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Review

Bone Turnover Markers: Basic Biology to Clinical Applications

Marian Schini,[1,2,*] Tatiane Vilaca,[1,*] Fatma Gossiel,[1] Syazrah Salam,[1,2] and Richard Eastell[1]

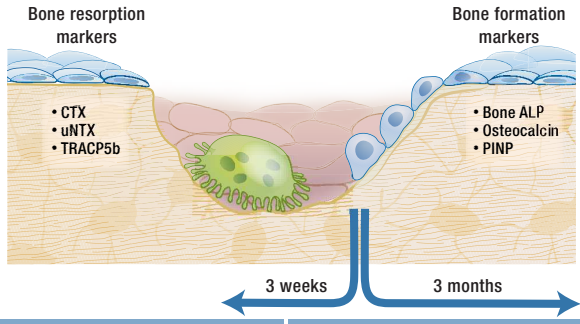
[1]Department of Oncology and Metabolism, University of Sheffield, Sheffield S10 2HQ, UK
[2]Metabolic Bone Centre, Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield S5 7AU, UK

Correspondence: Richard Eastell, MD, Department of Oncology and Metabolism, The University of Sheffield, Metabolic Bone Centre, Northern General Hospital, Herries Road, S5 7 AU Sheffield, UK. Email: r.eastell@sheffield.ac.uk.
* Bothauthors have made equal contributions

Abstract

Bone turnover markers (BTMs) are used widely, in both research and clinical practice. In the last 20 years, much experience has been gained in measurement and interpretation of these markers, which include commonly used bone formation markers (bone alkaline phosphatase, osteocalcin, and procollagen I N-propeptide); and commonly used resorption markers (serum C-telopeptides of type I collagen, urinary N-telopeptides of type I collagen, and tartrate-resistant acid phosphatase type 5b). BTMs are usually measured by enzyme-linked immunosorbent assay or automated immunoassay. Sources contributing to BTM variability include uncontrollable factors (eg, age, gender, ethnicity) and controllable factors, particularly relating to collection conditions (eg, fasting/feeding state, and timing relative to circadian rhythms, menstrual cycling, and exercise). Pregnancy, season, drugs, and recent fracture(s) can also affect BTMs. BTMs correlate with other methods of assessing bone turnover, such as bone biopsies and radiotracer kinetics, and can usefully contribute to diagnosis and management of several diseases such as osteoporosis, osteomalacia, Paget's disease, fibrous dysplasia, hypophosphatasia, primary hyperparathyroidism, and chronic kidney disease–mineral bone disorder.

Graphical Abstract



Methodology		Clinical use	
Main assays	• ELISA • Automated immunoassays	Diagnosis and/or management	• Osteoporosis • Primary hyperparathyroidism • Osteomalacia • Paget's disease • Fibrous dysplasia • Hypophosphatasia • Metastatic bone disease • Chronic kidney disease-mineral bone disorder
Sources of variability	• Age • Gender • Ethnicity • Lactation/pregnancy • Menstrual cycle phase • Fasting/feeding • Seasonal/Circadian rhythm • Exercise/immobilization • Recent fracture/drugs	Monitoring drug use	• Anti-osteoporotic drugs

CTX: C-telopeptide of type I collagen; TRACP5b:Tartrate resistant acid phosphatase type 5b; uNTX: Urinary N-telopeptide of type I collagen; Bone ALP: Bone isoform of alkaline phosphatase; PINP: Procollagen type I N-propeptide

Key Words: biochemical markers of bone turnover, osteocalcin, C-telopeptides of type I collagen, urinary N-telopeptides of type I collagen, tartrate-resistant acid phosphatase 5b, bone alkaline phosphatase

