Biochemical markers of bone turnover

A review

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A vast number of fractures result from low energy trauma in elderly people, especially women. The etiology is multifactorial and apart from the propensity to fall, a low bone mass and poor bone quality contribute to the fracture susceptibility. A low bone mass in turn is due to alterations in bone turnover, both as a natural cause of age or induced by diseases affecting bone metabolism. In orthopedics, the treatment of fragile bone merits specific consideration, but that is true also of less common diseases affecting the bone, such as Paget's disease, osteopetrosis or multiple myeloma. Arthrosis and rheumatoid arthritis also cause changes in bone turnover and it is of value to obtain knowledge of metabolic and qualitative properties of the bone into which orthopedic implants are anchored. Therefore it is desirable to develop easily accessible methods to assess bone metabolism.

Invasive methods to evaluate bone turnover, such as histomorphometry and calcium kinetic studies, are not usable on a routine basis, but are valuable in diagnosing certain metabolic bone disorders, especially osteomalacia. This has led to an intense search for non-invasive methods to measure bone turnover and bone mass during the past years (83). To determine bone mineral content, dual photon X-ray absorptiometry (DEXA) has become the preferred method, with excellent precision and accuracy (79), whereas biochemical markers provide information about total skeletal turnover which is a different aspect of bone metabolism. The search has generated new biochemical markers of bone metabolism that are more specific and sensitive than the first generation of markers, such as alkaline phosphatase and hydroxyproline. This opens possibilities to evaluate more discrete changes in bone metabolism, such as those occurring in osteoporosis which are necessary for adequate monitoring of medical interventions in bone metabolic disorders.

In humans, the cortical bone represents 80 percent of the total bone mass. The trabecular bone, on the other hand, is the most metabolically active. Age and disease will thus affect the compartments unequally. Bone turnover is initiated by the activation of the osteoblasts, which is probably stimulated by PTH, vitamin D, cytokines or load (91, 110) (Figure 1). The osteoblasts produce proteinases that can degrade the non-mineralized surface matrix (61, 90). The osteoblasts secondarily activate the osteoclasts which then adhere to the pre-activated surface on the trabecula. The recruitment and stimulation of osteoclasts are enhanced by PTH, vitamin D, several growth factors and cytokines (11, 13, 52). Non-collagenous matrix proteins may play a role in the osteoclasts' attachment to the bone, whereas an acidic environment is probably more important for dissolving the mineralized bone (62, 65). When the resorption is completed, the area is invaded by osteoblasts and the formation starts by the production of a non-mineralized collagenous matrix (osteoid). The osteoid consists of 90–95 percent type I collagen. The collagen fibrils are composed of collagen molecules with a triple helical structure, tightly bound together by covalent pyridinoline cross-links. The subsequent mineralization is probably initiated in the gap regions of the collagen fibers, but the trigger event is not exactly known (45). During adulthood, bone mass is normally maintained through an equilibrium between formation and resorption and the two processes are closely coupled. However, when resorption is relatively increased or when bone formation is also reduced, the resorbed area is incompletely filled, resulting in a loss of bone. Qualitatively deficient bone may be explained by defective mineralization, as in osteomalacia, when the amount of osteoid is relatively increased.

Bone formation

Biochemical markers of bone turnover consist of substances or molecules, which are released into the circulation during formation or resorption and are measurable in serum or in urine (Table 1). The markers

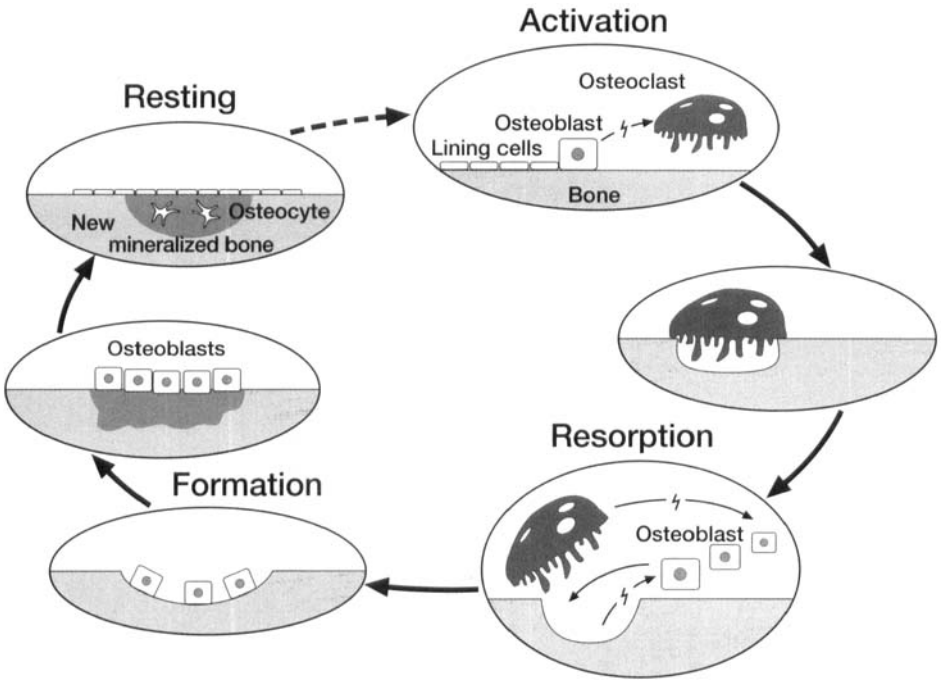

Figure 1. Schematic illustration of bone turnover and the normal remodeling of trabecular bone (Ill. B Henriksson).

Table 1. Biochemical markers of bone metabolism

Markers of bone formation	Markers of bone resorption	
Serum	*Urine*	*Serum*
Alkaline phosphatase (ALP)	Calcium	Cross-linked carboxyterminal telopeptide type I collagen (ICTP)
Bone-specific alkaline phosphatase	Hydroxyproline	
Osteocalcin	Pyridinoline and deoxypyridinoline	Other non-collagenous proteins (?)
Procollagen I C-terminal extension peptide (PICP)	Cross-linked aminoterminal telopeptide type I collagen (INTP)	
Other non-collagenous proteins (?)		

reflect the enzymatic activity in the bone cells as measured by phosphatase activity, excess products from formation, or degradation fragments from the matrix components.

Biochemical markers of bone metabolism provide information about the total skeletal bone turnover in comparison with histomorphometric analysis where only a specific site, usually the iliac crest, is evaluated imposing limitations on the interpretation. However, in the evaluation of biochemical markers, it is necessary to consider that the release and clearance may be affected by different systemic factors influencing the sensitivity and specificity of the markers.

