Bone turnover markers and prediction of bone loss in elderly women

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Dedicated to my parents

Contents

List of papers

This thesis is based on the following original publications, which are referred to in the text by Roman numerals (I–V):

I. Lenora J, Norrgren K, Thorsson O, Wollmer P, Obrant KJ, Ivaska KK. Bone turnover markers are correlated with total skeletal uptake of 99mTc-methylene diphosphonate (99mTc-MDP). BMC Med Phys. 2009 Mar 30;9:3.

II. Lenora J, Ivaska KK, Obrant KJ, Gerdhem P. Prediction of bone loss using biochemical markers of bone turnover. Osteoporos Int. 2007 Sep;18(9):1297-1305.

III. Lenora J, Gerdhem P, Obrant KJ, Ivaska KK. Bone turnover markers are correlated with quantitative ultrasound of the calcaneus: 5-year longitudinal data. Osteoporos Int. 2009 Jul;20(7):1225-1232

IV. Ivaska KK, Lenora J, Gerdhem P, Åkesson K, Väänänen HK, Obrant KJ. Serial assessment of serum bone metabolism markers identifies women with the highest rate of bone loss and osteoporosis risk. J Clin Endocrinol Metab. 2008 Jul;93(7):2622-32.

V. Lenora J, Åkesson K, Gerdhem P. Effect of precision on longitudinal follow-up of bone mineral density measurements in elderly women and men. Submitted.

Abbreviations

aBMD – areal bone mineral density

BMC – bone mineral content

BTMs – bone turnover markers

BUA – broadband ultrasound attenuation

CI – confidence interval

CV % – coefficient of variation

DXA – dual-energy X-ray absorptiometry

LSC – least significant change

OPRA – osteoporosis prospective risk assessment

QUS – quantitative ultrasound

S-bone ALP – serum bone-specific alkaline phosphatase

S-OC – serum osteocalcin

S-OC[1-49] – serum intact osteocalcin

S-cOC – serum carboxylated osteocalcin

S-Total OC – serum total osteocalcin

SD – standard deviation

SoS – speed of sound

S-TRACP5b – serum tartrate-resistant acid phosphatase 5b

TB – total body

TH – total hip

TSU – total skeletal uptake

^{99m}Tc-MDP – technetium 99m-labelled methylene diphosphanate

U-DPD/crea – urinary deoxypyridinoline to urinary creatinine ratio

U-LongOC/crea – urinary long osteocalcin to urinary creatinine ratio

U-MidOC/crea – urinary mid-osteocalcin to urinary creatinine ratio

U-TotalOC/crea – urinary total osteocalcin to urinary creatinine ratio

Introduction

Burden of osteoporosis

Osteoporosis is a disease characterised by low bone mass and micro-architectural deterioration of bone tissue, leading to enhanced bone fragility and thereby an increased fracture risk (1,2). It is mainly an age-related phenomenon, commonly occurring in postmenopausal women and in elderly men (3). It can also occur earlier in life secondary to other disease conditions or medications. Osteoporosis is a major healthcare problem, clinically manifested as fractures resulting from minor trauma acting on a skeleton that has reduced strength. The lifetime risk of hip, wrist or clinically diagnosed vertebral fractures in Caucasian women is about 35–40% and it is 13% for men (2,4,5). Risk of fragility fracture begins to rise rapidly after the age of 75 years (6). The remaining lifetime risk for a Caucasian woman at the age of 80 years is 70% (7,8). Osteoporotic fractures are a major burden to the global costs of healthcare (9,10). In Europe, it is estimated that 179,000 men and 611,000 women will suffer a hip fracture each year, and the related costs will probably reach 25 billion euros (11).

The most common sites of fracture are the thoracic and lumbar spine, proximal femur, distal radius, pelvis and humerus (2). Having sustained an osteoporotic fracture increases the risk of subsequent fractures (12). The fracture with the largest impact on quality of life is the hip fracture (13). It is often followed by morbidity and is associated with increased mortality. Most of the osteoporotic hip fractures result from simple falls. Up to 20% of hip fracture patients die between six and twelve months after the fracture event (14) and more than 50% of the survivors require long-term care (15). Only 20–50% of patients who were independent prior to fracture return to pre-fracture activity levels twelve months after hip fracture (15).

It has been estimated that nearly 15–20% of postmenopausal women sustain vertebral fractures due to osteoporosis, and these fractures often cause significant pain (4,16). About 20% of women with vertebral fractures sustain other fractures within one year (12). The incidence of distal forearm fractures begins to increase from 45–60 years and is followed by a plateau (17), and the incidence does not increase with aging – perhaps because of the altered neuromuscular reflexes with aging (18).

Pathogenesis of osteoporosis

The pathogenesis of osteoporosis is complex and includes both genetic and environmental factors. Osteoporosis results from imbalance in the normal mechanisms that control bone remodelling (19). In healthy adults, bone mass is maintained by continuous turnover of bone in the bone remodelling process (20). In this cycle,

resorptive activity of osteoclasts is tightly coupled to the formative activity of osteoblasts, ensuring that bone mass remains constant, but after menopause the rate of bone resorption is higher than that of bone formation. The rate of bone loss can be 2–5% per year during the first few years after menopause (21). The pathogenic causes of osteoporosis can be divided into three main categories. These are (i) failure to achieve optimal peak bone mass during growth, (ii) excessive bone resorption after reaching peak bone mass, and (iii) impaired bone formation responses during remodelling (19,22).

It is not only the decrease in bone mass that influences bone strength; trabecular microstructure is also an important factor (23-25). Osteoporotic bone has lost trabecular connectivity, in addition to trabecular bone mass, leading to trabecular thinning (Figure 1) (22). The accumulation of micro-damage with age causes an increase in risk of fracture throughout life, especially in women (26). The pathogenesis of bone loss in osteoporosis involves several risk factors that include gender, age, genetics, hormonal changes and environmental influences.

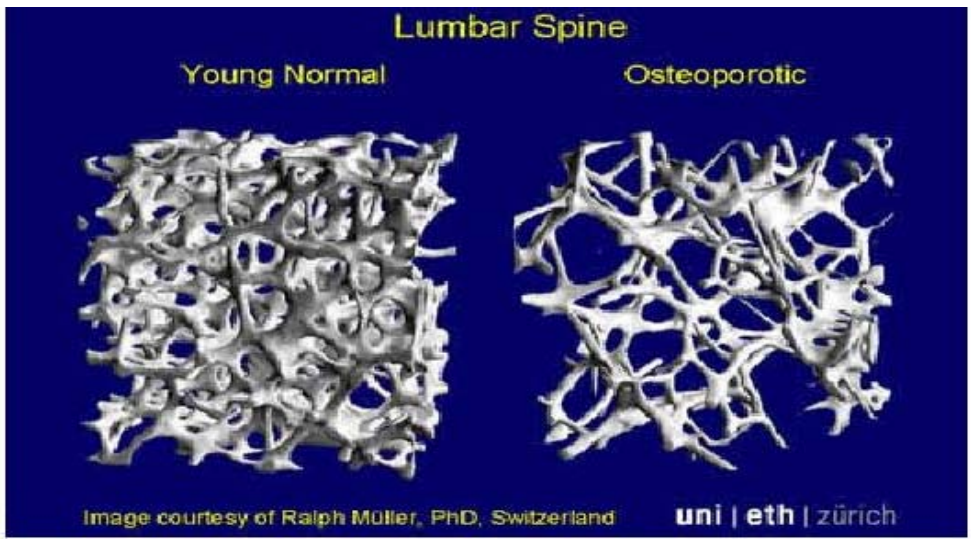

Figure 1. Trabecular bone structure in the lower spine of a young adult compared to that of an elderly osteoporotic adult. Reprinted by courtesy of Professor Ralph Muller, Switzerland.

Bone tissue: functions and microstructure

Bone tissue, together with cartilage, makes up the skeletal system in vertebrates. There are three major functions of the skeleton: (i) to provide mechanical support and muscle attachment for locomotion, (ii) to protect vital organs (with the bone marrow providing space for haematopoiesis in the medullary cavities), and (iii) to provide a metabolic function that is essential for the regulation of calcium and phosphate levels in the body (27). In addition, bone absorbs potentially hazardous trace elements such as toxins and heavy metals, thus minimising the harmful effects to other tissues of the body (28).

The long bones have epiphyses: a mid-shaft, which is called the diaphysis, and a development zone, the metaphysis. The growth plate lies between the metaphysis and the epiphysis. There are two types of bone tissue, cortical bone (compact bone) and trabecular bone. Although cellular and matrix components are similar in cortical and trabecular bones, they differ in structure and function. The external shell of the bone and also the diaphysis of long bones consist of cortical bone that is composed of lamellae, dense layers of calcified tissue arranged around Haversian canal forming osteons. Cortical bone contributes to about 85% of the whole skeleton, and it is mainly responsible for the mechanical strength and stiffness of bone (29,30).

Trabecular bone (cancellous or spongy bone), which makes up about 15% of the whole skeleton, is located in the epiphysis of long bones, in vertebral bodies and in the inner parts of the small bones. It is a rigid meshwork of thin, mineralised trabeculae that are less dense than cortical bone (Figure 1). Trabecular bone has both a mechanical and a metabolic role, acting as a reservoir for calcium and phosphorus (29). Despite their differences in structure, distribution and function, trabecular and cortical bone are produced by the same cell types and have the same overall matrix composition.

Bone matrix

Bone matrix consists of abundantly mineralised extracellular tissue and functionally distinct cell populations, osteoblasts, osteoclasts and osteocytes. Chemically, inorganic minerals – predominantly calcium phosphate – contribute to two-thirds of the composition of bone tissue and organic bone materials account for the remaining one third. More than 90% of the organic bone matrix consists of triple-helical type I collagen fibrils. The network of collagen fibres provides strength and also binds other proteins. The intrinsic properties of the collagen matrix and bone mineral also contribute to fracture resistance (together with the amount, architecture and rate of turnover of bone) (31). Collagen and most non-collagen matrix proteins are secreted by osteoblasts (32). After secretion of collagen fibrils, these undergo a series of post-translational

modifications and are arranged in triple helices (31). Osteocalcin is the most abundant non-collagen protein.