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Abbreviations: ADT, androgen deprivation therapy; AED, anti-epileptic drug; ALP, alkaline phosphatase; ASBMR, American Society of Bone Mineral Research; AUC, Area under the curve; AUROC, area under the receiver operating characteristic; BCE, bone collagen equivalents; BFR/BS, bone formation rate/bone surface; BMD, bone mineral density; BMU, bone multicellular units; Bone ALP, bone isoform of alkaline phosphatase; BTM, bone turnover marker; CKD, chronic kidney disease; CKD-MBD, chronic kidney disease–mineral bone disorder; COC, combined oral contraception pill; CTX, C-telopeptide of type I collagen; CV, coefficient of variation; CYP450, cytochrome P 450; DMPA, depot medroxyprogesterone acetate; ECTS, European Calcified Tissue Society; eGFR, Estimated glomerular filtration rate; ELISA, enzyme-linked immunoassay; FD, fibrous dysplasia; FGF23, fibroblast growth factor 23; FLEX, Fracture Intervention Trial Long-term Extension; FREEDOM, Fracture Reduction Evaluation of Denosumab in Osteoporosis Every 6 Months; GIO, glucocorticoid-induced osteoporosis; Gla, gamma-carboxy glutamic acid; GLP-2, glucagon-like peptide-2; GR, gradient of risk; HORIZON-PFT E1, Health Outcomes and Reduced Incidence with Zoledronic Acid Once Yearly-Pivotal Fracture Trial Extension I; HPLC, high-performance liquid chromatography; HPP, hypophosphatasia; HR, hazard ratio; HRT, hormone replacement treatment; IFCC, International Federation of Clinical Chemistry and Laboratory Medicine; IGF-I, insulin-like growth factor 1; IOF, International Osteoporosis Foundation; iPTH, Intact parathyroid hormone; KDIGO, Kidney Disease Improving Global Outcomes; LRP-5, low-density lipoprotein receptor-related protein 5; LSC, least significant change; M-CSF, macrophage colony stimulating factor; mAb, Monoclonal antibody; MAR, mineral apposition rate; MMP, matrix metalloproteinase; N-MID, N-terminal MID fragment; NPHPT, normocalcaemic hyperparathyroidism; NTX, N-telopeptide of type I collagen; OC, osteocalcin; PHPT, primary hyperparathyroidism; PICP, procollagen I carboxyterminal propeptide; PINP, procollagen type 1 N-propeptide; PLP, pyridoxal-5′-phosphate; PTH, parathyroid hormone; RA, rheumatoid arthritis; RANK, Receptor activator of nuclear factor kappa-B; RANKL, receptor activator of nuclear factor kappa-B ligand; RIA, radioimmunoassay; ROD, renal osteodystrophy; SHPT, secondary hyperparathyroidism; T1D, type 1 diabetes; T2D, type 2 diabetes; TNSALP, tissue-nonspecific alkaline phosphatase; TRACP5b, tartrate-resistant acid phosphatase type 5b; TRAP, tartrate-resistant acid phosphatase; uNTX, urinary N-telopeptides of type I collagen; XLH, X-linked hypophosphatemia.

ESSENTIAL POINTS

- Some bone turnover markers reflect bone formation (serum bone alkaline phosphatase, osteocalcin, and procollagen I N-propeptide [PINP]) while others (serum C-telopeptides of type I collagen [CTX], tartrate-resistant acid phosphatase type 5 b [TRACP5b], and urinary N-telopeptides of type I collagen [uNTX]) reflect bone resorption
- Bone turnover markers are usually measured by enzyme-linked immunosorbent assay (ELISA) or automated immunoassay analyzers and correlate with other methods of assessing bone turnover, such as bone biopsies and radiotracer kinetics
- Several sources of variability should be considered while interpreting bone turnover markers; some are uncontrollable such as age and gender but set conditions for sample collection such as fasting state and time of the day can reduce variability
- Bone turnover markers can be used in the diagnosis and management of several bone diseases and systemic diseases affecting the skeleton
- Bone turnover markers can be used in the management of osteoporosis and mineral bone disorder associated with chronic kidney disease

The use of bone turnover markers (BTMs) is widespread, both for research and for clinical practice. Much of the development of the markers commonly used today was completed more than 20 years ago. Since this time, a lot of experience has been gained on the sources of variability of these markers and their clinical utility. This review will consider the most used markers and how they compare with other approaches to studying bone turnover. The review will also consider how BTMs are best measured, the key sources of variability and how BTMs can be used in clinical practice.

The article begins with some considerations of bone physiology that are critical to an understanding of the clinical interpretation of bone turnover markers.

Part 1. Bone Turnover Markers: What They Are, How They Are Measured, and the Causes of their Variability

Bone Physiology

In the adult skeleton, bone is continually remodeled, with bone resorption by osteoclasts being followed by bone formation by osteoblasts (1). This remodeling occurs in an orderly process called *coupling*, in which resorption is followed by formation. Osteoclasts (bone resorbing cells) and osteoblasts (osteoid producing cells) and a third cell type, the osteocyte (mature long-living cell), signal to each other to promote this orderly process. The rate of bone remodeling is more rapid in trabecular bone than in cortical bone. The osteoblasts secrete bone matrix proteins that form the organic matrix of bone, or osteoid. This subsequently mineralizes to form the mineral phase of bone (1).

