]. Such different disease definitions are also likely to have different genetic architectures the description of which could support this notion. Indeed, for example, Castano-Betancourt et al. demonstrated that stratification of OA cases into more homogeneous endophenotypes showed different association between hip OA and BMD: subject with atrophic OA (degradation of cartilage in the absence of osteophyte formation) had systemically lower BMD as compared to those with normotrophic OA and the controls, while those with osteophytic OA had higher BMD. In addition, subjects with atrophic hip OA had an increased risk of osteoporotic fractures that is not fully explained by systemically lower BMD as compared to controls[71]. Panoutsopoulou et al. demonstrated that stratification of OA cases into subgroups can identify genes of potential functional importance otherwise obscured by disease heterogeneity[72]. And in this thesis, a much stronger associating between OA and fracture risk was observed in the subgroup of subjects with high BMD compared to the group with low BMD (**Chapter 2.1**).

Sex

Another relevant factor to examine when studying the role of bone in OA is the difference between sexes. Although in nearly all studies analyses are adjusted for sex, stratifying is the only way to get insight in differences between groups. The observed risk

of incidence and progression of knee OA in men with high BMD was more than twice the risk in women with high BMD (**Chapter 2.2**). The sex differences of prevalence, incidence and severity of osteoarthritis have been studied extensively in the past[73], and although estrogens play a role in that difference in the development of OA, their role is not clarified[74]. Alternatively, the differences between men and women influencing the development of OA can be explained by mechanical factors, like gait mechanics and joint loading[75], but also by a sex-associated systemic factor, such as leptin[76]. Further research focussing on sex-specific systemic factors, including analysis of differences in bone remodelling and turn over[74; 77] is needed to get insight in underlying factors explaining the differences in OA prevalence, incidence and severity of osteoarthritis between men and women.

Endophenotypes

Thus, stratification of OA cases into more homogeneous endophenotypes is needed to study factors possibly related to the development of OA (**Table 5.4.1**). A problem with stratifying between subgroups is the decreases in power and the number of tests performed. Therefore, subgroup analyses must by based on a previously defined hypothesis and done in a setting of sufficient power. Based on the observations in this thesis, sub-analysis stratifying for sex when studying the association between BMD and OA is advised, and stratifying between low and high BMD when studying the association between OA and fracture risk. To compensate for the loss of power by stratifying, multi-centred studies or meta-analyses are needed.

weightbearing	non - weightbearing
atrophic	hypertrophic
low BMD	high BMD
women	men
middle-aged	elderly
single joint OA	generalized OA

Table 5.4.1. Subgroups relevant for OA studies

Clinical implications

Apart from the aforementioned methodological recommendations, some clinical implications can by distilled out of the studies in this thesis. After investigating the role of BMD and vitamin D status as potential risk factors for different types of OA, no evidence was found that vitamin D status is of influence on the development of OA**.** It is therefore questionable whether further research should continue to examine a possible association, or even treatment directed at vitamin D. Since this thesis confirms that BMD is associated with the risk of OA, it seems more useful to further examine the way bone turnover influences the development of OA, and in specific relevant subgroups. Experimental and observational studies suggest that acting on bone can change progression of osteoarthritis[78]. This could implicate that drugs modifying bone turn-over might influence the development of OA[79]. Since bisphosphonates inhibit osteoclastic bone resorption, and reduce bone turnover, they could potentially decrease the progression of OA by inhibiting osteophyte formation. Studies on animals showed promising results[80; 81], but clinical, placebo-controlled studies on bisphosphonates have not shown a clear effect on the development of OA up to now[82; 83]. In their meta-analysis of randomized controlled trials on bisphosphonates therapy for osteoarthritis, Xing et al. found that bisphosphonates therapy improved pain, stiffness and function significantly in OA[84]. Bisphosphonates also reduced osteophyte score significantly, but no significant differences were found in subjective improvement, osteoarthritis progression, or joint replacement. All 15 in the meta-analysis included studies had radiographs available, but only 4 assessed for a specific OA subgroup (osteophyte formation), and no differentiation was made between men and women. Furthermore, the OA endpoints were heterogeneous (knee, hip, hand and spine OA), and they did not differentiate between weight-bearing and non- weightbearing joints. Thus, future meta-analyses should include analyses according to the aforementioned subgroups to avoid heterogeneity, and thereby weakening possible associations.

Another medicine modifying bone turn-over, strontium ranelate, has the ability to both increase action of osteoblasts as well as the resorption of bone by osteoclasts, improving trabecular and cortical intrinsic bone quality, a determinant of bone strength[85].

Its effect on subchondral bone makes it a promising disease-modifying osteoarthritis drug[86].

While bisphosphonates reduce bone-turnover by inhibiting osteoclastic bone resorption, teriparatide, the recombinant form of parathyroid hormone (PTH), is an anabolic drug, reducing turnover by promoting bone formation: it activates osteoblasts more than osteoclasts, which leads to an overall increase in bone. Therefore, teriparatide could also potentially decrease the progression of OA. Like in the studies on bisphosphonates, animal studies showed hopeful results[87-89], but in patients with OA, no positive results have been reported to this date[90].

The reason for the lack of a positive effect on OA of drugs that are modifying the bone turnover could be that these are only effective in a subgroup of OA, in particular the "bone-forming" subgroup[91]. Therefore, future studies on the effect of bisphosphonates should focus on these bone-formers. To identify this subgroup, radiographs can be used to discriminate between atrophic OA (no osteophytes) versus hypertrophic (distinct osteophytes) OA[71], but also MRI[92], biochemical markers (C-propeptide, cleavage by collagenase of type II)[69] or genetic markers involved in bone turnover (vitamin D receptor)[93].

One of the major opportunities for prediction of OA, and thus targeted interventions, lies in the genetic risk factors for OA. As for other common complex diseases, OA has a polygenetic architecture, involving many common genetic variants with small effect sizes. The most extensive GWAS study in OA was performed by the Genetics of OA (GO)–consortium[94]. In this study one hundred independent genetic loci associated with OA risk were recently identified, explaining approximately 6%–21% of the estimated heritability for different types of OA. These genetic variants can be used to quantify the individual's genetic OA risk in the form of polygenic risk scores (PRSs) and these could be used as a risk prediction tool in different settings, such as the clinical practice or in society[95; 96]. Depending on how much is known of the genetic architecture of a certain disease, the PRS can be a successful tool. For example, in breast cancer, the added value of the breast cancer PRS on prediction of breast cancer is evident and is now moving

into large scale clinical application for example in the population screening setting[97; 98]. For OA, PRS–based risk prediction can identify a significant fraction of the population at sufficiently increased risk for OA to be clinically relevant[99]. However, the added value for genetic prediction is dependent on the setting. For most patients with a joint complaint who come to the clinic, the OA process has progressed too far for efficient intervention. Therefore, it might be more effective to explore the added value of the genetic prediction for OA in a prevention setting in the population before the onset of disease. For future studies, PRSs might also be useful for discriminating different subgroups of OA, especially if pathway-specific PRSs can be built. While a given PRS contains the SNPs coming from all of the loci that were found to be associated with the particular endpoint, pathway-specific PRS-es are a subset of those and contain only SNPs located in areas containing genes related to a certain biological pathway. Examples of possible pathways that could be linked to specific subgroups in OA are: bone formation, cartilage degradation and inflammation pathway. Thus, certain subjects might carry more genetic risk variants (in their PRS) for one particular pathway compared to other pathways. In this way, future therapy could be tailor-made by utilizing genetic information from PRSs, and focusing on pathways that present the greatest genetic risk in one individual compared to the next.

References

1. Hunkin, S.J. (1909). Osteo-Arthritis. Cal State J Med 7, 204-206.

2. van Saase, J.L., van Romunde, L.K., Cats, A., Vandenbroucke, J.P., and Valkenburg, H.A. (1989). Epidemiology of osteoarthritis: Zoetermeer survey. Comparison of radiological osteoarthritis in a Dutch population with that in 10 other populations. Annals of the rheumatic diseases 48, 271-280.

3. Arden, N., and Nevitt, M.C. (2006). Osteoarthritis: epidemiology. Best practice & research Clinical rheumatology 20, 3-25.

4. Stamm, T.T. (1939). Aetiology and treatment of osteo-arthritis. Lancet 2, 754-756.

5. Lee, P., Rooney, P.J., Sturrock, R.D., Kennedy, A.C., and Dick, W.C. (1974). The etiology and pathogenesis of osteoarthrosis: a review. Semin Arthritis Rheum 3, 189-218.

6. Shane Anderson, A., and Loeser, R.F. (2010). Why is osteoarthritis an age-related disease? Best practice & research Clinical rheumatology 24, 15-26.

7. Silverwood, V., Blagojevic-Bucknall, M., Jinks, C., Jordan, J.L., Protheroe, J., and Jordan, K.P. (2015). Current evidence on risk factors for knee osteoarthritis in older adults: a systematic review and meta-analysis. Osteoarthritis and cartilage 23, 507-515.

8. O'Neill, T.W., McCabe, P.S., and McBeth, J. (2018). Update on the epidemiology, risk factors and disease outcomes of osteoarthritis. Best practice & research Clinical rheumatology 32, 312-326.

9. Aspden, R.M., and Saunders, F.R. (2019). Osteoarthritis as an organ disease: from the cradle to the grave. European cells & materials 37, 74-87.

10. Foss, M.V., and Byers, P.D. (1972). Bone density, osteoarthrosis of the hip, and fracture of the upper end of the femur. Annals of the rheumatic diseases 31, 259-264.

11. Carlsson, A., Nilsson, B.E., and Westlin, N.E. (1979). Bone mass in primary coxarthrosis. Acta Orthop Scand 50, 187-189.

12. Hannan, M.T., Anderson, J.J., Zhang, Y., Levy, D., and Felson, D.T. (1993). Bone mineral density and knee osteoarthritis in elderly men and women. The Framingham Study. Arthritis and rheumatism 36, 1671-1680.

13. Hart, D.J., Mootoosamy, I., Doyle, D.V., and Spector, T.D. (1994). The relationship between osteoarthritis and osteoporosis in the general population: the Chingford Study. Annals of the rheumatic diseases 53, 158-162.

14. Nevitt, M.C., Lane, N.E., Scott, J.C., Hochberg, M.C., Pressman, A.R., Genant, H.K., and Cummings, S.R. (1995). Radiographic osteoarthritis of the hip and bone mineral density. The Study of Osteoporotic Fractures Research Group. Arthritis and rheumatism 38, 907-916.