Calcium is the mineral component of bone tissue. Together with phosphorus, calcium forms hydroxy-appetite crystals ($Ca_{10}(PO_4)_6$ (OH_2)); these lie along the collagen fibrils embedded in an amorphous ground substance that, together with the bone cells, makes up bone. The ground substance is mainly composed of glycoproteins and proteoglycans (27). Bone acts as a calcium store for the body and is the major tissue involved in regulation of plasma calcium levels (33). Ninety-nine per cent of the body's calcium is found in the skeleton and teeth, with the remaining 1% being found in extracellular fluid, plasma and cell membranes (34). At birth, the human body contains 25 grams of calcium, and in an adult the body contains 1,000–1,300 g (35). High calcium intake during childhood results in high bone density (not above normal), while low calcium intake (< 500 mg per day) results in low bone density (36).

Bone cells

There are 3 types of cells in bone: osteoblasts, osteocytes and osteoclasts. These have different roles in keeping the bone metabolically active.

Osteoblasts

Osteoblasts are derived from the mesenchymal stem cells found in bone marrow and in the periosteum. Osteoblasts produce most of the matrix proteins in bone, and a mature osteoblast on the surface of bone secretes type I collagen and other bone matrix proteins during bone formation. Osteoblasts work in clusters along the bone surface, with around 100–400 cells per bone-forming site (37). Towards the end of the secreting period, the osteoblasts become either flat lining cells or osteocytes (27). When the bone is neither at a stage of formation nor resorption, there is a flattened, single layer of osteoblasts and these can differentiate into osteogenic precursors, and may be involved in the propagation of the activation signal that initiates bone resorption and bone remodelling (38).

Osteocytes

Osteocytes are the most abundant cells in mature bone, and they have reached the terminal differentiation stage in the osteoblast lineage (32). Osteocytes are important for structural and metabolic support. During the transformation from motile osteoblast to embedded osteocyte, the cell produces an extracellular matrix that is three times its own volume. The rounded osteoblast becomes a more dendritic-shaped osteocyte. Osteocytes connect to neighbouring osteocytes and to other cells on the surface of bone by thin and long processes containing microfilaments. These processes form a network

of canaliculi that permeate the entire bone matrix (39). The main function of this cell-to-cell contact is considered to be mechanosensory: transducing stress signals (stretching, bending) to mechanical loading. Osteocytes have a long lifespan, which can even be several decades (32).

Osteoclasts

Osteoclasts are the main bone-resorbing cells, and they represent the smallest proportion of bone cells. Osteoclasts are of haematopoietic origin. These multinucleated cells, containing 4–20 nuclei, are derived from bone marrow hematopoietic stem cells (40). They are usually found in contact with calcified bone surface and within lacunae. Osteoclasts have several specific features such as the capacity to resorb calcified bone and cartilage (40). Usually, 1–2 osteoclasts are found in one resorption site, but there can even be up to 5. They have a ruffled border, which is a deep sealed fold of the plasma membrane containing hundreds of motile microvilli that are directed at the resorbing surface. The osteoclast cytoplasm is "foamy", with many vacuoles containing enzymes such as tartrate-resistant acid phosphatase 5b (S-TRACP5b) and cathepsin K (27). Osteoclastogenesis needs the action of two cytokines: receptor for activator of nuclear factor-κB ligand (RANKL) and macrophage colony stimulating factor (M-CSF) (41,42). These proteins are secreted, for example, by marrow stromal cells and osteoblasts (41).

Bone remodelling

Bone is a dynamically and metabolically active organ that is continuously subjected to resorption and formation by the coordinated action of osteoclasts and osteoblasts on the surface of trabecular bone and in the Haversian canals (43). These two processes are collectively called bone turnover or bone remodelling. Due to the higher surface area of trabecular bone compared to cortical bone, most of the bone turnover takes place in trabecular bone. Osteoclasts degrade existing bone matrix and osteoblasts synthesise new bone matrix. About 10% of the skeleton is remodelled each year (44), allowing the it to adjust its strength to mechanical stress and to repair any damage (20,39). Bone remodelling is also necessary for maintenance of the metabolic function of the skeleton and for plasma calcium homeostasis (45).

The remodelling occurs in focal and discrete remodelling units on the surface of bone throughout the skeleton. The initial activation begins with interaction of osteoclast precursor cells and osteoblast precursor cells. This is followed by differentiation, migration and fusion of the large multinucleate osteoclasts. The mechanism behind this activation is still not known. These active osteoclasts attach to the surface of bone and secrete hydrogen ions and lysosomal enzymes (46). The resorptive phase stops after a certain volume of bone has been removed, which takes approximately 10 days. After the resorption phase, osteoblast precursors are recruited to the site (46). This occurs possibly

through signalling from the proteins released during the bone resorption in a paracrine fashion. They may also enhance cell proliferation. The formation phase of bone remodelling takes 3–4 months. The initial, rapid phase is followed by slow, passive mineralization; this continues for another 4–5 months until all the new bone is mineralized (47).

Many factors affect bone turnover, which can increase either the bone formation or the resorption, or both. Stimulation or inhibition of one of these two processes leads to uncoupling of bone turnover. During growth, bone size and strength increase. During the first three decades of life, bone formation predominates over bone resorption, until the maximum bone mass has been achieved. This maximum bone mass is referred to in the literature as "peak bone mass" (48,49). After reaching the peak bone mass, there is a state of equilibrium where the rate of bone formation equals the rate of bone resorption. After the age of 40 years, the remodelling process is not in balance and bone resorption predominates over bone formation (50). The aging process includes endosteal resorption, periosteal apposition, trabecularization of cortical bone (Figure 1) and increase in cortical porosity (50). In women, this process is accelerated in the first few years after the menopause (50). This process will lead to more fragile bone, and subsequently increased risk of fracture.

Bone turnover markers

Bone turnover markers – or biochemical markers of bone turnover – are bone tissue proteins or their fragments, or enzymes released from bone cells during bone turnover. Proteins can be by-products of collagen formation or products of collagen degradation, or non-collagenous proteins such as osteocalcin and bone sialoprotein. Enzymes such as bone-specific alkaline phosphatase and tartrate-resistant acid phosphatase 5b can also be used as markers of bone turnover. Bone turnover markers can be detected in serum or urine. Ideally, they should reflect only the activity of osteoblasts or osteoclasts. The bone turnover markers that are mainly released during bone formation or resorption are known as bone formation or resorption markers, respectively (Table 1). Formation and resorption are usually tightly coupled in time and space; thus, any such marker reflects the overall rate of bone turnover. Certain bone turnover markers may reflect different stages of formation and resorption, but they cannot reflect disease-specific processes and cannot distinguish between the activities at cortical or trabecular bone.

Assessment of bone turnover using bone turnover markers has the advantages of relatively low cost and non-invasive sample collection compared to the evaluation of bone turnover rate by histomorphometry in bone biopsies from the iliac crest. Although bone biopsy may give direct evidence concerning the aetiology, pathogenesis and progress of metabolic bone diseases, it has the disadvantage of being invasive and of giving information on bone turnover only concerning that specific skeletal region.

Table 1. Currently available, common bone turnover markers

	Tissue of origin	Specimen	Abbreviation
Bone formation markers			
Alkaline phosphatase	liver, bone, placenta, intestine, germ cells	Serum	S-ALP
Bone-specific alkaline phosphatase	bone (osteoblasts), platelets	Serum	S-Bone ALP
Osteocalcin (intact, total, carboxylated)	bone (osteoblasts)	Serum	S-OC
Procollagen I C-terminal extension peptide	bone, soft tissue, skin	Serum	S-PICP
Procollagen I N-terminal extension peptide	bone, soft tissue, skin	Serum	S-PINP
Bone resorption markers			
Tartrate-resistant acid phosphatase 5b	bone (osteoclasts)	Serum	S-TRACP5b
C-terminal cross-linking telopeptide of type I collagen	bone, soft tissue, skin	Serum/ Urine	S-CTX-I, U-CTX-I
N-terminal cross-linking telopeptide of type I collagen	bone, soft tissue, skin	Serum/ Urine	S-NTX-I, U-NTX-I
C-terminal cross-linking telopeptide of type I collagen, generated by metalloproteinases	bone, skin	Serum	S-ICTP or S-CTX-MMP
Deoxypyridinoline	bone, dentine	Urine	U-DPD
Pyridinoline	bone, cartilage, tendon, blood vessels	Urine	U-PYD
Osteocalcin	bone	Urine	U-OC

Bone formation markers

Total alkaline phosphatase (ALP) and bone-specific alkaline phosphatase (Bone ALP)

Alkaline phosphatase (ALP) is an enzyme located on the cell surface. Three different tissue-specific genes encode the intestinal, placental and germ-line enzymes, and the tissue-unspecific gene is expressed in numerous tissues, including bone and the liver. Tissue-unspecific ALPs are produced by the same gene but there are tissue-specific differences in their post-translational modification of the carbohydrate chains (51). The most common sources of elevated serum ALP levels are liver and bone. In bone, ALP is present on the cell surface of the osteoblasts, and it is probably cleaved off from the membrane and released into circulation. In healthy individuals, about half of the serum alkaline phosphatase is derived from bone. Thus, measurement of S-ALP can be used as a marker of bone turnover, but it lacks sensitivity and specificity, especially under conditions in which there is only a small increase in bone turnover. Measurement of the bone-specific isoform S-Bone ALP has better sensitivity for detecting changes in bone turnover. However, the S-Bone ALP assays that are currently available still have a cross-reactivity with the liver isoenzyme of 15–20% (52).

Osteocalcin (OC)

Osteocalcin (or bone Gla protein) is the most abundant non-collagenous matrix protein in bone. It forms about 1% of the organic component of bone. It is a low-molecular-weight protein consisting of 49 amino acids and is expressed by osteoblasts, osteocytes, odontoblasts and hypertrophic chondrocytes. Osteocalcin has a high affinity for Ca^{2+} in bone hydroxyapatite, due to the three γ-carboxy glutamic acid residues at positions 17, 21 and 24 (53). Part of the newly synthesized osteocalcin is incorporated into new bone matrix and part of it enters the circulation, where it can be detected. Serum osteocalcin is considered to be a specific marker of osteoblast activity, and its serum levels thus reflect the rate of bone formation. Circulating osteocalcin consists of different immune-reactive forms. Approximately one third of serum osteocalcin is intact, one third consists of the mid-molecule fragment 1-43 and one third is smaller fragments (54). It is not clear whether these fragments are by-products during the biosynthesis of OC, from proteolysis of osteocalcin in the circulation or whether they are released directly from bone during bone resorption. Different osteocalcin assays can detect different fragments of osteocalcin in serum. The presence of multiple isoforms of OC in serum and the differences between assays in detection of these isoforms limit the clinical usefulness of S-OC (55).