Alkaline phosphatase

Alkaline phosphatase (ALP) is a membrane protein with enzymatic activity. In adults the two main sources of ALP in serum are the endothelial cells of the liver and the osteoblasts, which make approximately equal contributions. The isoenzyme release from the small intestine, kidneys and placental cells is a minor source (69). An accumulation of ALP is seen in activated osteoblasts (25). Obstructions in the biliary system causes increases in the ALP levels. Similarly, medications affecting the liver may be responsible for augmentations. In children, ALP is mainly of skeletal origin. In women, a slight increase has been seen from age 30, with a more specific increase during the

menopause (21, 81). Despite evidence of the involvement of ALP in the mineralization process, its specific function remains unknown.

Total alkaline phosphatase measured in serum thus lacks both specificity and sensitivity, but it readily detects major increases in bone turnover, especially in high-turnover diseases, such as Paget's disease, advanced metastatic bone disease, but also during fracture healing (48, 84, 96). In osteoporosis where the changes are slight, the interpretation of ALP may be obscured by influences from other sources. To improve the specificity one can separate the skeletal isoenzyme of ALP through electrophoresis and recently immunological techniques have been developed which should further facilitate evaluation of the bone-specific alkaline phosphatase (40, 63).

Osteocalcin

Osteocalcin is the most abundant non-collagenous protein in the mineralized bone matrix. Osteocalcin is a small protein of 5.8 kDa, the intact molecule containing 49 amino acids. Three amino acids are γ-carboxylated through a vitamin K-dependent translation and this property is responsible for the affinity to hydroxyapatite (59, 99). Osteocalcin is bone-specific, as the small amount present in dentine is negligible. The protein synthesis in osteoblasts is stimulated by vitamin D, whereas PTH may be restrictive (3, 98). However abundant, an estimated 20 percent of the non-collagenous protein content, its precise function remains unknown. The measured serum osteocalcin represents the newly synthesized osteocalcin, of which about 15 percent is released into the circulation (92). Osteocalcin appears to be involved in the mineralization process and it is suggested that the release is associated with the initiation of mineralization (8, 60, 77). Further evidence of its relation to bone formation and mineralization has been presented, as osteocalcin relates to histological indices of bone formation (10, 130). However, functionally, osteocalcin is also a potent inhibitor of hydroxyapatite formation (97) and exhibits chemotactic potential on osteoclasts (46).

Osteocalcin is cleared through the kidneys and a reduced kidney function increases the serum levels. This should be considered especially in an elderly population (22). Osteocalcin is measured in serum samples, using a radioimmunoassay where a rabbit antiserum is raised against bovine osteocalcin (99).

Osteocalcin increases during childhood and adolescent growth spurts, however, it also increases after about the age of 30 and especially after the menopause in women (26, 31, 49, 124). In adults, osteocalcin is inversely related to bone mineral density (31, 121).

There is a seasonal variation in osteocalcin secretion, high during the winter months and low during summer (82, 127). A diurnal rhythm is also evident, the highest values being found after midnight and the lowest around noon (82).

Procollagen I C-terminal extension peptide

During collagen type I synthesis the C- and N-terminals of the procollagen molecule are split off as intact fragments extracellularly and released into the circulation before extracellular fibril formation (100). As collagen synthesis precedes mineralization and as the fragments represent collagen synthesis on an equimolar basis, the measurement of procollagen fragments should reflect the amount of mineralized bone (108). Immunoassays have been developed to identify both terminals but they have become commercially available only for the carboxyterminal (PICP) (80, 125). The PICP molecule is too large to be excreted through the kidneys. Instead it is cleared through the liver endothelial cells, which has to be taken into account, when evaluating the values in patients with liver disease (113). PICP increases by 20 percent directly after the menopause (56, 73). PICP shows a circadian variation with higher values at night (58). Using histomorphometry and calcium kinetics, PICP correlates with indices of bone formation (15, 32, 73, 122). Available studies indicate that PICP identifies obvious high and low turnover states, such as hyperparathyroidism, thyrotoxicosis, Paget's disease of the bone and myxedema, but it is less sensitive in identifying alterations in bone turnover that are more discrete (14, 15, 122).

Bone resorption

The serum calcium level is closely regulated by PTH and vitamin D, controlling intestinal absorption and kidney excretion, whereas the skeleton is the main reserve. Urinary calcium excretion, measured in a fasting morning sample of urine, may thus provide valuable information expressing the calcium excretion of a predominantly skeletal source, i.e., the resorption, as a consequence of the increased nocturnal bone turnover, but as a marker it lacks sensitivity.

Hydroxyproline

Urinary hydroxyproline is a collagen degradation product and is an indirect measure of the skeletal turnover, as turnover in bone is greater than in other collagen-containing tissues. Hydroxyproline is a non-essential amino acid derived from hydroxylation of proline during collagen synthesis. During collagen

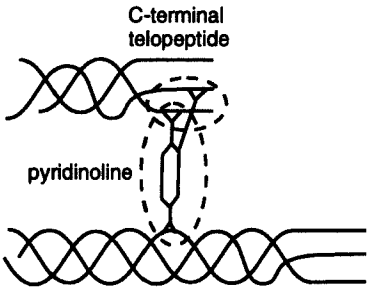

Figure 2. Schematic illustration of the cross-linking between two adjacent collagen molecules. The pyridinoline residues and the telopeptide regions are measurable as markers of bone resorption.

breakdown, hydroxyproline is liberated into the circulation in either free or peptide-bound form. Free hydroxyproline is reabsorbed by the kidneys and degraded in the liver, while the 10 percent of peptide-bound hydroxyproline is excreted without being metabolized via the kidneys (72). Since only 40 per cent of the total hydroxyproline is derived from bone matrix collagen and the levels are influenced by dietary intake, kidney excretion and the circumstances at the time of sampling, hydroxyproline as a diagnostic marker of bone resorption is unsatisfactory (23, 75).

Delmas et al. found that an age-related increase in hydroxyproline excretion occurred in women and hydroxyproline correlated with osteocalcin and ALP, but was inversely related to the bone mineral density (21). However, the correlation to bone resorptive indices, using histomorphometry or calcium kinetics, has been less certain (20). Due to the above-described variability of hydroxyproline measurements, it has limited clinical value, especially when changes in bone turnover are small.

Urinary pyridinoline

Pyridinoline derivatives, pyridinoline and deoxypyridinoline, are degradation products of mature extracellular collagen. Pyridinoline is present in collagen of bone and cartilage origin, whereas deoxypyridinoline is present in significant amounts only in bone and dentine and it appears to be the most discriminative marker (35, 116). The pyridinolines represent non-reducible covalent cross-links based on the aldehyde forms of hydroxylysine and lysine, interconnecting the collagen molecules and thereby increasing the tensile strength of the collagen fibers (4, 34) (Figure 2). The pyridinolines are excreted via the kidneys without further degradation, 40 percent in a free form and 60 percent peptide-bound. Initially, the urine measurement relied on high-performance liquid chromatography analysis (HPLC) after acid hydrolysis (39), but immunoassays have recently been developed detecting the free form of pyridinoline and showing a good correlation to the total measurement by HPLC (24, 33, 119). Total urinary pyridinoline and deoxypyridinoline levels are high during childhood and increase during adulthood, with a specific increase after the menopause (1, 24, 128). Increased levels are also noted in Paget's disease, other bone metabolic disorders as well as in hypercalcemia of malignancy (6, 24, 129).

