PREDICTION OF OSTEOPOROTIC FRACTURES

THE ROTTERDAM STUDY

P.L.A. van Daele

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"Voorspellen van fracturen: Het ERGO-onderzoek"

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Chapter 2.2 Van Daele PLA, Burger H, Algra D, Hofman A, Grobbee DE, Birkenhäger JC, Pols HAP. Age associated changes in ultrasound measurements of the calcaneus in men and women: The Rotterdam Study. J Bone Miner Res 1994;11:1751-1757

Chapter 2.3 Van Daele PLA, Burger H, De Laet CEDH, Hofman A, Grobbee DE, Birkenhäger JC, Pols HAP. Longitudinal changes in ultrasound parameters of the calcaneus. Submitted

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Chapter 3.1 Van Daele PLA, Stolk RP, Burger H, Algra D, Grobbee DE, Hofman A, Birkenhäger JC, Pols HAP. Bone density in non-insulin-dependent diabetes mellitus. The Rotterdam Study. Ann Intern Med 1995;122:409-414.

Chapter 3.2 Van Daele PLA, Birkenhäger JC, Pols HAP. Biochemical markers of bone turnover: an update. Neth J Med 1994;44:65-72

Chapter 3.3 Van Daele PLA, Seibel MJ, Burger H, Hofman A, Grobbee DE, Van Leeuwen JPTM, Birkenhäger JC, Pols HAP. Case-control analysis of bone resorption markers, disability and hip fracture risk: The Rotterdam Study. BMJ 1996;312:482-483

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CHAPTER 1

INTRODUCTION

Osteoporosis is defined as "a disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to increased bone fragility and a consequent increase in fracture risk".[1]
Fractures in general and hip fractures in particular contribute considerably to morbidity, mortality and financial costs. The risk for a 50 year old woman to sustain a hip fracture during the remainder of her life amounts to 16%.[2] Based on the aging of the population it has been estimated that the total number of hip fractures will be more than doubled by the year 2050.[3] In addition, the remaining lifetime risk of osteoporotic fractures in women at menopause is at least 30-40%.[4]
Prevention of fractures therefore is a major health goal but to implement preventive measures it is essential to be able to identify those subjects at greatest risk. Frequency of falling is by far the most important determinant of fracture risk.[5] However, whether a fall results in a fracture depends on a number of other factors such as the amount and quality of bone. Low bone density, as a reflection of the amount of bone, is associated with any type of fracture.[6] Nevertheless, bone density determines only in part the quality of bone. New techniques have been developed that may enhance our ability to get insight in the factors that determine bone strength and therefore fracture risk. However, the value of those techniques is not well established.
An additional putative determinant of bone density has been adressed by investigating the association between osteoporosis and diabetes mellitus. Conflicting results have been reported regarding the effect of non-insulin-dependent diabetes mellitus on bone mass and fracture risk.[7] The Rotterdam Study [8] provided an excellent opportunity to study this issue.

This thesis focuses on the application of new methods to predict fractures. Chapter 2 deals with ultrasound measurement as an emerging technique, with the potential advantage to detect qualitative deterioration of bone. In chapter 2.1, a review of ultrasound measurement is presented. Chapters 2.2

and 2.3 aim at age-related changes in ultrasound parameters. In chapter 2.4 the association between ultrasound parameters and fracture risk is discussed. Chapter 3 discusses metabolic factors of osteoporosis and fracture risk. In chapter 3.1, the association between non-insulin-dependent diabetes mellitus, bone density and fracture risk is discussed. Chapter 3.2 summarizes the currently available biochemical markers of bone turnover. In chapters 3.3 and 3.4 we discuss the use of biochemical markers in fracture prediction.
The thesis concludes with a general discussion including comments on clinical relevance and suggestions for further research in chapter 4.

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CHAPTER 2.1

ULTRASOUND MEASUREMENT OF BONE: A REVIEW

Introduction

Bone mass measurements are of major importance in the detection and follow-up of subjects with various metabolic bone diseases including osteoporosis. Currently available non-invasive techniques to measure bone mass include: single photon absorptiometry (SPA), dual energy x-ray absorptiometry (DXA) and computed tomography (QCT). However, all these techniques have the disadvantage to be expensive, time consuming and to be associated with exposure to ionizing radiation. Furthermore, none of these techniques gives insight into qualitative factors of bone tissue, such as trabecular bone structure. Qualitative aspects alone may account for up to 30% of the capability of bone to resist force. [1]
Ultrasonic assessment of bone has been proposed as an inexpensive and radiation free screening device for low bone mass. Furthermore, it has been suggested that by using ultrasonic techniques one may get insight into the qualitative aspects that determine bone strength.[2,3]

In this article we will first discuss briefly the fundamentals of ultrasound measurement of bone. Thereafter, we will summarize the present state of the art by reviewing the published literature.

Methods

Information for this paper was obtained from a search of the Medline Literature Database 1976 - June 1995 using Medical subject headings ultrasound, BUA, velocity, bone and fractures. The search was extended using lateral references.

Fundamentals of ultrasound measurement

The use of ultrasound in the context of metabolic bone disease is based largely on the postulated interaction of soundwaves with bone tissue. Transmission of sound through tissue leads to alterations in two acoustic

properties, namely wave velocity and wave amplitude (intensity). Alterations in the latter are usually called attenuation.

Velocity

The velocity (V) with which a sound wave travels through a material is a function of the modulus of elasticity (E) and density (ρ), such that $V=(E/\rho)^{½}$. E is the linear relationship of stress to strain at low loading levels; stress is defined as the force per unit area and strain is the measure of the deformation caused by that force (figure 1).

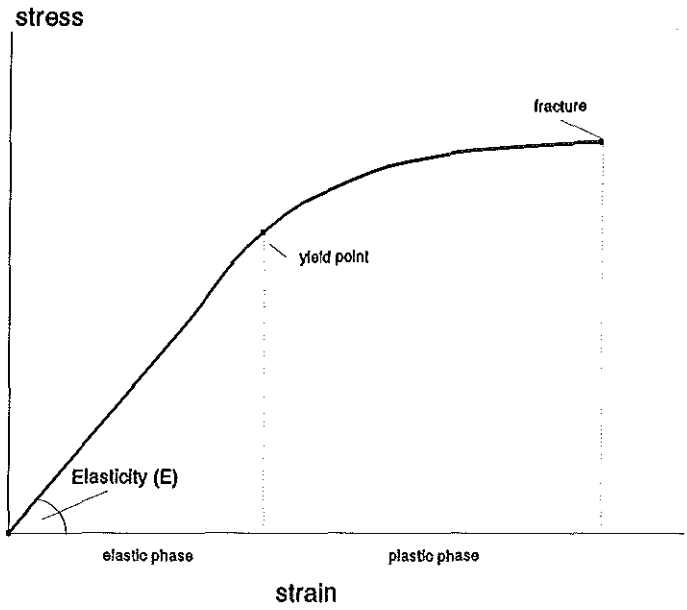

Figure 1: Stress-strain curve

At low levels of load, a deformation will be temporary, and the material will return to its original shape when the load is removed. This is called the elastic phase. If the load exceeds a certain level (yield point), deformation will be permanent (plastic phase) and finally the material will break. The more elastic (the steeper the line in figure 1 and therefore the higher the value for E), the more force a certain material can withstand before reaching the point of permanent deformation and

ultimately the point of breaking. [1,4]
In an inhomogeneous material such as bone, elasticity depends not only on the density of the material but also on a variety of other factors, including spatial orientation of the bony structures, inherent properties of the bony material and fatigue damage. [4-6]
It follows that $E=K\rho^2$, where K is a constant that incorporates these factors. The above mentioned formula may be rewritten to $V=(K\rho)^{½}$. Sound velocity through bone therefore appears to reflect both quantitative and qualitative aspects of bone. In vitro studies have shown that sound velocity is, indeed, highly correlated with ultimate strength of bone.[7,8]

There are several slightly different variants of sound velocity that can be measured:

1) true velocity: velocity of sound trough bone tissue only
2) heel velocity: velocity of sound through bone and soft tissue
3) time of flight: velocity of sound through coupling media, soft tissue and bone

However, as to the clinical outcomes all methods yield basically equivalent results. [9]

Attenuation
Attenuation of a sound wave, or loss of amplitude, results from two processes: scattering and absorption. Scattering occurs by definition when sound travels from one medium to another. Bone in general and cancellous bone in particular is an inhomogeneous material, leading to complex scattering. Absorption is basically the transformation of the energy of the propagating wave into heat.
Attenuation increases with increasing wave frequency. For many materials this relationship is linear over a wide range of frequencies. However, for bone tissue, the linear relationship is limited to a relatively narrow frequency range (< 1 MHz). Although one could argue, that it would

suffice to measure and present attenuation at a single frequency, attenuation is usually expressed as the slope of the relationship amplitude loss (in dB) over frequency (in MHz) after correction for the attenuation by the surrounding tissue and coupling media. This way of presenting data on attenuation is referred to as Broadband Ultrasonic Attenuation (BUA), a term which, for convenience, we will use further throughout this manuscript.[10-13]
Data from in vitro studies suggest that BUA is related to structural aspects of bone such as trabecular connectivity.[14,15] However, in contrast to sound velocity, no theoretical relationship between BUA and elasticity of bone has been established.

Commercially available devices are capable of measuring either or both ultrasonic parameters. An additional parameter which is sometimes reported is the so called stiffness. This parameter is formed by a combination of BUA and sound velocity. [2]

Ultrasonic examination of bone tissue can be performed either in a reflection mode (in which the same transducer acts both as transmitter and receiver) or in the transmission mode (in which two transducers are mounted opposite to each other on the tissue examined). The latter technique is most frequently used. To avoid interference of the ultrasound wave with air, a coupling media (either a waterbath or gel) is used between the transducers and the skin overlying the bone of interest.
The calcaneus, and to a lesser extent patella, tibia and phalanx of the finger are bones that are easily accessible and in use for measuring ultrasonic properties.

Both long and short term precision are regarded to be very good both for sound velocity and BUA, being at least as good as the precision for bone mineral density measurements. Coefficients of variations for repeated measurements range from approximately 1 to 3% for BUA and from 0.1 to 1.0% for sound velocity. [2] However, one has to bear in mind that a coefficient of variation is highly dependent on actual values and the range

of values of the measurement. As sound velocity has extremely high values with a range of approximately 15-20% of the minimum possible value (±1400 m/sec: speed of sound in soft tissue), the coefficient of variation will, by this fact alone, be low. While comparing precision of various measurements it is therefore advisable to use a standardized coefficient of variation (SCV), defined as the coefficient of variation (CV) divided by the 5 to 95% range (of the measurements) over mean ratio (SCV = CV / ((5-95% range)/mean)).[9] Applying this standardized coefficient of variation to ultrasound and bone mineral density measurements shows precision of BUA and SOS to be similar to precision of bone mineral density measurements of the proximal femur (own observations). Bone mineral density measurements of the lumbar spine show precision superior to either ultrasound measurement or bone density measurements of the proximal femur.

Review of previous studies

correlations

Although ultrasound measurement was supposed to give information beyond density, most attention has been focused on the ability of ultrasound measurement to detect subjects with low bone mass. In this respect, a considerable number of authors have reported correlations between bone density and ultrasound measurement, with varying results (Table 1).

In vitro, high correlations are found between bone mass and ultrasound measurements, which would indicate that bone mineral density and ultrasound measurements are two devices assessing the same. However, in vivo site matched ultrasound and bone mineral density measurements result in correlations amounting to a maximum of only 0.73, implying that just about half (53%) of the variability may be put down to the observed association. If bone density is measured at a site different from the one where ultrasound measurement is performed, correlations tend to be even less and slightly lower than the correlations between bone mineral density measurements at different sites.[32] Overall, correlations found do not

Table 1: correlations between ultrasound measurements and bone density in vitro and in vivo

site	sound velocity	BUA	references
in vitro	0.85 - 0.97	0.5 - 0.97	4, 10, 16, 17
in vivo			
- forearm	0.33 - 0.66	0.14 - 0.85	16, 18-23
- lumbar spine	0.33 - 0.63	0.25 - 0.83	16, 18, 21-29
- proximal femur	0.37 - 0.53	0.29 - 0.87	21-25, 27-29
- total body	0.57	0.54 - 0.59	21, 23
- calcaneus (site matched)	0.66	0.7 - 0.73	30, 31

warrant the use of ultrasound measurement as screening device for low bone density, but may indicate that different aspects of bone are determined.[23,24,28,29]

Relation with determinants of osteoporosis

A limited number of studies has been dedicated to determinants of ultrasound measurements and for most determinants examined, definite conclusions are hard to draw. However, a number of factors is known to influence ultrasound results. The most important determinant is undoubtedly gender. As for bone mineral density measurements, men show higher values than women. [28]

In cross-sectional studies, age has been demonstrated to be a determinant of both ultrasound velocity and BUA. In childhood there is an increase in both sound velocity and BUA with age both in boys and girls. This increase parallels bone mineral density measurements.[33] From adulthood until the age of approximately 50 ultrasound values remain relatively stable.[24,34-36] At older age, there appears to be a decrease which is somewhat more pronounced for sound velocity than for BUA and stronger in women than in men.[28,34,36,37] This suggests that postmenopausal status or lower estrogen level is a trigger for loss in ultrasound parameters, very much as it is a trigger for loss in bone mineral density.

In this respect it is interesting to see that postmenopausal use of estrogens is associated with higher values for sound velocity compared to non-use. [28,38] As far as we know, sodium fluoride is the only other treatment for osteoporosis that has been shown to influence ultrasound measurement.[39] This does not imply that other treatments do not influence ultrasound results, but merely that studies on this subjects are lacking.

One other important determinant of ultrasound measurement is mobility. Important because mobility is much to often overlooked as a way to influence skeletal status. It has been demonstrated that subjects that are less mobile show decreased ultrasound values. [28] Moreover, an intense program to improve mobility has been shown to increase BUA values up to 10% after one year in formerly sedentary but relatively young women, which is more than can be accomplished by any medical treatment.[40]

Assessment of fracture risk

Probably the most important question is whether ultrasound measurement of bone is capable to detect subjects at risk for fractures. Several cross-sectional studies have demonstrated that subjects with fractures have lower values for speed velocity and BUA than subjects without fractures.[41,42] Porter et al. demonstrated prospectively that subjects with a low BUA value in combination with a lower cognitive function were at an increased risk for hip fracture [43] and there are also some prospective data showing that those with lower ultrasound values have a higher risk for developing vertebral deformities. [44] Table 2 shows data that have been published on the relative risk (RR) of fracture per standard deviation decrease in either sound velocity or BUA. The relative risk for fracture per standard deviation decrease in either measurement is similar to the relative risk that has been reported per standard deviation decrease in bone mineral density measurements. [47]

The question arises whether the value of ultrasound measurements to predict fractures is a reflection of their ability to measure bone density or represents their potential ability to monitor quality of bone. Sofar prospective studies are lacking that examine this important issue. The

results from some cross-sectional studies indicate that the predictive value is at least partially independent of bone mass. [46,48] While combining ultrasound and bone mineral density measurements, both remained independent predictors of fracture risk. For sound velocity this is illustrated in figure 2.

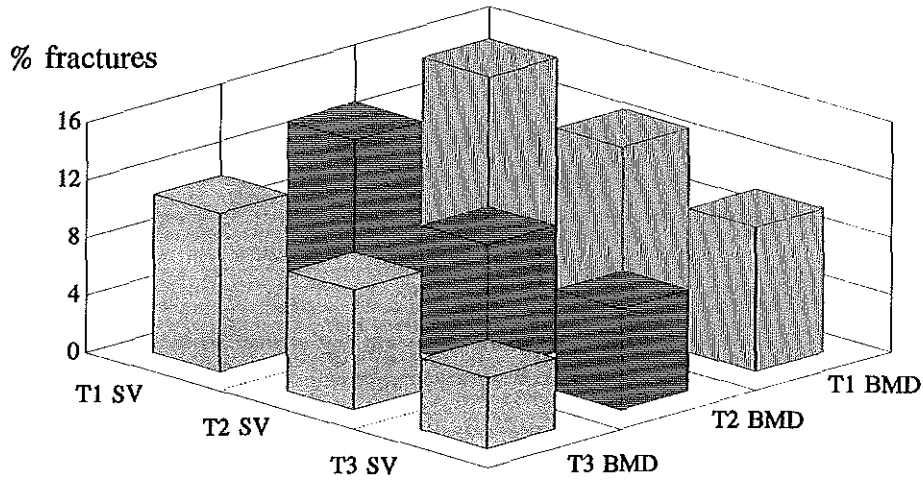

Figure 2: Frequency of fractures by tertiles bone density (BMD) and sound velocity (SV). For both measurements T1 is the lowest tertile and T3 the highest.

Nevertheless, it has to be emphasized that bone mineral density in those studies was measured at a site different from the one where the ultrasound measurement was done. Previous studies have shown that combining bone mineral density measurements from different sites improves the predictive value of these measurements. Subjects with low bone density at more than one site have a higher risk of any type of fracture than those with low bone density at just one single site. [49] This may be explained simply by the fact that the chance of misclassifying a subject with respect to the level of bone mass is smaller when using more

than one measurement. An alternative explanation is that the measurement at one site may reflect predominantly cortical bone mass (e.g. femoral neck), whereas the other site may be much more a reflection of the amount of trabecular bone (calcaneus). Combining measurements of both trabecular and cortical bone mass may therefore substantially increase the ability to predict whether a subject will get a fracture if we disregard the site. If one is interested in just one specific fracture type, for instance of the hip or the vertebrae, this added value of combining measurements may be less pronounced. The added value of ultrasound measurement that has been demonstrated does therefore not necessarily imply that ultrasound gives important information on the quality of bone. It may also indicate that, although in a different way, bone density is measured at more than one site, and/or that the measurements simply reflect different types of bone (trabecular or cortical).

Conclusions and further research

Ultrasonic assessment of bone appears potentially a useful tool in clinical practice. The assumption that ultrasound provides insight into quantitative as well as qualitative aspects of bone is supported by a theoretical background and factors like low correlations with bone density even in site-matched measurements. On the other hand, their similarity to bone mineral density measurements with respect to the factors by which they are influenced and with respect to their ability to predict fractures may suggest otherwise.

Therefore, some aspects of ultrasound still need further attention. With mainly cross-sectional data showing that ultrasound measurements predict fractures, more studies are needed that address this issue prospectively. In addition, further research must focus on the fact to what extent ultrasound measurement give insight into qualitative aspects of bone tissue that determine fracture risk. For this to be answered preferably site-matched ultrasound and bone densitometry measurements have to performed and used as predictors of fracture risk. Furthermore, the ability of ultrasound measurements to monitor the effect of treatment for metabolic bone disease deserves more attention.

Finally, the majority of clinical studies on ultrasound aim at osteoporosis as the metabolic bone disease of interest. Yet, studying patients with a variety of metabolic bone diseases, such as mineralization disorders or renal osteodystrophy may not only provide us with information about the usefulness of ultrasound in these diseases, but may also help in the understanding of the processes with which sound interacts with bone tissue.