Osteocalcin may also be released from the bone matrix during bone resorption (56). Osteocalcin that enters the circulation is rapidly degraded (57). Breakdown fragments are cleared via the liver and the kidneys, and immune-reactive osteocalcin fragments can also be detected in the urine (58). Urinary osteocalcin (U-OC) appears to be more related to bone resorption than bone formation (59,60).

Procollagen type I pro-peptides

During the extracellular processing of newly-synthesized type I collagen, the amino-terminal and carboxy-terminal extension peptides are cleaved before fibril formation (61). These extension peptides guide the helical folding of the collagen molecule and the released N- and C-terminal pro-peptides of type I collagen (PINP and PICP, respectively) can be detected in the circulation. PICP and PINP are considered to be quantitative measures of the newly formed type I collagen. Type I collagen is also a component of several soft tissues; thus, there is a possible contribution from sources other than bone. However, the rate of collagen turnover in bone is faster than in other tissues, and therefore the changes in S-PINP and S-PICP are assumed to primarily reflect changes in collagen synthesis in bone (61,62).

Bone resorption markers

Tartrate-resistant acid phosphatase 5b (TRACP5b)

Acid phosphatases are catalytic enzymes that act on phosphoesters in an acidic environment. Six isoenzymes of acid phosphatase have been identified in humans. Type 5 is expressed by osteoclasts and by alveolar and monocyte-derived macrophages, and is resistant to tartrate inhibition. Two isoforms of TRACP5 can be found in the human circulation. TRACP5a is sialylated and originates from macrophages and dendritic cells, whereas TRACP5b lacks sialic acid and is derived from osteoclasts. The two isoforms also have different pH optima. The biological function of S-TRACP 5b in osteoclasts remains elusive. It is believed to destroy the endocytosed bone matrix degradation products during trans-cytosis through the osteoclast. TRACP-containing vesicles are added to the trans-cytotic vesicles transporting matrix degradation products, and TRACP is believed to assist matrix degradation in vesicles by producing reactive oxygen species (ROS) (63). TRACP has been reported to reflect the bone resorption rate, but more recent data have shown that it more accurately reflects the number of osteoclasts rather than their activity (64). Circulating TRACP5b levels are not affected by the renal function, and the

effect of food intake is negligible (65). Furthermore, the level of TRACP5b is relatively stable in serum samples (66).

Collagen cross-links and cross-linked telopeptides

Collagen structure is stabilised by intra- and intermolecular cross-links. In bone, the predominant cross-links are pyridinoline (PYD) and deoxypyridinoline (DPD). Pyridinoline and deoxypyridinoline cross-links are released during bone resorption when type I collagen is degraded. PYD is more predominant in collagen while DPD is the minor component, but since DPD is most abundant in bone and dentin, it is considered to be a more bone-specific cross-link (67). Cross-links are cleared by the kidney, and they can be measured in serum or urine either as free cross-links or when bound to short collagen peptides.

Cross-linked telopeptides of type I collagen include the cross-linked N-terminal telopeptides (NTX) and cross-linked C-terminal telopeptides (CTX and ICTP). Fragments are generated by different collagenolytic pathways. NTX and CTX are released by cathepsin K cleavage and ICTP is a larger fragment produced by matrix metalloproteinases (68). CTX exists in an isomerised beta-CTX form and a non-isomerised alpha-CTX form. Isomerisation is associated with the aging of bone, and the assay for beta-CTX is therefore considered to measure the degradation of relatively old bone (69). Currently, beta-CTX-I is perhaps the most commonly used cross-link assay.

Use of bone turnover markers

Bone turnover markers assessed in serum or urine can be used in three main clinical areas, although individual patient management guidelines are still to come. The clinical areas are: (i) prediction of bone loss and the risk of developing osteoporosis, (ii) identification of individuals with a high risk of fracture, and (iii) monitoring of anabolic or anti-resorptive therapy.

Prediction of bone loss

Individuals with a high rate of bone loss are at risk of developing osteoporosis and fracture. High rate of bone loss is in itself a risk factor for fracture, independently of BMD (70). Accelerated bone turnover and subsequent bone loss cannot be assessed by DXA unless serial measurements are performed. Due to the imprecision of measurement and the relatively slow rate of change in bone density, it would take

years to detect a high turnover of bone with serial bone density measurements (71). The prediction of bone loss by measurement of bone turnover markers in single samples of serum or urine has been tested in many studies, and some of the studies are summarized in Table 2. In general, weak to modest correlations have been reported between bone turnover markers and bone loss at the lumbar spine and proximal femur. Women with high levels of bone turnover markers loose more bone than women with low levels of bone turnover markers (72-74). Accordingly, women with a high rate of bone loss have higher levels of bone turnover markers than women with a low rate of bone loss (75-77).

Table 2. Correlation between biochemical markers at baseline and subsequent bone loss. A. Correlation with aBMD changes in Hip or femoral neck. B. Correlation with aBMD changes in the lumbar spine. Significant correlation coefficients (r) are marked in bold, [a]p<0.05, [b]p<0.01, [c]p<0.001

A.

Bone turnover markers	R value	Reference	Number of participants	Mean age in years (SD or range)	Duration, years
Bone formation markers					
S-Bone ALP	0.14	(82)	188 women	59.2 (11.5)	3.0
	0.02	(73)	81 women	57.1 (26-86)	3.0
	-0.39[a]	(83)	59 women	46.8 (6.1)	3.0
	-0.38[a]	(80)	36 women	71.0 (4.0)	3.0
S-OC	-0.14	(82)	188 women	59.2 (11.5)	3.0
(all forms)	-0.13	(73)	81women	57.1 (26-86)	3.0
	-0.35[a]	(83)	59 women	46.8 (6.1)	3.0
	-0.20	(80)	36 women	71.0 (4.0)	3.0
S-PICP	**-0.19[a]**	(82)	188 women	59.2 (11.5)	3.0
	-0.30[b]	(73)	81 women	57.1 (26 -86)	3.0
	-0.20	(83)	59 women	46.8 (6.1)	3.0
Bone resorption markers					
S-TRACP5b	-0.03	(73)	81 women	57.1 (26-86)	3.0
S-ICTP	0.05	(82)	188 women	59.2 (11.5)	3.0
	-0.31[b]	(73)	81 women	57.1 (26-86)	3.0
U-NTX/ crea	-0.18	(83)	59 women	46.8 (6.1)	3.0
	-0.52[b]	(80)	36 women	71.0 (4.0)	3.0
U-CTX-I/crea	**-0.36[a]**	(83)	59 women	46.8 (6.1)	3.0
U-NTX/crea	**-0.20[a]**	(84)	143 women	65.6 (2.8)	4.0
U-DPD/crea	**-0.16[a]**	(82)	188 women	59.2 (11.5)	3.0
	-0.23	(73)	81 women	26-86	3.0
	-0.51[b]	(80)	36 women	71.0 (4.0)	3.0
U-Pyr/crea	**-0.35[b]**	(73)	81 women	57.1 (26-86)	3.0
	-0.44[b]	(80)	36 women	71.0 (4.0)	3.0

B.

Bone turnover markers	R value	Reference	Number of participants	Mean age in years (SD or range)	Duration, years
Bone formation markers					
S-Bone ALP	**0.20**[c]	(85)	603 Women	67.4 (6.8)	3.0
	0.11	(82)	188 women	59.2 (11.5)	3.0
	-0.19[a]	(86)	122 women	61.6 (0.6)	2.0
	-0.47[c]	(73)	81 women	57.1 (26-86)	3.0
	-0.36[a]	(83)	59 women	46.8 (6.1)	3.0
	-0.43[c]	(87)	60 women	57.0 (0.3)	4.0
	0.06	(80)	36 women	71.0 (4.0)	3.0
S-OC (all forms)	**0.09**[a]	(85)	603 women	67.4 (6.8)	3.0
	-0.15	(86)	122 women	61.6 (0.6)	2.0
	-0.40[c]	(73)	81 women	57.1 (26-86)	3.0
	-0.29[a]	(83)	59 women	46.8 (6.1)	3.0
	-0.42[b]	(87)	60 women	57.0 (0.3)	4.0
S-PICP	0.04	(82)	188 women	59.2 (11.5)	3.0
	-0.24[a]	(73)	81 women	57.1 (26-86)	3.0
	-0.13	(83)	59 women	46.8 (6.1)	3.0
S-PINP	**-0.53**[c]	(87)	57 women	57.0 (0.3)	4.0
Bone resorption markers					
S-TRACP5b	-0.20	(73)	81 women	57.1 (26-86)	3.0
S-ICTP	0.11	(82)	188 women	59.2 (11.5)	3.0
	-0.06	(73)	81 women	57.1 (26-86)	3.0
S-NTX					
U-NTX/ crea	**-0.42**[b]	(87)	60 women	57.0 (0.3)	4.0
	-0.09	(83)	59 women	46.8 (6.1)	3.0
U-CTX-I/crea	**-0.11**[a]	(85)	603 women	67.4 (6.8)	3.0
	-0.25	(83)	59 women	46.8 (6.1)	3.0
U-NTX/crea	**-0.21**[a]	(84)	143 women	65.6 (2.8)	4.0
U-DPD/crea	0.03	(82)	188 women	59.2 (11.5)	3.0
	-0.20[a]	(86)	122 women	61.6 (0.6)	2.0
	-0.19	(73)	81 women	57.1 (26-86)	3.0
	-0.35[b]	(87)	57 women	57.0 (0.3)	4.0
U-Pyr/crea	**-0.18**[a]	(82)	188 women	59.2 (11.5)	3.0
	-0.13	(86)	122 women	61.6 (0.6)	2.0
	-0.10	(73)	81 women	57.1 (26-86)	3.0

One study involving osteopenic elderly women showed that women in the high tertile of bone turnover markers (S-OC, S-Bone ALP, U-DPD/crea) were at a 4.3–6.4 times higher risk of developing osteoporosis in the lumbar spine after 3 years when compared to the women in the low tertile of bone turnover markers (78). In addition to bone loss at the lumbar spine and proximal femur, some studies have also reported an association between bone turnover markers and bone loss in the forearm (76,78,79) and calcaneus (77). The lack of association between levels of bone turnover markers and spinal bone loss in elderly women that has been observed in some studies (80) could be due to the difficulty in detecting spinal bone loss accurately in elderly women. An association between bone turnover markers and bone loss assessed by quantitative ultrasound of the calcaneus has been reported, but the data are still rather limited (81).