15. Lethbridge-Cejku, M., Tobin, J.D., Scott, W.W., Jr., Reichle, R., Roy, T.A., Plato, C.C., and Hochberg, M.C. (1996). Axial and hip bone mineral density and radiographic changes of osteoarthritis of the knee: data from the Baltimore Longitudinal Study of Aging. The Journal of rheumatology 23, 1943-1947.

16. Burger, H., van Daele, P.L., Odding, E., Valkenburg, H.A., Hofman, A., Grobbee, D.E., Schutte, H.E., Birkenhager, J.C., and Pols, H.A. (1996). Association of radiographically evident osteoarthritis with higher bone mineral density and increased bone loss with age. The Rotterdam Study. Arthritis and rheumatism 39, 81-86.

17. Sowers, M., Lachance, L., Jamadar, D., Hochberg, M.C., Hollis, B., Crutchfield, M., and Jannausch, M.L. (1999). The associations of bone mineral density and bone turnover markers with osteoarthritis of the hand and knee in pre- and perimenopausal women. Arthritis and rheumatism 42, 483-489.

18. Zhang, Y., Hannan, M.T., Chaisson, C.E., McAlindon, T.E., Evans, S.R., Aliabadi, P., Levy, D., and Felson, D.T. (2000). Bone mineral density and risk of incident and progressive radiographic knee osteoarthritis in women: the Framingham Study. The Journal of rheumatology 27, 1032-1037.

19. Hart, D.J., Cronin, C., Daniels, M., Worthy, T., Doyle, D.V., and Spector, T.D. (2002). The relationship of bone density and fracture to incident and progressive radiographic osteoarthritis of the knee: the Chingford Study. Arthritis and rheumatism 46, 92-99.

20. Nevitt, M.C., Zhang, Y., Javaid, M.K., Neogi, T., Curtis, J.R., Niu, J., McCulloch, C.E., Segal, N.A., and Felson, D.T. (2010). High systemic bone mineral density increases the risk of incident knee OA and joint space narrowing, but not radiographic progression of existing knee OA: the MOST study. Annals of the rheumatic diseases 69, 163-168.

21. Hartley, A., Hardcastle, S.A., Frysz, M., Parkinson, J., Paternoster, L., McCloskey, E., Poole, K.E.S., Javaid, M.K., Aye, M., Moss, K., et al. (2021). Increased development of radiographic hip osteoarthritis in individuals with high bone mass: a prospective cohort study. Arthritis research & therapy 23, 4.

22. Ding, M., Danielsen, C.C., and Hvid, I. (2001). Bone density does not reflect mechanical properties in early-stage arthrosis. Acta Orthop Scand 72, 181-185.

23. Ding, M., Odgaard, A., and Hvid, I. (2003). Changes in the three-dimensional microstructure of human tibial cancellous bone in early osteoarthritis. The Journal of bone and joint surgery British volume 85, 906-912.

24. Spector, T.D., and Campion, G.D. (1989). Generalised osteoarthritis: a hormonally mediated disease. Annals of the rheumatic diseases 48, 523-527.

25. Gorai, I., Chaki, O., Taguchi, Y., Nakayama, M., Osada, H., Suzuki, N., Katagiri, N., Misu, Y., and Minaguchi, H. (1999). Early postmenopausal bone loss is prevented by estrogen and partially by 1alpha-OH-vitamin D3: therapeutic effects of estrogen and/or 1alpha-OH-vitamin D3. Calcified tissue international 65, 16-22.

26. Khosla, S., Melton, L.J., 3rd, Atkinson, E.J., O'Fallon, W.M., Klee, G.G., and Riggs, B.L. (1998). Relationship of serum sex steroid levels and bone turnover markers with bone mineral density in men and women: a key role for bioavailable estrogen. The Journal of clinical endocrinology and metabolism 83, 2266-2274.

27. de Klerk, B.M., Schiphof, D., Groeneveld, F.P., Koes, B.W., van Osch, G.J., van Meurs, J.B., and Bierma-Zeinstra, S.M. (2009). No clear association between female hormonal aspects and osteoarthritis of the hand, hip and knee: a systematic review. Rheumatology 48, 1160-1165.

28. Kohler, M.J. (2019). Does menopausal hormonal therapy have a role in treatment of knee osteoarthritis? Menopause 26, 576-577.

29. Watt, F.E. (2016). Hand osteoarthritis, menopause and menopausal hormone therapy. Maturitas 83, 13-18.

30. Roman-Blas, J.A., Castaneda, S., Largo, R., and Herrero-Beaumont, G. (2009). Osteoarthritis associated with estrogen deficiency. Arthritis research & therapy 11, 241.

31. Davies, N.M., Holmes, M.V., and Davey Smith, G. (2018). Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. Bmj 362, k601.

32. Eriksson, A.L., Perry, J.R.B., Coviello, A.D., Delgado, G.E., Ferrucci, L., Hoffman, A.R., Huhtaniemi, I.T., Ikram, M.A., Karlsson, M.K., Kleber, M.E., et al. (2018). Genetic Determinants of Circulating Estrogen Levels and Evidence of a Causal Effect of Estradiol on Bone Density in Men. The Journal of clinical endocrinology and metabolism 103, 991-1004.

33. Zhu, J., Niu, Z., Alfredsson, L., Klareskog, L., Padyukov, L., and Jiang, X. (2021). Age at menarche, age at natural menopause, and risk of rheumatoid arthritis - a Mendelian randomization study. Arthritis research & therapy 23, 108.

34. Johnell, O., Kanis, J.A., Oden, A., Johansson, H., De Laet, C., Delmas, P., Eisman, J.A., Fujiwara, S., Kroger, H., Mellstrom, D., et al. (2005). Predictive value of BMD for hip and other fractures. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 20, 1185-1194.

35. Arden, N.K., Nevitt, M.C., Lane, N.E., Gore, L.R., Hochberg, M.C., Scott, J.C., Pressman, A.R., and Cummings, S.R. (1999). Osteoarthritis and risk of falls, rates of bone loss, and osteoporotic fractures. Study of Osteoporotic Fractures Research Group. Arthritis and rheumatism 42, 1378-1385.

36. Prieto-Alhambra, D., Nogues, X., Javaid, M.K., Wyman, A., Arden, N.K., Azagra, R., Cooper, C., Adachi, J.D., Boonen, S., Chapurlat, R.D., et al. (2013). An increased rate of falling leads to a rise in fracture risk in postmenopausal women with self-reported osteoarthritis: a prospective multinational cohort study (GLOW). Annals of the rheumatic diseases 72, 911-917.

37. Lips, P. (2001). Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. Endocrine reviews 22, 477-501.

38. Christodoulou, S., Goula, T., Ververidis, A., and Drosos, G. (2013). Vitamin D and bone disease. BioMed research international 2013, 396541.

39. Lips, P., and van Schoor, N.M. (2011). The effect of vitamin D on bone and osteoporosis. Best practice & research Clinical endocrinology & metabolism 25, 585-591.

40. Lips, P., Bilezikian, J.P., and Bouillon, R. (2020). Vitamin D: Giveth to Those Who Needeth. JBMR plus 4, e10232.

41. Heidari, B., Heidari, P., and Hajian-Tilaki, K. (2011). Association between serum vitamin D deficiency and knee osteoarthritis. International orthopaedics 35, 1627-1631.

42. Chaganti, R.K., Parimi, N., Cawthon, P., Dam, T.L., Nevitt, M.C., and Lane, N.E. (2010). Association of 25-hydroxyvitamin D with prevalent osteoarthritis of the hip in elderly men: the osteoporotic fractures in men study. Arthritis and rheumatism 62, 511-514.

43. Kalichman, L., and Kobyliansky, E. (2012). Association between circulatory levels of vitamin D and radiographic hand osteoarthritis. Rheumatology international 32, 253-257.

44. McAlindon, T.E., Felson, D.T., Zhang, Y., Hannan, M.T., Aliabadi, P., Weissman, B., Rush, D., Wilson, P.W., and Jacques, P. (1996). Relation of dietary intake and serum levels of vitamin D to

progression of osteoarthritis of the knee among participants in the Framingham Study. Annals of internal medicine 125, 353-359.

45. Lane, N.E., Gore, L.R., Cummings, S.R., Hochberg, M.C., Scott, J.C., Williams, E.N., and Nevitt, M.C. (1999). Serum vitamin D levels and incident changes of radiographic hip osteoarthritis: a longitudinal study. Study of Osteoporotic Fractures Research Group. Arthritis and rheumatism 42, 854-860.

46. Zhang, F.F., Driban, J.B., Lo, G.H., Price, L.L., Booth, S., Eaton, C.B., Lu, B., Nevitt, M., Jackson, B., Garganta, C., et al. (2014). Vitamin D deficiency is associated with progression of knee osteoarthritis. J Nutr 144, 2002-2008.

47. Felson, D.T., Niu, J., Clancy, M., Aliabadi, P., Sack, B., Guermazi, A., Hunter, D.J., Amin, S., Rogers, G., and Booth, S.L. (2007). Low levels of vitamin D and worsening of knee osteoarthritis: results of two longitudinal studies. Arthritis and rheumatism 56, 129-136.

48. Konstari, S., Paananen, M., Heliovaara, M., Knekt, P., Marniemi, J., Impivaara, O., Arokoski, J., and Karppinen, J. (2012). Association of 25-hydroxyvitamin D with the incidence of knee and hip osteoarthritis: a 22-year follow-up study. Scandinavian journal of rheumatology 41, 124-131.

49. Jiang, X., O'Reilly, P.F., Aschard, H., Hsu, Y.H., Richards, J.B., Dupuis, J., Ingelsson, E., Karasik, D., Pilz, S., Berry, D., et al. (2018). Genome-wide association study in 79,366 European-ancestry individuals informs the genetic architecture of 25-hydroxyvitamin D levels. Nature communications 9, 260.

50. Styrkarsdottir, U., Lund, S.H., Thorleifsson, G., Zink, F., Stefansson, O.A., Sigurdsson, J.K., Juliusson, K., Bjarnadottir, K., Sigurbjornsdottir, S., Jonsson, S., et al. (2018). Meta-analysis of Icelandic and UK data sets identifies missense variants in SMO, IL11, COL11A1 and 13 more new loci associated with osteoarthritis. Nature genetics 50, 1681-1687.