Table 2: Predictive value of ultrasound measurement (review of published studies)

fracture type	ultrasound measurement	N	gender	study type	outcome	Reference
vertebral	Sound velocity	130	women	Prospective	RR 2.11 (1.14 - 3.91)	45
hip	BUA	129	women	Cross-sectional	RR 3.7 (2.0 - 6.6)	46
hip	Sound velocity	129	women	Cross-sectional	RR 2.7 (4.5 - 1.7)	46
all fractures	Sound velocity	899	women	Cross-sectional	RR 1.24 (1.03 - 1.49)	20
all fractures	Sound velocity	529	men	Cross-sectional	RR 1.65 (1.31 - 2.06)	20
vertebral	BUA	443	women	Prospective	RR 1.8 (1.4 - 2.3)	44

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CHAPTER 2.2

AGE ASSOCIATED CHANGES IN ULTRASOUND MEASUREMENTS OF THE CALCANEUS IN MEN AND WOMEN: THE ROTTERDAM STUDY

Introduction

Although patients with osteoporotic fractures in general have lower bone mineral density (BMD), there is considerable overlap with subjects without fractures.[1] This suggests that qualitative factors, like an accumulated burden of fatigue damage or an ineffective trabecular bone structure, might play an important role as well.[2] Ultrasound measurements of the calcaneus (Speed of Sound (SOS) and Broadband Ultrasound Attenuation (BUA)) have been proposed as an inexpensive and radiation free screening device in osteoporosis, and may also provide a better insight in the extent to which the factors mentioned are of importance to the occurrence of fractures.[1-7]

Both SOS and BUA have been shown to be significantly decreased in women with osteoporosis and hip fractures.[2,8-11] Furthermore, Porter et al reported that BUA in combination with a low cognitive function, could predict hip fractures in a large prospective follow-up study of elderly women.[12]

The correlation between BMD and ultrasound measurements has been examined in several studies.[13-19] Depending on the population studied and on the site of measurement, correlations ranged from 0.29 to 0.90. This has led to conflicting conclusions whether ultrasound is able to predict BMD of the spine and hip. Unfortunately, most studies showing high correlations examined a relatively small and selected population.

Although several studies examined the relation between age and ultrasound results in women,[14-16,23,24] only very few investigators have looked at the age related changes in men. Furthermore, research has been limited with respect to other determinants of SOS and BUA.

In the present study we measured SOS and BUA in a large ambulatory population based cohort of men and women, and evaluated the effect of

age, body mass index and age of menopause as possible determinants. In addition, we examined the effect of smoking, current use of medication known to influence bone turnover (thiazides, loop-diuretics, estrogens and corticosteroids), and disability on SOS and BUA. Finally, we investigated whether ultrasound measurements of the calcaneus can be used to predict low BMD in hip and spine.

Methods

The Rotterdam Study is a prospective follow-up study of people aged 55 years or over, to investigate the incidence of, and risk factors for chronic disabling diseases.[20] The study has been approved by the Medical Ethics Committee of Erasmus University and written informed consent is obtained from all participants. All inhabitants aged 55 years or more, living at one point in time in the Rotterdam suburb of Ommoord were invited to participate. To obtain baseline data an initial home visit and interview by a trained research assistant was followed by further examination during two visits at the research centre. Interview data was collected for 78% of the eligible persons. The overall response rate for the centre visit was 69% (6,494 persons). Institutionalized persons (about 10%) were not eligible for examination at the research centre. For the evaluation of osteoporosis, Dual energy X-ray Absorptiometry (DXA) forms part of the routine examination at the research centre.

Since September 1992, ultrasound measurements form part of the Rotterdam Study protocol. In this cross-sectional study we present the data of all 1,405 persons (777 women and 628 men) on whom we performed ultrasound measurements of the calcaneus. The mean age of both men and women in this subsample (65.9, 66.4 resp.) was lower than the mean age of men and women in the total (including persons in nursing homes) invited population (69.3, 72.1 resp.), but differed only slightly from the mean age of all persons who visited the research centre (67.7, 68.6 resp.).

Speed of Sound (SOS) and Broadband Ultrasound Attenuation (BUA) at the right heel were measured using a Lunar Achilles Ultrasound Bone Densitometer (Lunar Radiation Corporation, Madison, WI). The system

consists of a water tank, containing two broadband ultrasonic transducers. The heel is placed in the water bath and after scanning, the SOS and net attenuation are calculated by a computer program, correcting for the influence of water.[4]

BMD measurements of the lumbar spine and hip were performed using a Lunar DPX-L densitometer. Standard positioning was used with anterior-posterior scans of the lumbar spine and the proximal femur. As for the femur, the right side was scanned unless there was a history of prosthesis implantation. In that case the left side was chosen. Using standard software we analyzed vertebrae L2 to L4 (L2-L4), the femoral neck, Ward's triangle and greater trochanter. The coefficient of variation of the ultrasound measurements, calculated from 13 participants measured twice on the same day, was 0.45% for SOS and 2.28% for BUA. For DXA the in vivo coefficient of variation, calculated from 12 cohort members scanned twice on the same day, was 3.2% in the femoral neck, 3.1% in Ward's triangle, 2.5% in the greater trochanter and 0.9% in the lumbar spine.

Quality assurance including calibration was performed routinely every morning for both DXA and ultrasound, using the standard provided by the manufacturer.

Height and weight were measured in standing position without shoes and the body mass index (BMI, kg/m^2) was calculated.

A medical history was obtained by an interviewer administered questionnaire. Medication use was validated by examination of pills during a home visit. For current or past smokers, the number of pack years was calculated as the number packs of cigarettes smoked per day times the total years of smoking. Disability was assessed by using a questionnaire modified from the Stanford Health Assessment Questionnaire (HAQ), which provides information over eight categories of activities of daily living (dressing, arising, eating, walking, hygiene, reaching, gripping and other activities (like getting in and out of a car)).[21] Each category has a score ranging from zero to three, zero meaning no impairment and three meaning unable to perform. Since mainly impairment in the function of the lower extremities will have an influence

on bone, a disability index was calculated for the lower limbs by calculating the mean of arising, walking, bending (which is part of the grip category) and getting in and out of a car (which is part of the activity category). Disability was then categorized in absent (0), mild (>0-1) and severe (>1).

Data analysis

Linear multiple regression analysis was used for the evaluation of the association between ultrasonic measurements and BMD with anthropometric parameters and age. Regression with polynomial models (quadratic and cubic) was performed to detect a possible non-linear trend in age-related changes in ultrasound measurements. The effect of menopause on BUA and SOS was assessed by adding "age of menopause" (AOM) to the model and not "years since menopause" (YSM), to avoid the problem of collinearity.[22] The effect of disability, current use of thiazides, loop-diuretics, corticosteroids and estrogens, and the effect of smoking on ultrasound was also studied using regression analysis.

A percentual apparent rate of loss was calculated by dividing the slope of the regression line by the mean value. On theoretical grounds SOS can not be smaller then 1400 m/sec. To calculate a clinically meaningful apparent rate of loss that can be compared to the apparent rates of loss of other measurements we, therefore, subtracted 1400 m/sec from the SOS.

The relationship between ultrasound and DXA was studied by calculating Pearson's product moment correlation coefficients. In accordance with Massie et al [15] quartiles of the study population measurements were calculated to examine the ability of ultrasound to identify those individuals with a low bone density.

All results are presented with two-sided p-values. The analyses were carried out for each sex separately.

Results

The characteristics of the study population are given in Table 1. All women were postmenopausal. BMI slightly increased with age in women

and decreased with age in men (data not shown).

Age and BMI

Figure 1a and 1b depict SOS and BUA by age in both sexes. Table 2 shows the results of both simple and multiple regression analysis. In both men and women a significant decline in SOS and BUA was observed. There was no evidence for a quadratic of cubic age-related decline in SOS or BUA, nor for periods of accelerated loss (results not shown). Unadjusted correlations between age and SOS were -0.39 for women and -0.11 for men. For the correlation between age and BUA these figures were -0.28 and -0.09 respectively. The apparent rate of loss, expressed as a percentage per year, was similar for both ultrasound measurements and the bone mass measurements of the proximal femur. In addition, the calculated rates of loss for SOS and BUA were, dependent upon the technique used, three to four times higher in women compared to men (Table 2). Excluding participants on medication known to affect bone metabolism did not change the results (data not shown).

BMI had a significant effect on BUA and SOS in women, but not in men (Table 2). The effect was substantially smaller than the effect on BMD. Adjusting for BMI did not significantly alter the associations between age and ultrasound measurements.

Other risk factors

Table 3 shows the effect of current use of estrogens, thiazides, loop-diuretics and corticosteroids, and the effect of disability and smoking on SOS and BUA after adjustment for age and BMI.

Current use of corticosteroids resulted in a significantly lower BUA in men (N=7). Furthermore, women who used estrogens had a borderline higher SOS.

In contrast to women, men with severe disability had significantly lower SOS and BUA. A similar pattern was found when we studied the effect of disability on BMD of the femoral measurement sites (data not shown).

Table 1: Characteristics of the study population by sex

	MEN		WOMEN	
	mean	SD	mean	SD
Number	628		777	
Age (yr)	65.9	7.4	66.4	7.3
Height (cm)	176.1	6.7	162.1	6.1
Weight (kg)	78.8	10.4	70.0	10.5
BMI (kg/m^2)	25.5	2.9	26.6	3.8
Age of menopause (years)			49.0	5.0
SOS (m/sec)	1541.1	36.5	1517.1	31.0
BUA (dB/MHz)	117.7	11.6	107.8	11.9
L2-L4 (g/cm^2)	1.158	0.179	1.037	0.174
Femoral neck (g/cm^2)	0.873	0.125	0.818	0.141
Ward's triangle (g/cm^2)	0.734	0.142	0.691	0.161
Trochanter (g/cm^2)	0.849	0.132	0.733	0.138
	N	%	N	%
Ever smoked	543	86.5	274	35.3
Current smokers	167	26.6	126	16.3
Pack years in smokers (years)	28.2		20.8	
Current use of thiazides	23	3.7	68	8.8
Current use of loop-diuretics	23	3.7	17	2.2
Current use of corticosteroids	7	1.1	14	1.8
Current use of estrogens			32	4.2
Disability mild	199	31.7	319	41.2
severe	27	4.3	66	8.5

Table 2: Results of univariate and multivariate regression analysis on ultrasound measurements and DXA

	UNIVARIATE				MULTIVARIATE				
MEN									
	i.c.*	age	%†	P	i.c.	age	P	BMI	P
SOS (m/sec)	1578.2	-0.56	-0.4	<0.01	1590.3	-0.58	<0.01	-0.42	0.4
BUA (dB/MHz)	127.3	-0.15	-0.1	0.02	118.8	-0.13	0.04	0.30	0.06
L2-L4 (g/cm²)	1.118	+0.0006	+0.1	0.5	0.661	+0.0014	0.15	0.0159	<0.01
Femoral neck (g/cm²)	1.075	-0.0031	-0.4	<0.01	0.723	-0.0025	<0.01	0.0123	<0.01
Ward's triangle (g/cm²)	1.036	-0.0046	-0.6	<0.01	0.693	-0.0040	<0.01	0.0120	<0.01
Trochanter (g/cm²)	1.035	-0.0028	-0.3	<0.01	0.530	-0.0020	<0.01	0.0177	<0.01
WOMEN									
SOS (m/sec)	1621.4	-1.56	-1.3	<0.01	1602.9	-1.59	<0.01	0.75	0.01
BUA (dB/MHz)	136.2	-0.43	-0.4	<0.01	122.4	-0.45	<0.01	0.56	<0.01
L2-L4 (g/cm²)	1.253	-0.0032	-0.3	<0.01	1.020	-0.0035	<0.01	0.0095	<0.01
Femoral neck (g/cm²)	1.194	-0.0057	-0.7	<0.01	0.923	-0.0060	<0.01	0.0111	<0.01
Ward's triangle (g/cm²)	1.155	-0.0070	-1.0	<0.01	0.847	-0.0073	<0.01	0.0125	<0.01
Trochanter (g/cm²)	0.999	-0.0040	-0.5	<0.01	0.573	-0.0045	<0.01	0.0174	<0.01

* i.c. = intercept † percentual rate of loss: ß/mean value (for SOS after subtraction of 1400 m/sec of initial mean value)

Table 3: Effect of different variables on ultrasound measurements

	WOMEN						MEN					
	SOS			BUA			SOS			BUA		
	ß*	SE	P	ß*	SE	P	ß*	SE	P	ß*	(SE)	P
Age of menopause	0.36	0.21	0.09	0.06	0.08	0.43						
Current use of estrogens	9.97	5.37	0.06	2.49	2.10	0.24						
Current use of thiazides	-1.31	3.87	0.74	1.39	1.51	0.36	4.03	7.76	0.60	-0.99	2.47	0.69
Current use of loop-diuretics	-2.65	7.28	0.72	-4.06	2.84	0.15	-3.55	7.84	0.65	0.82	2.50	0.74
Current use of corticosteroids	-5.00	7.93	0.53	4.17	3.10	0.18	-18.46	13.82	0.18	-9.30	4.39	0.03
Mild disability	-3.53	2.31	0.13	-0.80	0.90	0.38	-5.77	3.28	0.08	0.38	1.04	0.71
Severe disability	-6.35	4.29	0.14	-1.39	1.68	0.41	-17.31	7.42	0.02	-6.39	2.36	0.01
Pack years of smoking	-0.17	0.06	0.01	-0.02	0.03	0.39	-0.24	0.06	<0.01	-0.05	0.02	<0.01

* After adjustment for age and body mass index

ß = difference in outcome given the presence of the indicator variable, or the difference in outcome due to an increase of 1 of a continous variable

Table 4: Correlations between ultrasound and DXA

	Men (N=632)		Women (N=786)	
	SOS	BUA	SOS	BUA
L2-L4	0.33 (0.26 - 0.40)	0.32 (0.25 - 0.39)	0.42 (0.36 - 0.48)	0.37 (0.31 - 0.43)
Femoral neck	0.37 (0.30 - 0.44)	0.34 (0.27 - 0.41)	0.49 (0.44 - 0.54)	0.43 (0.37 - 0.49)
Ward's triangle	0.38 (0.31 - 0.44)	0.35 (0.28 - 0.42)	0.50 (0.45 - 0.55)	0.44 (0.38 - 0.49)
Trochanter	0.43 (0.36 - 0.49)	0.39 (0.32 - 0.45)	0.48 (0.42 - 0.53)	0.42 (0.36 - 0.48)

95% confidence interval between parentheses

Table 5: Percentual overlap in number of patients in the lowest quartile of SOS, BUA and BMD

	Men				Women			
	L2-L4	neck	Ward's	trochanter	L2-L4	neck	Ward's	trochanter
SOS	43.6	40.5	42.7	45.9	44.1	50.0	50.3	47.7
BUA	45.5	41.8	42.7	45.9	43.6	50.5	51.3	51.3

Menopause

As shown in Table 3, after adjustment for age and BMI, age of menopause had no significant effect on SOS and BUA.

Current smoking, as such, did not result in significantly different SOS and BUA (data not shown). However, with the exception of BUA results in women, the number of pack years of smoking did negatively influence ultrasound results.

Correlations between ultrasound and DXA are given in Table 4. All correlations were significantly different from zero at the level of $P<0.001$. Exclusion of subjects on medication known to affect bone metabolism did not change these correlations significantly. Correlations between BUA and SOS were 0.66 for men and 0.72 for women.

Table 5 shows the percentage overlap in number of patients in the lowest quartile of both DXA and ultrasound measurements.

Discussion

We were able to demonstrate a significant decrease in BUA with age in women aged 55 years and over (≈ 0.4%/year). Our results are similar to those of Yamazaki[23] who used the same method. Baran et al[16] and Waud et al [14] using a Walker Sonix densitometer found a steeper decline in BUA with age (-1%/year), studying a small and selected population. Damilakis et al [24] found a significant negative correlation ($r=-0.35$) between age and BUA, which is similar to the correlation we found, but they did not report the apparent annual change. On the other hand, Massie et al,[15] also using a Walker Sonix densitometer, did not find a correlation between BUA and age in a large cohort of perimenopausal women aged 44 to 50 years.

Much less has been published about the annual change for SOS. Again our results are similar to those of Yamazaki et al,[23] but the decrease appears less than reported by studies using the Walker Sonix. Not correcting for the absolute minimum value, Waud et al[14] reported an annual change of 0.3% in women. Using the same approach, the annual change was approximately 0.1% per year in the present study.

The difference in reported changes could be explained by a difference in scanning technique between the Walker Sonix and the Lunar Achilles densitometer. Also differences in study populations or in the selection of these populations could play an important role. With respect to the latter, most previous studies excluded patients with conditions known to affect bone metabolism. However, as those "conditions" are positively associated with age, and in most instances lead to bone loss, one would have expected to find a steeper decline in our study group. Furthermore, our results remained essentially unchanged after excluding participant on medication known to influence bone metabolism. Since this study concerns cross-sectional data, selective non-response of participants with low SOS or BUA in the older age group, might have biased the slope of the regression of SOS and BUA on age in upward direction. In this respect, it is important to notice that institutionalized persons were not eligible. Nevertheless, this does not explain the apparent steeper decline found in healthy subjects.

Increasing evidence suggests that osteoarthritis is associated with higher bone mass at several sites.[26,31] Given the fact that ultrasound measurements are partially dependent on bone mass, BUA and SOS may be elevated in participants with osteoarthritis. As osteoarthritis is positively related to age this may explain the smaller apparent rate of change we found compared to previous studies.

The difference between men and women in the apparent rate of change for the ultrasound measurements was higher than for the bone mass measurements. We suggest that this may be explained in the following way. With increasing age there is a loss of bone mass in both men and women. In women the postmenopausal loss leads to complete removal of some structural elements. Therefore, the elements that remain, are more widely separated and less well connected. In men, however, trabecular width decreases, but the trabecular structure stays intact.[25] Since trabecular connectivity is supposed to be related to BUA and SOS, these dissimilarities between men and women may explain the difference observed.

It has been suggested that shifts in bone size obscure the interaction

between age and ultrasound measurements, and that the results had to be corrected consequently. Unfortunately, heel width is not measured by the Achilles ultrasound densitometer. However, preliminary results suggest no age related changes in calcaneal width in adults (Mazess, personal communications). Furthermore, Miller et al suggested that correction for width does not improve precision.[33] On the contrary, it may even decrease precision.

We were not able to demonstrate an effect of age of menopause on ultrasound measurements. It is, however, difficult in a cross-sectional study to separate the menopause effect from that of age.[26] A follow-up study might give a better insight in the extent of changes related to the menopause. The effect of age of menopause on BMD was essentially similar to that reported earlier on a different subsample of the Rotterdam Study.[26]

Other studies have shown a positive effect of walking and mobility on both ultrasound and DXA results.[28,29] We showed a negative effect of disability on SOS, BUA and BMD results of the hip in men. We were not able to demonstrate such an effect in women. It might be that the effect of disability has a higher relative impact on mobility in men than in women because of a higher baseline level of physical activity (e.g. occupation). In addition, immobility, caused by disability might lead to a further thinning and even loss of trabeculae, of which the impact will be more pronounced in men than in women (see also above).

Of the different types of medication studied, only current corticosteroid use had a significantly effect on BUA. As the number of participants taking medication was relatively small, this does not rule out the possibility that there is some effect of other medication on SOS or BUA as well. Lehmann et al[32] found for instance a positive effect of post menopausal estrogen replacement therapy on SOS. Although not significant, our data point in the same direction.

The correlation between SOS and BUA was 0.66-0.72, which is slightly higher then the correlation reported by Rossman et al ($r=0.53$)[13]. The correlation we found between DXA and ultrasound measurements was 0.32-0.52, which is also reflected by the relatively small overlap in the

percentage of persons in the lowest quartile of either measurement. This is comparable to what Massie et al[15] found. Others however, reported correlations between 0.6 and 0.7.[8,9,16,30]

Although on theoretical grounds there has to be a relationship between SOS and BUA and BMD,[4] this study showed that BUA and SOS of the os calcis can not be used to identify subjects with a low spinal or hip BMD. Therefore, we have to conclude that ultrasound is not useful as a screenings device for low BMD. Of course, this does not exclude, maybe even indicate, that ultrasound provides other useful information. However, whether ultrasound measurements are related to bone quality and can be used to predict future fractures remains to be established.