Bone turnover markers and prediction of fracture

Osteoporosis is a silent disease. The end-stage of osteoporosis – fracture – is associated with reduced quality of life, shortened lifespan and large healthcare costs. Thus, the strategies of osteoporosis management are directed toward prevention of fracture. Prevention of fracture starts with early identification of fracture-prone individuals. Although a low BMD at the spine, proximal femur or forearm is associated with an increased risk of fracture (88), about half of such fractures occur in individuals who have a BMD above the level of the osteoporosis diagnostic threshold (6,89). Ideally, only those individuals who are at high risk of sustaining a fracture should receive anabolic or anti-resorptive treatment, and treatment should be targeted on the basis of risk assessment rather than the BMD measurement alone. Thus, improvement of fracture prediction is necessary. Increased levels of bone turnover markers have been found to be predictive of fractures independently of age, BMD and prior fracture (Table 3). Elevated levels of bone resorption markers such as S-TRACP5b, S-CTX-I, U-CTX-I, U-NTX-I and U-OC (90-98) have been shown to be associated with fracture risk; and of the bone formation markers tested, S-Bone ALP, S-OC, S-PICP and S-PINP (93,94,98-100), as well as 3-month change in S-OC (85), have been associated with fracture risk (Table 3).

Only a few studies have investigated the fracture predictability of bone turnover markers in men. Meier *et al.* showed that high baseline levels of S-ICTP, but not of S-CTX-I or S-PINP, were associated with fracture in elderly men during a 6-year follow-up (101). Luukinen *et al.* showed that baseline carboxylated osteocalcin and carboxylated to total osteocalcin ratio in serum were lower in men who had sustained a fragility fracture during a 5-year follow-up than in men who had not sustained a fracture (99). Szulc *et al.* reported that levels of S-OC, S-Bone ALP and S-CTX-I could be correlated to the rate of bone loss in the total body, the distal forearm or

Table 3. Summary of studies conducted to assess the possibility of predicting fracture using bone turnover markers. Bone turnover markers with and without statistically significant relative risk of sustaining a fracture are summarized. Relative risk is assessed for the high bone turnover group, when compared to the control group (low bone turnover group or all the other women, depending on the study)

Cohort	Mean age in years, (SD or range)	Follow-up, years	Analysis	Number of individuals with fractures	Bone turnover markers statistically significant	Bone turnover markers not statistically significant	Reference
322 Osteopenic women	62.9 (9.0)	9.1	highest quartile vs. all others	54 (any fracture)	S Bone ALP, S-OC and S-CTX-I	none	(98)
348 women	80.3 (5.6)	5.0 (up to 7.6)	highest quartile vs. others	83 (any fracture) 43 (non-vertebral fracture) 16 (hip fractures)	U-NTX/crea with non-vertebral fractures	S-ALP, S-OC with vertebral, hip or non-vertebral fracture U-NTX-I/crea with hip fracture	(90)
1040 women	75.0	3.0–6.5	highest quartile vs. all others	178 (any fracture) 49 (vertebral fracture)	S-TRACP5b, U-Long OC/crea, any fracture or vertebral fracture S-CTX-I, U-Mid OC/crea, vertebral fracture	S-Bone ALP, S-Total OC, S-OC[1-49], S-cOC, U-DPD/crea	(91)
491 women, 301 men	76.5 (4.9) 75.5 (4.9)	5.0	less than 1 SD below the mean compared to others; age and sex adjusted	84 (any fracture)	S-cOC S-cOC/S-Total OC ratio	S-Total OC	(99)
408 women	82.5 (4.5)	3.3	Above the upper limit of premenopausal range vs. others	115 (hip fractures) 293 (controls)	U-CTX-I/crea -morning sample U-DPD/crea- morning sample S-CTX-I –afternoon sample	S-CTX-I – morning sample	(92)
512 women	73.7 (5.2)	2.7	per 1 SD increase	55 (33 vertebral and 25 non-vertebral fractures)	S-Bone ALP U-CTX/crea	none	(93)
328 women	59 (40–80)	5.0	per 1 SD decrease, and adjusted to age and BMC	43 (any fractures)	S-PICP , S-ICTP	S-OC	(94)

Cohort	Mean age in years, (SD or range)	Follow-up, years	Analysis	Number of individuals with fractures	Bone turnover markers statistically significant	Bone turnover markers not statistically significant	Reference
435 women	64.4 (50–89)	5.1	highest quartile vs. others; adjusted for age, previous fractures and physical activity	55 (21 vertebral and 37 non-vertebral fractures)	S-Bone ALP S-CTX-I U-CTX-I/crea	S-OC, S-PICP, S-PINP, U-NTX-I/crea, U-DPD/crea	(95)
408 women	64.0 (50–89)	6.8	highest quartile vs. others; adjusted for age, prevalent fractures and physical activity	65 (16 vertebral and 55 non-vertebral fractures)	U-CTX-I/crea levels U α-L-CTX-I/crea U β-L-CTX-I/crea Total U-CTX-I/crea U-CTX-I ratios U-CTX-I α-L/ β-L U-CYX-I α-L/ α-D	U-CTX-I/crea levels U α-D-CTX-I/crea U β-D-CTX-I/crea U-CTX-I ratios U-CTX-I α-L/ β-D	(96)
603 women	67.4 (6.8)	3.0	highest quartile vs. lowest quartile	71 (vertebral fractures)	3-month change in S-OC	Baseline level of S-OC, Baseline or 3-month change in S-Bone ALP, U-CTX-I/crea,	(85)
401 women	82.5 (75–89)	1.9	per 1 SD increase	109 (hip fractures)	U-CTX-I/crea, U-DPD/crea	S-Bone ALP, S-OC, U-NTX/crea,	(97)
183 women	83.0 (70–97)	3.0	per 1 SD increase	30 (hip fractures)	Serum undercarbo xylated OC	S-cOC, S-ALP	(100)

the trochanter in men above the age of 50 years, but they were not predictive of fracture during 7.5 years of the study (102).

In general, the predictability of fracture from bone turnover markers is weak to modest (103), and is no better than the predictability of bone density measurement (104,105). Studies are sometimes inconsistent, and the same marker may not have the same predictability in different studies (Table 3). Different durations of follow-up, different age categories of individuals studied, different numbers of bone turnover markers used in each study and the variability of assays used may have contributed to this inconsistency.

Monitoring of anti-osteoporosis treatment

Monitoring of the efficacy of bone-active drugs is currently the most promising clinical application for bone turnover markers. When compared to imaging techniques such as DXA, the levels of bone turnover markers change much faster in response to therapeutic interventions. The changes in bone turnover markers also have a markedly wider range, compared with the imprecision of the assay, than changes in aBMD. Levels of bone turnover markers respond as early as 4–12 weeks after pharmacological treatment (106-114), while it may take years to detect a change in aBMD or to detect a reduction in fracture risk. Bone formation (S-Bone ALP and S-PINP) and resorption markers (S-TRACP5b, S-CTX-I, and U-NTX/crea) decrease by 30–70% after 4–12 weeks of treatment and remain low in osteoporotic women treated with bisphosphonate (108,109,111-113,115,116), hormone replacement therapy (109,117) or, more recently, denosumab, which is a monoclonal human antibody against receptor activator of nuclear factor kappa B ligand (RANKL) (107,108). In general, the change in level of resorption markers occurs slightly earlier than the change in formation marker levels. Treatment with parathyroid hormone, which is an anabolic agent, causes an increase in bone formation markers (S-Bone ALP, S-PINP and S-PICP) within one month, and this increase is followed by an increase in bone resorption markers (S-CTX-I, U-NTX-I and U-DPD). The bone turnover markers remain elevated during one year of treatment (106,118), indicating their different mode of action compared to anti-resorptive agents such as bisphosphonates.

Early change in bone turnover markers following pharmaceutical therapy has been found to correlate with an increase in hip and lumbar spine BMD over 1–3 years in osteopenic and osteoporotic women (106-109,111,112,115,119). This has been used in the development of pharmaceutical agents: to identify the differences in therapeutic response to the same pharmaceutical agent in dose-finding studies (111,120-122) and in the comparison of different pharmaceutical agents

(108,109,112,114,119). Thus, bone turnover markers can be used to identify the optimum therapeutic agent and optimum dose for individual patients.

In addition, bone turnover markers have been used not only to predict the therapeutic effect of medication on BMD, but also to assess the possibility of predicting fracture risk following treatment. Short-term decrease in bone turnover markers CTX-I and NTX-I has been shown to be associated with reduced incidence of vertebral and non-vertebral fractures over 1–3 years in women treated with alendronate, risedronate, and zoledronic acid (121,123-126), and with raloxifen (127). Greater early changes in bone turnover markers following pharmaceutical treatment have been associated with greater decrease in fracture risk following alendronate (123) and raloxifene (127).

Pre-treatment levels of bone turnover markers may be useful for identification of patients who would benefit the most from the treatment. Patients with high bone turnover before the start of therapy are likely to respond to anti-resorptive agents better than patients with low bone turnover. This has been shown in studies conducted in patients treated for 1–3 years with bisphosphonates (126,128,129), calcitonin (130), hormone replacement therapy (HRT) (117,131,132) and parathyroid hormone (106). In these studies, larger gain in BMD (106,117,126,129,131,132), in BMC (130), or lower incidence of fragility fractures (128) was seen in the group with high bone turnover before treatment when compared to the group with low bone marker levels before treatment.

Pre-analytical variability of bone turnover markers

Pre-analytical variability is one of the limitations affecting the clinical interpretation of bone marker measurement. Levels of markers are affected by diurnal, menstrual and seasonal variations, as well as food intake and the level of physical activity. Uncontrollable factors include, for example, age, gender, menopausal status, recent fracture, bed rest, metabolic bone disease and renal function (133,134). BTMs are higher in infants and children than in adults, and reach their highest levels during puberty. After puberty BTMs remain low and rise again after menopause in women, remaining elevated throughout the rest of life (135). There is a slow rise in elderly men (134). Bone turnover marker levels are highest in the early morning and lowest in the late afternoon and evening. The largest diurnal variation has been reported for urinary collagen cross-links and cross-linked telopeptides (136,137). Serum levels of bone turnover markers are less affected, with the exception of S-CTX-I, which can vary about 60% between morning and night (138). Food intake reduces the levels of most of the bone turnover markers, especially S-CTX-I (139). The effect of food intake is most probably mediated through glucagon-like peptide 2, the synthesis of which is stimulated by food intake (140). Because of this, it is recommended that

morning fasting samples should be used for bone marker assessment. Bone turnover markers can also be influenced by a recently sustained fracture. Retrospective studies have shown that bone turnover marker levels remain elevated for up to 2 years after a fracture (94,141). Changes in the level of physical activity have been shown to affect the bone formation markers S-OC and S-PINP, but S-CTX-I levels are less affected (142). However, contradictory data exist in which bone formation markers were found to be lower in trained athletes than in sedentary controls (143), or unchanged (144). There may be a small seasonal variation in bone turnover markers, with an increase in levels of these markers during winter compared to levels measured in summer (145). However, the influence of seasonal variation is modest at the level of the individual (134) and some studies have shown absence of such an effect (146,147).