In patients with vertebral osteoporosis, the pyridinoline cross-link excretion correlates with histological indices of bone resorption (24). The circadian variation of bone turnover is obvious from the variation seen in the pyridinoline excretion in normal, pre- and postmenopausal women. An even greater nocturnal increase is seen in osteoporotic women (27, 115).

Telopeptides

The non-helical terminal regions of the collagen molecule—the amino- and carboxy telopeptide region of type I collagen—are sites for the cross-linkage to the helical region of an adjacent collagen molecule by the pyridinolines (Figure 2). During degradation of bone tissue, these cross-linking peptide regions are liberated. Because of the specific amino acid sequences, they should be derived from bone collagen and therefore represent an index of bone resorption. The carboxyterminal telopeptide (ICTP) is released as an intact fragment and cleared from circulation through the kidneys. ICTP is measured in serum samples and recognized by a polyclonal antibody (106, 107). ICTP is not yet fully evaluated, but appears to correlate with histological and calcium kinetic indices of bone resorption, though less distinctly in normals and osteoporotic patients (15, 32).

In multiple myeloma, ICTP correlates strongly to disease severity and bone lesions (30). In another study, however, ICTP did not identify changes in bone turnover during estrogen therapy (57). ICTP and the marker of collagen formation, PICP, have not been shown to correlate, neither in normals nor in patients with bone metabolic disease (15, 30). This suggests the need for further studies, especially in normal populations, where balanced bone remodeling should be present.

The aminoterminal telopeptide region (INTP) is enriched in lysyl pyridinoline residues compared to the C-terminal telopeptide and contains 60 percent of the deoxypyridinoline linkage of bone collagen (87). The type of pyridinoline interaction between α-chains of adjacent collagen molecules appears to be bone-specific at this site (54). An inhibition ELISA-

immunoassay, using a monoclonal antibody recognizing the N-terminal telopeptide site of human bone, has been generated and the assay may be used in untreated urine samples (54). The INTP correlates with total urine deoxypyridinoline excretion and serum osteocalcin (41). During childhood and adolescence, the INTP excretion correlates to age-related changes in growth velocity (7). A postmenopausal increase in bone resorption, as measured by INTP, is evident and similar to the findings of total pyridinoline excretion, and a subsequent depression is found during antiresorptive therapy (41, 128).

The telopeptides may provide valuable information. However, further investigations are needed to evaluate their properties.

Other non-collagenous matrix proteins

Apart from osteocalcin, several other non-collagenous proteins have been identified, some of which are phosphorylated glycoproteins, with a specific capacity for binding to hydroxyapatite or with cell attachment properties. They are probably involved in the regulation and maintenance of the mineralization process (45, 126). These non-collagenous proteins include bone sialoprotein (BSP), osteonectin and osteopontin. BSP is synthesized by the osteoblasts and is a glycosylated phosphoprotein rich in sialic acid (47). BSP contains the amino-acid sequence Arg-Glu-Asp (RGD) which is responsible for the cell-adhesive properties (36, 38, 88). Immunostaining indicates that BSP is deposited in the newly synthesized osteoid and it has been suggested that it is involved in the regulation of bone turnover, especially in the initial phase of bone formation (37, 62, 65). In rheumatic patients higher BSP levels have been reported than in controls, however, the clinical evaluation of this protein as a marker of bone metabolism and especially formation is limited (114).

Other noncollagenous matrix proteins, including osteopontin and osteonectin may be synthesized by osteoblasts, but are also present in other connective tissues (62). Osteopontin binds to hydroxyapatite, but also to cells, and present data indicate a function during osteoclastic resorption, possibly via mediation of the binding of osteoclasts to the surface of the mineral phase (62, 65, 103). However, neither one of these proteins is today to be regarded as a biochemical marker of bone metabolism, as it is too early to ascribe to them a value of clinical significance.

Fracture

It is well known that fracture and fracture healing induce short- and long-term skeletal changes, as shown by histomorphometry and scintimetry (86, 131). DEXA has shown changes in bone mineral content up to 30 years after tibial shaft fractures in the involved limb (70).

In a population-based study of women, the biochemical markers were evaluated in relation to the occurrence of fracture. Women having sustained at least one fracture within 6 years preceding the measurement, had a 20 percent lower osteocalcin level independent of age and bone mineral content, indicating a decreased bone turnover (132).

In patients with diaphyseal fractures, osteocalcin levels increased during healing of the fracture (85). It would be of interest if biochemical markers could predict deficient fracture-healing. In one study of patients with closed fractures of the tibia diaphysis, the osteocalcin level was higher at onset and throughout the 4-month study period in patients with normal union, compared to those with delayed union (89). However, Haining et al., in a study of patients with diaphyseal fractures and established non-union 10 months after fracture, reported no evidence of abnormal bone turnover compared to normals (51). A possible explanation of this may be that the reparative process reaches its peak around 4 months, making discrepancies in healing more obvious at that time.

In hip fracture patients, the ALP level rises during the first 2 weeks after the fracture and the increased level persists for at least 3 months (64, 84, 120). Measurements of serum osteocalcin have given divergent results in hip fracture populations, decreased levels (2, 19), normal levels (76), but also increased levels have been found (123).

We have further evaluated women with hip fracture. Initially, no difference in the ALP levels was found compared to elderly controls, but at the end of the first week a significant rise was noted. On the other hand, using more sensitive biochemical markers, the osteocalcin level was 20 percent lower in women with hip fracture, whereas the bone resorption, as measured by the pyridinoline excretion, was 40 percent higher. Thus, elderly women with hip fracture presented biochemical evidence of an imbalance of bone turnover with decreased formation and increased resorption, a bone metabolic deficiency which may play a role in the development of a low bone mass (134). However, the ability to increase the bone turnover in response to the fracture-healing process was intact; osteocalcin and ALP were increased at 3–7.5 months after the fracture (133). It has been

proposed that patients with trochanteric fractures are more osteoporotic than those with cervical fractures (71, 118). At the initial measurement, we found no evidence of differences in bone metabolism between women with cervical and trochanteric fractures. At follow-up, ALP was higher in women with trochanteric fractures (133). It was not possible to identify defective fracture-healing or pseudoarthrosis in cervical fractures through biomarkers.

Fracture of the distal radius involve a small bone compared to that involved in a hip fracture, but nevertheless changes in bone formation have been found. Soon after the fracture, ALP increased, whereas the rise in the osteocalcin level was slower, although it remained elevated for up to one year (78).