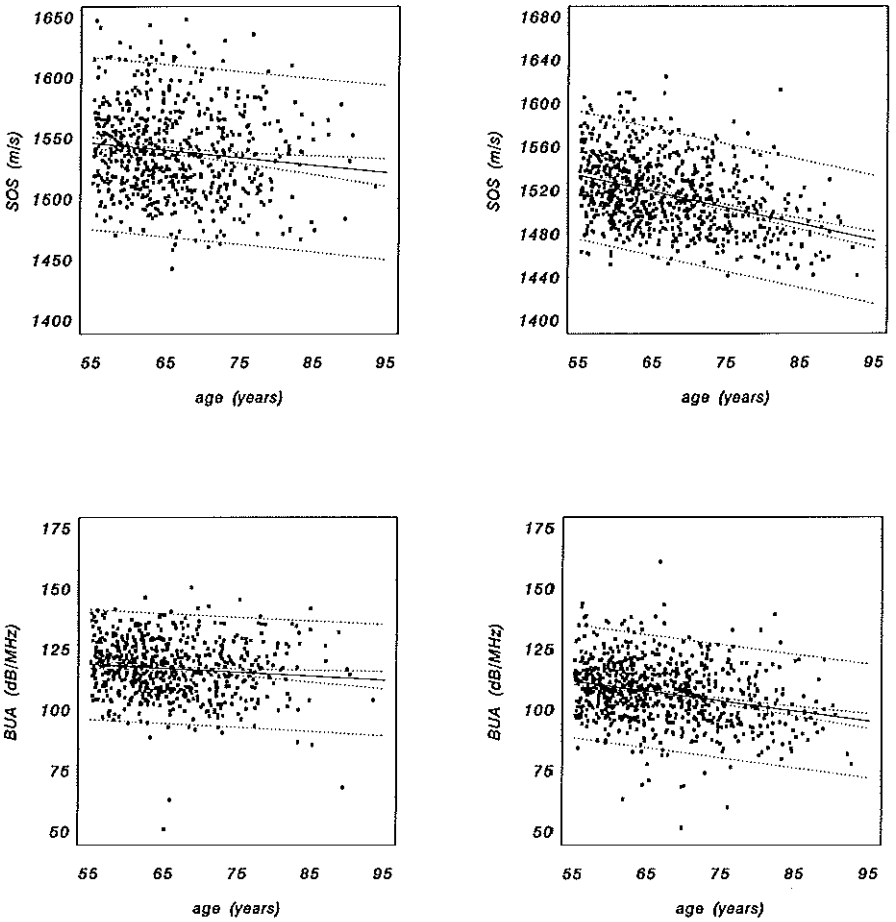

Figure 1: Age-associated changes of SOS and BUA in men (left) and women (right). The dotted bands reflect the prediction intervals (outer bands) and the confidence intervals for the means (inner bands). The solid band represent the regression line.

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CHAPTER 2.3

LONGITUDINAL CHANGES IN ULTRASOUND PARAMETERS OF THE CALCANEUS

Introduction

Osteoporosis is characterized by loss of bone quantity and bone quality, leading to fractures by low energy trauma.[1] It has been suggested that ultrasound parameters provide information on both qualitative and quantitative aspects of bone tissue.[2] This potential advantage together with the fact that ultrasound measurements are radiation-free and relatively inexpensive would make them valuable tools in assessing the risk of osteoporotic fractures.

Previous cross-sectional studies have indicated a substantial apparent decline in ultrasound parameters with age,[3-6] which is in accordance with the age-related decline in bone mass as observed by bone mineral mass measurements.[7] Cross-sectional studies however, may give a biased estimate of the true rate of loss.[8] Conflicting results from such studies may reflect cohort effects or survivor bias. Some of these biases may adequately be controlled for in longitudinal studies.

In the current study, we examined changes in ultrasound parameters (Speed of Sound (SOS) and Broadband Ultrasound Attenuation (BUA) determined by longitudinal measurements in a group of men and women aged 55 years or over and compared them to the cross-sectional estimated rate of change.

Material and methods

Population

The Rotterdam Study is a prospective cohort study of people aged 55 years or over; its intent is to investigate the incidence of and the risk factors for chronic disabling diseases. Its rationale and design have been described previously.[9] All 10,275 inhabitants, aged 55 years or over of a district in Rotterdam, The Netherlands, were invited to participate in this

study. The study at baseline consisted of an initial home interview by a trained research assistant and a series of medical examinations during two visits to the research centre. The study has been approved by the Medical Ethics Committee of the Erasmus University and written informed consent has been obtained from all participants. The overall response rate for the Rotterdam Study was 78%.

Measurements

Since September 1992, ultrasound measurements have formed part of the Rotterdam Study protocol. Follow-up ultrasound measurements were performed between March and December 1994. In the present study, longitudinal changes in ultrasound measurements of all 543 subjects (224 men, 319 women) on which we had follow-up ultrasound data will be described.

SOS and BUA at the right heel were measured using a Lunar Achilles Ultrasound bone densitometer. The system consists of a water tank containing two broadband ultrasonic transducers. The heel is placed in the water bath and after scanning, the SOS and net attenuation are calculated by a computer program, correcting for the influence of water. The coefficient of variation, calculated from 13 cohort members scanned twice on the same day, was 0.5% for SOS and 2.3% for BUA. The standardized coefficient of variation, defined as the coefficient of variation divided by the ratio of the range (5 to 95%) over the mean of the measurement[10] was 6.0% for SOS and 6.1% for BUA.

Calibration was performed daily. During follow-up there was an upward trend in the calibration values due to a slight narrowing of the distance between the transducers. However, after calibration no drift was detected in the ultrasound parameters of a phantom which was regularly scanned at room temperature. This indicates that the daily calibration corrected accurately for this narrowing in the distance between the transducers.

Data analysis

An annual change in SOS and BUA was calculated by subtracting the second measurements from the first and dividing the result by the follow-

up time in years. A percentual annual change was calculated by dividing the above results by the average of the first and the second measurement (for SOS after subtraction of 1400 m/s of initial value).[3] Linear regression analysis was performed to estimate an effect of age and body mass index on longitudinal rates of changes.

We calculated the percentage of subjects that had an absolute difference between the first and the second measurement larger than two standard deviations of the difference of two measurements performed on the same day (for SOS > 15.3 m/s and for BUA > 5.06 dB/MHz). This was done to estimate the percentage of subjects with an absolute rate of change above that which could easily be accounted for by measurement error. Using a similar approach, the follow-up time necessary to state that the direction of a change per year in an individual can not be accounted for by measurement error was calculated. In other words, how long will it take, for various rates of change, to get at least a difference between two measurements of 15.3 m/s for SOS and 5.06 dB/MHz for BUA. For reasons of simplicity we did not take into account in the analysis that the rates of change may vary with time and that the degree of measurement error may depend on the level of the parameter studied.

Total variability in the rates of change is the sum of true variability and variability due to measurement error ($Var_{tot} = Var_{true} + Var_{error}$). By dividing the measurement error variability by the total variability we estimated the percentage of the variability that could be explained by measurement error (% due to error = (Var_{error}/Var_{tot}) *100).

All data are presented with the 95% confidence interval between parentheses. Negative values for mean change represent loss.

Results

The characteristics of the study population are listed in Table 1. Subjects in the follow-up study of ultrasound parameters were approximately 4 years younger than the total population of the Rotterdam Study. They were less likely to report a history of non-vertebral fractures in the preceding 5 years and they were less disabled.[3] Median follow-up time between the first and second ultrasound measurements was 1.4 year

(range 1.0 to 2.0 years). Follow-up time was similar for men and women. For SOS there was a significant and substantial decline per year in both sexes of -2.5 m/s (Table 1). There was no difference in the rates of change between men and women.

For BUA, the longitudinally estimated rate of change was not significantly different from zero for both men and women. Both for SOS and BUA the longitudinally estimated rates of loss differed considerably from those estimated cross-sectionally, most pronounced for SOS in men (Table 1).

Table 1: Baseline and follow-up characteristics

	Men (N=224)		Women (N=323)	
	mean	95% CI	mean	95% CI
Age (years)	66.5	65.5 to 67.5	67.3	66.4 to 68.3
Body mass index (kg/m^2)	25.7	25.3 to 26.0	26.5	26.1 to 26.9
SOS-1 (m/s)	1536	1532 to 1540	1515	1511 to 1518
SOS-2 (m/s)	1532	1528 to 1537	1511	1508 to 1515
Δ-SOS (m/s/year)	-2.5	-4.0 to -1.1	-2.5	-3.6 to -1.4
% Δ-SOS (%/year)	-2.1	-3.3 to -1.0	-2.5	-3.5 to -1.5
cross-sectional (m/s/year)	-0.7	-1.2 to -0.1	-1.6	-1.9 to -1.2
BUA-1 (dB/MHz)	116.5	115.1 to 117.9	106.0	104.7 to 107.3
BUA-2 (dB/MHz)	116.6	115.1 to 118.0	106.0	104.7 to 107.3
Δ-BUA (dB/MHz/year)	0.11	-0.64 to 0.87	0.05	-0.67 to 0.77
% Δ-BUA (%/year)	0.08	-0.58 to 0.74	0.04	-0.68 to 0.76
cross-sectional (dB/MHz/year)	-0.24	-0.43 to -0.06	-0.34	-0.49 to -0.20

In men, but not in women, the rate of change tended to increase with age (figure 1). This trend was statistically significant in men if in the analysis subjects older than 80 (which are more likely to be subject to selection bias) were excluded (men: β=-0.26 m/s/year^2 (-0.50 to -0.02), women: β=-0.06 m/s/year^2 (-0.25 to 0.12)). Rates of change for BUA were not

significantly influenced by age in either sex, while body mass index did not significantly influence the rates of change for neither SOS nor BUA.

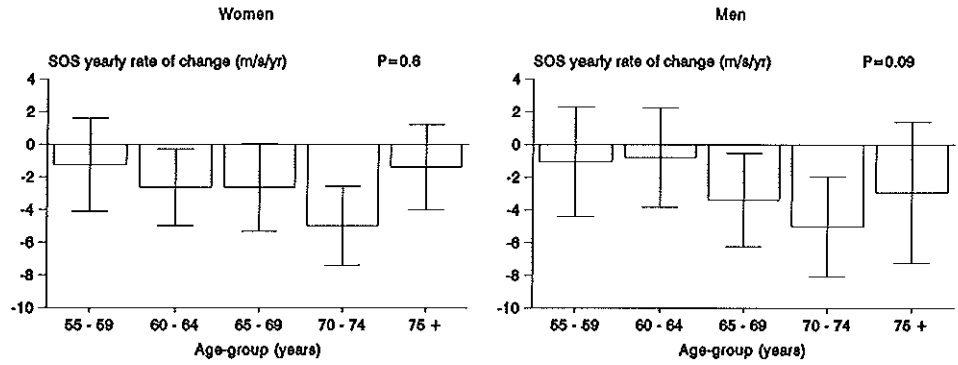

Figure 1: Mean rate of change per year (95% CI) for SOS according to age-group in women and men. P-values are for linear trends.

Both for SOS and BUA there was a substantial variation in the rate of change. With a median follow-up time of 1.4 years, approximately 27% of this variation in rates of change in SOS could be explained by measurement error. For BUA this percentage was 9%. Table 2 shows for SOS and BUA the percentage of subjects that had changes more than could be accounted for by measurement error.

Table 2: Percentage of subjects with changes larger than attributable to measurement error alone

	SOS decrease	SOS increase	BUA decrease	BUA increase
Men	16.5 %	10.3 %	13.8 %	14.7 %
Women	13.8 %	7.8 %	19.4 %	19.4 %

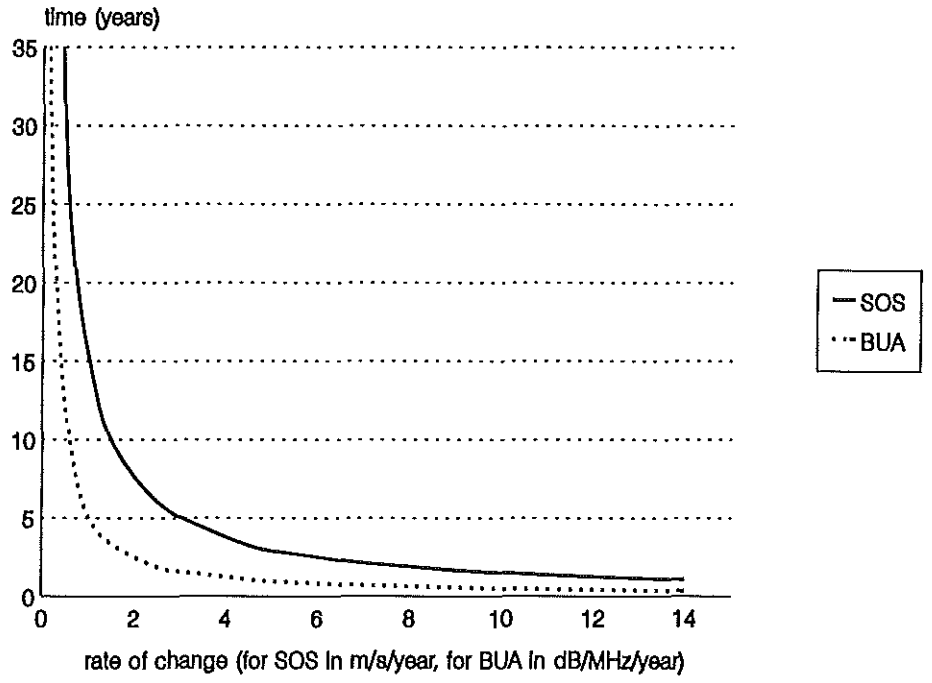

Figure 2: Number of years necessary to state that the direction of a change in an individual is not attributable to measurement error. Curves are estimated using a coefficient of variation of 0.5% for SOS and 2.3% for BUA with a mean value for SOS of 1530 m/s and a mean value for BUA of 110 dB/MHz.

Figure 2 shows the estimated number of years necessary to conclude that the direction of a change in an individual is not attributable to measurement error. Although the direction of the change is unlikely to be due to measurement error for any change at the right hand side of the curve, the exact magnitude of the change is still uncertain. For instance, if we find, after a follow-up of 4 years that a subject has a rate of loss of 10 m/s/year in SOS, this may easily vary between 6 and 14 m/s/year ((rate of loss per year * follow-up time $\pm$ 15.3)/follow-up time). With increasing interval time this uncertainty will become smaller. Figure 2 also shows the

relative value of a coefficient of variation, or rather its dependence on the minimum level of the parameter. Although the coefficient of variation is smaller for SOS than for BUA, it will take longer to conclude that a change in a certain direction is true for SOS than for BUA. For this reason, it is better to use the standardized coefficient of variation while comparing precision of various parameters.

Discussion

In the present study, a significant decline in SOS after a relatively short follow-up of 1.4 year in both men and women was found. For BUA no significant loss could be observed. As far as we know, this is the first study showing longitudinal data on annual changes in ultrasound parameters. Our findings may have been hampered by the fact that the subjects studied belong to a relatively healthy sample of the Rotterdam Study cohort. Nonetheless, the decrease in SOS per year was substantial in both sexes, and may, therefore, even be higher in the overall population.

Cross-sectional studies show that hip fracture risk more than doubles per standard deviation decrease in SOS.[11] A reduction in SOS of the magnitude found may therefore be associated with an increase of around 15% in fracture risk per year. This appears to be of the same magnitude as the observed increase in hip fracture risk per year in the Netherlands.[12]

Especially in men, the longitudinal changes in SOS are larger than those estimated from a cross-sectional analysis. Selection and survival bias or cohort effects may explain a difference between cross-sectional and longitudinal results. Non response and mortality in the oldest age group will tend to reduce the apparent effect of age on SOS. Furthermore, with an increasing rate of change with age, especially this elderly group of subjects influences the mean rate of loss in a longitudinal analysis.

One would have expected to find a similarly higher rate of change in BUA as compared to cross-sectional data. Instead, there even was a tendency to an increase in BUA in time. The reason for this discrepancy

is unclear. SOS and BUA reflect different aspects of bone. SOS is supposed to be related to elastic properties of bone, whereas BUA is thought to reflect structural aspects of bone tissue.[2]

The age dependent increase in the rate of change of SOS in men is in agreement with the increased rate of change in bone mineral density with age that has been reported by Jones et al.[8] However, we did not find a significant increase in the rate of change in ultrasound parameters with age in women. One has to bear in mind that although rates of change were assessed longitudinally, the analysis of rates of change with age is in fact cross-sectional, with the potential hazard of selection bias. Nevertheless, it is unlikely that selection bias will induce an increase in rates of change with age. Rather, selection of relatively healthy subjects in the older age groups, as may have been the case in the present study, will tend to decrease the rate of change with age.

The difference in age-related rates of bone loss between men and women might be explained as follows. As can be seen in figure 2, the increase in the rate of change with age in men appears to start around the age of 65, which is approximately the time that men retire in the Netherlands. Retirement might be related to a change in loading of the skeleton. We have recently shown that physical activity is especially an important determinant of SOS in men.[3] In contrast, women in this age group tend not to have outdoor jobs. Therefore, it is less likely that they will change their level of physical activity.

Short term precision of ultrasound measurements is reported to be very good.[13,14] Nevertheless, if the changes studied are small, even a measurement error of the size reported for SOS may seriously hamper the use of the measurement in the follow-up of individual patients. This is illustrated by the considerable part of the variation in the rates of change that can be attributed to measurement error. If follow-up time is short it will be almost impossible to judge what part of an observed change between two measurements is real and what part is due to imprecision error.[15,16] A way to reduce this problem would be to repeat the measurements on the same day and take the average of the

measurements. Overall, the average of the measurement will be closer to the true value and measurement error will thereby be smaller. With increasing follow-up time, variability due to imprecision relative to true variability will become smaller. We may, therefore, be able to demonstrate small changes per year in an individual if the interval between two measurements is long, as was illustrated in figure 2.

Taken together, for SOS, but not for BUA there is a significant change per year. Furthermore, in men there is an increasing rate of change in SOS with increasing age. Finally, the magnitude of the yearly changes in comparison with the precision of the measurements, may limit the use of ultrasound measurements as a follow-up tool in individuals rather than in populations.

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CHAPTER 2.4

THE ASSOCIATION BETWEEN ULTRASOUND MEASUREMENT OF BONE AND RECENT NON VERTEBRAL FRACTURES

Introduction

In recent years, identification of subjects at increased risk for fractures has received considerable attention.[1] Low bone mass has been shown to be associated with an increased fracture risk. At present, Dual energy X-ray Absorptiometry (DXA) is the most commonly used technique for measuring bone mass. Bone quality is an other factor besides bone mass that contributes to bone strength and thereby to fracture risk.[2]

Recently, ultrasound measurement of bone has been introduced. It has been postulated that the speed (speed of sound (SOS)) and the attenuation (Broadband Ultrasound Attenuation (BUA)) of ultrasound at various frequencies may give insight in both quantitative as well as qualitative aspects of bone. SOS may provide information about density and elasticity of bone. BUA on the other hand may be associated with bone density as well as with structural aspects such as connectivity.[4]

Low costs, absence of ionizing radiation and the fact that ultrasound measurement is easy to use, have been mentioned as valuable benefits.[5] Yet, there is little evidence that ultrasound measurement may be helpful in identifying subjects at risk for fracture.

We examined, in the open population, the association between ultrasound parameters of the calcaneus and recent non-vertebral fractures. In addition, we examined whether ultrasound parameters add to the predictive value of bone mineral density measurements of the femoral neck.

Methods

The Rotterdam Study is a prospective follow-up study of people aged 55 years or over to investigate the incidence of, and risk factors for chronic disabling diseases. Rationale and design have been described previously.[6,7] Invited to participate in the study were all 10,275 inhabitants (6325

women), aged 55 years or over of a district in Rotterdam, The Netherlands. The study consisted of an initial home interview by a trained research assistant and a series of medical examinations during two visits at the research centre. The study has been approved by the Medical Ethics Committee of the Erasmus University and written informed consent has been obtained from all participants. During the interview, all subjects were asked whether they had had a non-vertebral fracture within the preceding 5 years.

For the evaluation of osteoporosis, Dual energy X-ray Absorptiometry (DXA) forms part of the routine examination at the research centre.[8]

Since September 1992, ultrasound measurements form part of the Rotterdam Study protocol. In this cross-sectional study we present the data of 1,421 persons (790 women and 631 men) on whom we performed ultrasound measurements of the calcaneus.

Speed of Sound (SOS) and Broadband Ultrasound Attenuation (BUA) at the right heel were measured using a Lunar Achilles Ultrasound Bone Densitometer (Lunar Radiation Corporation, Madison, WI). The system consists of a water tank, containing two broadband ultrasonic transducers. The heel is placed in the water bath and after scanning, the SOS and net attenuation are calculated by a computer program, correcting for the influence of water.