To minimize pre-analytical variability, it is advisable to collect morning fasting samples. The patient should be asked to refrain from physical exercise prior to sampling. History of recent fracture should also be taken into account when interpreting the results.

Future markers of bone turnover

Although bone turnover markers have shown clinically interesting associations, those that are currently available also have some limitations. They reflect quantitative changes but do not provide information on structural abnormalities of bone or on the remodelling rate of different bone compartments. Some have high variability or are not bone-specific. Recent progress in the identification of important pathways in bone physiology has led to the development of new potential biochemical markers. These include osteoclastic enzymes, regulators of bone cell activity, non-collagenous matrix proteins or their fragments, and markers of bone matrix properties.

Cathepsin K is an osteoclastic enzyme of the cysteine protease family; it is secreted by active osteoclasts and it is needed for the cleavage of both helical and telopeptide regions of type I collagen. Recently, an assay has been developed to assess serum cathepsin K (148). So far, only preliminary studies have been performed using human samples (149). Further studies are needed to identify the potential use of cathepsin K in the clinical context.

Osteoprotegerin (OPG) and receptor activator for nuclear factor kappa B ligand (RANKL) are expressed by osteoblasts and are important regulators of bone turnover. Interaction of RANKL with receptor activator for nuclear factor kappa B (RANK) receptor on osteoclast precursor cells induces the differentiation and maturation of osteoclasts. OPG acts as a decoy receptor for RANKL, and prevents osteoclastogenesis by preventing the signals induced by RANKL-RANK interaction (150). OPG levels

increase with age; higher levels have been found in post-menopausal and elderly women, probably as a homeostatic response to limit the bone loss (151). Mezquita-Raya *et al.* found that OPG was independently associated with osteoporosis and prevalent vertebral fractures (152). Low levels of the soluble form of RANKL can be detected in the circulation, and immunoassays have been developed for its measurement (153). In one study, low levels of RANKL were shown to be predictive of fracture (154). However, further studies are required to confirm the possible clinical value of novel biochemical markers of bone remodelling.

Methods of bone mass measurement

The assessment of fracture risk relies to a great extent on the measurement of bone mass. There are many factors that contribute to bone strength. These include bone geometry, trabecular architecture, accumulation of microfractures, accumulation of cement lines, cortical porosity and microheterogeneity of mineralisation (155,156). Several methods are available for the purpose of bone mass measurement. Single-photon absorptiometry (SPA), dual-photon absorptiometry (DPA) and single-energy X-ray absorptiometry (SXA) are no longer used in clinical practice. Quantitative computed tomography (QCT) can be used to measure volumetric BMD (vBMD), but it has no clinical application. Peripheral quantitative computed tomography (pQCT) has a lower radiation dose than QCT, but it can only be used to measure peripheral parts such as the distal radius and the tibia. Dual-energy X-ray absorptiometry (DXA), which is the golden standard for use in clinical practice, and QUS, which is mostly used in screening programmes and research applications, are discussed in detail below.

Diagnosis of osteoporosis

In the past, the clinical diagnosis of osteoporosis relied on the presence of a fragility fracture. Today, diagnosis of osteoporosis is mainly done by measuring bone density by DXA. The T-score is defined as the number of standard deviations that the bone density differs from the reference mean for young adults.

According to WHO guidelines, results of bone mineral density measurement are divided into 4 groups (2):

1. **Normal** – aBMD (or BMC) above 1 standard deviation (SD) below the young adult reference mean value (T-score ≥ -1.00).
2. **Osteopenia –** aBMD (or BMC) between 1 and 2.5 SD below the young adult reference mean value (T score < -1.0 and > -2.5).

3. **Osteoporosis** – aBMD (or BMC) 2.5 SD or more below the young adult reference mean value (T-score $\leq$ -2.5).
4. **Severe osteoporosis** (established osteoporosis) – aBMD (or BMC) 2.5 SD or more below the young adult reference mean value (T $\leq$ -2.5) and the presence of one or more fragility fractures.

Dual-energy X-ray absorptiometry (DXA)

DXA technology, which has the advantage of high precision, accuracy, short scan times and low radiation dose, meets the requirements of a non-invasive method of diagnosis and follow-up of osteoporosis (2). DXA measures the bone mineral content (BMC, g) and areal bone mineral density (aBMD, g/cm^2). It involves transmission of two different X-ray energies through the patient, which are differentially attenuated by the soft and bone tissue. DXA is a safe investigation, because the radiation dose to the patient is less than the daily dose of radiation from natural background radiation (157). Two different techniques are used in DXA: the pencil beam and the fan beam techniques. In the pencil beam technique, the image of the scanned region is achieved by multiple scans over the region of interest while the fan beam technique requires only a few sweeps over the region (158-160). The fan beam technique shortens the scan time required to 10–30 seconds for the hip or spine, as compared to 5–10 min for the pencil beam technique. The radiation dose in the pencil beam technique is 0.3–1.9 mSv, but with the fan beam it can increase by at least tenfold: up to 5-30 mSv depending on the site being measured (158).

The regions most often measured with DXA are the proximal femur (femoral neck or total hip) and lumbar spine (from the first or second lumbar vertebra to the fourth lumbar vertebra, depending on the manufacturer), but DXA can also be used to measure other sites such as the radius, the calcaneus and the total body. From a total body scan, lean mass, fat mass, BMC and aBMD of the total body or a particular region such as the arms, legs, head or trunk can be obtained. The hip is the best site for predicting hip fracture whereas the spine is the best site for prediction of vertebral fracture (161). It has been found that each standard deviation (SD) decrease in femoral neck BMD is associated with an increase in the relative risk of sustaining a femoral neck fracture of 2.6 times (104,105). Similarly, one SD decrease in spine BMD is associated with a 2.3-fold increase in the relative risk of vertebral fracture (104,105).

Quantitative ultrasound of the calcaneus

Quantitative ultrasound (QUS) of the calcaneus has been developed as a non-ionizing technique to measure bone mass in peripheral skeletal regions. The QUS technique has some advantages over the DXA technique such as lower cost, lack of X-ray radiation and the possibility of using a portable QUS device in field studies or in screening processes (162). QUS measures bone with two variables: speed of sound (SoS) and broadband ultrasound attenuation (BUA). In addition, a third variable called stiffness index, which is independent of mechanical stiffness of the bone, has been derived from SoS and BUA in Lunar Achilles® machines (162). QUS variables correlate to axial aBMD to approximately the same degree as to peripheral aBMD (162), and they have been shown to predict fractures independently of bone mineral density measured by DXA (163-166). In the QUS technique, bone strength is evaluated by analysing the alteration of an ultrasonic wave after penetration of an irregularly shaped non-homogeneous propagation medium, including both trabecular network and cortical shell (167,168). Thus, the ultrasonic impulse received may result from a combination of multiple waves, transmitted through different pathways in the bone, in the trabecular network as well as in the cortical shell of the bone, resulting in a complex signal. Ultrasonic wave propagation through bone depends on bone mass and on other material properties such as microarchitecture and tissue elasticity (169-171). The correlation between QUS and axial or site-matched DXA-based aBMD measurement was shown to range from moderate to strong in earlier studies (169), including the Malmö OPRA cohort study (172).

Scintigraphy

Technetium-99m (^{99m}Tc)-labelled diphosphonates are commonly used bone-seeking substances in scintigraphic procedures; they are used to detect lesions in conditions such as cancer metastasis, occult fractures and osteomyelitis, due to their high affinity to metabolically active sites in bone. Studies with ^{99m}Tc-labelled methylene diphosphonate (^{99m}Tc-MDP) suggest that skeletal uptake of MDP reflects a combination of skeletal blood flow and osteoblastic activity (173,174). In these procedures, the skeletal or extra-osseous accumulation of ^{99m}Tc-MDP is used to identify the lesions as "hot spots" (175,176). In earlier studies, the measurement of 24-hour whole body retention of ^{99m}Tc-MDP was used to assess the skeletal metabolism (177,178), before introduction of the regional quantification of ^{99m}Tc-MDP activity by D'Addabbo *et al.* (179) and Brenner *et al.* (180). These techniques have been found to be useful for estimation of skeletal turnover rate at the time of the measurement. The regional quantification after 5 hours has the advantage over 24-hour retention that it directly gives a measure of skeletal uptake, and a shorter time period is needed. Studies have shown that total skeletal uptake of ^{99m}Tc-MDP correlates with aBMD and bone turnover markers (178,181-183).

Aims of the study

In this thesis, the following specific questions were set for the study:

- Is bone turnover, as assessed by total skeletal uptake of Technetium 99-labelled methylene diphosphonate, correlated more to bone formation markers or to resorption markers?

- Can baseline levels of bone turnover markers predict changes in aBMD?

- Can serial measurement of bone turnover markers improve the ability of these markers to predict changes in aBMD?

- Can baseline levels of bone turnover markers predict changes in quantitative ultrasound variables?

- Does the precision error affect the assessment of repeated bone densitometry in elderly women and men?

Materials and methods

Participants in paper I

For this study, we recruited 22 women from the registers of the orthopaedic clinic at Malmö University Hospital. The median age of the women was 65 years (range 52–80). The inclusion criteria were women who had sought medical advice or treatment for complaints such as non-fracture trauma, back pain, vertebral fractures or ankle fractures at least 6 months before the recruitment. By the time the study was started, they were free from the condition that had originally brought them to the clinic. Women who had ever been treated with bisphosphanates or women who had been treated with oestrogens or corticosteroids within the previous year were excluded. Patients with primary hyper-parathyroidism, hyper-thyroidism, osteomalacia, chronic malnutrition, any malignancy, hepatic cirrhosis or a joint prosthesis were also excluded.

Participants from the Malmö OPRA cohort, Papers II–V

The Malmö Osteoporosis Prospective Risk Assessment (OPRA) cohort consists of elderly women who were randomly recruited from the population registry of Malmö. For the baseline investigation, 1,604 women were invited by mail one week after their seventy-fifth birthday. Baseline recruitments took place between November 1995 and May 1999. Of the 1,604 women invited, 1,044 (65%) participated at baseline. Of the 560 women who did not participate, 13 had died shortly after the invitation, 139 could not come because of illness, 376 were not interested or could not attend for reasons other than illness, and 32 women could not be reached despite repeated letters and phone calls. Baseline DXA was performed on 995 individuals. The women were invited for prospective follow-up visits after 1, 3 and 5 years. At the 5-year follow-up, 691 had second aBMD measurements performed at at least one site, and 551 women had completed both baseline and 5-year QUS measurements.