It appears to be essential to apply a time perspective whenever relating biochemical markers of bone metabolism to fractures. Immediately after a major fracture, posttraumatic systemic effects as well as changes induced by surgery, fluid substitution, etc., affect the marker levels. This has to be borne in mind when designating baseline levels in the individual patient. Changes in bone turnover one month to approximately one year after fracture, are reflected by increased indices of bone formation, but on long-term they reflect a decreased bone formation.

One of the goals for further development of biochemical markers with clinical applications in orthopedics, should be the provision of markers sensitive enough to identify at an earlier stage fractures with a propensity for deficient fracture-healing. Another desirable objective would be to detect patients at risk of developing pathologic posttraumatic osteopenia, as seen in reflex sympatethic dystrophy.

Joint disease

Rheumatoid arthritis is associated with generalized osteoporosis as well as periarticular osteopenia. The etiology of the osteoporosis is multifactorial and is influenced by disease activity and duration, medications and physical activity level (18, 74, 101). Measurements of biochemical markers of bone metabolism have given divergent results. Higher levels of osteocalcin (42, 43, 44), normal (94) and low levels have been found, with the lowest levels in the corticosteroid-treated patients (29). Kröger et al. found low osteocalcin and PICP levels in rheumatic patients, especially in those with a recent onset of the disease and in the age groups 60–70 years (73). Neither marker correlated to the disease activity. Treatment with NSAID drugs alone did not alter osteocalcin or PICP levels (29, 95), whereas secondary line treatment increased osteocalcin levels and simultaneously reduced the indices of disease activity (29). Bone resorption, measured by urinary pyridinoline, and deoxypyridinoline excretion are increased in rheumatoid arthritis, and the pyridinoline level correlates to the disease activity, as measured by the CRP and erythrocyte sedimentation rate (5, 109, 117).

Arthrosis, on the other hand, is linked to an increase in bone mineral content (12, 111, 112) and increased levels of osteocalcin have been found (42, 43). Bone resorption, measured by pyridinoline excretion, is equally increased (109, 117). In patients undergoing total hip replacement because of primary arthrosis, the osteocalcin level was normal, while the surgical trauma induced a steep decrease in the serum osteocalcin. The baseline level was reestablished after 3 months (28).

Osteoporosis

Osteoporosis is defined as "a disease characterized by low bone mass, microarchitectural deterioration of bone tissue, leading to enhanced bone fragility, and a consequent increase in fracture risk" (104). Osteoporosis is partly an age-related disease, with a decrease of bone mass in the natural course of aging but, especially in women, the bone loss may be accelerated and lead to a further enhancement of bone fragility and fracture risk. Low peak bone mass and a rapid loss during estrogen withdrawal at menopause potentially predispose to osteoporosis, but are genetically influenced and modified by constitutional and lifestyle factors.

In postmenopausal osteoporosis, the evaluation of bone metabolic properties has given divergent results, probably reflecting the heterogeneity of the disease. Assessment of bone turnover by biochemical markers confirms this variability. Brown et al. (9) showed similar values of osteocalcin in postmenopausal vertebral osteoporosis, compared to normal women and simultaneously confirmed by histomorphometric evaluation that osteocalcin reflects bone formation. Osteocalcin also identified women with histological evidence of high bone turnover (10). Generally, a wider scatter of individual osteocalcin values is found in an osteoporotic population than in normals, which has been demonstrated by both normal and increased osteocalcin levels (21, 50). In postmenopausal vertebral osteoporosis, it is often possible to identify one group of women with a high turnover, representing about 30 percent of the patients (16). This condition is characterized by increased formative and resorptive indices and an

accelerated rate of bone loss after menopause, hence this subpopulation of women has been designated "fast losers" (16, 53).

In one study, women with high-turnover osteoporosis and subsequently increased baseline levels of serum osteocalcin and hydroxyproline, calcitononin therapy had a greater effect, with an increase in bone mineral content, in contrast to those with normal bone turnover (17). Estrogen treatment is an antiresorptive therapy with advantageous effects on the bone mass (105). In a European multicenter study, postmenopausal estrogen treatment reduced the relative risk of hip fracture to 0.6 and was, in comparison to other medical interventions, the most efficient treatment for preventing hip fracture (68). It is possible to evaluate the response and efficacy of treatment in postmenopausal osteoporosis by biochemical markers of bone metabolism. Positive effects are evident, such as a decrease in osteocalcin (67) and PICP (55). A concomitant decrease in resorptive markers has been described, especially in the urinary pyridinoline excretion, where deoxypyridinoline appears to be the most discriminative marker, (116, 128) but INTP also decreased with antiresorptive therapy (41).

Secondary osteoporosis, most commonly glucocorticosteroid-induced, is often seen in patients with chronic pulmonary obstructive disease and chronic inflammatory conditions and in transplanted patients. Osteoporosis may develop even after very short-term exposure, possibly due to variable individual sensitivity. Glucocorticoids have a direct inhibitory effect on the osteoblasts and thus the mechanism for glucocorticoid-induced osteoporosis appears to differ from the changes in postmenopausal osteoporosis. Serum osteocalcin seems to be a sensitive marker of steroid-induced osteoporosis and the effect on the serum level is a dose-dependent suppression (93, 102). Low doses of glucocorticoids (2.5–10 mg) appear to have a relatively small effect. Inhaled glucocorticoids, today the most prevalent therapy for asthma, may also have less adverse effects on bone turnover, but only a few studies have been reported (66).

Conclusions

The newer biochemical markers of bone metabolism appear to have the specificity and sensitivity to detect even small changes in bone turnover. The main potential for clinical use lies in their ability to identify, in combination with bone mineral measurements, women with an increased risk of developing severe postmenopausal osteoporosis. Furthermore, as the pharmacological possibilities for efficient treatment of osteoporosis are improving, the demand for safe monitoring increases. Bone mineral measurements, using the DEXA technique, have good precision but, because of the low normal bone turnover, it takes at least a few years before changes in bone mass can be detected. The biochemical markers of bone metabolism have the advantage of reflecting alterations in bone turnover within weeks or months, which allows for rapid evaluation and adjustment of the therapy.

Moreover, in clinical orthopedic practice it would be advantageous if the biochemical markers could detect adverse changes in relation to fracture healing, including reflex sympathetic dystrophy or bone resorptive activity in relation to prosthetic implant loosening.