Bone mineral density measurements of the femoral neck were performed using a Lunar DPX-L densitometer.[8]

Statistical analyses

Mean values for ultrasound parameters were compared between subjects with and without a recent non-vertebral fracture. Values for SOS, BUA and bone mineral density of the femoral neck were expressed as z-scores (number of standard deviations below or above the age-standardized average value).

Subsequently we calculated the relative risk for fracture per tertile SOS and BUA after adjusting for age and body mass index. This was done for men and women separately. We subdivided in fractures of the upper and lower limb. While calculating relative risks for fractures of the upper limb,

subjects with a fracture of the lower limb were excluded. Additionally, we examined within tertiles of bone density whether there was a trend in the frequency of fractures within tertiles of SOS and BUA. All results are presented with the 95% confidence interval between parentheses.

Results

Baseline characteristics for the study population are shown in table 1.

Table 1: Baseline characteristics of the study population

	Men		Women	
	mean	SD	mean	SD
N	631		790	
age (years)	65.9	7.4	66.4	7.3
height (cm)	176.1	6.7	162.1	6.1
weight (kg)	78.8	10.4	70.0	10.5
body mass index (kg/m^2)	25.5	2.9	26.6	3.8
SOS (m/s)*	1541	37	1518	31
BUA (dB/MHz)*	117.7	11.6	107.8	11.9
Bone mineral density (g/cm^2)*	0.88	0.13	0.82	0.13

* after adjustment for age and body mass index

After adjustment for age and body mass index, men had higher values for SOS and BUA than women. Women reported more fractures in the preceding 5 years than men. Especially fractures of the upper limb were more frequently reported by women (table 2).

After correction for age and body mass index, subjects with a fracture history had significantly lower values for SOS (men: -17.1 m/s (-28.0 to -6.3), women: -10.6 m/s (-16.8 to -4.3)) and for BUA (men: -4.4 dB/MHz (-7.9 to -1.0), women: -4.6 dB/MHz (-7.1 to -2.2)). However, as illustrated in figure 1, there was almost complete overlap in the values between subjects with and without a fracture.

Subjects with a value for SOS and BUA in the lowest tertile reported

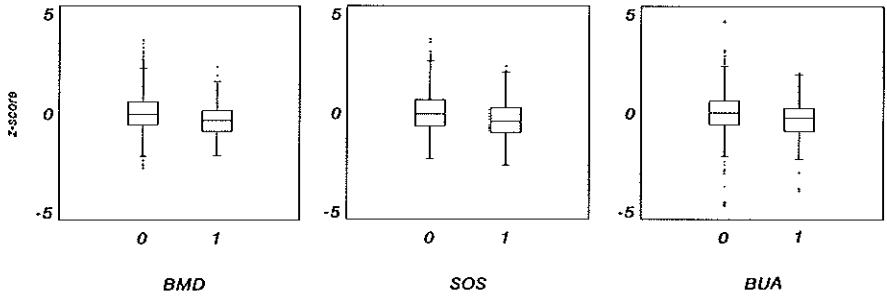

Figure 1: Box- and Whiskerplots of bone mineral density (BMD), SOS and BUA (all expressed as z-scores) in subjects with (1) and without (0) a fracture. The upper and lower lines of each box represent 25th and 75th percentile, respectively. The line in the middle of the box represent the 50th percentile (median). The vertical bars extend to the 2.5th (lower) and 97.5th (upper) percentile. Outliers are shown as a dot.

approximately 2 to 3 times more often a fracture in the preceding 5 years than subjects with a value for SOS and BUA in the highest tertile (table 3). In comparison, men with a bone mineral density of the femoral neck within the lowest tertile reported 3.0 times (1.3 to 6.7) and women 2.1 times (1.2 to 3.8) more fractures than those with a value in the highest tertile.

Within tertiles of bone mineral density, subjects with a value for SOS in the lowest tertile invariably reported a higher frequency of fractures than those with a values for SOS in the middle or highest tertile (figure 2), even after correction for residual variation in bone mineral density between tertiles (P for trend = 0.02). For BUA a similar, although less pronounced trend was visible (figure 2) (P for trend = 0.02).

Discussion

In the present study, we found a 2 to 3 times higher frequency of self reported fractures amongst subjects with a value for speed of sound (SOS) and Broadband Ultrasound Attenuation (BUA) in the lowest tertile compared to those with a value within the highest tertile. Thereby, ultrasound parameters had a similar "predictive value" as bone mineral density of the femoral neck. Furthermore, within tertiles of bone mineral

Table 2: Number of fractures by sex

	Men		Women	
	N	%	N	%
fracture in preceding 5 years	46	7.3	97	12.5
fracture upper extremities	29	4.6	67	8.6
upper arm	9		16	
wrist	10		52	
hand	12		7	
fracture lower extremities	20	3.2	35	4.5
hip	2		6	
upper leg	2		5	
lower leg	6		5	
ankle/foot	13		19	

density there was a trend in which subjects with lower values for ultrasound parameters reported more fractures.

Self reporting has been shown to be a reliable method to get information about fracture frequency.[9,10] The fact that the ultrasound measurements were performed after the fracture may have influenced the results. Fracture related immobility is associated with a considerable decrease in bone mineral density.[11] Furthermore, immobility has been shown to influence ultrasound parameters.[12] Especially for fractures of the lower limb, lower values for SOS and BUA might therefore be a consequence rather than a causal factor for fractures. Fractures of the upper limb will have no or a less pronounced effect on mobility and therefore on ultrasound parameters of the calcaneus. Finding similar relative risks for fractures of the upper and lower limb suggests that changes in mobility due to the fracture has not influenced our results considerably.

Finding a pattern in which, within tertiles of bone mineral density, those with lower values for ultrasound parameters report a higher fracture frequency suggests that ultrasound measurements may have additional

value. As mentioned previously, they might provide information about qualitative aspects of bone tissue. Higher elasticity and a more intens trabecular connectivity appears to be associated with a lower fracture risk. Our results corroborate the findings of two non-population based cross-sectional studies, that examined the association between ultrasound parameters and hip and vertebral fractures.[13,14]

Despite the fact that subjects with lower values for SOS and BUA, reported a fracture more frequently, there was almost complete overlap in the values between subjects with and without a fracture. A similar overlap was found for bone mineral density of the proximal femur and has repeatedly been mentioned in medical literature.[15,16] This underlines once again the importance of other factors in the occurrence of fractures, such as trauma.

Conclusion

The results of this study show that subjects with lower values for SOS and BUA are at increased risk for fractures. Furthermore, the combination of ultrasound and bone mineral density measurements appears to have additional value as compared to either of them separately.

Table 3a: Association between SOS and recent non-vertebral fractures

	T1	T2
men		
fracture upper or lower	2.0 (1.0 - 4.1)	0.8 (0.3 - 1.8)
fracture upper	2.2 (0.9 - 5.3)	0.8 (0.3 - 2.2)
fracture lower extremities	2.3 (0.8 - 6.7)	0.8 (0.2 - 3.0)
women		
fracture upper or lower	3.0 (1.6 - 5.5)	2.4 (1.3 - 4.4)
fracture upper	3.0 (1.1 - 5.0)	2.4 (1.1 - 5.0)
fracture lower extremities	3.4 (1.2 - 9.1)	2.4 (0.9 - 6.3)

Table 3b: Association between BUA and recent non-vertebral fractures

	T1	T2
men		
fracture upper or lower	2.1 (1.0 - 4.5)	1.3 (0.6 - 3.0)
fracture upper	3.0 (1.0 - 8.5)	2.3 (0.8 - 6.8)
fracture lower extremities	1.9 (0.7 - 5.2)	0.5 (0.1 - 2.0)
women		
fracture upper or lower	2.7 (1.5 - 4.8)	1.7 (0.9 - 3.0)
fracture upper	2.6 (1.3 - 5.2)	1.8 (0.9 - 3.6)
fracture lower extremities	2.4 (1.0 - 5.8)	1.4 (0.6 - 3.6)

Expressed is the risk for the lowest (T1) and second (T2) tertile relative to the risk for the upper tertile (T3), where the risk in the upper tertile is regarded to be 1.

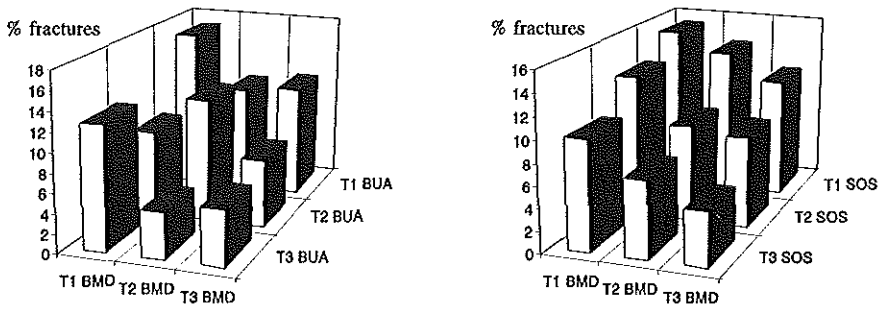

Figure 2: Frequency of fractures by the combination of tertiles of bone mineral density (BMD) and BUA and SOS, respectively. For all measurement T1 is the lowest and T3 is the highest tertile.

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CHAPTER 3.1

BONE DENSITY IN NON-INSULIN-DEPENDENT DIABETES MELLITUS: THE ROTTERDAM STUDY

Introduction

Low bone mineral density is often mentioned as a complication of diabetes mellitus. For insulin dependent diabetes mellitus most studies point in this direction, especially in patients with poor metabolic control.[1] However, conflicting findings have been reported in patients with non-insulin-dependent diabetes mellitus, where some authors reported elevated,[2-6] some decreased [7-9] and others unaltered [10-12] bone density. Heterogeneity of the cases (in- or exclusion of patients on insulin), differences in measurement techniques and region of interest, the use of an inappropriate group of subjects for comparison and the absence of control for several possible confounders may explain the dissimilarities in the results. Another reason for the conflicting results may be that several previous studies were restricted to patients with already diagnosed non-insulin-dependent diabetes mellitus. To address the effect of non-insulin-dependent diabetes mellitus on bone density, studies should preferably include untreated non-insulin-dependent diabetes mellitus cases, because studies not including previously undiagnosed patients may address the effect of antidiabetic treatment rather than the effect of non-insulin-dependent diabetes mellitus. In addition, to our knowledge no study has been conducted that has linked the effect of non-insulin-dependent diabetes mellitus on bone mineral density to the occurrence of fractures. We investigated the associations of non-insulin-dependent diabetes mellitus, bone mineral density and fractures in 5,931 unselected men and women, aged 55 years or over, who were examined in the population-based Rotterdam Study.

Methods

Study population

The Rotterdam Study is a prospective follow-up study of people aged 55

years or over to investigate the incidence of, and risk factors for chronic disabling diseases. Rationale and design have been described previously.[13] Invited to participate in the study were all 10,275 inhabitants, aged 55 years or over of a district in Rotterdam, The Netherlands. The study consisted of an initial home interview by a trained research assistant and a series of medical examinations during two visits at the research center. The study has been approved by the Medical Ethics Committee of the Erasmus University and written informed consent has been obtained from all participants. For the present study, institutionalized people (1,114 persons) were not eligible for examination at the research center. Interview data were collected for 77% of the remaining eligible persons. The overall response rate for the Rotterdam Study was 78%. Bone mineral density was measured in 2,481 men and 3,450 women. Scan data were not available for 563 persons. This was mainly due to the fact that they took part in a pilot study (N=338), in which no scans were made. No scanning because of maintenance procedures accounted for the remaining missing scan data.

Measurements

During the home interview, a medical history was obtained by an interviewer administered questionnaire and the medication use was validated by examination of pills. To estimate the frequency of fractures, information was asked about the number and type of fractures during the preceding 5 years.

At the research center all participants, except for those using antidiabetic medication, were given a non-fasting 37.5% oral glucose solution (75 g of glucose). Before and after two hours, venous glucose levels were determined. The diagnosis of non-insulin-dependent diabetes mellitus was made if one or both glucose levels were at least 11.1 mmol/L, or if the participant used antidiabetic medication.

Bone mineral density was measured at the lumbar spine and proximal femur using a Lunar DPX-L densitometer (Lunar Corp. Madison WI). Standard positioning was used with anterior-posterior scans of the lumbar spine and proximal femur. As for the proximal femur, the right side was

scanned unless there was a history of prosthesis implantation, in which case the left side was chosen. The in vivo coefficient of variation for the bone mineral density, calculated from 12 participants scanned twice on the same day, was 3.2% in the femoral neck, 3.1% in Ward's triangle, 2.5% in the greater trochanter and 0.9% in the lumbar spine. Quality assurance, including calibration, was performed routinely every morning, using the standard provided by the manufacturer.

Height and weight were measured with participants in indoor clothing and without shoes. Body mass index (weight/height^2 (kg/m^2)) was calculated as a measure of obesity. The waist-hip ratio was used as an indicator of central obesity. Measurement of the waist circumference was done midway between the lower rib margin and the iliac crest. Measurement of the hip circumference was done at the point resulting in the maximum circumference over the buttocks with the tape held horizontally.[14]

Osteoarthritis was scored on standard X-rays of the hips in a random sub-sample of 1,136 men and 1,697 women, according to Kellgren.[15] Participants were classified as having osteoarthritis with a Kellgren score of at least 2 at the right hip, or when they had a hip prosthesis due to osteoarthritis.

For current and past smokers, the number of pack-years was calculated as the average number of packs of cigarettes smoked per day times the total years of smoking. Falling was recorded as never, less than once per month, less than once per week but more than once per month and more than once per week. However, in the analysis falling was regarded as a dichotomous variable. Impairment in activities of daily living (ADL) was assessed by using a questionnaire modified from the Stanford Health Assessment Questionnaire.[16] As it is in particular impairment in the function of the lower extremities that will have an influence on bone mineral density, a disability index was calculated for the lower limbs by calculating the mean of arising, walking, bending and getting in and out of a car. Each of these four categories has a score ranging from zero to three: zero indicating no impairment, and three indicating inability to perform. Disability was then categorized in absent (0), mild (>0-1) and

severe (>1).

Data analysis

Men and women were analyzed separately. Newly diagnosed patients with non-insulin-dependent diabetes mellitus and those already treated were analyzed combined and separately. Furthermore, analyses were performed with and without patients on insulin treatment. A chi-square test statistic was calculated for differences in proportions. For continuous variables a t-test was used. Multiple linear regression analysis was used to evaluate the association of non-insulin-dependent diabetes mellitus with bone mineral density and to control for possible confounders. Effect modification by age was studied by including an interaction term for age and the presence of non-insulin-dependent diabetes mellitus in the model.

To examine selective non-response to the center visit of subjects with diabetes, we calculated the age-adjusted prevalence of antidiabetic medication use in participants with interview data only, and compared this prevalence with that in participants who had both had the interview and the examination at the research center. In women there appeared to be no selective non-response as the age adjusted prevalence of current antidiabetic medication use was similar for those with and without a center visit (5.0% vs 4.9%, P=0.9). In men, however, this prevalence was significantly higher in participants without a center visit (4.2% vs 10.0%, P=0.0001).

Logistic regression was used to study the association between non-insulin-dependent diabetes mellitus and a history of a fracture (irrespective of cause) in the preceding 5 years. Vertebral fractures were excluded from the analyses, because participants were commonly unaware of this type of fracture. All other self reported fractures were included in the analyses. The association of diabetes and fracture frequency was expressed as an odds ratio with the 95% confidence interval. For all analyses two-sided p-values were calculated. Analysis of variance and covariance was used to estimate multiple adjusted mean values.

Results

Among the 2,481 men and 3,450 women with bone mineral density measurements, 243 men and 335 women were classified as having non-insulin-dependent diabetes mellitus. 94 men and 140 women were already treated for diabetes with antidiabetic medication. Table 1 shows the characteristics of the men and women with and without non-insulin-dependent diabetes mellitus. As expected, subjects with non-insulin-dependent diabetes mellitus were older than subjects without diabetes mellitus. The body mass index of women, but not of men, with non-insulin-dependent diabetes mellitus was substantially higher compared to those without diabetes. Among subjects with non-insulin-dependent diabetes mellitus were more current users of loop diuretics. Furthermore, among women with non-insulin-dependent diabetes mellitus there was a higher percentage of current thiazide users. Serum creatinine was substantially higher in diabetic men but not in women, as compared to subjects without diabetes. Both men and women with non-insulin-dependent diabetes mellitus reported a higher frequency of impairment in function of the lower extremities. Although the difference was not significant, men with non-insulin-dependent diabetes mellitus tended to fall more often than men without diabetes.

Table 2 shows bone mineral density by diabetic status in men and women. After adjustment for age and body mass index, both men and women with non-insulin-dependent diabetes mellitus had substantially higher bone mineral density values at all sites compared to participants without diabetes. The difference in bone mineral density amounted to approximately 3% and was similar for men and women. There was no interaction between the effect of diabetes and age. Adjustment for all potential confounders measured, including age, body mass index, waist/hip ratio, current use of thiazides, loop diuretics and estrogens, smoking, serum creatinine and impairment in ADL, did not substantially alter our findings (Table 2). The subgroup for whom pelvic x-rays were available had similar bone mineral density values as compared to the total group. Age and body mass adjusted prevalence of osteoarthritis was about

11.5% and similar for subjects with and without non-insulin-dependent diabetes mellitus. Subjects with osteoarthritis had an approximately 3% higher boner mineral density at the proximal femur. After further adjustment for osteoarthritis, subjects with non-insulin-dependent diabetes mellitus in this subset still had higher bone mineral density at all foursites compared to subjects with a normal glucose tolerance.

In men, the higher bone mineral density was present at all four sites measured, both for subjects with newly diagnosed and already treated non-insulin-dependent diabetes mellitus. For instance, bone mineral density at the femoral neck was 0.873 g/cm^2 for non diabetics, 0.898 g/cm^2 for newly diagnosed and 0.908 g/cm^2 for subjects already treated. Women on oral antidiabetic treatment did not differ from subjects without diabetes in bone mineral density measured at Ward's triangle, but did have higher values at the three other sites. Women with newly diagnosed non-insulin-dependent diabetes mellitus had higher values at all four sites measured. Patients treated with insulin had no substantially different bone mineral density compared to controls (men: femoral neck bone mineral density 0.878 g/cm^2, women: femoral neck bone mineral density 0.796 g/cm^2), but this group comprised only 25 men and 31 women.