In **Paper II,** 601 women who had attended both the baseline and the 5-year DXA measurements were included. These women had baseline serum and/or urine samples available and had not taken hormone replacement therapy or bisphosphonates during the study period.

In **Paper III,** 506 women who had attended both the baseline and the 5-year QUS measurements were included. These women had baseline serum and/or urine samples available and had not taken hormone replacement therapy or bisphosphonates during the study period.

In **Paper IV,** 573 women were included. They attended both the baseline and the 5-year DXA measurements, and had given serum and/or urine samples at baseline and at the

1-, 3- and 5-year follow-ups. The women included had not taken hormone replacement therapy or bisphosphonates during the study period.

In **Paper V,** 691 women were included. These women had a baseline and 5-year follow-up DXA measurements available. In addition, 211 men from the Malmö part of the MrOs study who attended DXA measurements at baseline and at the 5-year follow-up were included. The MrOs study is an international multi-centre study on risk factors for osteoporosis and fracture in elderly men. The men in the Malmö cohort of the MrOs study were recruited from the population registers of Malmö city.

Bone density measurements

Dual-energy X-ray absorptiometry

The total body, the total hip, the femoral neck and the lumbar spine aBMD measurements in the women were performed by using a Lunar DPX-L scanner (Lunar DPX-L; Lunar Corporation, Madison, USA) at baseline (**Papers I–V**) and after 5 years **(Papers II, III, IV and V)**. Scan analysis at baseline was done with software versions 1.33 or 1.35, except for hip scans, which were analyzed with 4.7b. Follow-up scans at 5 years were done with software version 4.7b. Men were measured at the same regions of interest using a Lunar Prodigy scanner (Lunar Prodigy, Madison, USA), which uses the fan beam technique. Software version 2.05 was used for scan analysis at baseline and at five years.

Quantitative ultrasound of the calcaneus

Ultrasound measurements were performed in elderly women at baseline and after five years with a Lunar Achilles® scanner (Lunar Corporation, Madison, USA) for the right calcaneus. (If there was a history of previous injury or fracture on the right side, the left calcaneus was used instead). The results were obtained as speed of sound (SoS), broadband ultrasound attenuation (BUA) and the stiffness index **(Paper III)**.

Serum and urine samples

Serum and urine samples were collected for the analysis of markers of bone turnover at baseline (age 75 years, **Papers II, III and IV**), and follow-ups after 1, 3, and 5 years (**Paper IV**). Non-fasting blood samples were collected between 08.00 and 13.00, and serum was separated and stored within 2 hours. First morning void urine

samples were also collected. Serum and urine samples were stored at -80°C. For **Paper I**, non-fasting serum and urine samples were collected at 09.00. The analyses for each bone metabolic marker were done at the same time in order to minimise inter-assay variability.

Measurement of bone turnover markers

Markers of bone formation

Bone-specific alkaline phosphatase (S-Bone ALP) was determined by using Metra BAP immunoassay (Quidel Corporation), with an intra- and inter-assay coefficient of variation (CV) of 3.6% and 4.4%, respectively. Serum intact and N-mid osteocalcin (S-Total OC(N-Mid®)) were determined by using the Elecsys N-MID Osteocalcin Immunoassay (S-Total OC; N-MID®; Roche Diagnostics), with intra- and inter-assay CV of 2.3% and 2.4%, respectively. Serum intact osteocalcin (S-OC[1-49]), serum total osteocalcin (S-Total OC) and serum total carboxylated osteocalcin (S-cOC) were determined by previously described, in-house protocols with intra- and inter-assay CV of less than 5% and 8%, respectively, for all the assays (184). Briefly, protocols are two-site assays based on two monoclonal antibodies (Mabs) in the combinations 3G8/2H9 (for S-OC[1-49]), 2H9/6F9 (for S-TotalOC) and 6F9/3H8 (for S-cOC). Mab 3G8 is specific for intact OC, Mab 6H9 binds to fragment Gly^{7}-Arg^{19}, Mab 2H9 recognizes fragment Arg^{20}-Arg^{43} and Mab 3H8 binds to the same fragment (Arg^{20}-Arg^{43}) but prefers OC-containing gamma-carboxyglutamic acid (Gla), with only 9% cross-reactivity with non-Gla-containing OC (185).

Markers of bone resorption

Serum C-terminal cross-linking telopeptides of type I collagen (S-CTX-I) was determined by Elecsys β-Cross Laps® immunoassay (Roche Diagnostics) with intra- and inter-assay CV of 5.9% and 5.8%, respectively. Serum tartrate-resistant acid phosphatase 5b (S-TRACP5b) was assessed by a solid phase, immunofixed enzyme activity assay as described earlier (186) with an intra- and inter-assay CV of 1.8% and 2.2%, respectively.

Urinary deoxypyridinoline (U-DPD) was measured by the Metra DPD Immunoassay (Quidel Corporation, San Diego, CA, USA) with an intra- and inter-assay CV of less than 12% and 10%, respectively.

Urinary osteocalcin

Urinary osteocalcin (U-OC) consists of fragments less than thirty residues in length from the middle region of the molecule (58). Three assays for the detection of various molecular forms of U-OC were analysed as described previously (59). Assays were based on the same Mabs as the assays for serum OC (see details above). Briefly, the two-site assay U-MidOC consisted of Mabs 6F9 and 3H8 and recognized the most abundant mid-molecule fragments of U-OC (spanning residues 7–31, 7–29, 6–29, 9–31, 7–32 and 7–33). Two-site assay U-LongOC (2H9/6F9) detects only the longest U-OC fragments (7–32, 7–33) with low affinity. Competitive assay U-TotalOC (3H8) also measures (in addition to the same mid-molecule fragments) more truncated U-OC fragments, starting from residue Asp^{14}. The intra- and inter-assay CVs were 1.7% and < 12% (for U-MidOC), 4.3% and < 14% (for U-LongOC), and 14% and < 27% (for U-TotalOC), respectively (59).

Urinary creatinine

Urinary creatinine was measured by the kinetic Jaffe reaction with a Beckman synchron LX20-4, with CVs of 3% or less. All the measurements of urinary bone markers were corrected for urinary creatinine and expressed as ratios (**Papers I, II, III, and IV**).

Bone scintigraphy

Bone scintigraphy procedure was performed within 28 days after the DXA scanning (**Paper I**) according to a method described by Brenner *et al.* (180). An intravenous injection of 520 (517 ± 15) MBq of ^{99m}Tc-MDP (Medronate®, Amersham International) was given at 09.00 h. Whole body imaging was performed directly (3 minutes) after injection and 5 hours after injection (14:00 h). The radioactivity was measured in the syringe both before and after injection, to enable an accurate determination of injected activity. A double-headed gamma camera system (Siemens Multispect 2) equipped with low-energy high-resolution collimators was used for the scan. The scan speed was 40 cm/min for the image at 3 minutes and 15 cm/min for the image after 5 hours.

Regions of interest (ROIs) were drawn on the anterior and posterior images to quantify the activity in the whole body, the urinary bladder, and the adductor muscles of both thighs, as described by Brenner *et al.* (180). The geometric mean of the anterior and posterior image was used in the calculation of activity content and the 3-minute image was used as a reference to calculate the percentage uptake in the later

image. For all data, the numbers of counts in the regions were corrected for decay of ^{99m}Tc. The soft tissue activity was calculated from the adductor compartment of both thighs as follows: activity of adductor muscles at 5 hours divided by the activity of adductor muscles at 3 minutes and multiplied by whole body activity at 3 minutes. All activity was considered to be excreted from the body, only via urine. The excretion was calculated from the difference in whole body activity between two imaging times. Correction for radioactive decay and scan speed was done. The total skeletal uptake (TSU) of ^{99m}Tc-MDP was calculated as whole body radioactivity at 3 min minus urinary excretion minus soft tissue uptake at 5 hours, all divided by whole body radioactivity at 3 min and expressed as a percentage (multiplied by 100) (180).

Summary of papers

Paper I

Bone turnover markers are correlated with total skeletal uptake of ^{99m}Tc-methylene diphosphonate (99mTc-MDP)

Is there a correlation between total skeletal uptake of ^{99m}Tc-MDP and bone turnover markers? Does total skeletal uptake of ^{99m}Tc-MDP correlate more to bone formation markers or to bone resorption markers?

Background: The skeletal uptake of ^{99m}Tc-MDP is regularly used to produce images of pathological bone uptake. In a clinical context, incorporation of ^{99m}Tc-MDP reflects bone turnover. This study was done to validate a panel of biochemical markers of bone formation and resorption with total skeletal uptake of ^{99m}Tc-MDP in a sample of post-menopausal women.

Methods: Twenty-two post-menopausal women (aged 52–80 years) volunteered to participate. The total body aBMD was measured by DXA. Scintigraphy was performed by injecting 520 MBq of ^{99m}Tc-MDP. Whole body images were taken 3 minutes and 5 hours after injection, to obtain whole body radioactivity. The TSU of ^{99m}Tc-MDP after 5 hours was calculated by subtracting the urinary loss and the soft tissue uptake from the first image and was expressed as a percentage of the radioactivity of the 3-minute image.

Nine BTMs including bone formation markers (S-Bone ALP and three different assays for S-OC), bone resorption markers (S-TRACP5b and S-CTX-I) and three different assays for U-OC were analysed.

Results: The median TSU of ^{99m}Tc-MDP was 23% (range 5–48%). There was a significant correlation between all bone turnover markers, with r-values from 0.52 ($p = 0.013$) to 0.90 ($p < 0.001$). The two bone resorption markers had numerically higher correlations (S-TRACP5b: $r = 0.90$; and S-CTX-I: $r = 0.80$) than the bone formation markers (S-Total OC: $r = 0.72$; and S-Bone ALP: $r = 0.66$), but the differences were not statistically significant. There was no correlation between the TSU of ^{99m}Tc-MDP and age, weight, body mass index or total body BMD.

Conclusions: There was a strong correlation between biochemical markers of bone turnover and skeletal metabolism as measured by TSU of ^{99m}Tc-MDP. There were no significant differences in correlations of ^{99m}Tc-MDP with bone formation markers and with bone resorption markers. This is probably due to the tight coupling between formation and resorption taking place in post-menopausal women.