References

1. Beardsworth L J, Eyre D R, Dickson I R. Changes with age in the urinary excretion of lysyl- and hydroxylysyl-pyridinoline, two new markers of bone collagen turnover. J Bone Min 1990; 5: 671–6.
2. Benhamou C L, Popelier M, Gauvain J B, et al. Métabolisme phospho-calcique et hormones calciotropes dans la fracture du col du fémur après 70 ans. Rev Rhumatisme 1989; 56 (12): 835–41.
3. Beresford J N, Gallagher J A, Poser J W, Russell R G G. Production of osteocalcin by human bone cells in vitro. Effects of 1,25 (OH)2D3, 24,25 (OH)2D3, parathyroid hormone, and glucocorticoids. Metab Bone Dis Relat Res 1984; 5: 229–34.
4. Black D, Duncan A, Robins S P. Quantitative analysis of the pyridinium cross-links of collagen in urine, using ion-paired reversed-phase high-performance liquid chromatography. Analyt Biochem 1988; 169: 197–203.
5. Black D, Marabani M, Sturrock R D, Robins S P. Urinary execretion of the hydroxypyridinium cross-links of collagen in patients with rheumatoid arthritis. Ann Rheum Dis 1989; 48: 641–4.
6. Body J J, Delmas P D. Urinary pyridinium cross-links as markers of bone resorption in tumor-associated hypercalcemia. J Clin Endocrinol Metab 1992; 74 (3): 471–5.
7. Bollen A-M, Eyre R. Bone resorption rates in children monitored by the urinary assay of collagen type I cross-linked peptides. Bone 1994; 15 (1): 31–4.
8. Boskey A L. Noncollagenous matrix proteins and their role in mineralization. Bone Min 1989; 6: 111–23.
9. Brown J P, Delmas P D, Arlot M, Meunier J P. Active bone turnover of the cortico-endosteal envelope in postmenopausal osteoporosis. J Clin Endocrinol Metab 1987; 64 (5): 95–9.
10. Brown J P, Malaval L, Chapuy M C, Delmas P D, Eduoard C, Meunier P J. Serum bone Gla-protein: a specific marker for bone formation in postmenopausal osteoporosis. Lancet 1984; i: 1091–3.

11. Burger E H, Van der Meer J W M, Nijweide P J. Osteoclast formation from mononuclear phagocytes: role of bone-forming cells. J Cell Biol 1984; 99: 1901–5.
12. Carlsson Å S, Nilsson B E, Westlin N E. Bone mass in primary coxarthrosis. Acta Orthop Scand 1979; 50: 187–9.
13. Chambers T J, Fuller K. Bone cells predispose bone surfaces to resorption by exposure of mineral to osteoclastic contact. J Cell Sci 1985; 76: 155–65.
14. Charles P, Hasling C, Risteli J, Risteli L, Mosekilde L, Eriksen E F. Assessment of bone formation by biochemical markers in metabolic bone disease: separation between osteoblastic activity at the cell and tissue level. Calcif Tissue Int 1992; 51: 406–11.
15. Charles P, Mosekilde L, Risteli L, Risteli J, Eriksen E F. Assessment of bone remodeling using biochemical indicators of type I collagen synthesis and degradation: relation to calcium kinetics. Bone Min 1994; 24: 81–94.
16. Christiansen C, Riis B J, Rodbro P. Prediction of rapid bone loss in postmenopausal women. Lancet 1987; (i): 1105–8.
17. Civitelli R, Gonnelli S, Zacchei F, et al. Bone turnover in postmenopausal osteoporosis. Effect of calcitonin treatment. J Clin Invest 1988; 82: 1268–74.
18. Compstone J E, Crawley E I, Evans B, O'Sullivan M M. Spinal trabecular bone mineral content in patients with non-steroid-treated rheumatoid arthritis. Ann Rheum Dis 1988; 47: 660–4.
19. Cooper C, Mclaren M, Wood P J, Coulton L, Kanis J A. Indices of calcium metabolism in women with hip fracture. Bone Min 1989; 5: 193–200.
20. Delmas P. Biochemical markers of bone turnover in osteoporosis. In: Osteoporosis: Etiology, Diagnosis and Management (Eds. Riggs L B and Melton J L). Raven Press, New York 1988: 297–316.
21. Delmas P, Stenner D, Wahner H W, Mann K G, Riggs B L. Increase in serum bone gamma-carboxyglutamic acid protein with aging in women. Implications for the mechanism of age-related bone loss. J Clin Invest 1983; 71: 1316–21.
22. Delmas P, Wilson D M, Mann K G, Riggs B L. Effect of renal function on plasma levels of bone Gla-protein. J Clin Endocrinol Metab 1983; 57 (5): 1028–30.
23. Delmas P D. Biochemical markers of bone turnover for the clinical investigation of osteoporosis. Osteoporosis Int (Suppl) 1993; 1: 81–6.
24. Delmas P D, Gineyts E, Bertholin A, Garnero P, Marchand F. Immunoassay of pyridinoline cross-link excretion in normal adults and in Paget's disease. J Bone Min Res 1993; 8 (5): 643–8.
25. Doty S B, Schofield B H. Enzyme histochemistry of bone and cartilage cells. Prog Histochem Cytochem 1976; 8: 1–38.
26. Duda R J, O'Brien J F, Katzmann J A, Peterson J M, Mann K G, Riggs B L. Concurrent assays of circulating bone Gla-protein and bone alkaline phosphatase: Effect of sex, age and metabolic bone disease. J Clin Endocrinol Metab 1988; 66 (5): 951–7.
27. Eastell R, Calvo M S, Burritt M F, et al. Abnormalities in circadian patterns of bone resorption and renal calcium conservation in type I osteoporosis. J Clin Endocrinol Metab 1992; 74 (3): 487–94.
28. Ekenstam af E. Influence of inflammation and corticosteroid treatment on indices of bone turnover and parathyroid function. Thesis, Uppsala University, Sweden, 1990.
29. Ekenstam af E, Ljunghall S, Hällgren R. Serum osteocalcin in rheumatoid arthritis and other inflammatory arthritides: relation between inflammatory activity and the effect of glucocorticoids and remission-inducing drugs. Ann Rheum Dis 1986; 45: 484–90.
30. Elomaa I, Virkkunen P, Risteli L, Risteli J. Serum concentration of the cross-linked carboxyterminal telopeptide of type I collagen (ICTP) is a useful prognostic indicator in multiple myeloma. Brit J Cancer 1992; 66: 337–41.
31. Epstein S, McClintock R, Bryce G, Poser J, Johnston C C J, Hui S. Differences in serum bone Gla-protein with age and sex. Lancet 1984; (i): 307–10.
32. Eriksen E F, Charles P, Melsen F, Mosekilde L, Risteli L, Risteli J. Serum markers of type I collagen formation and degradation in metabolic bone disease: Correlation with bone histomorphometry. J Bone Min Res 1993; 8 (2): 127–32.
33. Eyre D R. Collagen cross-linking amino acids. In: Methods in enzymology (Ed. Cunningham L W). New York: Academic Press Inc, 1987; 144: 115–139.
34. Eyre D R, Dickson I R, Ness van K. Collagen cross-linking in human bone and articular cartilage. Biochem J 1988; 252: 495–500.
35. Eyre D R, Koob T J, Ness van K P. Quantification of hydroxypyridinium cross-links in collagen by high-performance liquid chromatography. Analyt Biochem 1984; 137: 380–8.
36. Fisher L W, McBride O W, Termine J D, Young M F. Human bone sialoprotein. Deduced protein sequence and chromosomal localization. J Biol Chem 1990; 265 (4): 2347–51.
37. Fisher L W, Whitson S W, Avioli L V, Termine J D. Matrix sialoprotein of developing bone. J Biol Chem 1983; 258: 12723–7.
38. Franzén A, Heinegård D. In: Chemistry and Biology of Mineralized Tissues (Ed. Butler W T), EBSCO, Birmingham, England 1985: 132–41.
39. Fujimoto D. Aging and cross-linking in human aorta. Biochem Biophys Res Commun 1982; 109: 1264–9.
40. Garnero P, Delmas P D. Assessment of the serum levels of bone alkaline phosphatase with a new immunoradiometric assay in patients with metabolic bone disease. J Clin Endocrinol Metab 1993; 77 (4): 1046–53.
41. Gertz B J, Shao P, Hanson D A, et al. Monitoring bone resorption in early postmenopausal women by an immunoassay for cross-linked collagen peptides in urine. J Bone Min Res 1994; 9 (2): 135–41.
42. Geusens P, Vanderschueren D, Verstraeten A, Dequeker J, Devos P, Bouillon R. Short-term course of 1,25 (OH)2D3 stimulates osteoblasts but not osteoclasts in osteoporosis and osteoarthritis. Calcif Tiss Int 1991; 49: 168–73.
43. Gevers G, Dequeker J, Geusens P, Verstraeten A, Vandershueren D. Serum bone Gla protein (osteocalcin) and other markers of bone mineral metabolism in postmenopausal osteoporosis and osteoarthritis. J Orthop Rheum 1988; 1: 22–7.