The difference in the percentage of subjects with at least one reported non-vertebral fracture in the preceding 5 years between those with and without non-insulin-dependent diabetes mellitus is shown in figure 1. 22 men with non-insulin-dependent diabetes mellitus and 222 men without non-insulin-dependent diabetes mellitus reported to have had at least one fracture in the preceding 5 years. In women, 38 subjects with non-insulin-dependent diabetes mellitus reported at least one fracture compared to 493 subjects without non-insulin-dependent diabetes mellitus. For men, ankle and foot fractures were reported most frequently (33%), followed by fractures of the wrist and forearm (32%). For women wrist and forearm fractures were the most often reported fracture types (60%), followed by ankle and foot fractures (27%). After adjustment for age and body mass index, women with non-insulin-dependent diabetes mellitus had a lower frequency of fractures (odds ratio 0.63 (0.44-0.90)). Results

Table 1: Characteristics of men and women with and without non-insulin-dependent diabetes mellitus (NIDDM)

	Men				Women			
	NIDDM	No NIDDM	difference	95% CI	NIDDM	No NIDDM	difference	95% CI
Number	243	2238			335	3115		
Age (years)	69.6	67.3	2.3	1.3 - 3.3	72.3	68.0	4.3	3.4 - 5.2
Body mass index (kg/m^2)	25.8	25.7	0.1	-0.3 - 0.5	27.8	26.6	1.2	0.8 - 1.7
Waist/hip ratio	0.97	0.96	0.01	0.00 - 0.02	0.90	0.86	0.04	0.03 - 0.05
Age of menopause (years)					49.0	48.8	0.2	-0.4 - 0.8
Current use of thiazides (%)*	4.7	5.0	0.3	-2.6 - 3.2	14.8	10.4	4.4	0.8 - 8.0
Current use of loop-diuretics (%)*	8.7	2.6	6.1	3.7 - 8.5	7.2	3.8	3.4	1.1 - 5.7
Current use of estrogens (%)*					2.3	2.8	0.5	-1.1 - 2.4
Current smokers (%)*	31.4	29.0	2.4	-3.8 - 8.6	19.6	19.3	0.3	-4.3 - 4.9
Pack years in smokers	37.6	32.5	5.1	1.8 - 8.4	22.9	21.3	1.6	-0.6 - 3.8
Serum creatinine (µmol/L) †	96.2	89.3	6.9	4.5 - 9.3	79.0	77.3	1.7	-0.6 - 4.0
Severe disability (%)†	8.2	5.0	3.2	0.2 - 6.2	15.8	10.5	5.3	1.8 - 8.8
Falling (%)†	12.1	9.1	3.0	-0.9 - 6.9	18.3	20.1	1.7	-2.9 - 6.5

Values are means.
* age adjusted
† age and body mass index adjusted

Table 2: Mean bone mineral density (g/cm^2) at the lumbar spine and proximal femur in men and women with and without non-insulin-dependent diabetes mellitus

	Men				Women			
	NIDDM	no NIDDM			NIDDM	no NIDDM		
Site	mean	mean	% difference	95% CI	mean	mean	% difference	95% CI
Adjusted for age and body mass index								
L2-L4	1.196	1.161	3.0	0.7 - 5.3	1.069	1.033	3.5	1.5 - 5.5
Femoral neck	0.901	0.873	3.2	0.9 - 5.5	0.831	0.808	2.8	1.2 - 4.5
Ward's triangle	0.749	0.725	3.3	0.5 - 6.1	0.694	0.673	3.0	1.0 - 5.2
Trochanter	0.869	0.845	2.8	0.5 - 5.2	0.743	0.723	2.8	0.9 - 4.7
Site								
Adjusted for a full set of measured confounders								
L2-L4	1.200	1.162	3.3	0.9 - 5.6	1.068	1.032	3.5	1.5 - 5.5
Femoral neck	0.906	0.873	3.8	1.5 - 6.1	0.832	0.808	3.0	1.3 - 4.7
Ward's triangle	0.754	0.725	4.0	1.2 - 6.8	0.697	0.673	3.6	0.6 - 6.6
Trochanter	0.874	0.846	3.3	1.0 - 5.7	0.744	0.723	2.9	1.0 - 4.8

* P value for difference

† Including: age, body mass index, waist/hip ratio, use of benzothiadiazides, use of loop-diuretics, use of estrogen, smoking, renal function and impairment in activities of daily living

were similar for newly diagnosed non-insulin-dependent diabetes mellitus patients as compared to those already treated (odds ratio 0.62 (0.35-1.10)). Adding bone mineral density to the model did not substantially influence the odds ratio. Although the percentage of men who reported a non-vertebral fracture was slightly lower for those who had non-insulin-dependent diabetes mellitus, after adjustment no significantly different fracture frequency remained compared to men without diabetes (odds ratio 0.96 (0.60-1.52)).

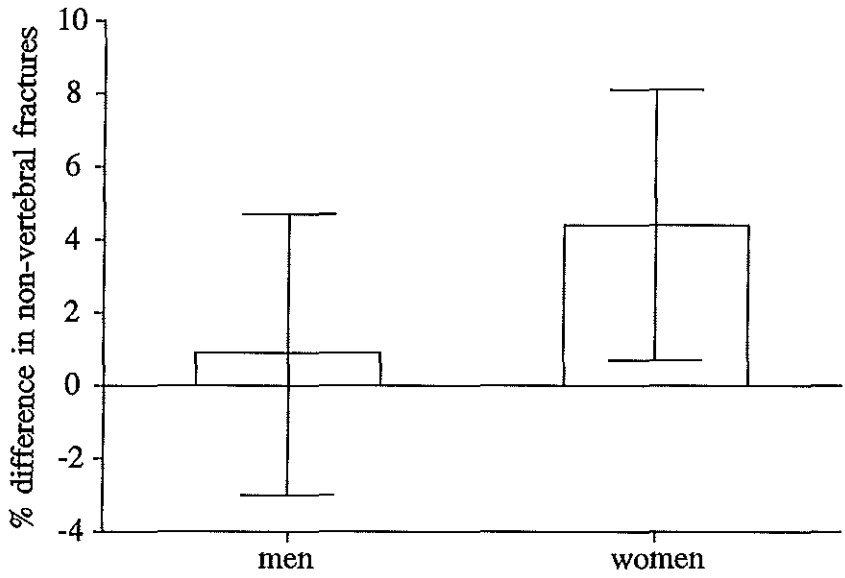

Figure 1: Difference in the percentage of subjects with at least one reported non-vertebral fracture in the preceding 5 years between those with and without NIDDM and the 95% CI for the difference.

Discussion

This cross-sectional study provides evidence for an association between

non-insulin-dependent diabetes mellitus and elevated bone density at the proximal femur and lumbar spine in both men and women. The observed difference in bone mineral density could not be explained by differences in age, body mass index, current use of medication known to influence bone metabolism, smoking, osteoarthritis, renal function or impairment in ADL, factors all known to influence bone mass. In addition, women with non-insulin-dependent diabetes mellitus reported a lower frequency of fractures in the preceding 5 years.

In this large epidemiologic study we were not able to perform a fasting oral glucose tolerance test. Therefore, in accordance with the guidelines of the WHO Study Group on diabetes mellitus for epidemiological studies, we used the 2-hours value.[17] The prevalence values obtained for non-insulin-dependent diabetes mellitus are consistent with what is known about the prevalence in the Netherlands.[18] Nevertheless, it remains possible that some patients were misclassified, most likely as false negatives. However, rather than inducing a difference this would have diluted the association of non-insulin-dependent diabetes mellitus and bone mineral density.

We found a higher percentage of known diabetics among the non-attending men compared to those that did visit the center. It is possible that diabetic patients that do not respond have a poorer health and are less active than responders, and therefore have a lower bone mineral density. However, it seems unlikely that this has biased the results. Bias would only have occurred if inactive diabetics were more likely to be non responders than inactive non-diabetics. More importing, selective non-response is unlikely in newly diagnosed diabetics, and we observed similar results if we restricted the analyses to this group. For the same reason it is unlikely that our observations are explained by an increased mortality of osteoporotic diabetic patients.

Aortic calcifications and osteophytes are known to give a spuriously elevated bone mineral density at the lumbar spine. Especially aortic calcifications may be present more often in diabetics as compared to non diabetics. However, this would not explain the elevated bone mineral density at the proximal femur.

The fractures in the preceding 5 years were self-reported. Self-report has shown to be an accurate and reliable method to calculate fracture rates especially for major osteoporotic fractures, including those of the hip, wrist and humerus, at least for time periods up to one year.[19,20] Recall over longer time periods may lead to a certain degree of misclassification. However, this misclassification is unlikely to be systematically different for subjects with or without non-insulin-dependent diabetes mellitus. Furthermore, the frequencies of fractures we found were similar to those observed in previous studies in which the fractures where radiographically verified.[21] The lower frequency of fractures in women with non-insulin-dependent diabetes mellitus, as observed in our study, is in accordance with findings of Heath et al.[22]

Our results differ from several previous studies. For instance, Levin et al [7] found a lower bone mineral density in patients with non-insulin-dependent diabetes mellitus. However, in their group a number of patients with insulin dependent diabetes mellitus might have been included, given the age distribution and the proportion of persons using insulin. Furthermore, their study was not population-based, and not able to estimate the effect in undiagnosed cases. Other investigators found results which are more in agreement with ours.[4,5] In particular, Barrett-Connor et al,[2] in a similar but smaller population based study of elderly subjects, recently reported a higher bone mass in women with non-insulin-dependent diabetes mellitus, but not in men. These investigators suggested that this apparent sex difference may be explained by the greater androgenicity reported in women with hyperglycemic and hyperinsulinemic conditions. However, the similarly elevated bone mineral density we observed in both men and women with non-insulin-dependent diabetes mellitus makes this hypothesis less likely.

The findings of our study are more in support for an anabolic effect of insulin on bone tissue as suggested by Weinstock et al.[11] Non-insulin-dependent diabetes mellitus is preceded by a period of insulin resistance causing hyperinsulinemia before the onset of diabetes.[23] It has been suggested that this insulin resistance is restricted to the effect of insulin on glucose transport.[24] Hyperinsulinemia will still lead to a stimulation of

the unopposed mitogenic and anabolic actions of insulin. Indicative for this mechanism is the finding of already elevated bone mineral density in newly diagnosed diabetics. Ovarian thecal hyperplasia and vascular endothelial proliferation developing in insulin resistant subjects are other examples of the stimulating effect of hyperinsulinemia.[24,25] Insulin may exhibit its action on bone either directly or by binding to the receptor of insulin-like growth factor-I.[26] Interestingly, some,[25,27] but not all[28,29] investigators found a positive association between circulating insulin levels and bone mineral density. An alternative pathway, by which hyperinsulinemia may lead to an increase of bone mineral density, is by its negative effect on sex hormone binding globulin.[30-33] Van Hemert et al[34] found a strong negative correlation between serum sex hormone binding globulin levels and bone mineral density in postmenopausal women. Lower sex hormone binding globulin levels may lead to higher free serum oestradiol and testosterone levels. Subsequently, the higher free sex hormone levels could protect from the age related bone loss that occurs in both men and women.[35,36] Nevertheless, we can only speculate on the mechanism underlying the elevated bone mineral density in non-insulin-dependent diabetes mellitus.

Caution is needed in trying to explain the apparent lower fracture frequency in women with non-insulin-dependent diabetes mellitus, in particular because at least in newly diagnosed diabetic patients fractures occurred before non-insulin-dependent diabetes mellitus became apparent. Bone mineral density is an important determinant of fracture risk,[37] and the higher bone mineral density in women with non-insulin-dependent diabetes mellitus may give rise to their lower reported fracture frequency. Nevertheless, the fact that the "protective effect" remained after correcting for bone mineral density is at least curious. Alternatively, as women with non-insulin-dependent diabetes mellitus are significantly more obese than women with normal glucose tolerance one could also have attributed this to an energy absorbing effect of their fat tissue.[38] However, the effect persisted after adjustment for body mass index. Whether the absence of a "protection" in men is perhaps due to selection or due to the fact that diabetic complications like neuropathy or

retinopathy lead to a more frequent falling and therefore to an increase in fracture frequency needs to be elucidated.

In conclusion, we present evidence for an increased bone mineral density in patients with non-insulin-dependent diabetes mellitus. In women non-insulin-dependent diabetes mellitus appears to be accompanied by a lower frequency of non-vertebral fractures.

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CHAPTER 3.2

BIOCHEMICAL MARKERS OF BONE TURNOVER: AN UPDATE

Introduction

Bone turnover or remodelling in vivo can be studied by histomorphometric, radiokinetic or biochemical methods. Bone histomorphometry on iliac crest biopsy allows both a qualitative and a quantitative assessment of bone turnover at the cell and tissue level. However, this method requires an invasive procedure and is subject to substantial sampling variations in individuals. Therefore, histomorphometry is more useful for the study of pathogenetic or therapeutic mechanisms in groups of patients.

Radiokinetic studies with radioactive calcium isotopes are expensive, time consuming and need simultaneous measurement of external calcium balance to obtain separate measurements of bone resorption and formation.

Given these considerations it is not surprising that the biochemical methods are by far the most commonly used parameters to obtain insight in the process of bone turnover. These so-called biomarkers of bone turnover can be measured in blood and urine samples and can provide independent assessment of bone resorption and formation.

Until recently, the only available markers of bone turnover were serum alkaline phosphatase for monitoring bone formation and urinary hydroxyproline for monitoring bone resorption. Because these assays are not specific for bone and are unable to detect subtle changes in bone turnover (for instance in patients with osteoporosis) much effort has been made to develop new and more specific markers. At present several bone specific markers are available which reflect several aspects of the complex mechanisms of bone formation and resorption. In the present paper the clinical relevance of both the conventional and newly discovered biochemical markers will be discussed. First the organization of bone remodelling will be reviewed, because knowledge of this process is important for a proper interpretation of the biomarkers of bone turnover.

Bone remodelling

Bone remodelling or turnover is a cyclical process, which is occurring dyssynchronously at thousands of sites in the human skeleton at all times. Each cycle is initiated by the recruitment of a team of osteoclasts to a targeted site of the bone surface. A cavity excavated by these osteoclasts is refilled by a subsequently appearing team of osteoblasts. This process is called coupling. Circulating hormones may act on bone cells either directly or indirectly, modulating the synthesis or effects of local growth factors which in turn stimulate or inhibit bone formation or resorption. These bone derived growth factors appear to play an important role in the coupling of bone formation to bone resorption and possibly in pathophysiological processes.[1]

Because of its high surface to volume ratio, many more remodelling sites are to be found per unit volume of trabecular or cancellous bone than cortical bone. Therefore, disorders of remodelling become earlier manifest in trabecular bone than in cortical bone.

The rate of formation and resorption of bone can be assessed by measuring an enzymatic activity related to bone-forming (alkaline phosphatase) or resorbing (acid phosphatase) cells. An alternative is to measure bone matrix components released into the circulation, either after their secretion by osteoblasts (osteocalcin, procollagen type I) or after their release (as breakdown products) during osteoclastic bone resorption (urinary hydroxyproline, pyridinoline cross-links). It has to be emphasized that the level of biochemical markers of bone formation and resorption represents primarily the whole body rate of bone remodelling. They give little information about the function of individual teams of osteoclasts and osteoblasts, and none at all about the activity of individual cells.[2] In other words, in disease states in which resorption and formation are coupled and in balance either of the markers will reflect the overall rate of bone turnover. These markers are presented in table I.

Biochemical markers of bone formation

Alkaline Phosphatase (AP) is probably the best known marker for bone turnover.[3,4] It is one of the most frequently performed assays in clinical

chemistry and its elevation in the circulation in various skeletal disorders has been recognized for decades. However, its specificity and sensitivity in clinical practice remains troublesome. This is due to the fact that AP is not only produced by osteoblasts but also by other tissues, including liver, intestines, kidneys and placenta. Alterations in blood AP levels, therefore, not always reflect metabolic bone disease. In patients with osteoporosis, AP values are normal or slightly elevated and correlate poorly with bone formation as determined by iliac crest bone biopsy. On the other hand in Paget disease AP is a much better parameter of disease activity than osteocalcin.[3,5,6,7] Most interpretation problems arise when AP is slightly elevated in elderly patients; it may reflect a mineralization defect, but in most cases effects of medication on the hepatic isoenzyme can not be excluded.

Efforts have been made to distinguish between the several isoenzymes of AP, for instance by the use of differentially effective activators and inhibitors (heat, urea and phenylalanine), through separation by electrophoresis or, more recently, by the use of monoclonal antibodies.[22] A newly developed immunoradiometric assay (IRMA) for skelet alkaline phosphatase has been shown to have a low cross-reactivity with the liver isoenzyme, and appears to be more sensitive than total alkaline phosphatase for the clinical investigation of patients with osteoporosis and other metabolic bone diseases.[61]

Serum Osteocalcin

Osteocalcin, a vitamin K dependent protein that contains three residues of gamma-carboxyglutamic acid, is synthesized only by the osteoblasts.[8,9,10] Its synthesis is stimulated by the active metabolite of vitamin D, 1,25-dihydroxyvitamin D_3.

In bone osteocalcin constitutes 1 to 2% of the total protein and about 25% of the non-collagenous matrix proteins. Although its exact function is unknown, it has been postulated that osteocalcin plays a regulatory role in both bone resorption and mineralization.[4] During production a certain amount of osteocalcin leaks into the circulation, where it can be

Table 1: Markers of bone metabolism

Markers of bone formation
(Bone specific) Alkaline phosphatase
Osteocalcin
Collagen type I extension peptides
Markers of bone resorption
Urinary Hydroxyproline/creatinine ratio
Hydroxy Pyridinium crosslinks of collagen
Cross-linked telopeptide of type I collagen (ICTP)
Serum Tartrate-Resistant Acid Phosphatase (STRAP)
Serum Alkaline Phosphatase

measured by radioimmunoassay. Theoretically serum osteocalcin could originate from bone matrix degradation but several studies have suggested that most osteocalcin originates from new cellular synthesis.[11,12] This implies that the osteocalcin level may be a valid marker of bone turnover if resorption and formation are coupled and specific markers of bone formation if they are uncoupled. This is illustrated in patients with multiple myeloma (uncoupling of formation and resorption), where an inverse relation between osteocalcin levels and the severity of the disease has been found, with the lowest values being observed in patients with extensive lytic lesions (frequently associated with hypercalcemia), suggesting the presence of a strong osteoblastic inhibition. During remission, osteocalcin levels return to normal values.[13]

Since vitamin K is essential for its carboxylation,[10,14,15] the use of vitamin K antagonists inhibits the normal carboxylation of osteocalcin, resulting in an increased proportion of serum noncarboxylated osteocalcin to total osteocalcin.[16] Undercarboxylation of osteocalcin was also found in elderly women,[15,18] despite normal levels of vitamin K. Daily administration of vitamin K, however, increased osteocalcin concentration as well as hydroxylapatite binding capacity.[15] The exact mechanism of this undercarboxylation is still unclear. It has been suggested that it might play a role in osteoporosis, since only fully carboxylated osteocalcin is

incorporated into bone tissue.[17] In this respect, Sculz et al found that an increased level of undercarboxylated osteocalcin was associated with lower bone density and an increased hip fracture risk in institutionalized elderly women.[62] On the other hand patients receiving long-term maintenance therapy with vitamin K antagonists have normal bone density.[58]

The pathways involved in the metabolic clearance of osteocalcin are unknown, but it is known that the serum osteocalcin level varies reciprocally with the glomerular filtration rate.[11] As for most biochemical markers of bone turnover, serum osteocalcin has a circadian rhythm with a nightly peak at 4 a.m., reflecting increased bone turnover at night.[21]

Osteocalcin has been shown to be a sensitive marker in several metabolic bone diseases like osteoporosis, hyperparathyroidism, and hyperthyroidism, correlating well with histomorphometric measurements of bone turnover.[9,12,19-24] It has also been shown that in untreated postmenopausal women, serum osteocalcin is the best single biochemical marker reflecting the spontaneous rate of bone loss as assessed by repeated bone mass measurements over a period of 2-4 years.[25]

In Paget's disease, however, the correlation between osteocalcin and histomorphometric findings is low. This is probably due to an increased binding or trapping of the protein by the woven bone matrix.[6]

Serum Procollagen 1 Extension Peptides

Collagen type I is the major collagen product produced by the osteoblasts and represents more than 90% of the organic matrix. Therefore, it is clear that markers of bone collagen synthesis may be of crucial importance as biochemical markers.

Collagen type I is synthesized and secreted as procollagen with large amino- and carboxyterminal extension peptides. Before formation of collagen fibrils, these extension peptides are cleaved from the molecules by endopeptidases in a 1 : 1 molar ratio to newly formed collagen. The extension peptides are then released into the extracellular fluid.[26,27] It should be noted, however, that a fraction of the aminoterminal extension peptide is incorporated into bone matrix, where it has been identified as the 24 K phosphoprotein of bone.[28]

Both the carboxy-terminal propeptide (PICP) and the amino-terminal

propeptide (PINP) can be measured by recently developed radio-immuno-assays.[29,30] Despite the fact that the assays for PICP and PINP both measure native procollagen similarly, and competitive binding curves for standard preparations and for serum are parallel for both assays, the serum concentration of PINP is almost 100-fold higher than that of PICP.[30] This strongly suggest that there is a preferential release of PINP from bone or that plasma clearance of PINP is retarded compared to PICP, or both.[30]

In clinical practice measurement of PICP seems to agree much better with the results expected in certain metabolic bone diseases than PINP assays.[30-33] Moreover, the agreement between PICP and PINP values has been poor and even deviations in opposite directions have been reported.[30]

In most studies a relatively high correlation between PICP and other markers of bone formation have been found.[31,32] However, Parfitt et al did not find a significant correlation between PICP and tetracycline-based bone formation rates determined in iliac crest bone biopsies.[32]

Because of its high molecular weight (approximately 100,000) PICP is unlikely to be filtered by the renal glomeruli. Smedsrød et al found that PICP is mainly eliminated by the liver.[34] That is probably why increased serum levels have been found in subjects with alcoholic liver disease, though this might also be a result of increased type I collagen synthesis in an inflamed or newly fibrosed liver.[31]

Like OC, PICP concentration shows a significant circadian rhythm, with about 20% higher values at night than in the afternoon.[35]

In summary, the currently available assays for procollagen I extension peptides seem not to have a clear advantage over the more widely used assays for serum osteocalcin.