BTMs reflect the work of osteoblasts and osteoclasts. The production of osteoid by osteoblasts is reflected by the production of bone alkaline phosphatase (bone ALP), osteocalcin (OC) and procollagen I N-propeptide (PINP). The removal of bone organic matrix of bone following enzymatic digestion is reflected by the production of fragments of the degradation of type I collagen (N- and C-telopeptides of type I collagen, or NTX and CTX) and by release of the enzyme tartrate-resistant acid phosphatase type 5 b (TRACP5b) (2).

Bone Cells

Osteoclasts originate from hematopoietic stem cells in the marrow or bloodstream. The monocyte-macrophage lineage differentiates into osteoclast precursors and these mature into osteoclasts. The fate of the osteoclast is usually programmed cell death, or apoptosis. These stages of the osteoclast are under local and endocrine control, and many of these actions are mediated by the osteoblast (1).

Osteoblasts originate from mesenchymal stem cells, arriving from the bone marrow or the bloodstream (3). The osteoblast progenitors and pre-osteoblasts differentiate into osteoblasts. The fate of the osteoblasts is three-fold, 1) into osteocytes; 2) into lining cells); 3) to undergo apoptosis. The immature osteoblast synthesizes alkaline phosphatase and type I collagen whereas the mature osteoblast synthesizes osteocalcin.

Osteocytes differentiate from osteoblasts, but they are not believed to be the direct source of bone turnover markers. Osteocytes are an important source of receptor activator of

nuclear factor kappa-B ligand (RANKL), the key regulator of bone resorption (see below). Fatigue damage is associated with osteocyte apoptosis (4), and this is detected by other osteocytes that signal to replace the damaged bone (5). Finally, they regulate phosphate metabolism through production of the hormone fibroblast growth factor-23 (FGF-23).

Regulation of bone cells

There are many hormones that regulate osteoclast differentiation, activity, and apoptosis, including parathyroid hormone (PTH), thyroid hormones, insulin-like growth factor 1 (IGF-1). They do so indirectly through actions on the osteoblast (6). The osteoblast and osteocyte produce RANKL (7, 8) which binds to the receptor activator of nuclear factor kappa-B (RANK) receptor on osteoclasts (Fig. 1). Macrophage colony stimulating factor (M-CSF) is also important in promoting the differentiation of osteoclasts from pre-osteoclasts (9). There is a decoy receptor, osteoprotegerin (OPG), and this also binds to RANKL and stops it binding to RANK; this is also produced by the osteoblast in response to changes in the hormone environment (Fig. 1). The RANK signaling pathway has proven to be very important and a target for drug development (6). A monoclonal antibody has been developed against RANKL (denosumab, an antiresorptive therapy).

The osteoclast is able to regulate the osteoblast (10). It does this in 3 ways: 1) it resorbs bone and releases proteins which are activated and affect osteoblasts eg, transforming growth factor beta (TGF-b); 2) it produces clastokines that affect osteoblasts, such as Wnt and sphingosine-1-phosphate (11); and 3) it is in contact with osteoblasts in a closed space with a canopy and signals through membrane-bound molecules, the ephrins. The Wnt signaling pathway has proven to be very important and a target for drug development. Wnts bind to a receptor ("frizzled") and to co-receptors such as low-density lipoprotein receptor-related protein 5 (LRP-5) (12). Sclerostin inhibits Wnt signaling by binding to the LRP-5. A monoclonal antibody has been developed against sclerostin that has proven to be anabolic (romosozumab). It is also antiresorptive as it stimulates the production of osteoprotegerin.

Bone Remodeling

In adulthood, bone is constantly remodeled, with removal (resorption) and replacement (formation) of bone tissue taking place at a rate of about 5% in the mature skeleton (eg, age 40 years). This remodeling takes place at discrete locations in bone, the bone multicellular units (BMU) (9). At any given time, there are more than 1 million BMUs in the adult skeleton. The remodeling follows an orderly sequence, the bone remodeling cycle (Fig. 2). The lining cells on the surface of bone retract to leave bone exposed and osteoclasts are attracted to this location and resorb bone for a period of about 3 weeks. They resorb bone by secreting acid from their ruffled border and this dissolves the calcium hydroxyapatite, allowing the release of calcium into the circulation. They also release enzymes (eg, cathepsin K) that are active at low pH and digest the bone proteins, the most abundant of which is type I collagen. The enzymes release fragments of type I collagen (eg, CTX and NTX) which enter the circulation and then are excreted in the urine; these can be measured in serum or urine as biochemical markers of bone resorption (2). The osteoclasts also release enzymes (eg, TRACP5b) that can also be measured as biochemical markers of bone resorption.