44. Gevers G, Devos P, de Roo M, Dequeker J. Increased levels of osteocalcin (serum bone Gla-protein) in rheumatoid arthritis. Brit J Rheum 1986; 25: 260–2.
45. Glimcher M J. Recent studies of the mineral phase in bone and its possible linkage to the organic matrix by protein-bound phosphate bonds. Phil Trans Roy Soc B 1984; 304: 479–508.
46. Glowacki J, Lian J B. Impaired recruitment of osteoclast progenitors by osteocalcin-deficient bone implants. Cell Diff 1987; 21: 247–54.
47. Gotoh Y, Gerstenfeld L C, Glimcher M J. Identification and characterization of the major chicken bone phosphoprotein. Analysis of its synthesis by culture embryonic chick osteoblasts. Eur J Biochem 1990; 187: 49–58.
48. Gundberg C M. Alkaline phosphatase and osteocalcin. In: Favus MJ, ed. Primer on the metabolic diseases and disorders of mineral metabolism. Am Soc Bone Min 1990: 74–6.
49. Gundberg C M, Lian J B, Gallop P M. Measurements of gamma-carboxyglutamate and circulating osteocalcin in normal children and adults. Clin Chim Acta 1983; 128: 1–8.
50. Gundberg C M, Lian J B, Gallop P M, Steinberg J J. Urinary gamma-carboxyglutamic acid and serum osteocalcin as bone markers; studies in osteoporosis and Paget's disease. J Clin Endocrinol Metab 1983; 57 (6): 1221–5.
51. Haining S A, Atkins R M, Guilland-Cumming D F, Sharrard W J W, Russel R G G, Kanis J A. Vitamin D metabolites in patients with established non-union of fracture. Bone Min 1986; 1: 205–9.
52. Hall A K, Dickson I R. The effect of parathyroid hormone on osteoblast-like cells from embryonic chick calvaria. Acta Endocrinol 1985; 108: 217–23.
53. Hansen M A, Overgaard K, Riis B, Christansen C. Role of peak bone mass and bone loss in postmenopausal osteoporosis: 12-year study. Brit Med J 1991; 303: 961–4.
54. Hanson D A, Weis M A E, Bollen A-M, Maslan S L, Singer F R, Eyre D R. A specific immunoassay for monitoring human bone resorption: Quantitation of type I collagen cross-linked N-telopeptides in urine. J Bone Min Res 1992; 7 (11): 1251–8.
55. Hasling C, Eriksen E F, Melkko J, et al. Effects of a combined estrogen-gestagen regimen on serum levels of the carboxy-terminal propeptide of human type I procollagen in osteoporosis. J Bone Min Res 1991; 6 (12): 1295–1300.
56. Hassager C, Fabbri-Mabelli G, Christiansen C. The effect of the menopause and hormone replacement therapy on serum carboxyterminal propeptide of type I collagen. Osteoporosis Int 1993; 3: 50–2.
57. Hassager C, Jensen L T, Pødenphant J, Thomsen K, Christiansen C. The carboxy-terminal pyridinoline cross-linked telopeptide of type I collagen in serum as a marker of bone resorption: The effect of nandrolone decanoate and hormone replacement therapy. Calcif Tiss Int 1994; 54: 30–3.
58. Hassager C, Risteli J, Risteli L, Jensen S B, Christiansen C. Diurnal variation in serum markers of type I collagen synthesis and degradation in healthy premenopausal women. J Bone Min Res 1992; 7 (11): 1992.
59. Hauschka P V, Lian J B, Gallop P M. Direct identification of the calcium-binding amino acid, gamma-carboxyglutamate, in mineralized tissue. Proc Natl Acad Sci USA 1975; 72 (10): 3925–9.
60. Hauschka P V, Reid M L. Vitamin D dependence of a calcium-binding protein containing gamma-carboxyglutamic acid in chicken bone. J Biol Chem 1978; 253: 9063.
61. Heath J K, Atkinson S J, Meikle M C, Reynolds J J. Mouse osteoblast synthesis of collagenase in response to bone resorbing agents. Biochim Biophys Acta 1984; 800: 151–4.
62. Heinegård D, Oldberg Å. Glycosylated matrix proteins. In: Connective Tissue and its Heritable Disorders (Eds. Royce P M, Steinmann B). Wiley-Liss, Inc., New York 1993: 189–209.
63. Hill C S, Wolfert R L. The preparation of monoclonal antibiodies which react preferentially with human bone alkaline phosphatase and not liver alkaline phosphatase. Clin Chim Acta 1989; 186: 315–20.
64. Hordon L D, Peacock M. Osteomalacia and osteoporosis in femoral neck fracture. Bone Min 1990; 11: 247–59.
65. Hultenby K, Reinholt F P, Norgård M, Oldberg Å, Wendel M, Heinegård D. Distribution and synthesis of bone sialoprotein in metaphyseal bone of young rats show a distinctly different pattern from that of osteopontin. Eur J Cellbiol 1994; 63: 230–9.
66. Jennings B H, Andersson K E, Johansson S A. Assessment of systemic effects of inhaled glucocorticosteroids: comparison of the effects of inhaled budesonide and oral prednisolone on adrenal function and markers of bone turnover. Eur J Clin Pharmacol 1991; 40 (1): 77–82.
67. Johansen J S, Riis B J, Delmas P D, Christiansen C. Plasma BGP: an indicator of spontaneous bone loss and of the effect of oestrogen treatment in postmenopausal women. Eur J Clin Invest 1988; 18: 191–5.
68. Kanis J A, Johnell O, Gullberg B et al. Evidence for efficacy of drugs affecting bone metabolism in preventing hip fracture. B Med J 1992; 305 (7):1124–8.
69. Kaplan M M. Alkaline phosphatase. N Engl J Med 1972; 286: 200.
70. Karlsson M, Nilsson B, Obrant K J. Fracture incidence after tibial shaft fractures—a 30-year follow-up. Clin Orthop Rel Res 1993; 287: 87–9.
71. Karlsson M K, Johnell O, Nilsson B E, Sernbo I, Obrant K J. Bone mineral mass in hip fracture patients. Bone 1993; 14: 161–5.
72. Kivirikko K I. Excretion of urinary hydroxyproline peptide in the assessment of bone collagen deposition and resorption. In: Clinical Disorders of Bone and Mineral Metabolism (Eds. Frame B, Potts J T). Excerpta Medica, Amsterdam 1983: 105–7.
73. Kröger H, Risteli J, Risteli L, Penttilä I, Alhava E. Serum osteocalcin and carboxyterminal propeptide of type I procollagen in rheumatoid arthritis. Ann Rheum Dis 1993; 53: 338–42.
74. Laan R F J M, van Riel P L C M, van Erning L, Lemmens J A M, Ruijs S H J, van de Pute L B A. Vertebral osteoporosis in rheumatoid arthritis patients: effect of low dose prednisolone therapy. Brit J Rheumatol 1992; 31: 91–6.