51. Trajanoska, K., Morris, J.A., Oei, L., Zheng, H.F., Evans, D.M., Kiel, D.P., Ohlsson, C., Richards, J.B., Rivadeneira, F., consortium, G.G., et al. (2018). Assessment of the genetic and clinical determinants of fracture risk: genome wide association and mendelian randomisation study. Bmj 362, k3225.

52. Revez, J.A., Lin, T., Qiao, Z., Xue, A., Holtz, Y., Zhu, Z., Zeng, J., Wang, H., Sidorenko, J., Kemper, K.E., et al. (2020). Genome-wide association study identifies 143 loci associated with 25 hydroxyvitamin D concentration. Nature communications 11, 1647.

53. Mangge, H., Weghuber, D., Prassl, R., Haara, A., Schnedl, W., Postolache, T.T., and Fuchs, D. (2015). The Role of Vitamin D in Atherosclerosis Inflammation Revisited: More a Bystander than a Player? Current vascular pharmacology 13, 392-398.

54. Wang, C.C., Tzeng, I.S., Su, W.C., Li, C.H., Lin, H.H., Yang, C.C., and Kao, J.H. (2020). The association of vitamin D with hepatitis B virus replication: Bystander rather than offender. Journal of the Formosan Medical Association = Taiwan yi zhi 119, 1634-1641.

55. Gois, P.H.F., Ferreira, D., Olenski, S., and Seguro, A.C. (2017). Vitamin D and Infectious Diseases: Simple Bystander or Contributing Factor? Nutrients 9.

56. Arden, N.K., Cro, S., Sheard, S., Dore, C.J., Bara, A., Tebbs, S.A., Hunter, D.J., James, S., Cooper, C., O'Neill, T.W., et al. (2016). The effect of vitamin D supplementation on knee osteoarthritis, the VIDEO study: a randomised controlled trial. Osteoarthritis and cartilage 24, 1858-1866.

57. McAlindon, T., LaValley, M., Schneider, E., Nuite, M., Lee, J.Y., Price, L.L., Lo, G., and Dawson-Hughes, B. (2013). Effect of vitamin D supplementation on progression of knee pain and cartilage volume loss in patients with symptomatic osteoarthritis: a randomized controlled trial. Jama 309, 155-162.

58. Gao, X.R., Chen, Y.S., and Deng, W. (2017). The effect of vitamin D supplementation on knee osteoarthritis: A meta-analysis of randomized controlled trials. Int J Surg 46, 14-20.

59. Halfon, M., Phan, O., and Teta, D. (2015). Vitamin D: a review on its effects on muscle strength, the risk of fall, and frailty. BioMed research international 2015, 953241.

60. Beaudart, C., Buckinx, F., Rabenda, V., Gillain, S., Cavalier, E., Slomian, J., Petermans, J., Reginster, J.Y., and Bruyere, O. (2014). The effects of vitamin D on skeletal muscle strength, muscle mass, and muscle power: a systematic review and meta-analysis of randomized controlled trials. The Journal of clinical endocrinology and metabolism 99, 4336-4345.

61. Diamond, T., Wong, Y.K., and Golombick, T. (2013). Effect of oral cholecalciferol 2,000 versus 5,000 IU on serum vitamin D, PTH, bone and muscle strength in patients with vitamin D deficiency. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 24, 1101-1105.

62. Tomlinson, P.B., Joseph, C., and Angioi, M. (2015). Effects of vitamin D supplementation on upper and lower body muscle strength levels in healthy individuals. A systematic review with meta-analysis. Journal of science and medicine in sport 18, 575-580.

63. Castaneda, S., Roman-Blas, J.A., Largo, R., and Herrero-Beaumont, G. (2014). Osteoarthritis: a progressive disease with changing phenotypes. Rheumatology 53, 1-3.

64. Deveza, L.A., Melo, L., Yamato, T.P., Mills, K., Ravi, V., and Hunter, D.J. (2017). Knee osteoarthritis phenotypes and their relevance for outcomes: a systematic review. Osteoarthritis and cartilage 25, 1926-1941.

65. Bierma-Zeinstra, S.M., and van Middelkoop, M. (2017). Osteoarthritis: In search of phenotypes. Nature reviews Rheumatology 13, 705-706.

66. Funck-Brentano, T., Nethander, M., Moverare-Skrtic, S., Richette, P., and Ohlsson, C. (2019). Causal Factors for Knee, Hip, and Hand Osteoarthritis: A Mendelian Randomization Study in the UK Biobank. Arthritis & rheumatology 71, 1634-1641.

67. Visser, A.W., de Mutsert, R., le Cessie, S., den Heijer, M., Rosendaal, F.R., Kloppenburg, M., and Group, N.E.O.S. (2015). The relative contribution of mechanical stress and systemic processes in different types of osteoarthritis: the NEO study. Annals of the rheumatic diseases 74, 1842-1847.

68. Conrozier, T., Merle-Vincent, F., Mathieu, P., Richard, M., Favret, H., Piperno, M., Caton, J., and Vignon, E. (2004). Epidemiological, clinical, biological and radiological differences between atrophic and hypertrophic patterns of hip osteoarthritis: a case-control study. Clinical and experimental rheumatology 22, 403-408.

69. Conrozier, T., Ferrand, F., Poole, A.R., Verret, C., Mathieu, P., Ionescu, M., Vincent, F., Piperno, M., Spiegel, A., and Vignon, E. (2007). Differences in biomarkers of type II collagen in atrophic and hypertrophic osteoarthritis of the hip: implications for the differing pathobiologies. Osteoarthritis and cartilage 15, 462-467.

70. Schnitzler, C.M., Mesquita, J.M., and Wane, L. (1992). Bone histomorphometry of the iliac crest, and spinal fracture prevalence in atrophic and hypertrophic osteoarthritis of the hip. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 2, 186-194.

71. Castano-Betancourt, M.C., Rivadeneira, F., Bierma-Zeinstra, S., Kerkhof, H.J., Hofman, A., Uitterlinden, A.G., and van Meurs, J.B. (2013). Bone parameters across different types of hip osteoarthritis and their relationship to osteoporotic fracture risk. Arthritis and rheumatism 65, 693-700.

72. Panoutsopoulou, K., Thiagarajah, S., Zengini, E., Day-Williams, A.G., Ramos, Y.F., Meessen, J.M., Huetink, K., Nelissen, R.G., Southam, L., Rayner, N.W., et al. (2017). Radiographic endophenotyping in hip osteoarthritis improves the precision of genetic association analysis. Annals of the rheumatic diseases 76, 1199-1206.

73. Srikanth, V.K., Fryer, J.L., Zhai, G., Winzenberg, T.M., Hosmer, D., and Jones, G. (2005). A meta-analysis of sex differences prevalence, incidence and severity of osteoarthritis. Osteoarthritis and cartilage 13, 769-781.

74. Christgau, S., and Cloos, P.A. (2005). Sex hormones in the regulation of bone and cartilage metabolism: an old paradigm and a new challenge. Minerva Ginecol 57, 611-617.

75. Sims, E.L., Carland, J.M., Keefe, F.J., Kraus, V.B., Guilak, F., and Schmitt, D. (2009). Sex differences in biomechanics associated with knee osteoarthritis. J Women Aging 21, 159-170.

76. Teichtahl, A.J., Wluka, A.E., Proietto, J., and Cicuttini, F.M. (2005). Obesity and the female sex, risk factors for knee osteoarthritis that may be attributable to systemic or local leptin biosynthesis and its cellular effects. Med Hypotheses 65, 312-315.

77. Grumbach, M.M. (2000). Estrogen, bone, growth and sex: a sea change in conventional wisdom. J Pediatr Endocrinol Metab 13, 1439-1455.

78. Roux, C., and Richette, P. (2012). Impact of treatments for osteoporosis on osteoarthritis progression. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 23 Suppl 8, S881-883.

79. Lane, N.E. (2018). Osteoarthritis: Bisphosphonates and OA - is there a bone and joint connection? Nature reviews Rheumatology 14, 185-186.

80. Hayami, T., Pickarski, M., Wesolowski, G.A., McLane, J., Bone, A., Destefano, J., Rodan, G.A., and Duong le, T. (2004). The role of subchondral bone remodeling in osteoarthritis: reduction of cartilage degeneration and prevention of osteophyte formation by alendronate in the rat anterior cruciate ligament transection model. Arthritis and rheumatism 50, 1193-1206.

81. Strassle, B.W., Mark, L., Leventhal, L., Piesla, M.J., Jian Li, X., Kennedy, J.D., Glasson, S.S., and Whiteside, G.T. (2010). Inhibition of osteoclasts prevents cartilage loss and pain in a rat model of degenerative joint disease. Osteoarthritis and cartilage 18, 1319-1328.

82. Spector, T.D., Conaghan, P.G., Buckland-Wright, J.C., Garnero, P., Cline, G.A., Beary, J.F., Valent, D.J., and Meyer, J.M. (2005). Effect of risedronate on joint structure and symptoms of knee

osteoarthritis: results of the BRISK randomized, controlled trial [ISRCTN01928173]. Arthritis research & therapy 7, R625-633.

83. Bingham, C.O., 3rd, Buckland-Wright, J.C., Garnero, P., Cohen, S.B., Dougados, M., Adami, S., Clauw, D.J., Spector, T.D., Pelletier, J.P., Raynauld, J.P., et al. (2006). Risedronate decreases biochemical markers of cartilage degradation but does not decrease symptoms or slow radiographic progression in patients with medial compartment osteoarthritis of the knee: results of the two-year multinational knee osteoarthritis structural arthritis study. Arthritis and rheumatism 54, 3494-3507.

84. Xing, R.L., Zhao, L.R., and Wang, P.M. (2016). Bisphosphonates therapy for osteoarthritis: a meta-analysis of randomized controlled trials. Springerplus 5, 1704.

85. Ammann, P., Badoud, I., Barraud, S., Dayer, R., and Rizzoli, R. (2007). Strontium ranelate treatment improves trabecular and cortical intrinsic bone tissue quality, a determinant of bone strength. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 22, 1419-1425.

86. Rodrigues, T.A., Freire, A.O., Bonfim, B.F., Cartagenes, M.S.S., and Garcia, J.B.S. (2018). Strontium ranelate as a possible disease-modifying osteoarthritis drug: a systematic review. Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas 51, e7440.