The period of bone resorption is then followed by a period of bone formation, characterized by matrix synthesis and mineralization (13). The matrix is synthesized by osteoblasts that release proteins (eg, OC) or fragments of proteins (eg, PINP) that can be measured in the circulation as biochemical markers of bone formation. The osteoblasts also release enzymes (eg, bone ALP) that promote mineralization and can also be measured as a biochemical markers of bone formation (from the osteocyte). The bone formation period takes typically 3 months.

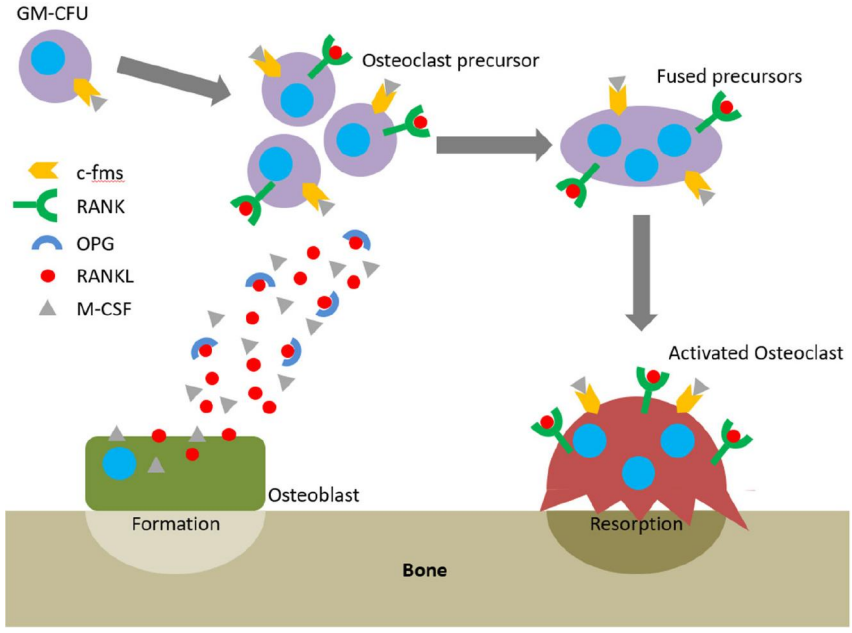


Figure 1. The RANKL/RANK/OPG axis and M-CSF direct osteoclastogenesis and activation. Abbreviations: c-fms, colony stimulating factor 1 receptor; GM-CFU, granulocyte-macrophage colony forming unit; M-CSF, macrophage colony stimulating factor; OPG, osteoprotegerin; RANK, receptor activator of nuclear factor kappa-B; RANKL, receptor activator of nuclear factor kappa-B ligand. © 2018 Owen and Reilly (1).