75. Laitinen O. Clinical applications of urinary hydroxyproline determination. Acta Med Scand (Suppl 577) 1974.
76. Lamberg-Allardt C, von Knorring J, Slätis P, Holmström T. Vitamin D status and concentration of serum vitamin D metabolites and osteocalcin in elderly patients with femoral neck fracture—a follow-up study. Eur J Clin Nutr 1989; 43: 355–61.
77. Lian J B, Carnes D L, Glimcher M J. Bone and serum concentrations of osteocalcin as a function of 1.25-dihydroxyvitamin D3 circulating levels in bone disorders in rats. Endocrinol 1987; 120: 2123–30.
78. Mallmin H. Fracture of the distal forearm. Epidemiological and clinical studies. Thesis, Uppsala University, Sweden, 1992.
79. Mazess R B, Barden H S, Bisek J P, Hanson J. Dual-energy X-ray absorptiometry for total body and regional bone-mineral and soft-tissue composition. Am J Clin Nutr 1990; 51: 1106–12.
80. Melkko J, Niemi S, Risteli L, Risteli J. Radioimmunoassay of the carboxyterminal propeptide of human type I procollagen. Clin Chem 1990; 36 (7): 1328–32.
81. Moss D W. Alkaline phosphatase isoenzymes. Clin Chem 1982; 28 (10): 2007–16.
82. Nielsen H K, Brixen K, Mosekilde L. Diurnal rhythm and 24-hour integrated concentrations of serum osteocalcin in normals: influence of age, sex, season and smoking habits. Calcif Tiss Int 1990; 47: 284–90.
83. Nilsson B E, Johnell O, Petersson C. In vivo bone-mineral measurement. How and why—review. Acta Orthop Scand 1990; 61 (3): 275–81.
84. Nilsson B E, Westlin N E. The plasma concentration of alkaline phosphatase, phosphorus and calcium following femoral neck fracture. Acta Orthop Scand 1972; 43: 504–10.
85. Obrant K J, Merle B, Bejui J, Delmas P D. Serum bone Gla-protein after fracture. Clin Orthop Rel Res 1990; 258: 300–3.
86. Obrant K J, Nilsson B E. Histomorphologic changes in the tibial epiphysis after diaphyseal fracture. Clin Orthop 1984; 185: 270–5.
87. Ogawa T, Ono T, Tsuda M, Kawaniski Y. A novel fluor in insoluble collagen: A cross-linking moiety in collagen molecule. Biochem Biophys Res Commun 1982; 107: 1252–7.
88. Oldberg Å, Franzén A, Heinegård D, Pierschbacher M, Ruoslathi E. Identification of a bone sialoprotein receptor in osteosarcoma cells. J Biol Chem 1988; 263 (36): 19433–6.
89. Oni O O A, Mahabir J P, Iqbal S J, Gregg P J. Serum osteocalcin and total alkaline phosphatase levels as prognostic indicators in tibial shaft fractures. Injury 1989; 20 (1): 37–8.
90. Otsuka K, Sodek J, Limeback H F. Collagenase synthesis by osteoblast-like cells. Calcif Tiss Int 1984; 36: 722–4.
91. Parfitt A M. The cellular basis of bone remodeling: the quantum concept reexamined in the light of recent advances in the cell biology of bone. Calcif Tiss Int 1984; 36: 37–45.
92. Parfitt A M, Kleerekoper M. Diagnostic value of bone histomorphometry and comparison of histologic measurements and biochemical indices of bone remodeling. In: Osteoporosis (Eds. Christiansen C, Arnaud C D, Nordin B E C, Parfitt A M, Peck W A, Riggs B L). Glosotrup, Denmark: Aalborg Stiftsbogtrykkeri, 1984: 111–120.
93. Peretz A, Praet J P, Bosson D, Rozenberg S, Bourdoux P. Serum osteocalcin in the assessment of corticosteroid-induced osteoporosis. Effect of long- and short-term corticosteroid treatment. J Rheumatol 1989; 16 (3): 363–7.
94. Peretz A, Praet J P, Rozenberg S, Bosson D, Famaey J P, Bourdoux P. Osteocalcin and bone mineral content in rheumatoid arthritis. Clin Rheum 1989; 8 (1): 42–8.
95. Pitt P, Compston J, Trivedi P, et al. Systemic bone changes accompanying early rheumatoid arthritis in patients treated with nonsteroidal antiinflammatory drugs alone. Clin Orthop Rel Res 1994; (298): 250–8.
96. Posen S, Cornish C, Kleerekoper M. Alkaline phosphatase and metabolic bone disorders. In: Metabolic Bone Disease (Eds. Avioli L V, Krane S M). Academic Press, New York 1982: 14–81.
97. Price P A. Vitamin K-dependent formation of bone Gla protein (osteocalcin) and its function. Vitam Horm 1985; 42: 65–107.
98. Price P A, Baukol S A. 1,25-dihydroxyvitamin D3 increases the synthesis of the vitamin K-dependent bone protein by osteosarcoma cells. J Biol Chem 1980; 255: 11660–3.
99. Price P A, Nishimoto S K. Radioimmunoassay for the vitamin K-dependent protein of bone and its discovery in plasma. Proc Natl Acad Sci USA 1980; 77: 2234–81.
100. Prockop D J, Kivirikko K I, Tuderman L, Guzman N A. The biosynthesis of collagen and its disorders. N Engl J Med 1979; 301: 13–23.
101. Reid D M, Kennedy N S J, Smith M A, Tothill P, Nuki G. Total body calcium in rheumatoid arthritis: effects of disease activity and corticosteroid treatment. Br Med J 1982; 285: 330–2.
102. Reid I R, Chapman G E, Fraser T R C, et al. Low serum osteocalcin levels in glucocorticoid-treated asthmatics. J Clin Endocrinol Metab 1986; 62 (2): 379–83.
103. Reinholt F P, Hultenby K, Oldberg A, Heinegård D. Osteopontin—a possible anchor of osteoclasts to bone. Proc Natl Acad Sci USA 1990; 87: 4473–5.
104. Report. Consensus development conference: Prophylaxis and treatment of osteoporosis. Copenhagen: Am J Med 1990; 90: 107–10.
105. Riis B J, Johansen J S, Christiansen C. Continuous oestrogen-progestogen treatment and bone metabolism in postmenopausal women. Maturitas 1988; 10: 51–88.
106. Risteli J, Elomaa I, Niemi S, Novamo A, Risteli L. Radioimmunoassay for the pyridinoline cross-linked carboxy-terminal telopeptide of type I collagen: A new serum marker of bone collagen degradation. Clin Chem 1993; 39 (4): 635–40.
107. Risteli J, Melkko J, Niemi S, Risteli L. Use of a marker of collagen formation in osteoporosis studies. Calcif Tiss Int (Suppl 49) 1991; 24–5.