87. Chang, J.K., Chang, L.H., Hung, S.H., Wu, S.C., Lee, H.Y., Lin, Y.S., Chen, C.H., Fu, Y.C., Wang, G.J., and Ho, M.L. (2009). Parathyroid hormone 1-34 inhibits terminal differentiation of human articular chondrocytes and osteoarthritis progression in rats. Arthritis and rheumatism 60, 3049-3060.

88. Morita, Y., Ito, H., Ishikawa, M., Fujii, T., Furu, M., Azukizawa, M., Okahata, A., Tomizawa, T., Kuriyama, S., Nakamura, S., et al. (2018). Subchondral bone fragility with meniscal tear accelerates and parathyroid hormone decelerates articular cartilage degeneration in rat osteoarthritis model. Journal of orthopaedic research : official publication of the Orthopaedic Research Society 36, 1959-1968.

89. Yan, J.Y., Tian, F.M., Wang, W.Y., Cheng, Y., Song, H.P., Zhang, Y.Z., and Zhang, L. (2014). Parathyroid hormone (1-34) prevents cartilage degradation and preserves subchondral bone micro-architecture in guinea pigs with spontaneous osteoarthritis. Osteoarthritis and cartilage 22, 1869-1877.

90. Lee, S. (2016). Endogenous parathyroid hormone and knee osteoarthritis: a cross-sectional study. International journal of rheumatic diseases 19, 248-254.

91. Hardcastle, S.A., Dieppe, P., Gregson, C.L., Davey Smith, G., and Tobias, J.H. (2015). Osteoarthritis and bone mineral density: are strong bones bad for joints? BoneKEy reports 4, 624.

92. Roemer, F.W., Guermazi, A., Niu, J., Zhang, Y., Mohr, A., and Felson, D.T. (2012). Prevalence of magnetic resonance imaging-defined atrophic and hypertrophic phenotypes of knee osteoarthritis in a population-based cohort. Arthritis and rheumatism 64, 429-437.

93. Sheehan, D., Bennett, T., and Cashman, K.D. (2001). An assessment of genetic markers as predictors of bone turnover in healthy adults. Journal of endocrinological investigation 24, 236-245.

94. Boer, C.G., Hatzikotoulas, K., Southam, L., Stefansdottir, L., Zhang, Y., Coutinho de Almeida, R., Wu, T.T., Zheng, J., Hartley, A., Teder-Laving, M., et al. (2021). Deciphering osteoarthritis genetics across 826,690 individuals from 9 populations. Cell 184, 4784-4818 e4717.

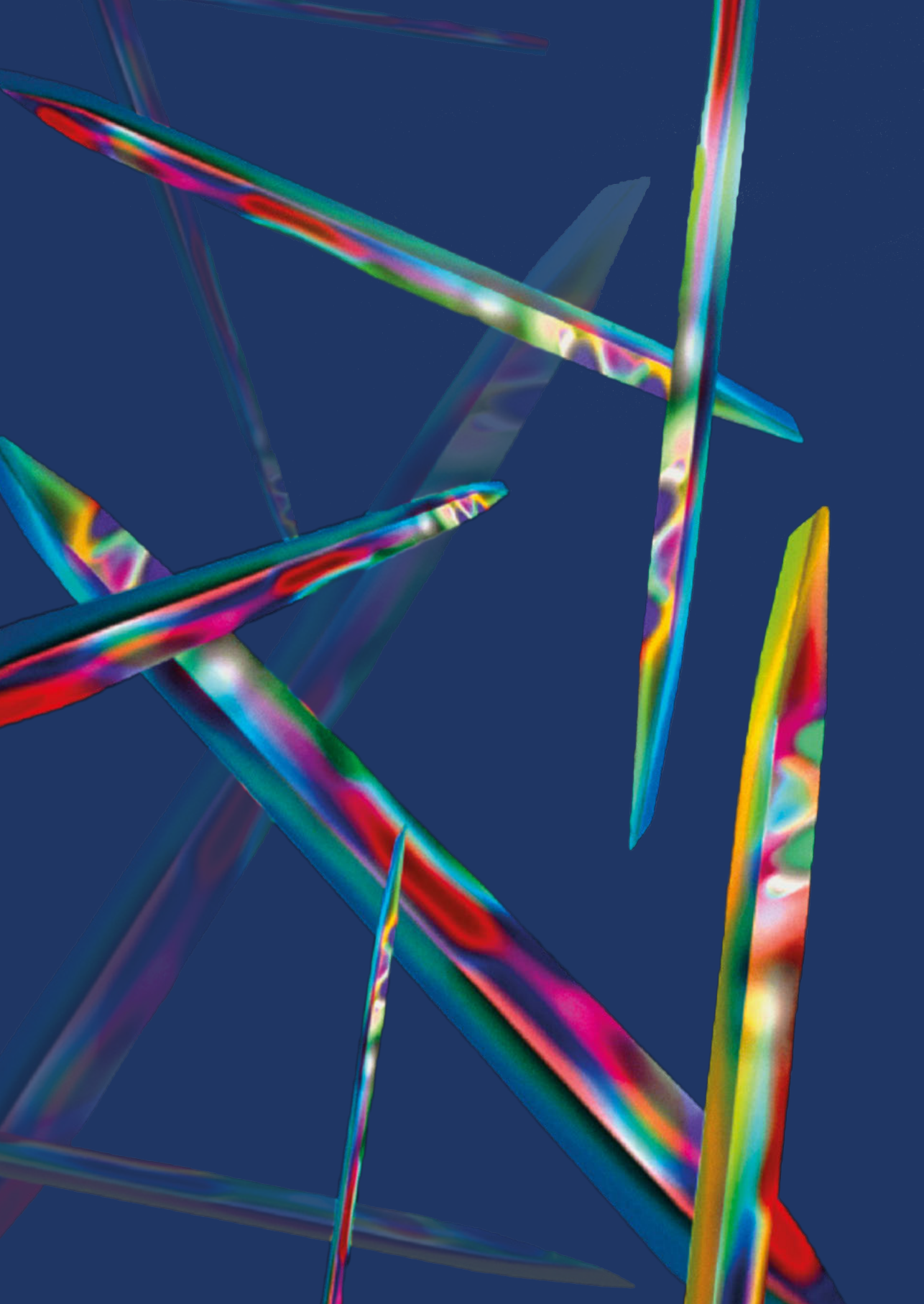
95. Torkamani, A., Wineinger, N.E., and Topol, E.J. (2018). The personal and clinical utility of polygenic risk scores. Nature reviews Genetics 19, 581-590.

96. Visscher, P.M., Wray, N.R., Zhang, Q., Sklar, P., McCarthy, M.I., Brown, M.A., and Yang, J. (2017). 10 Years of GWAS Discovery: Biology, Function, and Translation. American journal of human genetics 101, 5-22.

97. Lakeman, I.M.M., Rodriguez-Girondo, M., Lee, A., Ruiter, R., Stricker, B.H., Wijnant, S.R.A., Kavousi, M., Antoniou, A.C., Schmidt, M.K., Uitterlinden, A.G., et al. (2020). Validation of the BOADICEA model and a 313-variant polygenic risk score for breast cancer risk prediction in a Dutch prospective cohort. Genetics in medicine : official journal of the American College of Medical Genetics 22, 1803-1811.

98. Mavaddat, N., Michailidou, K., Dennis, J., Lush, M., Fachal, L., Lee, A., Tyrer, J.P., Chen, T.H., Wang, Q., Bolla, M.K., et al. (2019). Polygenic Risk Scores for Prediction of Breast Cancer and Breast Cancer Subtypes. American journal of human genetics 104, 21-34.

99. Khera, A.V., Chaffin, M., Aragam, K.G., Haas, M.E., Roselli, C., Choi, S.H., Natarajan, P., Lander, E.S., Lubitz, S.A., Ellinor, P.T., et al. (2018). Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. Nature genetics 50, 1219-1224.



6

SUMMARY

6.1 Summary

Osteoarthritis (OA) is a disabling joint disease that results from degeneration of joint cartilage and underlying bone. It is the most common form of arthritis, and the leading cause of disability in the elderly. Despite numerous studies, the etiology of OA is still unclear, leaving much questions how to treat and prevent OA unanswered. In this thesis, the possible role of several bone quality related factors on the development and progression of different types of OA is investigated. The studies were mainly performed within the Rotterdam Study, a large prospective population-based cohort study in the Netherlands.

First, the association between OA and fracture risk was examined. In **Chapter 2.1** the results of a longitudinal study among 2,773 men and women are shown. Despite a higher bone mineral density (BMD), subjects with prevalent radiographic OA (ROA) had a 2 times increased risk for an incident vertebral fracture compared to subjects without ROA, and a 50% increased risk for a non-vertebral fracture. Stratification in subgroups showed that this risk was even higher in subjects with high BMD compared to those with low BMD. Adjustment for potential confounding factors, including parameters of postural stability, did not change the results.

Chapter 2.2 describes the results of a study among 1,403 subjects on the association between BMD, fractures and ROA-risk. High systemic BMD at baseline was associated with increased incidence of knee ROA in men and women: subjects in the highest femoral neck BMD (FN-BMD) quartile had a nearly 3 times increased risk of incident knee ROA, and the risk of those in the highest lumbar spine BMD (LS-BMD) almost 5 times, compared to subjects in the lowest quartiles. We also examined whether having a fracture at baseline changed the risk for OA. We observed that the presence of a vertebral fracture had a protective effect on the incidence and progression of knee ROA.

Over time, more data became available: the study population expanded from 1,403 to 4,154 participants, while also a longer follow-up time was available. In addition, data on the development of hip and hand ROA, besides knee ROA, were collected. **Chapter 2.3**

shows the results of this expanded study. It confirmed that high baseline BMD is a risk factor for the development of knee OA, and also showed it to be a risk factor for the development of hip OA; subjects in the highest quartile of FN-BMD had a 60% increased risk of incident knee and of incident hip ROA as well compared to the lowest quartile. No relation between high BMD and progression of knee or hip ROA, or incident hand ROA, was found. And, in contrast to the findings of **Chapter 2.2**, a protective effect of a prevalent fracture on development knee ROA was not observed anymore. On the contrary, a vertebral fracture was found to be a risk factor for incident hand ROA: a subject with a vertebral fracture at baseline had a more than 70% increased risk of developing hand OA. Thus, the association between baseline BMD as a marker for bone quality and the development of hip and knee OA seem robust, but the association between fractures and OA is less clear.