However in contrast to osteocalcin, the serum antigen of PICP is stable during storage and repeated thawing.[29]

Biochemical markers of bone resorption

Urinary Hydroxyproline

Hydroxyproline (OH-P) is mainly found in collagens and represents about 13% of the total amino acid content of mature collagen. OH-P released

during degradation of collagens can not be reutilized in collagen synthesis and is excreted in the urine. Since half of the amount of human collagen is found in bone, where its turnover is probably faster than in other soft tissues, urinary hydroxyproline is often used as a marker of bone resorption.

Urinary OH-P has provided useful information during the last decades but suffers from several disadvantages.[3,4] It has been recognized that several sources of OH-P in addition to bone resorption, including the diet (gelatin), turnover of soft connective tissues (acromegaly, hyperthyroidism, certain skin diseases), C1q and other serum proteins, and degradation of the aminoterminal propeptides from collagen biosynthesis, all contribute to urinary OH-P.[36] Furthermore, only 10% of OH-P released by the breakdown of collagen circulates in the peptide bound form and is excreted in urine without further metabolism. The free imino-acids are almost entirely reabsorbed by the kidney and metabolized in the liver to carbon dioxide and urea.[3,4]

Although dietary influences can be circumvented by using a gelatin-free diet or by measuring the OH-P/creatinine ratio in an early morning specimen after an overnight fast,[37] most of the problems with the measurement of urinary OH-P as mentioned above still remain. Therefore, the use of urinary OH-P measurements is mainly useful as an index of greatly increased rates of bone resorption seen, for example, in Paget's disease and hypercalcemia of malignancy. For the more subtle changes in bone resorption more sensitive assays are needed.

Urinary Excretion of Collagen Pyridinium Crosslinks

Collagen fibrils are stabilized not only by hydrophobic and electrostatic forces, but also by the development of covalent intra- and intermolecular crosslinks. After collagen formation, the enzyme lysyl oxidase converts the amine side chains of specific lysine and hydroxylysine residues at the two telopeptide regions of the collagen molecules into aldehydes. These aldehydes react with lysine, hydroxylysine of glycosylated hydroxylysine on the helical part of close by collagen molecules to form an instable Schiff base, which matures to pyridinium crosslinks.

The urinary excretion of two of these crosslinks, pyridinoline, also called

hydroxylysylpyridinoline (HP) and deoxy-pyridinoline, also called lysyl-pyridinoline (LP), have recently been introduced as indices of bone resorption.[38-40] As both crosslinks result from a posttranslational modification of collagen molecules already secreted and incorporated into the extracellular matrix, they can not be reutilized during collagen synthesis.[40] Their distribution shows a certain degree of tissue specificity, attributing to the tissue specific functional diversity of collagen. However, neither HP nor LP is restricted to one particular tissue or collagen type. Bone type 1 collagen contains a higher proportion of the LP form of the crosslinks than any other connective tissue, showing a molar ratio between HP:LP of about 3.5:1 in adult human bone. In all other human connective tissues this molar ratio is at least 10:1.[40] Given the high turnover of bone tissue, compared to other connective tissues, the pool of pyridinoline crosslinks is primarily believed to be bone derived. Furthermore, the measurement of urinary crosslinks appears to have several potential advantages over hydroxyproline: they are relatively specific for bone, they do not appear to be metabolized in vivo prior to their urinary excretion, and their excretion is not influenced by the intestinal absorption of denatured collagen in the form of cooked meat or gelatin.[41] HP and LP are excreted in urine in the free form (about 40 %) and in peptide bound form (around 60 %), and the ratio of the free and bound fractions is assumed to remain constant in normal adults and in patients with metabolic bone disease. The total amount can be measured by fluorometry after reversed phase HPLC of cellulose bound extracts of hydrolysed urine.[42,43]

Like the other biochemical markers mentioned above, the excretion of crosslinks shows a circadian rhythm, with peak levels between 0500 and 0800 h, a steep fall during the morning hours and nadirs between 1400 and 2300 h.[44] This makes controlling of the time period of urine collection important.[40] Mostly 24 hour urine is used for measuring crosslinks, whereby the data have to be corrected for creatinine levels, although one can also use an overnight or a 2 hour fasting urinary sample.

Although HP is more abundant, which could be an advantage in the case of a low bone turnover, the specificity of LP is higher. The fact that almost a stoichiometric relation has been found between the rate of LP excretion and the whole body rate of bone resorption[39] underlines this high specificity. The

urinary excretion of crosslinks appears a more sensitive marker of bone resorption than hydroxyproline in patients with postmenopausal osteoporosis,[45] Paget disease[46] and primary hyperparathyroidism.[47] Elevations in the excretion of both crosslinks of on the average 2-3 fold have been found in patients with malignant hypercalcemia,[48] but also in patients with growth hormone (GH) deficiency after 4 months treatment with GH.[49] Finally the urinary crosslink excretion is increased in osteomalacia, in patients with hyperthyroidism[50] and appears to be a sensitive index of bone metabolism in hypothyroid patients treated with L-thyroxine.[51]

Unfortunately, the measurement of urinary crosslinks has also some disadvantages. The current chromatographic method of analysis is complex and there exist differences in standardization between laboratories. In this respect newly developed immunoassays to measure crosslinks in urine,[52,53] and the measurement of the carboxy-terminal cross-linked telopeptide of collagen type 1 in serum[54] could provide simpler and more convenient methods to measure crosslinks. Assays have been developed that measure either peptide bound (CrossLaps, NTX) or free (Pyrilinks-D) urinary crosslinks. Since the metabolic origin of the urinary free and bound crosslinks are largely unknown, there is a possibility that the analysis of the free or peptide-bound fractions will give different information as compared to the total excretion, while studying bone resorption or the effect of antiresorptive treatment.[63]

Serum Tartrate-resistant Acid Phosphatase

Acid phosphatase is released by the osteoclasts during bone resorption, but is also present in prostate, platelets, erythrocytes and the spleen. Different isoenzymes can be separated. In serum there are two main isoenzymes of acid phosphatase, namely 3 and 5. Isoenzyme 5 is the one produced by the osteoclasts. The bone acid phosphatase is resistant to tartrate, whereas isoenzyme 3 is inhibited.[3,55] Its function is still unclear. Elevated levels have been demonstrated in a number of metabolic bone diseases, including osteoporosis, correlating well with other markers of bone resorption.[55] It is, however, not clear wether this marker is actually any better than OH-P.[55,56]

Conclusions

The currently available biomarkers have considerably improved the possibilities to monitor changes in bone turnover. The assays for carboxy-terminal procollagen I fragments, osteocalcin and the bone specific isoenzyme of alkaline phosphatase allow a more precise assessment of the complex osteoblastic functions in health and disease, whereby osteocalcin and bone specific alkaline phosphatase appears at present the most satisfactory ones.

With respect to bone resorption the measurement of urinary pyridinoline crosslinks seems to be the most reliable assay.

Biochemical markers are useful in monitoring the severity of a certain disease or the effect of treatment, and it is even suggested that they might be useful in predicting bone loss in for instance osteoporosis,[57,59,60] but it is still impossible to make a diagnosis based solely on the level of certain biochemical markers. Finally, it has to be emphasized that a single marker may be of value in some diseases and not in others, while the time course of markers during treatment can show differences, dependent on the phase of bone remodelling.

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CHAPTER 3.3

BONE RESORPTION MARKERS, DISABILITY AND HIP FRACTURE RISK: THE ROTTERDAM STUDY

Introduction

Several factors besides bone mineral mass have been related to the risk of hip fracture. Bone quality, the rate of bone loss and non-skeletal factors have been identified as important risk factors.[1,2] High rates of bone resorption may be associated with disruption of the trabecular network as well as with an increased rate of bone loss. Furthermore, disability related immobility induces bone resorption not followed by an increased bone formation.[3]

We investigated prospectively whether urinary pyridinium crosslinks, as markers of bone resorption, were associated with the risk of hip fractures. In addition, we examined whether such association was attributable to disability.

Subjects, methods, and results

This nested case-control analysis was conducted as part of the Rotterdam Study, a prospective cohort study to investigate the incidence of, and risk factors for chronic disabling diseases.[4] Briefly, all 10,275 inhabitants of a district in Rotterdam who were aged 55 years or more were invited to participate in this study, which consisted of an initial home interview and a series of medical examinations made during two visits to our research centre. The study was approved by the Medical Ethics Committee of Erasmus University, and written informed consent was obtained from all participants. The overall response rate was 78%.

In the period between January 1990 and February 1994, all 17 independently living women with an incident hip fracture following baseline assessments were selected using the computerized, general practitioner based, follow-up register. Each case was individually matched with three randomly selected independently living controls in the same one-year age group. All fractures were radiographically verified.

At baseline, all participants brought one overnight urine sample to the research centre. These samples were stored frozen at minus 20° C until analysis. Analyses were performed without prior knowledge of the fracture status. Urinary samples were unavailable for five controls. Urinary creatinine was measured using standard laboratory methods. Both total and free urinary pyridinoline and deoxypyridinoline crosslinks were measured by high-performance liquid chromatography (HPLC) analysis and corrected for creatinine excretion. Free deoxypyridinoline was also measured by a recently develop enzyme-linked immunoassay (ELISA).

We measured bone mineral density at the femoral neck using dual energy x-ray absorptiometry.

Disability was assessed by means of the Disability Index of the Stanford Health Assessment Questionnaire.[5] The lower limb disability index was composed of the mean score (0 indicating no impairment, 3 indicating unable to perform) for six component questions on arising, walking, bending and getting in and out of a car.

The associations of the levels of urinary (deoxy)pyridinoline crosslinks with incident hip fractures were expressed as relative risks, using conditional logistic regression. In an additional analysis we adjusted for disability.

Mean age of the women with a hip fracture was 80.5 years (SD 8.6 years). Table 1 shows the relative risk for the several biochemical markers. One standard deviation increase in total pyridinoline and free pyridinoline was associated with a substantially higher risk for hip fracture. Total an free deoxypyridinoline levels as measured by HPLC were not significantly associated with hip fracture risk. However, a significant relation was observed for the free deoxypyridinoline measured with the ELISA. A higher disability score was similarly associated with a considerably higher risk of hip fracture. Adjustment for disability resulted in substantially lower odds ratios for all urinary crosslinks. Although cases had lower bone mineral density as compared to controls (0.72 vs 0.76 g/cm^2 (SD: 0.14 g/cm^2), relative risk per standard deviation decrease 1.3 (0.6 - 2.7) this difference was not statistically significant. Adjustment for bone mineral density did not affect the risk estimates.

Table 1: Relative risks (RR) for hip fractures

	cases mean (SD)	controls mean (SD)	age adjusted		age and disability adjusted	
	nmol/mmol creat	nmol/mmol creat	RR	95 % CI	RR	95% CI
total pyridinoline	42.8 (14.3)	34.0 (12.1)	3.3	(1.3 - 8.6)	1.6	(0.4 - 5.9)
free pyridinoline	24.6 (10.1)	18.0 (7.5)	3.0	(1.2 - 7.2)	1.9	(0.6 - 5.6)
total deoxypyridinoline	9.9 (3.3)	8.5 (3.2)	2.2	(0.8 - 6.0)	1.0	(0.3 - 3.8)
free deoxypyridinoline	5.5 (1.6)	4.4 (1.8)	1.8	(0.8 - 4.1)	1.2	(0.5 - 3.2)
free deoxypyridinoline (ELISA)	7.6 (2.6)	6.4 (1.8)	10.2	(1.4 - 74.6)	4.5	(0.4 - 46.8)
	score median (95% range)	score median (95% range)				
disability	1.2 (0.2 to 2.2)	0.5 (0.2 - 2.3)	8.2	(1.6 - 42.3)		

Relative risks are presented per standard deviation increase in the level of the various biochemical markers. For disability the relative risk is presented per one point increase in the disability index.

Comment

A considerable body of evidence has accumulated over the past few years that confirms the validity of pyridinium crosslinks as parameters of primarily bone resorption. The present results show for most of the pyridinium crosslinks measured a significant association with the risk of hip fracture, although there was a considerable difference in the gradient of risk associated with each of the resorption markers measured. This is probably due to the relatively small sample size and the fact that the various markers do measure slightly different aspects of collagen degradation. The association between urinary pyridinium crosslinks and hip fracture risk appeared to be related to disability at baseline. Other investigators have shown that immobility increases bone resorption.[3] This study provides evidence that disability and the consequent immobility is followed by increased bone resorption, which then leads to increased bone fragility.

We conclude that urinary pyridinium crosslinks can be used in the prediction of hip fractures. Nevertheless, it remains to be seen whether for the identification of subjects at risk simple measurements of risk factors for hip fracture[1] are not more cost effective compared to the relatively expensive measurements of resorption markers.

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CHAPTER 3.4

THE USE OF BIOCHEMICAL MARKERS OF BONE TURNOVER IN THE PREDICTION OF NON-VERTEBRAL FRACTURES.

Introduction

Aging of the Western population leads to a considerable increase in the number of osteoporotic fractures.[1] Whether a subject will develop a fracture after minimal trauma depends both on quantitative as well as qualitative aspects of bone.[2] These aspects are in turn presumed to be related to the interplay of bone formation and bone resorption.[3] An imbalance between bone formation and bone resorption in favour of bone resorption may not only lead to loss in bone quantity but also bone quality.

Several biochemical markers are proposed as markers of bone turnover.[4,5] Osteocalcin and skeletal alkaline phosphatase are supposed to reflect bone formation, although their exact function is largely unknown. Urinary pyridinoline and deoxypyridinoline crosslinks are regarded as sensitive markers of bone resorption.[6] During collagen degradation both crosslinks are released in serum and excreted in urine. Recent data suggest that an increased bone resorption, as measured by these crosslinks, is associated with an increased hip fracture risk.[7,8] Nevertheless, hitherto prospective data on the association between bone turnover markers and fracture risk are scarce.

The objective of the present study was to examine whether various biochemical markers of both bone formation and bone resorption are associated with the occurrence of non-vertebral fractures in the elderly.

Methods

Population:

This study was performed as a nested case control study within the Rotterdam Study.

The Rotterdam Study is a large follow-up study of persons aged 55 years

or more; its intent is to investigate the incidence of and the risk factors for chronic disabling diseases. Its rationale and design have been described previously.[9] All 10275 inhabitants of a district in Rotterdam who were aged 55 years or more were invited to participate in this study. The baseline survey consisted of an initial home interview by a trained research assistant and a series of medical examinations made during two visits to the research centre. The study was approved by the Medical Ethics Committee of the Erasmus University, and written informed consent was obtained from all participants. The overall response rate for the Rotterdam Study was 78%.

Cases were all subjects with a fracture between baseline interview and January 6th 1995. They were selected using the computerized, general practitioner based, follow-up register. Controls were subjects from the same general practitioners without a fracture. Selection of controls was performed after frequency matching for age (5 year age strata) and sex. Median follow-up time for cases and controls was 2.3 years.

Measurements

At baseline, the participants were asked to bring one overnight urine sample to the research centre. These samples were stored frozen at minus 20^0 C until analysis. Serum samples were collected during the day. Blood samples were allowed to clot for approximately 30 to 45 minutes before being centrifuged. They were stored frozen at minus 70^0 C until analysis. Analyses were performed without prior knowledge of the fracture status.

Serum osteocalcin was measured using a commercially available radio immuno assay (Incstar Corp, Stillwater, Minnesota). Serum skeletal alkaline phosphatase (sAP) was determined using the Tandem-R Ostase assay (Hybritech Europe S.A., Belgium).

For the concentrations found in the present study, the intra- and interassay coefficients of variations for osteocalcin and sAP were all below 10%.

Total and free pyridinoline (total PYD and free PYD) and deoxypyridinoline (total DPD and free DPD) crosslinks were measured using High Performance Liquid Chromatography, as described

previously.[10] The coefficient of variation for the HPLC measurements was 3% for the HPLC step alone and 10% for the overall measurements including hydrolysis and freeze-drying. In addition, we measured free deoxypyridinolines using a competitive enzyme immuno assay (Pyrilinks-D, Metra biosystems, Palo Alto). The intra- and interassay coefficient of variation were 9.5% and 8.1%, respectively.

Bone mineral density (BMD) of the femoral neck was measured using Dual energy X-ray Absorptiometry (Lunar DPX-L) as described previously.[11]

Disability was assessed by means of the Disability Index of the Stanford Health Assessment Questionnaire.[12] The lower limb disability was composed of the mean score (0 indicating no impairment, 3 indicating unable to perform) for six component questions on arising, walking, bending and getting in and out of car.

Data analysis

We used multiple linear regression to examine the association between various biochemical markers of bone turnover and bone mineral density measured at the femoral neck.

Logistic regression was performed to estimate the association between the various biochemical markers and fracture risk. Odds ratios were calculated as an estimate of relative risk. Biochemical markers were entered into the model, either as a continuous variable or dichotomized (below or above the median). Men and women were analyzed separately. A subgroup analyses was performed for women with hip fractures. The number of hip fractures in men was too small to perform a meaningful subgroup analysis. In the subgroup analysis we excluded subjects with fractures other than a hip fracture. In all analyses we adjusted for age at baseline. Further adjustments for bone mineral density and lower limb disability was done for markers that appeared to be associated with fracture risk. As bone mineral density was not measured in subjects who at baseline were living in nursing homes, this adjusted analysis was restricted to the independently living. Data are presented with the 95% confidence interval between parentheses.

Table 1: Baseline characteristics of the study population by fracture status

		women				men			
		N	fracture mean (SD)	N	no fracture mean (SD)	N	fracture mean (SD)	N	no fracture mean (SD)
age (years)		166	74.6 (9.5)	163	73.8 (8.9)	23	76.1 (9.6)	31	74.9 (8.5)
body mass index (kg/cm^2)		157	26.6 (3.9)	155	27.0 (4.3)	22	24.8 (3.9)	30	25.7 (2.9)
bone mineral density (g/cm^2)		117	0.74 (0.12)	117	0.79 (0.13)	15	0.84 (0.16)	24	0.87 (0.14)
	median					median			
osteocalcin (µg/l)	5.9	160	6.2 (2.1)	152	6.3 (2.1)	5.2	21	5.2 (1.7)	30	5.5 (2.0)
sAP (µg/l)	11.1	160	11.9 (4.3)	152	12.1 (5.5)	9.2	21	10.9 (3.8)	30	10.7 (10.5)
Pyrilinks-D (nmol/mmol creat)	6.3	135	6.7 (2.4)	142	6.5 (2.8)	4.8	17	5.0 (1.7)	27	5.2 (2.0)
total PYD (nmol/mmol creat)	37.5	134	43.5 (20.0)	143	41.6 (23.7)	29.5	16	34.8 (19.4)	27	33.7 (19.2)
free PYD (nmol/mmol creat)	16.6	135	18.1 (6.7)	143	17.6 (7.8)	12.4	17	12.3 (4.3)	27	13.7 (4.4)
total DPD (nmol/mmol creat)	11.7	134	13.3 (5.4)	143	12.3 (6.0)	8.1	16	9.8 (5.1)	27	9.7 (5.6)
free DPD (nmol/mmol creat)	4.9	135	5.4 (2.2)	143	5.2 (2.3)	3.5	17	3.5 (1.3)	27	4.0 (1.3)

Medians are calculated while combining subjects with and without fractures

Results

In total, 237 subjects were identified with a non-vertebral fracture following their baseline home interview. An equal number of controls was selected. For the cases, serum and/or urinary samples were available for 204 subjects. Samples were available for 194 control subjects. Subjects for whom samples were unavailable were slightly older and more disabled than those with available samples. We excluded 9 cases, because we were not able to verify their fracture. Six cases were excluded as their fracture occurred before blood and urinary samples were collected. Table 1 shows baseline characteristics of the subjects with and without fractures. Table 2 shows the types and frequencies of the various fractures reported. Subjects with a hip fracture were at baseline older than those with other types of fractures (hip fracture: 80.1 year (SD: 8.7), other types of fractures: 73.1 year (SD: 9.1)).