108. Risteli L, Risteli J. Carboxyterminal propeptide of type I procollagen. A new direct indicator of bone matrix formation. Ital J Min & Electrol Metab 1992; 6 (1): 1–6.

109. Robins S P, Stewart P, Astbury C, Bird H A. Measurements of the cross-linking compound, pyridinoline, in urine as an index of collagen degradation in joint disease. Ann Rheum Dis 1986; 45: 969–73.

110. Rodan G A, Martin T J. Role of osteoclasts in hormonal control of bone resorption—a hypothesis. Calcif Tiss Int 1981; 33: 349–51.

111. Roh Y S, Dequeker J, Mulier J C. Bone mass in osteoarthrosis, measured in vivo by photon absorption. J Bone Joint Surg (Am) 1974; 56 (3): 587–9.

112. Roh Y S, Dequeker J, Mulier J C. Cortical bone remodeling and bone mass in primary osteoarthrosis of the hip. Invest Radiol 1973; 8: 251–4.

113. Savolainen E-R, Goldberg B, Leo M A, Valez M, Lieber C S. Diagnostic value of serum procollagen peptide measurements in alcoholic liver disease. Alcohol Clin Exp Res 1984; 8: 384–9.

114. Saxne T, Heinegård D. Increased synovial fluid content of bone sialoprotein reflects tissue destruction in rheumatoid arthritis. Transactions of the 39th Annual Meeting, Orthopaedic Research Society. San Francisco, USA, 1993: 216.

115. Schlemmer A, Hassager C, Jensen S B, Christiansen C. Marked diurnal variation in urinary excretion of pyridinium cross-links in premenopausal women. J Clin Endocrinol Metab 1992; 74 (3): 476–80.

116. Seibel M J, Cosman F, Shen V, et al. Urinary hydroxypyridinium cross-links of collagen as markers of bone resorption and estrogen efficacy in postmenopausal osteoporosis. J Bone Min Res 1993; 8 (7): 881–9.

117. Seibel M J, Duncan A, Robins S P. Urinary hydroxypyridinium cross-links provide indices of cartilage and bone involvement in arthritic diseases. J Rheumatol 1989; 16 (7): 964–70.

118. Sernbo I. Hip fracture. Thesis, Malmö General Hospital, Lund University, Sweden, 1988.

119. Seyedin S M, Kung V T, Daniloff Y N, et al. Immunoassay for urinary pyridinoline: The new marker of bone resorption. J Bone Min Res 1993; 8 (5): 635–41.

120. Sharland D E, Overstall P W. Alkaline phosphatase: changes in serum levels after a fracture. Brit Med J 1978; (i): 620.

121. Sherman S S, Jordan D T, Hollis B W, Gundberg C M, Roy T A, Plato C C. Biochemical parameters associated with low bone density in healthy men and women. J Bone Min Res 1992; 7 (10): 1123–30.

122. Simon L S, Krane S M, Wortman P D, Krane I M, Kovitz K L. Serum levels of type I and III procollagen fragments in Paget's disease of bone. J Clin Endocrinol Metab 1984; 58 (1): 110–9.

123. Slovik D M, Gundberg C M, Neer R M, Lian J B. Clinical evaluation of bone turnover by serum osteocalcin measurements in a hospital setting. J Clin Endocrinol Metab 1984; 59 (2): 228–30.

124. Tarallo P, Henny J, Fournier B, Siest G. Plasma osteocalcin: biological variations and reference limits. Scand J Clin Lab Invest 1990; 50: 649–55.

125. Taubman M B, Goldberg B, Sherr C J. Radioimmunoassay for human procollagen. Science 1974; 186: 1115–7.

126. Termine J D. Non-collagen proteins in bone. Ciba Found Symp 1988; 136: 178–202.

127. Thomsen K, Eriksen E F, Jorgensen J C R, Charles P, Mosekilde L. Seasonal variation of serum bone GLA protein. Scand J Clin Lab Invest 1989; 49: 605–11.

128. Uebelhart D, Schlemmer A, Johansen J S, Gineyts E, Christiansen C, Delmas P D. Effect of menopause and hormone replacement therapy on the urinary excretion of pyridinium cross-links. J Clin Endocrinol Metab 1991 72 (2): 367–73.

129. Uebelhart P J M, Gineyts E, Chapuy M-C, Delmas P D. Urinary excretion of pyridinium cross-links: a new marker of bone resorption in metabolic bone disease. Bone Min 1990; 8: 87–96.

130. Weinstein R S, Gundberg C M. Serum osteocalcin levels reflect bone mineralization in osteopenic patients. Clin Res 1983; 31: 1983.

131. Wendeberg B. Mineral metabolism of fractures of the tibia in man studied with external counting of Sr85. Acta Orthop Scand (Suppl 52) 1961.

132. Åkesson K, Ljunghall S, Gärdsell P, Sernbo I, Obrant K J. Serum osteocalcin and fracture susceptibility in elderly women. Calcif Tiss Int 1993; 53: 86–90.

133. Åkesson K, Vergnaud P, Gineyts E, Delmas P, Obrant K J, eds. Biochemical evidence for decreased bone formation and increased bone formation and increased bone resorption in women with hip fracture. In: Osteoporosis (Eds. Christiansen C, Overgaard K). Osteopress APS, Copenhagen, Denmark 1990: 362–3.

134. Åkesson K, Vergnaud P, Gineyts E, Delmas P D, Obrant K J. Impairment of bone turnover in elderly women with hip fracture. Calcif Tiss Int 1993; 53: 162–9.