Another factor influencing bone quality is vitamin D status. In **Chapter 3.1** the results of our study on the association between vitamin D status, BMD and the development of knee ROA are presented. The mean vitamin D intake was 64 IU/day and the mean 25(OH) vitamin D level 66 nmol/l, the mean follow-up time was 6.5 years. Progressive knee ROA occurred in 5.1% of the subjects in the highest tertile of vitamin D intake against 12.6% in the lowest tertile, resulting in a crude odds ratio (OR) of 2.7 (95% confidence interval (CI) 0.8 to 8.8), and an adjusted OR of 7.7 (95% CI 1.3 to 43.5). No association between vitamin D intake and incident knee ROA was seen. However, we found a significant interaction between vitamin D intake and BMD in the association with incident knee ROA. In subjects with low LS-BMD at baseline we observed an increasing incidence of knee ROA with decreasing vitamin D intake and serum levels. Vitamin D levels were not associated with incident or progressive knee OA. Thus, in this study we could confirm the results of earlier studies that found an association of progressive knee OA with vitamin D intake, but not with 25(OH)vitamin D levels. In addition, the observed interaction between vitamin D intake and BMD in the association with incident knee ROA could indicate that subgroup analyses (in this case subjects with low baseline BMD) might lead to altered insights.

The association between vitamin D intake and OA is more prone to confounding by lifestyle influences than between vitamin D levels and OA. In addition, sample size and rep-

lication of association across different cohorts can result in more firm and robust conclusions. Therefore, we conducted a meta-analysis on the relation between vitamin D levels and OA of the knee, hip and hand, with up-dated and expanded results of our previous study (**Chapter 3.2).** In this meta-analysis, 6 cross-sectional and 6 longitudinal studies were included. No clear association between vitamin serum levels and prevalent, incident or progressive knee, hip or hand OA was observed. However, the quality of most studies was low, and the results conflicting. Meta-analysis of three cross-sectional studies on vitamin D levels and knee joint space narrowing (JSN) showed an increased risk of prevalent JSN with decreasing vitamin D levels (OR 1.52, 95% CI 1.15 to 2.01). The association observed in the meta-analysis of three studies on low vitamin D levels and incident and progressive knee OA was not significant (OR 1.37, 95% CI 0.97 to 1.92), however when considering solely progressive knee OA, the risk was significantly increased (OR 2.40, 95% CI 1.22 to 4.72). In conclusion, this meta-analysis did not provide evidence of an independent association between 25(OH) vitamin D serum levels with hip or hand OA. When analyzing subgroups of knee OA, significant associations of low vitamin D levels with prevalent knee JSN and with progressive knee OA were observed.

Since the role of vitamin D in the development of OA remained unclear, a two sample Mendelian randomization (MR) study was conducted to investigate the causal relationship between genetically determined serum vitamin D levels and hip and knee OA (**Chapter 4.1).** MR is a study design in which genetic variants are used as proxy for modifiable exposure to test the unconfounded effect of the exposure on a specific outcome. Six single nucleotide polymorphisms (SNPs) associated with vitamin D levels were selected as instrumental variables. Summary statistics of the SNPs effects on OA were derived from the Iceland and UK Biobank, comprising 23,877 knee OA cases, 17,151 hip OA cases and over 562,000 controls. The control samples match the osteoarthritis cases in age, sex, and county of origin. The MR analyses showed no causal association between genetically determined vitamin D levels and knee OA (OR 1.03, 95% CI 0.84 to 1.26) or hip OA (OR 1.06, 95% CI 0.83 to 1.35). So, genetic variations associated with low vitamin D serum levels are not associated with increased risk of OA of hip or knee. This suggests that lifetime decreased vitamin D levels do not increase the risk of knee or hip OA.

In **Chapter 5**, the results of our investigations are discussed. We observed that OA is a risk factor for future fracture, despite high BMD, suggesting altered bone quality in the presence of OA. Another finding was that high systemic BMD increases the risk of developing knee and hip OA, also implying that higher BMD does not always reflect better bone quality. When studying the possible association of vitamin D status and the development of OA in various ways (prospective epidemiological study, meta-analysis and MR), no consistent association was found. This implicates that supplementing vitamin D does not prevent the onset or worsening of OA. While studying the possible association between different bone-related factors and OA, the importance of considering analysis of subgroups became clear: stratification of OA cases into more homogeneous phenotypes is needed to identify specific factors possibly related to the development of OA. Furthermore, genetic studies like MR are useful to to test the unconfounded effect of a possible factor on the development op OA, or to exclude its effect on OA. When underlying factors associated with OA can be identified, subgroups-specific pharmacotherapeutic treatments could be developed to prevent development or worsening of OA at an early stage.

6.2 Samenvatting

Artrose is een invaliderende gewrichtsziekte die het gevolg is van degeneratie van gewrichtskraakbeen en onderliggend bot. Het is de meest voorkomende vorm van artritis en de belangrijkste oorzaak van invaliditeit bij ouderen. Ondanks talrijke onderzoeken is de etiologie van artrose nog steeds onduidelijk, waardoor ook veel vragen over de behandeling en preventie van artrose onbeantwoord blijven. In dit proefschrift wordt de mogelijke rol van diverse factoren die samenhangen met botkwaliteit op de incidentie (het ontstaan) en progressie (verergering) van verschillende typen artrose onderzocht. De onderzoeken zijn voornamelijk uitgevoerd binnen de Rotterdam Studie, een grote prospectieve populatie-gebaseerde cohortstudie in Nederland.

Eerst werd het verband tussen artrose en risico op fracturen onderzocht. In **Hoofdstuk 2.1** worden de resultaten getoond van een longitudinaal onderzoek bij 2773 mannen en vrouwen. Ondanks een hogere botmineraaldichtheid (BMD) hadden deelnemers met prevalente (reeds aanwezige) radiologische artrose (artrose gediagnosticeerd met röntgenfoto's) een 2 keer verhoogd risico op het krijgen van een wervelfractuur in vergelijking met deelnemers zonder artrose, en een 50% verhoogd risico van een niet-wervelfractuur. Stratificatie (onderverdeling) in subgroepen toonde aan dat dit risico zelfs hoger was bij deelnemers met een hoge BMD dan bij degenen met een lage BMD. Correctie voor mogelijke verstorende factoren, waaronder parameters gerelateerd aan houdingsstabiliteit, veranderde de resultaten niet.

Hoofdstuk 2.2 beschrijft de resultaten van een onderzoek onder 1403 proefpersonen naar de associatie tussen BMD, fracturen en radiologische artrose-risico. Een hoge BMD bij aanvang was geassocieerd met een verhoogde incidentie van knie-artrose bij mannen en vrouwen: deelnemers in het hoogste femurhals-BMD (FH-BMD) kwartiel hadden een bijna driemaal verhoogd risico op incidente knie-artrose, en het risico van degenen in de hoogste lumbale wervelkolom BMD (LW-BMD) was bijna 5 keer verhoogd, vergeleken met deelnemers in de laagste kwartielen. We onderzochten ook of het hebben van een prevalente fractuur het risico op artrose veranderde. We zagen dat de aanwezigheid

van een wervelfractuur een beschermend effect had op de incidentie en progressie van knie-artrose.

In de loop van de tijd kwam er meer data beschikbaar: de onderzoekspopulatie breidde zich uit van 1403 naar 4154 deelnemers, terwijl ook een langere follow-up tijd beschikbaar was. Daarnaast werden gegevens over het ontstaan van heup- en hand-artrose, naast knie-artrose, verzameld. **Hoofdstuk 2.3** laat de resultaten zien van deze uitgebreide studie. Deze bevestigde dat een hoge BMD een risicofactor is voor de ontwikkeling van artrose van de knie, en toonde ook aan dat het een risicofactor is voor de ontwikkeling van heup-artrose; deelnemers in het hoogste kwartiel van FH-BMD hadden zowel een 60% verhoogd risico op incidente knie-artrose als op incidente heup-artrose in vergelijking met het laagste kwartiel. Er werd geen verband gevonden tussen hoge BMD en verergering van knie- of heup-artrose, of het ontstaan van hand- artrose. En, in tegenstelling tot de bevindingen van **Hoofdstuk 2.2**, werd een beschermend effect van een prevalente fractuur op de ontwikkeling van de knie-artrose niet meer waargenomen. Integendeel, wervelfracturen bleken een risicofactor te zijn voor incidente hand-artrose: een deelnemer met een prevalente wervelfractuur had een meer dan 70% verhoogd risico op het ontwikkelen van hand-artrose. De associatie tussen BMD als een marker voor botkwaliteit en de ontwikkeling van heup- en knie-artrose lijkt robuust, maar de associatie tussen fracturen en artrose is minder duidelijk.

Een andere factor die de botkwaliteit beïnvloedt, is vitamine D-status. In **Hoofdstuk 3.1** worden de resultaten gepresenteerd van onze studie naar de associatie tussen vitamine D-status, BMD en de ontwikkeling van knie-artrose in een cohort van 1248 mannen en vrouwen. De gemiddelde vitamine D-inname was 64 IE/dag en de gemiddelde 25(OH) vitamine D-spiegel in het bloed 66 nmol/l, de gemiddelde follow-upduur was 6.5 jaar. Progressieve knie-artrose trad op bij 5.1% van de deelnemers in het hoogste tertiel van vitamine D-inname tegen 12.6% in het laagste tertiel, wat resulteerde in een ongecorrigeerde odds ratio (OR) van 2.7 (95% betrouwbaarheidsinterval (BI) 0.8 tot 8.8), en een gecorrigeerde OR van 7.7 (95% BI 1.3 tot 43.5). Er werd geen verband gezien tussen vitamine D-inname en incidente knie-artrose. We vonden echter een significante interactie

tussen vitamine D-inname en BMD in de associatie met incidente knie-artrose. Bij deelnemers met een lage LW-BMD zagen we een toenemende incidentie van knie-artrose met afnemende vitamine D-inname en -spiegels. Vitamine D-spiegels waren niet geassocieerd met incidente of progressieve knie-artrose. Met deze studie konden we dus de resultaten bevestigen van eerdere studies die een associatie vonden van progressieve knie-artrose met vitamine D-inname, maar niet met vitamine D-spiegels. De waargenomen interactie tussen vitamine D en BMD in de associatie met incidente knie-artrose zou er daarnaast op kunnen wijzen dat subgroep-analyses (in dit geval voor deelnemers met een lage baseline BMD) tot andere inzichten zouden kunnen leiden.