Table 2: Types and numbers of fractures

Fracture type	Women	Men
Nasal bone	3	1
Mandibula	1	
sternum	1	
Clavicula	2	2
Scapula	2	
Rib	2	1
Pelvis	7	1
Upper arm	24	4
Lower arm/Forearm	53	1
Hand/Foot	25	3
Femoral neck*	36	10
Femoral shaft	1	
Patella	1	
Tibia/fibula	3	
Ankle	8	1

* included are 3 women and 1 men with a hip fracture after a previous other incident fracture.

There was a weak correlation between markers of bone formation and markers of bone resorption (table 3). For all correlations P was smaller than 0.001. The correlation between osteocalcin and sAP was 0.40. Correlation between the various resorption markers ranged from 0.59 to 0.88. Correlations were similar for those with and without fractures.
Both in men and women the various biochemical markers, except sAP, increased substantially with age. For women this is illustrated in figure 1. After age-adjustment, bone mineral density of the femoral neck was inversely associated with both markers of bone formation and bone resorption in women (table 4). In men, a significant positive association between most bone resorption markers and bone mineral density of the femoral neck was observed. However, the number of subjects was relatively small. We did not find an association between markers of bone formation and bone mineral density in men.

All fractures combined
In women, but not in men, a level of total PYD, total DPD and Pyrilinks-D above the median was significantly associated with an increased risk of all types of fractures combined (table 5). Adjustment for disability status and bone mineral density did not influence the results. Neither osteocalcin nor sAP was associated with total fracture risk.
Excluding those fractures that are clearly not related to osteoporosis (nasal bone, mandibula, patella) did not influence the results.

Hip fractures
Serum osteocalcin level was significantly associated with hip fracture risk in women (N= 36 cases, 132 controls). The odds ratio per 1 point increase in osteocalcin was 0.8 (0.7 - 1.0). Especially women with an osteocalcin level in the lowest tertile had an increased fracture risk (odds ratio: 2.4 (1.0 - 5.6)) compared to those with higher values. Women with an osteocalcin level below the median had a 1.5 (0.7 - 3.4) higher risk of hip fracture than those with a level above the median. For the independently living women, the risk for fracture was 3.1 (1.0 - 9.2) times higher among those with an osteocalcin below the median than for those

with an osteocalcin level above the median (table 6). Adjustment for bone density did not influence this result. Adjustment for disability resulted in a somewhat lower and no longer significant odds ratio (2.5 (0.8 - 7.7)).

No significant association was found for sAP, analysed in a similar way. Women with a urinary Pyrilinks-D level above the median had a 2.5 (1.0 - 6.5) times higher risk for hip fractures than those with a Pyrilinks-D level below the median. For free PYD and free DPD levels measured by HPLC, similar results were obtained. Total PYD and DPD crosslinks were not significantly associated with fracture risk. However, for total PYD, there was a tendency towards an increased risk of hip fracture for subjects with a level above the median (OR: 2.1 (0.8 - 5.2)). We found no "dose-response" relationship between the various resorption markers and hip fracture risk in the total group. However, there was a dose-response for free PYD (OR: 1.1 (1.0 - 1.2)) and free DPD (OR: 1.3 (1.0 - 1.6)) when restricting to the independently living elderly women.
Table 6 shows the association between the various biochemical markers and hip fracture risk for independently living women with complete data on disability and bone mineral density. Adjustment for bone mineral density measured at the femoral neck did not influence the results substantially. Adjustment for lower limb disability had limited effect on the results.

From the women with a hip fracture and a Pyrilinks-D level above the median 44.4% (8/18) had an osteocalcin level in the lowest tertile. From the women without a hip fracture, 15.8% (9/57) of those with a Pyrilinks-D above the median had an osteocalcin level in the lowest tertile (P for difference = 0.01). If osteocalcin (lowest tertile) and deoxypyridinoline (above the median) were entered in the logistic model together, both were independent predictors of hip fracture risk (osteocalcin: OR 4.5 (1.5 - 13.3), Pyrilinks-D: OR 3.3 (1.2 - 9.1)). For the free and total crosslinks, measured using HPLC, similar results were obtained.

Table 3: Correlations between markers of bone resorption and bone formation (N=300).

	Osteocalcin	sAP	Pyrilinks-D	total PYD	free PYD	total DPD
sAP	0.40					
Pyrilinks-D	0.36	0.29				
total PYD	0.32	0.27	0.60			
free PYD	0.29	0.25	0.74	0.73		
total DPD	0.42	0.32	0.64	0.86	0.59	
free DPD	0.32	0.24	0.79	0.62	0.88	0.62

Table 4: Association between markers of bone turnover and bone mineral density measured at the femoral neck in men and women.

	women			men		
	N	β	(95% CI)	N	β	(95% CI)
osteocalcin	221	-0.013	(-0.021 to -0.005)	37	-0.004	(-0.031 to 0.022)
sAP	221	-0.004	(-0.007 to -0.001)	37	0.004	(-0.001 to 0.009)
Pyrilinks-D	226	-0.006	(-0.012 to -0.000)	38	0.032	(0.005 to 0.058)
total Pyd	225	-0.001	(-0.002 to -0.000)	37	0.003	(0.000 to 0.006)
free Pyd	226	-0.002	(-0.005 to 0.000)	38	0.007	(-0.007 to 0.020)
total DPD	225	-0.004	(-0.007 to -0.001)	37	0.013	(0.004 to 0.022)
free DPD	226	-0.004	(-0.011 to 0.003)	38	0.047	(0.010 to 0.084)

Legend to table 4: β expressed per one point increase in the various biochemical markers.

Table 5: Association between various biochemical markers and all fractures combined

	women		men	
	Odds ratio	95% CI	Odds ratio	95% CI
Osteocalcin*	1.0	(0.9 - 1.1)	0.9	(0.6 - 1.2)
Osteocalcin below median	0.9	(0.6 - 1.4)	1.3	(0.4 - 4.1)
sAP*	1.0	(0.9 - 1.1)	1.0	(0.9 - 1.1)
sAP below median	0.9	(0.6 - 1.5)	0.5	(0.1 - 1.4)
Pyrilinks-D*	1.0	(0.9 - 1.1)	0.9	(0.7 - 1.3)
Pyrilinks-D above median	1.7	(1.0 - 2.7)	0.6	(0.2 - 2.2)
total PYD*	1.0	(0.9 - 1.1)	1.0	(0.9 - 1.1)
total PYD above median	1.8	(1.1 - 2.9)	1.8	(0.5 - 6.6)
free PYD*	1.0	(0.9 - 1.1)	0.9	(0.8 - 1.1)
free PYD above median	1.2	(0.7 - 1.9)	0.4	(0.1 - 1.5)
total DPD*	1.0	(0.9 - 1.1)	1.0	(0.9 - 1.1)
total DPD above median	1.6	(1.0 - 2.6)	1.1	(0.3 - 4.0)
free DPD*	1.0	(0.9 - 1.2)	0.7	(0.4 - 1.2)
free DPD above median	1.3	(0.8 - 2.1)	0.6	(0.2 - 1.9)

* Biochemical marker entered as a continuous explanatory variable into the logistic model.

Table 6: Association between hip fracture risk and various biochemical markers in independently living women

	cases/controls	Odds ratio	95% confidence interval
Osteocalcin (age adjusted)	24/106	3.1	1.0 - 9.2
- BMD adjusted		3.2	1.1 - 9.4
- disability adjusted		2.5	0.8 - 7.7
sAP (age adjusted)	24/106	1.0	0.4 - 2.8
- BMD adjusted		1.2	0.4 - 3.2
- disability adjusted		1.0	0.4 - 2.7
Pyrilinks-D (age adjusted)	21/115	3.9	1.3 - 11.9
- BMD adjusted		3.8	1.2 - 11.6
- disability adjusted		3.5	1.1 - 10.8
total PYD (age adjusted)	21/115	2.6	0.9 - 7.6
- BMD adjusted		2.4	0.8 - 7.0
- disability adjusted		2.0	0.6 - 6.2
free PYD (age adjusted)	21/115	3.7	1.2 - 11.3
- BMD adjusted		3.5	1.1 - 10.9
- disability adjusted		3.0	0.9 - 9.5
total DPD	21/115	1.4	0.5 - 4.0
- BMD adjusted		1.3	0.5 - 3.7
- disability adjusted		1.3	0.5 - 3.7
free DPD (age adjusted)	21/115	3.4	1.1 - 10.6
- BMD adjusted		3.2	1.0 - 10.0
- disability adjusted		3.6	1.1 - 11.6

For osteocalcin and sAP odds ratios represent the risk for having a level below the median relative to having a level above the median. For Pyrilinks-D, total PYD, free PYD, total DPD and free DPD the odds ratios indicate the risk for having a level above the median relative to having a level below the median.

Discussion

In the present population based study, an association between higher levels of urinary bone resorption markers and the risk of subsequent hip and non-hip fractures was found in women. We also observed an association between low osteocalcin levels and hip fracture risk in women. No significant association was found between bone turnover markers and fracture risk in men. The total number of fractures in men was small and, therefore, the finding of a significant association is less likely. However, it is well possible that bone turnover is less important in the occurrence of fractures in men. Despite a larger skeleton, men showed lower levels of bone turnover markers.

Cross-sectional studies in which bone turnover markers were measured shortly after the fracture showed that bone resorption was increased.[13,14] This indicates that bone resorption may already have been increased prior to the fracture. However, conclusions from cross-sectional studies may be severely hampered by the impact of the fracture on biochemical markers of bone turnover.[15,16] A prospective study is therefore necessary.

The association found between resorption markers and hip fracture risk in the current study appears to be less pronounced than the association that we reported previously in a smaller group of independently living elderly women.[7] Yet, the confidence intervals for the association are wide in bothstudies, indicating that the point estimate is rather imprecise. However, in the present study again, the association appears to be most prominent in independently living elderly women. A possible explanation may be that in subjects living in homes for the elderly, hip fractures risk is already high for other reasons, such as a higher propensity to fall. In the previous study, we reported that the association was in part attributable to baseline disability. In the present study, some, but not all of the association could be attributed to baseline disability. Recently the positive association between resorption markers and hip fracture risk in women has been corroborated in a different population.[8]

It is remarkable that predominantly total pyridinium crosslinks were associated with total fracture risk, whereas for hip fractures the association was most pronounced for free pyridinium crosslinks. Recent

studies suggest that free and peptide bound crosslinks may reflect differences in collagen degradation.[17] Whether differences in the pattern of collagen degradation may explain the different association of free and total pyridinium crosslinks with hip and non-hip fracture risk, or whether this is merely due to chance has to be elucidated in larger studies.

An association between decreased bone formation markers and fracture risk has also been suggested in cross-sectional studies,[13,14,18,19] although not invariably.[20] No prospective studies have confirmed this association sofar. In fact, in one prospective study among institutionalized elderly women, there was a not statistically significant higher osteocalcin level among those with a hip fracture.[21] Another prospective study[19], however, showed non-significantly lower osteocalcin levels previous to fracture. In the present study we observed an association between low osteocalcin and hip fracture risk. This is surprising realizing that, at least in women, there is an inverse association between osteocalcin level and bone mineral density, a well established risk factor for fractures. Presumably this inverse relationship represents coupling between bone resorption and bone formation. An increased bone resorption is followed by an increase in bone formation. In subjects with hip fractures, coupling of formation to resorption may be disturbed, with a subsequent decrease in osteocalcin levels. Consequently, this could result in a change of bone quality, which is not necessarily reflected in bone mineral density. This hypothesis is supported by the finding that in the group of women with hip fractures a significantly higher concomitance of increased resorption markers and low osteocalcin was observed compared to those without fractures.

No association was found between hip fracture risk and sAP, which is an other bone formation marker. It has been suggested that osteocalcin and sAP reflect different phases in the process of bone formation.[22] In vitro, sAP is produced mainly during osteoblast proliferation, whereas osteocalcin is mainly produced during matrix mineralization. Apparently, the disturbance of bone formation is restricted to the process involving osteocalcin.

It remains possible that our observations are a reflection of vitamin D deficiency in subjects with hip fractures. Lips et al found that vitamin D

levels were lower in subjects with a recent hip fracture.[23] Nevertheless, preliminary results obtained in our study did not show a difference in 25-hydroxy-vitamin D levels between subjects with and without a hip fracture.[24]

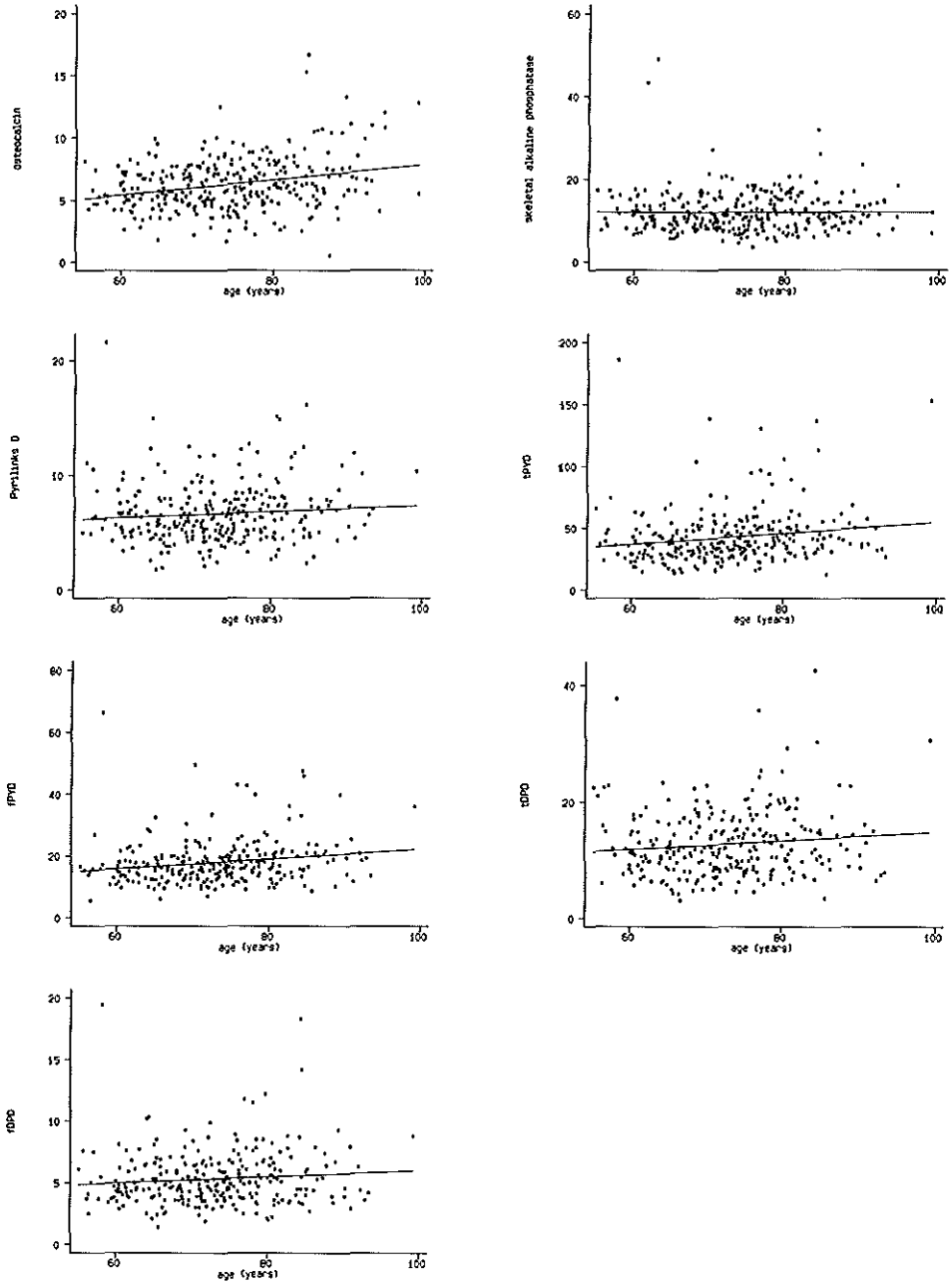
An alternative explanation can be found in a decreased mobility. It is known that immobility is associated with increased bone resorption and decreased bone formation.[25] As mentioned, some although not all of the association between bone turnover markers and hip fracture risk could be attributed to baseline disability.

Hip fractures have a substantial physical and psychological impact and are responsible for a considerable amount of cost. Preventive strategies should therefore aim primarily at avoiding this kind of fracture. The present findings may have several implications for preventive strategies.

If reduced mobility is causing a disturbance in the coupling of bone resorption and formation, a decrease in hip fracture incidence should preferably be obtained by programs to enhance mobility in the elderly. The increase of markers of bone resorption even at advanced age suggests that prescribing inhibitors of bone resorption to elderly subjects, may also be a valuable approach to prevent future fractures. By doing this, therapy is targeted on those subjects which are most at risk, which in turn will help compliance. Nevertheless, the longterm effects of interfering with the remodeling cycle by antiresorptive agents in elderly subjects are still not known.

If vitamin D deficiency is causing disturbances in bone turnover, administrating vitamin D may be a useful approach to reduce the number of fractures. Several studies have shown that vitamin D administration in the elderly reduces the number of hip fractures.[26,27] However, a large prospective study in the Netherlands did not show an effect of this agent on hip fracture incidence.[28]

Figure 1: Association between various biochemical markers and age in women.



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CHAPTER 4.1

GENERAL DISCUSSION

In this thesis developments in the field of fracture prediction were discussed. This chapter will focus on the main findings and the clinical relevance of the studies described. Furthermore, suggestions for further research will be given.

Main findings

Osteoporosis is defined as "a disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to increased bone fragility and a consequent increase in fracture risk".[1] The direct consequence of osteoporosis, fractures, is a major concern for public health. Especially hip fractures are responsible for substantial physical and social problems and bring about a considerable amount of cost.[2] But also other types of fractures, for instance of wrist, ankle and humerus, add to the osteoporosis related discomfort.[3] Vertebral fractures often do not reach clinical attention, which may suggest that they are not a problem. However cross-sectional studies have suggested that multiple severe vertebral deformities cause substantial pain and disability.[4] In addition, Burger et al found that vertebral deformities are associated with an increased risk of other non-vertebral fractures.[5]

With the ageing of the population osteoporosis related fractures will become more and more a problem. Fracture preventive measures seem therefore warranted, but in order to be cost-effective, need to focus on those at highest risk.

Measurement of ultrasound parameters forms a potential alternative for bone mineral density measurements in detecting subjects at increased risk of osteoporosis related fractures. It is a relatively inexpensive method which does not require the use of ionizing radiation.[6] In a cross-sectional analysis, we found that ultrasound parameters measured at the calcaneus are influenced by factors that also determine bone density, like for instance age, body mass index and mobility. Cross-sectional analyses, however, may

provide biased estimates of an association. In general one tends to underestimate the impact of a factor that has a negative effect on the parameter of interest if this factor is positively associated with either mortality or non-response. This may explain why the age-effect estimated from the longitudinal analyses (chapter 2.3) differed considerably from that estimated cross-sectionally (chapter 2.2). It seems plausible that in the older age groups, selection of relatively healthy subjects has occurred.

It was observed that the rates of loss in ultrasound parameters remain relatively stable until old age in women and appear to accelerate with age in men. One has to bear in mind, however, that although rates of change were assessed longitudinally, the analysis of rates of change with age was in fact cross-sectional, with the potential hazards of selection bias and cohort effects. It is unlikely that selection bias will induce an increase in rates of change with age. Rather, selection of relatively healthy subjects in the older age groups, would have led to a decrease in the rate of change with age. A cohort effect, however, can not be ruled out.