Paper II.
Prediction of bone loss using biochemical markers of bone turnover

Is it possible to predict change in areal bone mineral density over 5 years, using a single measurement of bone turnover markers?

Background: Low bone mass is the most important risk factor for fragility fractures. Identification of individuals with a high rate of bone loss is also important to prevent them from developing osteoporosis. Although one measurement with DXA can help diagnose individuals with osteoporosis, at least two measurements of DXA are needed with several years apart to detect those with rapid bone loss. Prediction of bone loss with bone turnover markers has been investigated in many studies and there have been conflicting results. There is limited information in this respect regarding the elderly.

Methods: Eleven BTMs (S-Bone ALP, four assays for S-OC, S-TRACP5b, S-CTX-I, U-DPD, and three assays for U-OC) were analysed in 75-year old women (n = 601) and prospectively compared to the annual rate of change in aBMD over 5 years in seven skeletal regions, using standardized regression coefficients ($Beta_{std}$), with and without adjustment for baseline total body BMC.

Results: Annual change in aBMD varied between +0.4% (spine) and -2.0% (femoral neck). Significant associations ($p < 0.01$) in the aBMD change of the leg region (derived from the total body measurement) were found for four different S-OCs (standardized regression coefficient -0.20 to -0.22), U-DPD (-0.19), S-TRACP5b (-0.19), S-CTX-I (-0.21), two of the three U-OC/crea (-0.16).

After adjustment for baseline total body BMC, associations were found for all S-OC:s (-0.11 to -0.15), two of the three U-OC:s (-0.14 to -0.16) and aBMD change at the total hip, and for three of the four S-OC:s (-0.14 to -0.15), S-TRACP5b (-0.11), two of the three U-OC:s (-0.14 to -0.15) and aBMD rate of change at the femoral neck. There were no significant results concerning change in aBMD at the lumbar spine.

Conclusion: In summary, we conclude that biochemical markers of bone turnover are associated with change in aBMD at some skeletal sites. However, there was no clear prediction of bone loss at clinically important sites such as the total hip, femoral neck and lumbar spine, thus limiting the usefulness of bone turnover markers as predictors of bone loss.

Paper III.

Bone turnover markers are correlated with quantitative ultrasound of the calcaneus: 5-year longitudinal data

Is it possible to predict ultrasound changes of the calcaneus over 5 years, using a single measurement of bone turnover markers?

Background: Ultrasound wave propagation through the bone, which is measured by QUS, depends on structural properties of the bone such as bone mass, micro-architecture and tissue elasticity. Studies have shown that QUS predict fracture, independently of aBMD. Our knowledge of the association between BTMs and QUS variables is limited.

Methods: Eight BTMs (S-Bone ALP, three S-OCs, S-TRACP5b, S-CTX-I, U-DPD and U-MidOC) were analysed in 506 75-year-old women in the OPRA study and compared to baseline and 5-year change in calcaneal QUS. The associations between bone turnover markers and QUS were evaluated by using standardised regression coefficients ($Beta_{std}$), with and without adjustment for baseline body weight.

Results: There was a correlation between all markers and baseline QUS measurements ($Beta_{std}$ values from -0.07 [$p < 0.05$] to -0.23 [$p < 0.001$]). When we evaluated the correlations between the baseline bone markers and 5-year prospective changes in QUS, all three serum osteocalcins showed correlations with changes of SoS and stiffness index (unadjusted and adjusted for baseline body weight) ($Beta_{std}$ = –0.10 [$p < 0.05$] to -0.17 [$p < 0.001$]). S-CTX-I showed a correlation with changes in SoS (unadjusted and adjusted for weight) and unadjusted stiffness index ($Beta_{std}$ = –0.09 to –0.10 [$p < 0.05$]). S-TRACP 5b and U-MidOC/crea showed correlations with unadjusted changes in SoS ($Beta_{std}$ = -0.10 [$p < 0.05$]). S-Bone ALP did not show any correlation with any of the prospective changes in QUS, and none of the bone turnover markers correlated with prospective changes in BUA before or after adjustment of baseline body weight.

Conclusions: Bone turnover, as assessed at baseline with bone turnover markers, correlates with concomitantly assessed quantitative ultrasound of the calcaneus, as well as with 5 year prospective changes in ultrasound variables.

Paper IV.
Serial assessment of serum bone turnover markers identifies women with the highest rate of bone loss and osteoporosis risk

Can serial assessment of bone turnover markers improve our ability to predict bone loss?

Background: A single measurement of bone turnover markers has shown some degree of correlation with bone loss. We attempted to evaluate whether assessment of bone turnover markers on multiple occasions could improve the identification of women with rapid bone loss.

Methods: Women participating in OPRA study who had given serum and/or urine samples on all four occasions (at baseline and at the 1-, 3- and 5-year follow-ups) were included in this study. After exclusion of women taking hormone replacement therapy or bisphosphonates, 573 women were eligible for this analysis.

Eight BTMs (S-Bone ALP, three different assays for S-OC, S-TRACP5b, S-CTX-I, U-DPD and U-MidOC) were used to assess the bone turnover at baseline and at the 1-, 3- and 5-year follow-ups. Standardised linear regression coefficients ($Beta_{sd}$) were determined between BTMs and the change in total body aBMD over 5 years (percentage of baseline aBMD). BTMs were introduced into analyses as single measurements (baseline) or as the average of two (baseline and 1-year), three (baseline, 1-year and 3-year) or four (baseline, 1-year, 3-year and 5-year) measurements of the same BTM.

Results: Baseline BTMs showed a weak correlation with change in total body aBMD, but the association was more pronounced when we used the average of two measurements of each marker (standardised regression coefficient from –0.12 to –0.23, $p < 0.01$). Adding a third and a fourth measurement further strengthened the correlation (with coefficients of up to -0.30, $p < 0.001$). Changes in BTMs did not correlate to bone loss as strongly as the average values. Women with constantly high turnover lost significantly more bone at total body (-2.6%) than women with intermediate (-1.6%) or low turnover (-0.2%, p for trend < 0.001). They also had greater bone loss at the hip (-8.3%, -6.0% and -5.1%, respectively; $p = 0.01$). Results were similar in the subgroup of women with osteopenia.

Conclusion: Consecutive assessment of bone turnover may improve the identification of women with high bone loss, particularly osteopenic women at high risk of developing osteoporosis, and may assist in targeting pharmacological treatment.

Paper V.
Effect of precision on longitudinal follow-up of bone mineral density measurements in elderly women and men

How does the precision error of DXA influence longitudinal monitoring of elderly individuals? Are the monitoring time intervals used in clinical practice long enough to identify a true change in bone mineral density?

Background: Repeated bone densitometry is used to assess treatment effects and to monitor bone loss. In common practice, individuals with risk of developing osteoporosis are followed up with DXA with durations of 1–2 years. The precision error influences the least significant change (LSC) detectable.

Methods: We assessed the capacity to detect changes in bone density over a 5-year period in two population-based cohorts of elderly individuals. Six hundred and ninety women from the Malmö OPRA study, with a mean age of 75.2 years (SD = 0.1), were measured using Lunar DPX-L. In addition, 211 men from the MrOs study in Malmö, mean age 74.7 years (SD = 3.2), were measured using Lunar Prodigy at baseline. For both cohorts, follow-up DXA was performed 5 years later. Precision error was determined for Lunar DPX-L by performing duplicate measurements on 30 elderly women. For Lunar Prodigy, triplicate measurements on 15 elderly women and duplicate measurements on 30 elderly men were performed. Individuals were repositioned between measurements. The number of individuals whose aBMD changed more than the LSC (defined as 2.77 × precision error) was calculated at the follow-up.

Results: At baseline, aBMD (SD) in g/cm^2 for women was: total body (TB) 1.008 (0.093), total hip (TH) 0.857 (0.147) and lumbar spine (LS) 0.987 (0.190); in men, TB 1.187 (0.097), TH 0.982 (0.138) and LS 1.240 (0.190). Precision error (in g/cm^2) for Lunar DPX-L in women was 0.010 (TB), 0.028 (TH) and 0.016 (LS). Precision error using Lunar Prodigy for women was 0.009 (TB), 0.009 (TH) and 0.039 (LS). Precision error using Lunar Prodigy for men was 0.007 (TB), 0.014 (TH), and 0.031 (LS).

Mean change in aBMD (in g/cm^2) per year in women was, for TB -0.003 (0.007), for TH -0.011 (0.016) and for LS 0.004 (0.015). Corresponding results in men were -0.003 (0.006), -0.006 (0.009) and 0.005 (0.016) at TB, TH and LS respectively.
The number of individuals with 5-year aBMD change at TB that exceeded the LSC was 244 women (38.6%) and 73 men (35.6%). The corresponding results at TH were 265 women (41.4%) and 78 men (38.6%); at LS the numbers were 303 women (45.0%) and 51 men (24.6%).

Monitoring time interval (i.e. LSC/median rate of change in aBMD) for both populations was 8 years (for TH aBMD) and 13 years (for LS aBMD). Based on

Prodigy precision data, the monitoring time intervals for women were 3 and 32 years for TH and LS, respectively.

Conclusions: Precision has an influence on the shortest follow-up time between repeated scans. In these population-based cohorts, several years appear to be needed to be able to detect a significant change between measurements. A shorter follow-up time can be used only when a high degree of bone loss is expected.

General discussion

The overall aim of the work described in this thesis was to improve the prevention of fragility fractures in the future. There are numerous risk factors for fragility fracture. Bone mineral density is one of the most important risk factors that is potentially modifiable. For diagnostic purposes, a diagnostic threshold is used for bone density test results, below which the term osteoporosis is used. However, a large proportion of individuals who sustain a fragility fracture are not osteoporotic (6,89,187). Apart from the fact that they do not take other risk factors into account, bone density test results only reveal the current situation. They do not show the ongoing bone turnover; thus, they do not provide information on future changes in bone density.

There are several reasons for the development and use of bone turnover markers. The work in this thesis illustrates efforts to find ways of assessing future bone loss by the measurement of bone turnover markers (**Papers II and III**), of how to improve this assessment (**Paper IV**), and to investigate whether some markers are more specific than others (**Papers I–IV**). Since the time required to assess bone density changes with bone density equipment is very long (Paper V), it seems unreasonable to follow up compliance and effect of anti-osteoporotic medication by repeated bone density measurements.