De associatie tussen vitamine D-inname en artrose is vatbaarder voor 'confounding'* door levensstijl-invloeden dan de associatie tussen vitamine D-spiegels en artrose. Daarnaast kunnen steekproefomvang en replicatie van associaties tussen verschillende cohorten resulteren in meer stevige en robuuste conclusies. Daarom hebben we een meta-analyse uitgevoerd om de relatie tussen vitamine D-spiegels en artrose van de knie, heup en hand te onderzoeken, met bijgewerkte en uitgebreide resultaten van onze vorige studie (**Hoofdstuk 3.2**). In deze meta-analyse zijn 6 cross-sectionele en 6 longitudinale studies opgenomen. Er werd geen duidelijk verband waargenomen tussen vitamine-spiegels en prevalente, incidente of progressieve knie-, heup- of hand-artrose. De kwaliteit van de meeste onderzoeken was echter laag en de resultaten waren tegenstrijdig. Meta-analyse van drie cross-sectionele onderzoeken naar vitamine D-spiegels en gewrichtsspleetversmalling (GSV) van de knie toonde een verhoogd risico op prevalente GSV met afnemende vitamine D-spiegels (OR 1.52, 95% BI 1.15 tot 2.01). De associatie die werd waargenomen in de meta-analyse van drie onderzoeken naar lage vitamine D-spiegels en incidente en progressieve knie-artrose was niet significant (OR 1.37, 95% BI 0.97 tot 1.92), maar wanneer alleen naar progressieve knie-artrose werd gekeken, was het risico significant verhoogd (OR 2.40, 95% BI 1.22 tot 4.72). Al met al leverde deze meta-analyse geen bewijs voor een onafhankelijke associatie tussen 25(OH) vitamine D-spiegels met heup- of hand-artrose.

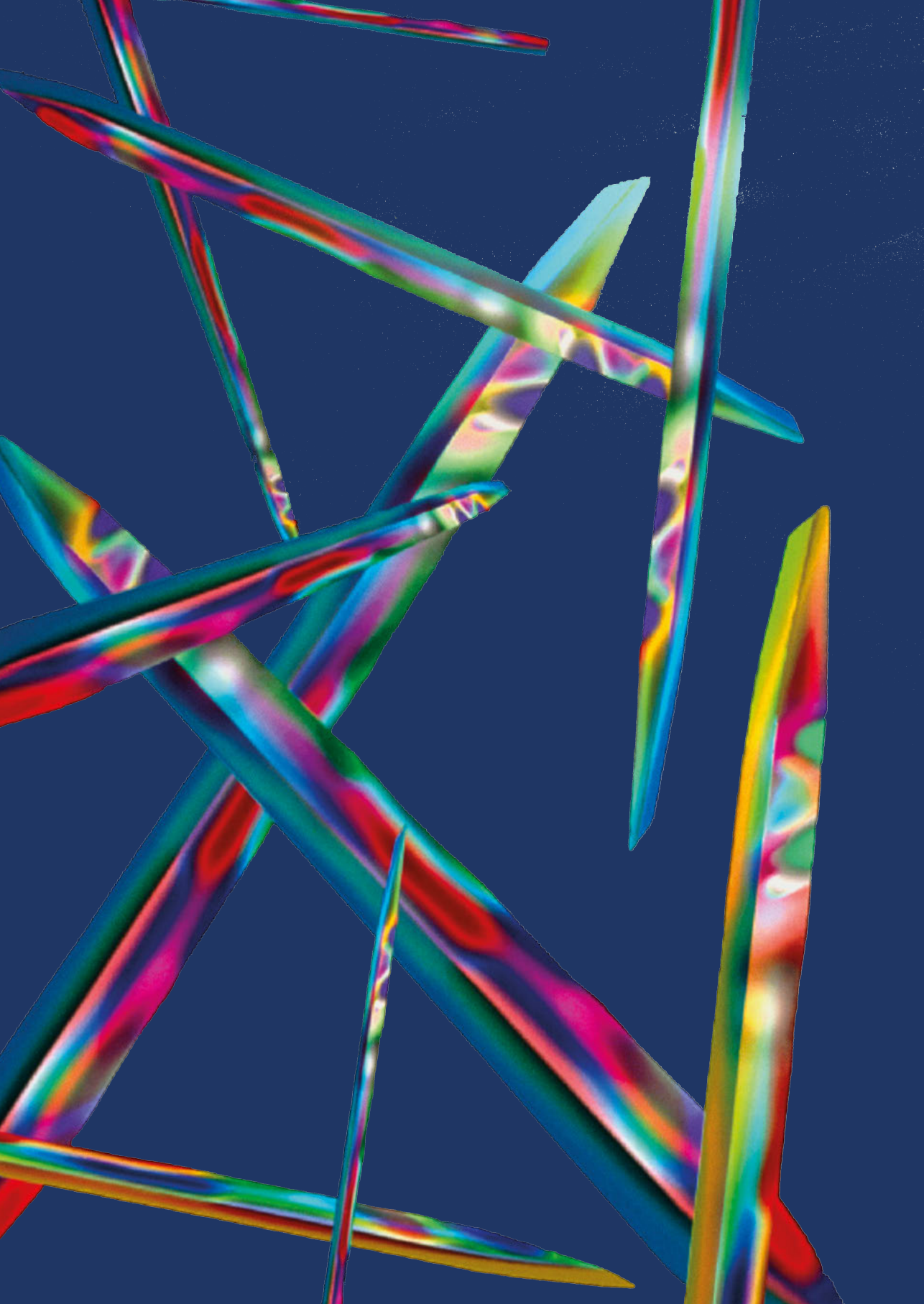
*een confounder is een variabele die zowel de afhankelijke (exposure) variabele als de onafhankelijke variabele (uitkomst) beïnvloedt, waardoor een valse associatie ontstaat

Bij het analyseren van subgroepen van knie-artrose werden significante associaties van lage vitamine D-spiegels met prevalente GSV van de knie en met progressieve knie-artrose waargenomen.

Omdat de rol van vitamine D bij het ontstaan van artrose nog steeds niet duidelijk was, werd een Mendeliaanse Randomisatie (MR) studie uitgevoerd om een eventuele causale relatie tussen genetisch bepaalde vitamine D-spiegels en heup- en knie-artrose te onderzoeken (**Hoofdstuk 4.1**). MR is een onderzoeksopzet waarin genetische varianten worden gebruikt als proxy (afgeleide) voor een bepaalde invloed ('exposure') om het effect van die exposure op een specifieke uitkomst te testen zonder 'confounding'. Zes single nucleotide polymorfismen (SNP's) geassocieerd met vitamine D-spiegels werden hiervoor gebruikt, en de effecten van SNP's op artrose waren afgeleid van de Iceland and UK Biobank studies, bestaande uit 23877 knie-artrose-cases, 17151 heup-artrose-cases en meer dan 562000 controles. De MR-analyses lieten geen causaal verband zien tussen genetisch bepaalde vitamine D-spiegels en artrose van de knie (OR 1.03, 95% BI 0.84 tot 1.26) of heup-artrose (OR 1.06, 95% BI 0.83 tot 1.35). Dus, de genetische variaties geassocieerd met lage vitamine D-spiegels zijn niet geassocieerd met een verhoogd risico op artrose van heup of knie. Dit suggereert dat levenslang verlaagde vitamine D-spiegels het risico op knie- of heup-artrose niet verhogen.

In **Hoofdstuk 5** worden de resultaten van ons onderzoek besproken. We zagen dat artrose een risicofactor is voor toekomstige fracturen, ondanks een hoge BMD, wat wijst op een veranderde botkwaliteit in de aanwezigheid van artrose. Een andere bevinding was dat een hoge BMD het risico op het ontwikkelen van knie- en heup-artrose verhoogt, wat ook impliceert dat een hogere BMD niet altijd een betere botkwaliteit weerspiegelt. Bij het bestuderen van de mogelijke associatie van vitamine D-status en het ontstaan van artrose op verschillende manieren (prospectief epidemiologisch onderzoek, meta-analyse en MR) werd geen consistente associatie gevonden. Dit impliceert dat suppletie met vitamine D het ontstaan of het verergeren van artrose niet voorkomt. Tijdens het bestuderen van de mogelijke associatie tussen verschillende bot-gerelateerde factoren en artrose, werd het belang van het in overweging nemen van subgroep-analyse duidelijk: stratificatie

van artrosegevallen naar meer homogene fenotypes is nodig om specifieke factoren te identificeren die mogelijk verband houden met de ontwikkeling van artrose. Verder zijn genetische studies zoals MR nuttig om het effect van een mogelijke factor op de ontwikkeling van artrose te testen zonder de invloed van confounding. Wanneer de onderliggende factoren geassocieerd met artrose kunnen worden geïdentificeerd, zouden subgroep-specifieke farmacotherapeutische behandelingen kunnen worden ontwikkeld om de ontwikkeling artrose in een vroeg stadium te voorkomen.



DANKWOORD

Dankwoord

Uiteindelijk heeft Sisyphus dan toch die steen over de top geduwd. Dat kon hij niet alleen, er zijn velen die hiervoor bedankt moeten worden.

Te beginnen bij een hele belangrijke groep; de muzikanten, van Koos Alberts via Jovink en Sinatra tot ZZ-Top, zonder wie de totstandkoming van dit proefschrift niet mogelijk was geweest. Met name de blues, en nummers als 'My way', en 'It's a long way to the top (if you wanna rock 'n' roll)', hebben me gesteund in meerdere fases van het promotie-traject

Een andere groep mensen zonder wie dit proefschrift er niet zou zijn en ik hartelijk wil bedanken, bestaat uit alle medewerkers en deelnemers van de ERGO-studie. Daarnaast ook dank aan iedereen werkzaam bij het Genetic Laboratory of Internal Medicine voor alle bepalingen die nodig waren voor de analyses in dit proefschrift.

Dan het sociale netwerk, een niet minder belangrijke groep, hoewel deze mogelijk ook een wat vertragend effect op het hele traject heeft gehad. Middelbare schoolkameraden, studievrienden, opleidingsmaatjes en bijbehorende bazen, Bikkels, dispuutsgenoten van M.H.D. Cambrinus, de vriendenclub van Dei (en ook van mij), tennismakkers, personal trainers, ouders van het schoolplein en de hockeyclub, buren: zonder jullie geen leven, en dus ook geen promotie. Dank daarom, en voor het altijd omzichtig informeren naar het p-woord.