Ultrasound measurement is regarded to be highly precise. Nevertheless, in chapter 2.3 it was demonstrated that despite its precision, ultrasound parameters are not very useful in the yearly follow-up of individual patients, given the rates of change per year that were observed.

It appears obvious that for judgement of causality to be reasonable, it should be clear that the exposure of interest preceded the outcome by a period of time consistent with any proposed biologic mechanism. In cross-sectional studies the determinant and outcome are measured at the same time, which often makes it difficult to assess causality. In chapter 2.4 we reported that subjects with a recent non-vertebral fracture had on average lower ultrasound values than those without fractures. However, all fractures preceded the measurement. Concluding that low ultrasound parameters are causing fractures therefore seems unwarranted. Theoretically, lower ultrasound values may have resulted from the fracture rather than preceded it, for instance due to fracture related immobility. An immobility related effect will be most prominent for fractures of the lower limb. As a similar association between low ultrasound parameters and fractures was found for upper and lower limb fractures, a causal relationship nevertheless seems

possible.

It has been suggested that ultrasound parameters may measure aspects of bone strength different from density,[6] like elasticity and trabecular structure. When combining ultrasound and bone mineral density measurements it appeared that ultrasound parameters measured at the calcaneus did provide additional information, as the frequency of fractures within strata of bone density differed according to the level of the ultrasound parameter. Nevertheless, it has to be emphasized that bone mineral density in this study was measured at another site (femoral neck) than where the ultrasound measurement (calcaneus) was performed. Previous studies have shown that combining bone mineral density measurements from different sites improves the predictive value of these measurements.[7] Subjects with low bone density at more than one site have a higher risk of any type of fracture than those with low bone density at just one single site. This may be explained by the fact that the chance of misclassifying a subject with respect to the level of bone mass is smaller when using more than one measurement. An alternative explanation is that the measurement at the femoral neck reflects predominantly cortical bone mass, whereas the calcaneus is much more a reflection of the amount of trabecular bone. Combining measurements of both trabecular and cortical bone mass may subsequently increase the ability to predict a fracture. The added value of ultrasound measurement that has been demonstrated in this study does, therefore, not necessarily imply that ultrasound gives important information on the quality of bone. It may also indicate that, although in a different way, bone density is measured at more than one site, and/or that the measurements simply reflect different types of bone (trabecular or cortical).

In chapter 3.1 the association between non-insulin-dependent diabetes mellitus (NIDDM), bone mineral density and fractures was studied cross-sectionally. In medical textbooks, diabetes mellitus is mentioned as a cause for low bone mass.[8] In contrast, an approximately 3% higher bone density was observed in men and women with NIDDM. In cohort studies, selective non-response of unhealthy subjects is liable. Subjects with NIDDM who do not respond probably have a poorer health and are less active than those that are responders. Non-responding diabetics will presumably also have a

lower bone mineral density due to their inactivity. However, it seems unlikely that this has biased the results. Bias will only occur if inactive diabetics are more likely to be non responders than inactive non-diabetics.
A nested case-control design was chosen to study the association between bone turnover markers and osteoporotic fractures (chapter 3.3 and 3.4). This approach is a particularly efficient way to deal with relatively expensive laboratory test that would have to be made on thousands of individuals if a basic cohort design would have been chosen. It was found that higher levels of urinary pyridinoline crosslinks (reflecting bone resorption) and lower osteocalcin levels were associated with an increased hip fracture risk in independently living elderly women. The results of these studies suggest that in elderly subjects the normal coupling of bone resorption and bone formation may be disturbed leading to an increased fracture risk. As the number of cases in the two studies described was relatively small, the estimate of the magnitude of the association is still rather imprecise. Rather than just focusing on prediction, the study question both in chapter 3.3 and 3.4 was etiologic in origin. In etiologic research it is important to consider all potential confounding factors. Age is a manifest confounder as it is associated both with the level of the various biochemical markers and with the outcome (i.e. the frequency of fractures). All analyses were therefore adjusted for age. Is disability a confounder? Disability is associated with an increase in bone resorption and a decrease in bone formation markers.[9] In addition, subjects that are disabled have an increased fracture risk.[10] Adjustment for disability status did influence the association between bone turnover markers and fracture risk. Rather than being a confounder, disability may also have been the factor that set the train on running. Disability leads to immobility, which in turn leads to alterations in bone turnover and thereby to bone loss and a higher susceptibility to fractures.

Clinical relevance

The studies described in this thesis show that ultrasound measurement at the calcaneus may be a useful tool in detecting subjects at increased risk for osteoporosis related fractures. In this respect, it performs equally well as bone mineral density measurements. In addition, it has the advantage to be

radiation free and relatively inexpensive. We have estimated that the overall cost of an ultrasound measurement of the calcaneus is about half the cost of a bone mineral density measurement. It has to be emphasized that low ultrasound values are a risk factor, rather than a diagnostic tool. In this respect, similarities exist with hypertension as a risk factor for stroke. A substantial number of subjects will have low ultrasound values without ever suffering from a fracture. On the other hand, fractures will occur without low ultrasound values being present.

Ultrasound measurement at the calcaneus can not be used to identify subjects with a low bone density at either proximal femur or lumbar spine.

In general practice, bone mass measurements are performed in subjects at increased risk for secondary osteoporosis. In this respect, non-insulin-dependent diabetes mellitus can no be longer be regarded as a risk factor for low bone mass.

Based on the studies on rates of change with age in ultrasound parameters and on the results concerning bone turnover markers and fracture risk, it is suggested that bone loss continues with increasing age. Recent studies in other populations support these observations.[11,12] It is therefore no longer warranted to consider bone loss a problem of only perimenopausal women and to restrict intervention to this group of subjects. In fact, targeting intervention to elderly subjects may be a more valuable approach to prevent future fractures. By doing this, therapy is targeted to the period at which people are most at risk, which in turn might have a positive impact on compliance.

Further research

Overall, research in osteoporosis has to focus on two broad topics. First of all, what determines fracture risk, and secondly, which strategies or interventions can (cost-effectively) reduce the number of osteoporosis related fractures.

In light of these two topics, several issues, closely related to subjects described in this thesis, deserve further attention.

Bone strength is important for fracture susceptibility. Strength is determined by both the amount and the quality of bone. As already mentioned, the

results of chapter 2.4 suggest that ultrasound parameters may provide information on qualitative aspects of bone that are independent of bone density. However, for a definite answer, prospective studies in which bone density and ultrasound measurements are site-matched are essential.

With respect to the association between non-insulin-dependent diabetes mellitus and bone mass, it is important to identify the factor responsible for the increased bone density in subjects with NIDDM. We have hypothesized that hyperinsulinemia may lead to a stimulation of the mitogenic and anabolic actions of insulin on the osteoblast. Studies in which both insulin levels and markers of bone turnover are measured provide an opportunity to unravel the underlying mechanisms. Furthermore, it is important to examine prospectively whether the increase in bone mass is indeed associated with a decrease in the number of fractures, as was suggested by the lower frequency of recent non-vertebral fractures in women with NIDDM. Theoretically, a higher bone mass in NIDDM may have resulted from a state of low bone turnover which in turn may lead to a reduction in the quality of bone, due to the fact that old bone is not renewed. Thereby the number of (stress) fractures (fatigue damage) may in fact increase.

Presumably the most important issue that has to be addressed in future research concerns the association between bone turnover markers and fracture risk. Reduced mobility and/or vitamin D deficiency are factors that may induce an effect as was observed. Elucidating what causes the disturbances in bone turnover may give clues for intervention strategies. This may ultimately lead to the core of osteoporosis related research: The prevention of fractures.

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CHAPTER 5

SUMMARY

Osteoporosis related fractures in general and hip fractures in particular are a major public health problem in the elderly. Preventive strategies may therefore be warranted, but in order to be cost-effective should aim at subjects at highest risk. This thesis focuses on the developments in the field of fracture prediction. All studies described in this thesis were conducted as part of the Rotterdam Study, a large population based cohort study of determinants of disease in subjects aged 55 years and over.

Chapter 2.1 gives a review of ultrasound measurement of bone. The theoretical background behind the use of Speed of Sound (SOS) and Broadband Ultrasound Attenuation (BUA) is discussed. Briefly, SOS is regarded to reflect bone density as well as bone elasticity. BUA is thought to represent bone density and structural aspects of bone. Furthermore, in this chapter an overview is given of recent studies concerning ultrasound parameters.

In chapter 2.2 the cross-sectional changes of ultrasound parameters with age are described in a sample of 1,405 men and women. There was a significant decline with age in SOS and BUA in men (-0.4 and -0.1%/year, respectively) and women (-1.3 and -0.4%/year, respectively). Furthermore, a number of determinants known to influence bone metabolism was studied. Of those, only smoking, severe disability and the use of corticosteroids appeared to be associated with ultrasound parameters. In addition, we studied the association between ultrasound parameters and bone mineral density measured at the lumbar spine and proximal femur. Modest correlations were observed ranging from 0.33 to 0.50, which indicate that ultrasound parameters are not useful to predict low bone mineral density in either spine or hip.

The cross-sectionally estimated rates of change in ultrasound parameters may have been affected by factors like selection bias. In order to put limits to these effects, the rates of change were calculated longitudinally in a sample of 543 subjects on whom follow-up ultrasound measurements were

available. SOS fell by -2.5 m/s/year (comparable to -2.1%/year in men and -2.5%/year in women) in both sexes (95% CI -4.0 to -1.1 m/s/year in men and -3.6 to -1.4 m/s/year in women). In men the rate of change in SOS tended to increase with age. BUA did not change significantly during follow-up in either sex. Thereby, the prospectively assessed rates of loss differed considerably from those observed cross-sectionally, especially for SOS in men.

Based on the precision of the ultrasound measurement and the rate of change observed it could be estimated that ultrasound parameters are not very useful in the yearly follow-up of individual patients.

In chapter 2.4, the association between ultrasound parameters and recent non-vertebral fractures was discussed. Subjects with a value for either SOS or BUA within the lowest tertile were found to report three times as many fractures when they were compared to subjects with values for SOS and BUA in the highest tertile. A similar pattern was found for bone mineral density of the proximal femur. Within tertiles of bone mineral density, subjects with a low SOS reported more fractures than subjects with a high SOS. For BUA a similar but less pronounced pattern was visible. This made us conclude that ultrasound parameters may measure aspects of bone strength different from density. In chapter 3.1 the association between non-insulin-dependent diabetes mellitus (NIDDM), bone mineral density measured at the lumbar spine and hip and recent non-vertebral fractures was examined. Bone mineral density was measured in 2481 men and 3450 women. Of those, 243 men and 335 women had NIDDM. Both men and women with NIDDM had an approximately 3% higher bone mineral density at all sites measured than subjects with a normal glucose tolerance. Furthermore, in women, NIDDM appeared to be associated with a lower frequency of non-vertebral fractures.

In chapter 3.2 an overview was given of the currently available biochemical markers of bone turnover. Assays for carboxy-terminal procollagen I fragments, osteocalcin and the bone-specific isoenzyme of alkaline phosphatase allow a more precise assessment of the complex osteoblastic functions in health and disease. Osteocalcin and skeletal alkaline phosphatase appear at present to be the most satisfactory ones. With respect

to bone resorption, the measurement of urinary pyridinoline crosslinks seems to be the most reliable assay.

We found that higher urinary pyridinoline crosslinks are associated with an increased risk of hip fracture in independently living women, as was described in chapter 3.3. Most likely, the higher urinary pyridinoline crosslinks result from a immobility related bone loss. We therefore concluded that urinary pyridinium crosslinks can be used in the prediction of hip fractures. Nevertheless, it remains to be seen whether for the identification of subjects at risk simple measurements of risk factors for hip fracture are not more cost effective compared to the relatively expensive measurements of resorption markers.

In chapter 3.4 the results of the previous study were in part corroborated in a larger sample of subjects. In addition, we observed an association between a lower osteocalcin level and increased hip fracture risk. Taken together, this suggest that at advanced age a potential uncoupling of bone resorption and bone formation, may lead to an increased bone fragility.

The clinical relevance of the studies presented in this thesis is discussed in chapter 4. Furthermore, suggestion for future research are given.

CHAPTER 6

SAMENVATTING

Met osteoporose samenhangende fracturen in het algemeen en heupfracturen in het bijzonder vormen een aanzienlijk probleem voor de volksgezondheid bij ouderen. Preventieve maatregelen lijken noodzakelijk, maar dienen, om kosten-effectief te zijn, toegepast te worden bij personen met het hoogste risico op fracturen. Dit proefschrift richt zich met name op recente ontwikkelingen op het gebied van de voorspelling van fracturen. Alle onderzoeken die in dit proefschrift worden beschreven werden uitgevoerd binnen het ERGO onderzoek, een vervolg-onderzoek in de open populatie met als doel het opsporen van determinanten van ziekten bij personen ouder dan 55 jaar.
Hoofdstuk 2.1 geeft een overzicht omtrent ultrageluidsmetingen van botweefsel. De theorie achter het gebruik van geluidssnelheid (Speed of Sound (SOS)) en geluidsverzwakking (Broadband Ultrasound Attenuation (BUA)) worden besproken. Kort samengevat wordt aangenomen dat SOS informatie geeft over botdichtheid en botelasticiteit. BUA zou inzicht geven in dichtheid en structurele aspecten van botweefsel. Verder wordt in hoofdstuk 2.1 een overzicht gegeven van recente onderzoeken die op het gebied van de ultrageluidsmetingen van bot zijn verricht.
In hoofdstuk 2.2 worden de met de leeftijd samenhangende veranderingen in ultrageluidsparameters besproken. Hiertoe werd een dwarsdoorsnede-onderzoek uitgevoerd bij een groep van 1405 mannen en vrouwen. Met toenemende leeftijd was er een afname in zowel SOS als BUA bij mannen (respectievelijk -0.4% en -0.1% per jaar) en bij vrouwen (respectievelijk -1.3% en -0.4% per jaar). Ook werd het effect van determinanten van de botstofwisseling op ultrageluidsparameters onderzocht. Roken, het hebben van een ernstige lichamelijke handicap en het gebruik van corticosteroïden bleken een invloed te hebben op de ultrageluidsparameters.
Daarnaast werd bekeken wat de relatie was tussen ultrageluidsparameters en botmineraaldichtheid van zowel de lumbale wervelkolom als de heup. Er werd een matige correlatie gevonden tussen beide typen metingen variërend tussen 0.33 en 0.50. Dit wijst erop dat voor het voorspellen van een lage

botmineraaldichtheid van wervelkolom of heup ultrageluidsmetingen weinig zinvol zijn.
Als gevolg van factoren zoals selectie-bias kunnen de in een dwarsdoorsnede-onderzoek geschatte jaarlijkse veranderingen een vertrokken beeld geven. Daarom werden de jaarlijkse veranderingen ook berekend in een vervolg-onderzoek. Bij 543 personen werden twee metingen verricht met een gemiddelde tijdsduur tussen de metingen van 1.4 jaar. Zowel bij mannen als bij vrouwen daalde de SOS met 2.5 m/s/jaar (dit komt overeen met een daling van 2.1% per jaar bij mannen en 2.5% per jaar bij vrouwen). Het 95% betrouwbaarheidsinterval bedroeg bij mannen -4.0 tot -1.1 m/s/jaar en bij vrouwen -3.6 tot -1.4 m/s/jaar. Bij mannen was er sprake van een toenemend verlies met het voortschrijden van de leeftijd. BUA veranderde noch bij mannen, noch bij vrouwen. De in het vervolg-onderzoek berekende jaarlijkse veranderingen in de ultrageluidsparameters waren aanzienlijk anders dan de veranderingen berekend uit het dwarsdoorsnede onderzoek.
Op grond van de precisie van de ultrageluidsmetingen en op grond van de waargenomen jaarlijkse veranderingen kon berekend worden dat ultrageluidsmetingen weinig zinvol zijn voor het vervolgen van individuele patiënten.
In hoofdstuk 2.4 werd de associatie besproken tussen ultrageluidsparameters en recente niet-wervel fracturen. Personen met een waarde voor SOS of BUA in het laagste tertiel rapporteerden driemaal vaker een fractuur dan personen met een waarde in het hoogste tertiel. Voor de botmineraaldichtheid gemeten aan de heup werden vergelijkbare getallen gevonden. Binnen tertielen van botmineraaldichtheid rapporteerden personen met een lage SOS steeds een hogere fractuurfrequentie. Voor BUA was een vergelijkbare, maar op het oog minder uitgesproken trend zichtbaar. Wij concludeerden hieruit dat ultrageluidsparameters wellicht inzicht geven in aspecten van de botsterkte die niet samenhangen met de botdichtheid.
In hoofdstuk 3.1 werd de associatie onderzocht tussen niet van insuline afhankelijke diabetes mellitus (NIDDM), botmineraaldichtheid van heup en wervelkolom, en recente niet-wervel fracturen. De botmineraaldichtheid werd bepaald bij 2481 mannen en bij 3450 vrouwen. Hiervan hadden 243 mannen en 335 vrouwen NIDDM. Zowel mannen als vrouwen met NIDDM

hadden een ongeveer 3% hogere botmineraaldichtheid dan personen met een normale glucose-tolerantie op alle plaatsen waar gemeten werd. Bovendien leek NIDDM bij vrouwen samen te gaan met een lagere frequentie van niet-wervel fracturen.

In hoofdstuk 3.2 werd een overzicht gegeven van de biochemische markers van de botombouw die momenteel in gebruik zijn. Recent ontwikkelde markers geven een beter inzicht in de complexe functies van de osteoblast onder normale omstandigheden maar ook tijdens ziekte. Osteocalcine en het bot-specifiek alkalische phosphatase lijken op dit moment de meest veelbelovende markers voor botaanmaak. Voor het meten van de botafbraak lijken de pyridinoline crosslinks, bepaald in de urine, het meest aangewezen.

In hoofdstuk 3.3 werd beschreven dat hogere pyridinoline crosslinks spiegels in de urine samengaan met een toegenomen heupfractuur kans in nog zelfstandig wonende oudere vrouwen. Waarschijnlijk zijn de hogere spiegels een gevolg van een toegenomen botverlies als gevolg van een verminderde mobiliteit. We concludeerden dat in de urine bepaalde pyridinoline crosslinks wellicht bruikbaar zijn voor het voorspellen van heupfracturen. Echter, het is de vraag of voor het identificeren van personen met een verhoogd kans op het krijgen van een heupfractuur het meten van eenvoudig te bepalen risicofactoren niet een meer kosten-effectieve aanpak is.

In hoofdstuk 3.4 werden de resultaten uit hoofdstuk 3.3 nogmaals bevestigd. Bovendien werd gevonden dat een lage osteocalcine spiegel samengaat met een verhoogd heupfractuur risico. Deze twee bevindingen tezamen doen vermoeden dat op oudere leeftijd een ontkoppeling van de botaanmaak en -afbraak leidt tot een toegenomen broosheid van de botten.

De klinische relevantie van de in dit proefschrift gepresenteerde studies worden bediscussieerd in hoofdstuk 4. In dit hoofdstuk worden ook suggesties gegeven voor verder onderzoek.

DANKWOORD

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Paul van Daele was born on November 19, 1964 in Heerlen, The Netherlands. He graduated in 1983 at "St Willibrord Gymnasium" in Deurne. Subsequently, he started his medical training at the Catholic University of Nijmegen. He received his medical degree in 1990. Thereafter he worked for 3 month at the University Lung Centre - Medical Centre Dekkerswald in Groesbeek. After his military service at her Majesties Royal Navy, he worked for a brief period at Leyenburgh Hospital in The Hague. In 1992 he started to work on his thesis at the department of Epidemiology & Biostatistics (head: Prof A. Hofman) and the Department of Internal Medicine III (head: Prof J.C. Birkenhäger). In January 1996 he started his training in internal medicine at "Merwede Hospital" in Dordrecht. He is married to Mirèse van Daele-Swinkels.