Currently, bone turnover markers are being used extensively in research applications and also being tested as tools for the management of metabolic bone diseases such as osteoporosis and Paget's disease in clinical practice, because these markers are non-invasive and relatively inexpensive. Monitoring of the efficacy of bone-active drugs is currently the most promising clinical application of bone turnover markers, because of the possibility of detecting a change in the levels of bone turnover markers within a few weeks of treatment (106-114). Some markers, particularly resorption markers such as S-TRACP5b, S-CTX-I, U-CTX-I, U-NTX-I and U-DPD, and some bone formation markers such as S-bone ALP and S-OC, have shown some degree of fracture predictability in different populations (Table 3), but the prediction is not strong enough to use in individual patients. The fracture predictability afforded by bone turnover markers is weaker than the predictability afforded by DXA (104,105), but it is somewhat inconsistent between studies (85,90-100).

A high rate of bone turnover is associated with a high rate of bone loss and osteoporosis (Table 2) (188,189). Early detection of individuals who are at high risk of developing osteoporosis could be important for clinical decision-making. In particular, individuals with osteopenia and individuals with a high rate of bone loss may need more careful follow-up.

In **Paper II** and **III**, baseline bone turnover markers, in particular S-OCs, U-DPD/crea, S-TRACP5b, S-CTX-I, U-LongOC/cea and U-MidOC/crea could be

correlated to rate of change of aBMD in the legs. To some degree, there were correlations with rate of change of aBMD in the arms, in the total body, in part of the body, in the total hip and in the femoral neck. None of the markers were found to be correlated to rate of change of aBMD at the lumbar spine; nor did S-Bone ALP and U-TotalOC/crea show any correlation with rate of change of aBMD. When the correlation between bone turnover markers and 5-year change of QUS variables was examined, all markers except S-Bone ALP showed correlations with changes in SoS, while none of the markers showed any correlation with changes in BUA **(Paper III)**. When the mean of serial measurement of bone turnover markers was used instead of baseline measurement, the correlations became stronger as the number of samples used increased, and the women with constantly elevated levels of bone turnover markers had a significantly higher rate of bone loss **(Paper IV)**.

In general, the correlation between bone turnover markers and the change in aBMD was not strong. The strongest correlation coefficients were 0.22 when the baseline levels were used, and they were 0.32 when the mean of four serial measurements was used. None of the markers proved to be superior to the others. Bone formation and resorption markers had almost similar magnitudes of correlations. This could be due to the tight coupling of bone formation and resorption. This idea is supported by the results of **Paper I**, in which no difference between bone formation markers and resorption markers in TSU of ^{99m}Tc-MDP was found. Bone turnover markers are released from the whole skeleton. This may be the reason for higher correlations with bone turnover markers at large skeletal sites including the total body, the partial body and the legs, than smaller sites such as the femoral neck and the lumbar spine (**Papers II and IV**).

Many other factors also affect the clinical usefulness of bone turnover markers. Pre-analytical conditions affecting bone turnover markers such as age, gender, menopausal state, ethnicity and recent fracture are not controllable, whereas other factors such as the effect of food intake, physical activity and circadian rhythm can be controlled (134). The OPRA study was designed to control for factors such as age, gender, ethnicity and menstrual status. Samples were taken in the morning in the non-fasting state, which could have affected the results, mainly the S-CTX-I levels (139). Many other factors such as time of the day, recent fracture and level of physical activity may have an effect on bone turnover markers. The study design was deliberately not changed during the study period, and all samples were collected in the same manner to make comparisons possible within the cohort.

Bone density has a smaller annual change or response to anti-resorptive and anabolic treatment compared to the response of bone turnover markers. Precision has an effect on the shortest follow-up interval between repeated scans. In the population-based cohorts in **Paper V**, several years were needed to detect a significant change between measurements. The estimated monitoring time intervals (i.e. least significant change/median rate of change in aBMD) were between 3 and 32 years, depending on

the site of measurement and the equipment used. Only when a high degree of bone loss is expected may a shorter follow-up time be useful. Thus, DXA has shortcomings in detecting rapid losers and individuals with a high risk of developing osteoporosis.

Single measurements of bone turnover markers and follow-up measurements of DXA both have limitations in their ability to detect individuals with rapid bone loss. Serial assessments of bone turnover markers can substantially improve the ability to find individuals with increased loss of bone density. Whether or not intervals shorter than one year could be used to improve the predictive ability of bone turnover markers remains to be evaluated.

To the best of my knowledge, the Malmö OPRA study has been the largest study in elderly women to assess the ability to predict bone loss over several years. The design of the OPRA study has several advantages: it has (i) a well-defined population, (ii) a high attendance rate, (iii) a thorough ascertainment of fracture, (iv) a long follow-up, and (v) the use of novel and established bone turnover markers.

Conclusions

There is a correlation between levels of bone turnover markers and the rate of bone loss in elderly women, with varying degrees of correlation coefficients at different skeletal regions. In general, bone turnover markers correlate better with change in aBMD at large skeletal sites, such as the total body, and weight-bearing sites such as the legs, than with aBMD change at specific clinically important regions such as the femoral neck and the total hip. Correlations between bone turnover markers and rate of bone loss become stronger when serial measurements of bone turnover markers are used. The individuals with constantly high levels of bone turnover markers have higher change in aBMD. However, these correlations may not be strong enough to be predictive of bone loss at the level of the individual patient. DXA is used to monitor change in aBMD to aid in treatment decisions. However, long durations of follow-up are needed to detect aBMD changes in elderly women and men that exceed the least significant change. DXA is therefore of limited use in the longitudinal monitoring of bone loss.

Future perspectives

Bone turnover markers are used extensively in research applications, and also as tools for the management of skeletal disorders in clinical practice. Novel, more specific markers and improvements in and standardization of measurement techniques should enhance the reliability of, and facilitate the use of bone turnover markers in clinical practice. It is reasonable to believe that bone turnover markers will replace part of the DXA-based treatment monitoring in the future. Bone turnover markers may also assist in the decision of whom to treat with anti-osteoporotic medication. Improvement of the precision of DXA measurements by using improved techniques and standardized guidelines could shorten the intervals required for monitoring.

Sammanfattning

Årligen får sig 70000 svenskar en s.k. osteoporosfraktur, en fraktur som åtminstone delvis beror på att skelettet är skört. Hälften av alla 50-åriga kvinnor kommer under sin återstående livstid att drabbas av en sådan fraktur. Osteoporos diagnostiseras genom en speciell röntgenteknik, s.k. DXA-teknik. Denna teknik har den begränsningen att den bara kan ge besked hur skelettets tillstånd är just vid mättillfället men den kan inte hjälpa oss att förutsäga vem som kommer att drabbas av osteoporos.

Benmarkörer är kemiska substanser som ständigt frisätts från det ”levande” skelettet. Skelettet förnyas hela tiden tack vare att vissa celler bildar och andra celler förstör benvävnad. I tider av tillväxt, som under barnaåren, överväger förstås nybildningen, i ung vuxen ålder är skelettet i balans men senare i livet överväger nedbrytningen och vi kan riskera att drabbas av osteoporos. Benmarkörer som kan mätas i blod eller urin kan hjälpa oss att avgöra hur benomsättningen är hos en enskild patient. Tidigare vetenskapliga studier har visat att vi med hjälp av sådana benmarkörer i viss mån kan förutsäga vilka patienter som inte bara kommer att få osteoporos utan också kommer att få fraktur, nämligen de patienter som förlorar benvävnad med onaturligt snabb hastighet.

I denna avhandling har ett stort antal benmarkörer analyserats hos sammanlagt över 1000 kvinnor. I **arbete 1** var vi intresserade att se om det finns en samvariation mellan benmarkörer och upptaget av ett radioaktivt ämne i benvävnaden. I en grupp av 22 kvinnor samvarierade de analyserade benmarkörerna mycket kraftfullt med upptaget av det radioaktiva ämnet. Både markörerna för bennybildning och markörerna för bennedbrytning samvarierade lika mycket med upptaget av det radioaktiva ämnet. Det finns en stark koppling mellan nybildning och nedbrytning av benvävnad även hos äldre kvinnor.

I **arbete 2** undersöktes om benmarkörer kunde förutse framtida benförlust hos en stor grupp av 75-åriga kvinnor. Blodprov och urinprov samlades in vid starten för undersökningen. Bentätheten uppmättes med DXA-teknik vid starten och efter 5 år. Vi fann ett statistiskt säkerställt samband mellan halten av vissa specifika benmarkörer och benförlust över de kommande 5 åren.

Förutom att mäta bentäthet med hjälp av DXA-teknik så kan man mäta bentäthet med ultraljudsteknik. Detta sker då vanligtvis i hälbenet. Ultraljudsmätning har både för- och nackdelar jämfört med DXA-mätning. Utmärkande för ultraljudsmätning är att man hävdat att det mätvärde som detta genererar skulle avspegla benvävnadens kvalitet snarare än mängden benvävnad. Eftersom det också hävdats att benmarkörer avspeglar benkvalitet så ville vi i **arbete 3** upprepa undersökningsförfarandet från

arbete 2 men byta ut DXA-mätning mot ultraljudsmätning. Liksom i arbete 2 fanns ett säkerställt samband mellan halten av vissa specifika benmarkörer och förändring av ultraljudsvärdena över 5 år. I väsentliga drag var det samma benmarkörer som visade sig fungera som i det föregående arbetet. Dessutom var markörernas förutsägbarhet för benförlust uppmätt med DXA-teknik eller med ultraljud väsentligen densamma.

I en strävan att med hjälp av benmarkörer försöka förbättra förutsägbarheten för framtida benförlust gjordes i **arbete 4** upprepade blod- och urinprovstagningar under 5-årsperioden. Då genomsnittshalten av dessa benmarkörer bestämdes så kunde en förbättrad förutsägbarhet för benförlust fås. Kvinnor med konstant höga halter i blod eller urin hade en större förlusthastighet av benvävnad under de 5 åren.

Det finns en relativt stor teknikosäkerhet när man analyserar benmarkörer. D.v.s. att även om man utför analysproceduren på identiskt sätt och från samma prov så kan man få olika resultat. Om det dessutom är två olika prover, men fortfarande från samma patient, så varierar utfallet ännu mer. Detta är en svaghet för den kliniska användbarheten av befintliga benmarkörer. Mätning av bentäthet med hjälp av DXA-teknik har hävdats ha betydligt mindre teknikosäkerhet vid upprepade mätningar. I **arbete 5** undersökte vi vilket mätintervall som kan rekommenderas. I denna grupp av äldre individer behövdes ett mätintervall på flera år för att med DXA-teknik säkert kunna upptäcka en benförlust. Bara hos de individer där man har särskild anledning att misstänka en ovanligt snabb förlust kan det vara motiverat att upprepa en bentäthetsmätning inom ett par år.

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