De laatste groep die ik wil noemen valt eigenlijk ook onder bovenstaande groep, namelijk iedereen bij OCON, waar het onderscheid tussen collega en vriend vervaagt. Ik ben blij dat ik onderdeel ben van deze mooie organisatie.

Vervolgens, in niet geheel willekeurige volgorde, enkele persoonsgerichte dankwoorden:

Dr. B.R.H. Jansen, nestor voor vele assistenten orthopedie, met een bepalende invloed op mij, zowel op orthopedisch als sociaal vlak. Deze markante opleider was degene die mij iets zeer waardevols gaf: vertrouwen in een toekomst als orthopeed. Hij introduceerde me bij professor Verhaar, en stond daarmee aan de basis van dit proefschrift. Hij is nog regelmatig in mijn gedachten, en ik hoop deze woorden hem bereiken in Frankrijk.

Prof.dr. J.A.N. Verhaar, beste Jan, ook bij jou stond vertrouwen centraal, mede jouw vertrouwen in het uiteindelijk afronden van mijn promotie, wat een enorme motivatie voor deze master procrastinator geweest is om door te zetten. Dank hiervoor, en ook jouw rol als mijn opleider. Gedurende die opleiding ging ik steeds meer beseffen wat een enorme hoeveelheid werk je heb verricht op de meest uiteenlopende vlakken, voor en achter de schermen. Het blijkt dat jouw vertrouwen terecht was, heel mooi dat je nu na al die jaren in mijn promotiecommissie zit.

Prof.dr. H.A.P. Pols, beste Huib, dank voor het toelaten van deze orthopedische eend in de internistische bijt. Ondanks je overvolle agenda was je altijd bereid om geduldig beschouwende kennis over te dragen aan mijn meer op snijden ingestelde brein, desnoods om half 7 's ochtends. Je maakte tijd om bij ons huwelijk in Delft te zijn, en ik kon zelfs vaderlijk kledingadvies van je krijgen.

Prof.dr. J.P.T.M. van Leeuwen, beste Hans, dank voor al je steun en advies op vitamine D vlak, maar ook voor je vrolijke noot tijdens congressen en vergaderingen, met name ook samen met Huib en André. Fijn dat je deel uit wil maken van de commissie.

Prof.dr. A.G. Uitterlinden, beste André, ontelbare keren hebben we de afgelopen 2 decennia overleg gehad, eerst in de hoogbouw in Rotterdam, later via videobellen vanuit Hengelo. Altijd waren dit plezierige en nuttige bijeenkomsten, door je omvangrijk kennis op gebied van genetica, maar ook daarbuiten; never a dull moment door jouw brede interesses, originele gezichtspunten, door ons gedeelde gevoel voor humor en waardering

van Monty Python. Dank voor je steun vanaf het eerste uur, en dat je naast Joyce mijn promotor bent.

Prof.dr. J.B.J. van Meurs, beste Joyce, in 1999 tegelijk begonnen met mij op dezelfde afdeling, de cirkel is rond nu ik jouw eerste promovendus mag zijn. Jij was mijn rots in de branding vanaf het prille begin en met jou heb ik de laatste jaren het meeste gespard over inhoud van dit proefschrift. Je bereidheid om de laatste jaren tweewekelijks ruim een half uur samen met André over mijn vorderingen (of gebrek daaraan), maar over ook vele niet direct hieraan gerelateerde zaken, te praten is de reden dat dit proefschrift hier nu ligt. Jouw uitgebreide expertise op gebied van artrose, analytische kijk op de zaken, kritische blik en geduld worden enorm door mij gewaardeerd. Bij jouw oratie op 2 december zal ik het hardst voor je klappen.

Prof.dr.ir. C.P.G.M. de Groot, prof.dr. H.B.J. Karperien, prof.dr. F.U.S. Mattace-Raso, prof.dr. S.M.A. Bierma-Zeinstra, dr.ir. R.G. Voortman, hartelijk dank voor het beoordelen van dit proefschrift en jullie bereidheid zitting te nemen in de promotiecommissie.

Kamergenoten van het eerste uur op de 21e, Angelique Weel, Marjolein van der Klift en Stephanie Schuit. Dank dat jullie mij in meerdere opzichten hebben opgevangen in de betonnen omgeving van de faculteitstoren. Marjolein, dank voor jouw gezelschap op de kamer, bij congressen, vergaderingen en cursussen, maar ook voor je uitleg van zaken die ik niet begreep, voor de etentjes met Tom en jou, en mijn eerste ervaring als paranimf bij jou; het was een hele mooie tijd.

Jessica Ekkel, dank voor je hulp bij verspreiden van de uitnodigingen en boekjes, maar ook voor al het andere dat je betekent voor iedereen bij OCON.

Wendy Bour, dank voor je mooie lay-out werk, en voor je altijd snelle reacties op mijn mails; het was een hele klus, maar het resultaat mag er zijn.

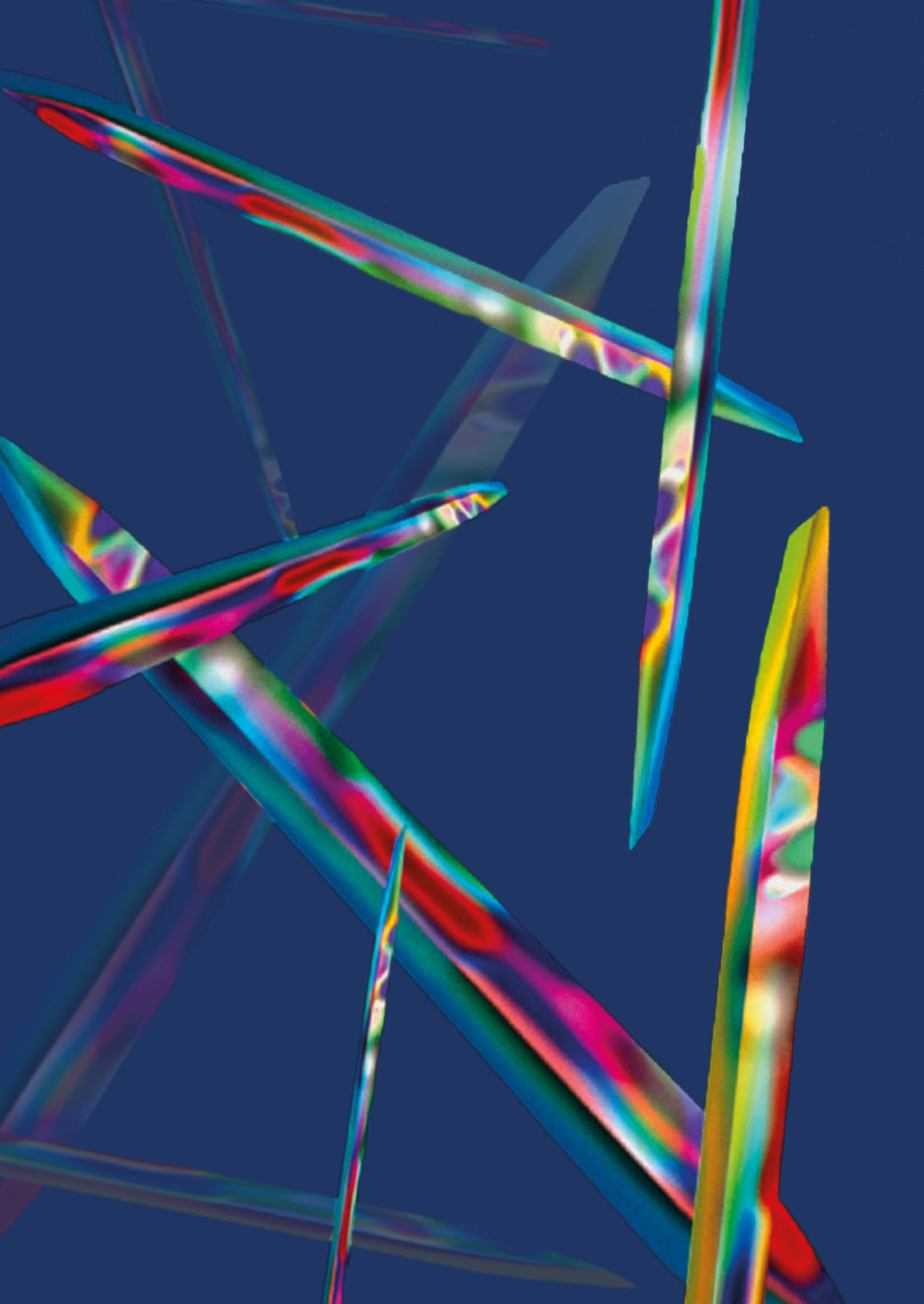
Eline van Veen, enorm bedankt voor je altijd opgewekte steun en Rotterdamse humor bij de speurtochten door de regels van het Erasmus en Hora Finita, zonder jou was ik hopeloos verdwaald. In WP drinken we er een op.

Paranimfen Peter Mensink en Maarten Schurink, makkers, mede-Cambrinianen. Bij jullie beiden ben ik zelf paranimf geweest, jullie hebben me laten zien hoe het kan, dat het kan. Dank voor jullie support en vriendschap, op naar een mooie dag, en daarna weer gezellig verder.

Lieve papa en mam, zonder jullie geen promovendus, en geen boekje. De basis van wie ik ben en waar ik nu mag zijn door jullie is gelegd. Dank voor de mooie jeugd die jullie me samen met mijn broertje Martin hebben gegeven, voor jullie Twentse nuchterheid, voor jullie optimisme en de warme belangstelling die wij allemaal nog steeds krijgen. Wat fijn dat jullie er 16 november bij zijn.

Dank lieve dochters, die zo lang ze leven niet beter weten dan dat ik met een of ander boekje bezig ben. Jullie geven de zin tussen alle onzinnige zaken, maar vooral ook veel lol. Marein, Eefke en Ilse, wat een leuke individuen zijn jullie, het is machtig mooi om met jullie in één gezin te zitten.

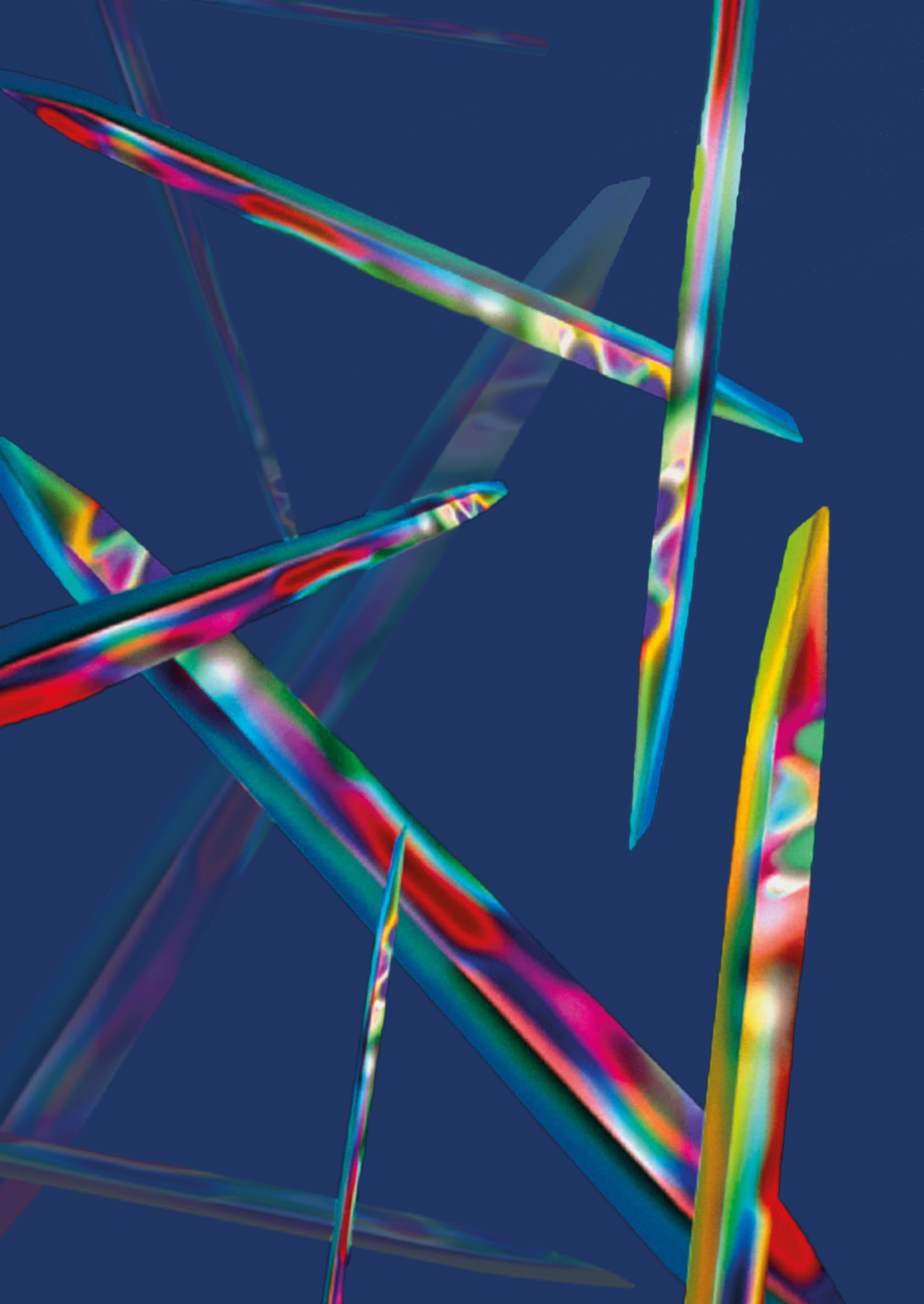
Lieve Dei, jij bent belangrijker is geweest voor de afronding van dit proefschrift dan alle muzikanten bij elkaar, en zo belangrijk op zo veel vlakken meer, niet in een paar regels te vatten. Ik ben heel blij met je, op naar een prachtig post-promotie tijdperk!



CURRICULUM VITAE

Curriculum vitae

Arjan Peter Bergink was born on the 15th of November 1969 in Hengelo, Overijssel, the Netherlands. He attended secondary school at the OSG 'De Bataafse Kamp' and finished gymnasium β in 1988. Then he went to Groningen to study medicine, and subsequently he moved to Amsterdam for his internships, which sparked his interest in orthopedics. After his graduation in 1996, he started as a resident in surgery and orthopedics at the BovenIJ Hospital. Shortly afterwards he moved to Delft, where he could start as an orthopedic resident at the Reinier de Graaf Hospital under the inspiring guidance of dr. B.R.H. Jansen. In 1999 he started a research project at the Academic Hospital Dijkzigt Rotterdam, initiated by prof. dr. J.A.N. Verhaar and prof. H.A.P. Pols, under the direct supervision dr. (now prof. dr.) A.G. Uitterlinden, ultimately resulting in this thesis. As part of the project, he obtained his Master of Science degree in Genetic Epidemiology in 2001. The next year, he started his 2-year training in general surgery at the Sint Franciscus Gasthuis, Rotterdam (dr. J.C.J. Wereldsma), and continued orthopedic surgery training in Rotterdam at the Academic Hospital Dijkzigt, Rotterdam (prof. dr. J.A.N. Verhaar) and the Haga Hospital, The Hague (dr. L.N.J.E.M. Coene). After his registration in 2008, he returned to his home region, first as an orthopedic surgeon at Hospital Group Twente, Almelo, and since 2011 at OCON orthopedic clinic, Hengelo, were he lives with his wife Deirdre and his daughters Marein, Eefke and Ilse.



LIST OF PUBLICATIONS

List of publications

Bergink AP, Trajanoska K, Uitterlinden AG, van Meurs JBJ. Mendelian randomization study on vitamin D levels and osteoarthritis risk: a concise report. Rheumatology. 2021 Jul 1;60(7):3409-12. PubMed PMID: 33463692

Bergink AP, Rivadeneira F, Bierma-Zeinstra SM, Zillikens MC, Ikram MA, Uitterlinden AG, et al. Are Bone Mineral Density and Fractures Related to the Incidence and Progression of Radiographic Osteoarthritis of the Knee, Hip, and Hand in Elderly Men and Women? The Rotterdam Study. Arthritis & rheumatology. 2019 Mar;71(3):361-9. PubMed PMID: 30264891

Bergink AP, Zillikens MC, Van Leeuwen JP, Hofman A, Uitterlinden AG, van Meurs JB. 25-Hydroxyvitamin D and osteoarthritis: A meta-analysis including new data. Semin Arthritis Rheum. 2016 Apr;45(5):539-46. PubMed PMID: 26522138

Bergink AP, Uitterlinden AG, Van Leeuwen JP, Buurman CJ, Hofman A, Verhaar JA, et al. Vitamin D status, bone mineral density, and the development of radiographic osteoarthritis of the knee: The Rotterdam Study. J Clin Rheumatol. 2009 Aug;15(5):230-7. PubMed PMID: 19654490

Reijman M, Pols HA, **Bergink AP**, Hazes JM, Belo JN, Lievense AM, et al. Body mass index associated with onset and progression of osteoarthritis of the knee but not of the hip: the Rotterdam Study. Annals of the rheumatic diseases. 2007 Feb;66(2):158-62. PubMed PMID: 16837490

Brouwer GM, van Tol AW, **Bergink AP**, Belo JN, Bernsen RM, Reijman M, et al. Association between valgus and varus alignment and the development and progression of radiographic osteoarthritis of the knee. Arthritis and rheumatism. 2007 Apr;56(4):1204-11. PubMed PMID: 17393449

Bergink AP, Uitterlinden AG, Van Leeuwen JP, Hofman A, Verhaar JA, Pols HA. Bone mineral density and vertebral fracture history are associated with incident and progressive radiographic knee osteoarthritis in elderly men and women: the Rotterdam Study. Bone. 2005 Oct;37(4):446-56. PubMed PMID: 16027057

Schuit SC, van Meurs JB, **Bergink AP**, van der Klift M, Fang Y, Leusink G, et al. Height in pre- and postmenopausal women is influenced by estrogen receptor alpha gene polymorphisms. J Clin Endocrinol Metab. 2004 Jan;89(1):303-9. PubMed PMID: 14715865

Bergink AP, van der Klift M, Hofman A, Verhaar JA, van Leeuwen JP, Uitterlinden AG, et al. Osteoarthritis of the knee is associated with vertebral and nonvertebral fractures in the elderly: the Rotterdam Study. Arthritis and rheumatism. 2003 Oct 15;49(5):648-57. PubMed PMID: 14558050

Bergink AP, van Meurs JB, Loughlin J, Arp PP, Fang Y, Hofman A, et al. Estrogen receptor alpha gene haplotype is associated with radiographic osteoarthritis of the knee in elderly men and women. Arthritis and rheumatism. 2003 Jul;48(7):1913-22. PubMed PMID: 12847685

van Meurs JB, Schuit SC, Weel AE, van der Klift M, **Bergink AP**, Arp PP, et al. Association of 5' estrogen receptor alpha gene polymorphisms with bone mineral density, vertebral bone area and fracture risk. Hum Mol Genet. 2003 Jul 15;12(14):1745-54. PubMed PMID: 12837697

Fang Y, van Meurs JB, **Bergink AP**, Hofman A, van Duijn CM, van Leeuwen JP, et al. Cdx-2 polymorphism in the promoter region of the human vitamin D receptor gene determines susceptibility to fracture in the elderly. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2003 Sep;18(9):1632-41. PubMed PMID: 12968672

Colin EM, Uitterlinden AG, Meurs JB, **Bergink AP**, van de Klift M, Fang Y, et al. Interaction between vitamin D receptor genotype and estrogen receptor alpha genotype influences vertebral fracture risk. J Clin Endocrinol Metab. 2003 Aug;88(8):3777-84. PubMed PMID: 12915669

Uitterlinden AG, Fang Y, **Bergink AP**, van Meurs JB, van Leeuwen HP, Pols HA. The role of vitamin D receptor gene polymorphisms in bone biology. Mol Cell Endocrinol. 2002 Nov 29;197(1-2):15-21. PubMed PMID: 12431791

Kann P, **Bergink AP**, Fang Y, Van Daele PL, Hofman A, Van Leeuwen JP, et al. The collagen Ia1 SP1 polymorphism is associated with differences in ultrasound transmission velocity in the calcaneus in postmenopausal women. Calcified tissue international. 2002 Jun;70(6):450-6. PubMed PMID: 12016466

Kann P, **Bergink AP**, Beyer J. Determination of the modulus of elasticity of bone material by an acoustical approach at the forearm distinguishes women with and without vertebral fractures independent of bone mineral density. Exp Clin Endocrinol Diabetes. 2002 Jun;110(4):161-5. PubMed PMID: 12058338