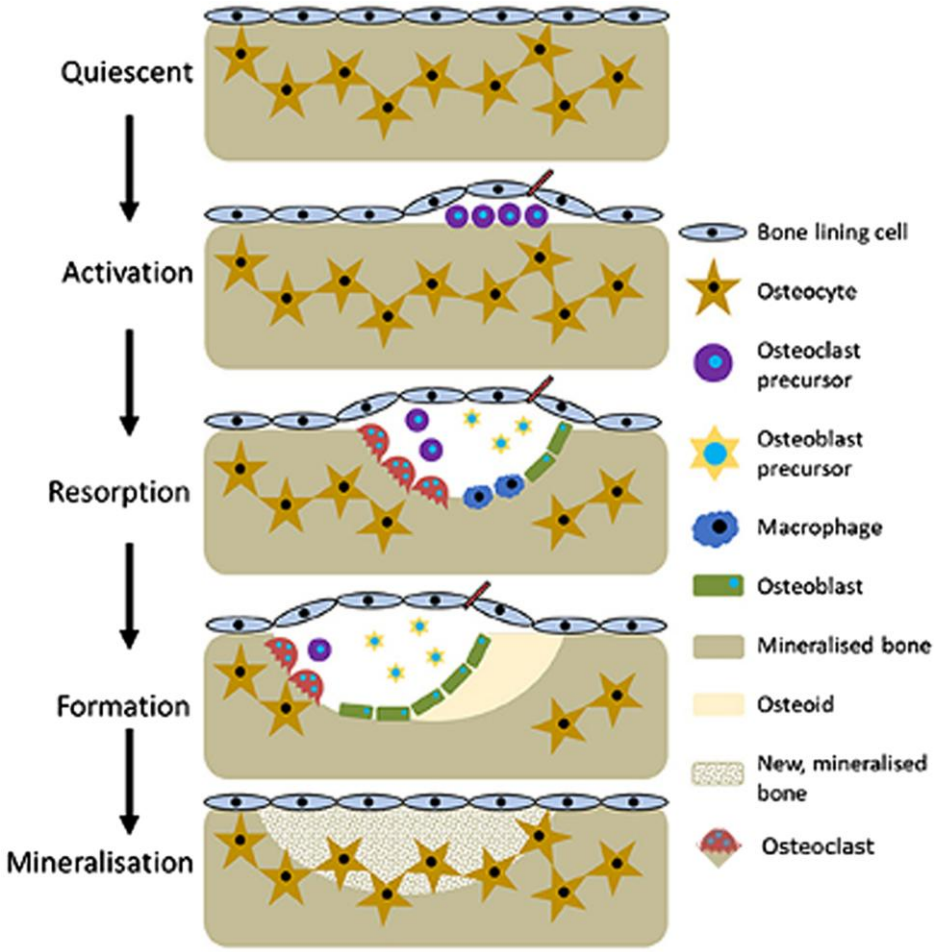


Figure 2. The 5 stages of bone remodeling. In the quiescent phase the bone is covered by lining cells; during activation osteoclasts and their precursors attach to the surface of bone; during resorption the osteoclasts dig a resorption pit and the debris is removed by macrophages; during formation the osteoblasts produce bone matrix. Note how the osteoclasts are in close proximity to the osteoblasts so as to allow "coupling"; during mineralization, the osteoid is mineralized and in young healthy people the amount of mineralized bone formed equals the amount resorbed and the processes of resorption and formation are in balance. © 2018 Owen and Reilly (1).

The rate of bone remodeling increases at the menopause, and due to coupling, the increase in bone resorption is followed by an increase in bone formation (14, 15). Similarly, when antiresorptive drugs are given for the treatment of osteoporosis, the reduction in bone resorption is followed a few weeks later by a reduction in bone formation (16).

Bone loss in the adult

In the young adult skeleton (around age 40), the amount of bone removed during bone resorption in the BMU is equal to the amount of bone formed, so there is "remodeling balance." In the older adult skeleton (after age 50 years), the amount of bone removed no longer matches the amount of bone formed and so there is "negative remodeling imbalance" (17). The decrease in bone formation could be a result of decreased precursors or osteoblast number or decreased osteoid synthesis (18). This reduction in bone formation is a key mechanism for age-related bone loss. In women, the rate of bone turnover increases by 50% to 100% (19, 20) at the menopause due to estrogen deficiency. This doubles the number of BMUs and contributes further to the bone loss of aging. This explains why women have greater bone loss than men. Bone remodeling rate also differs depending on the type of bone and whether the bone marrow is cellular or not. Thus, bone turnover is higher in the trabecular bone of the spine and pelvis (cellular marrow) and lowest in the cortical bone of the limbs (fatty marrow). It has been estimated that the rate of bone turnover is 4-times greater in trabecular than cortical bone, but since the skeleton is composed of 4-times more cortical bone than trabecular bone, the total contributions of trabecular and cortical bone are equal (21).

Throughout life, the bone changes shape, with overall wider bones with age. This is due to net resorption at the endosteal surface of cortical bone and net formation at the periosteal surface of cortical bone (22). This change in dimensions is a form of "modeling." The consequence of this is for the long bones to increase slowly in diameter. This would be a favorable adaptation; however, the rate of periosteal bone formation is insufficient to compensate bone resorption after the menopause in women and in older men. The result is a decrease in cortical width and reduced bone strength (23). In the clinical interpretation of bone turnover marker measurements in the adult, consideration needs to be given to the contributions of age, menopause, and the contributions from remodeling (in the trabecular and cortical envelopes) and modeling (both from the endosteum and periosteal surfaces).

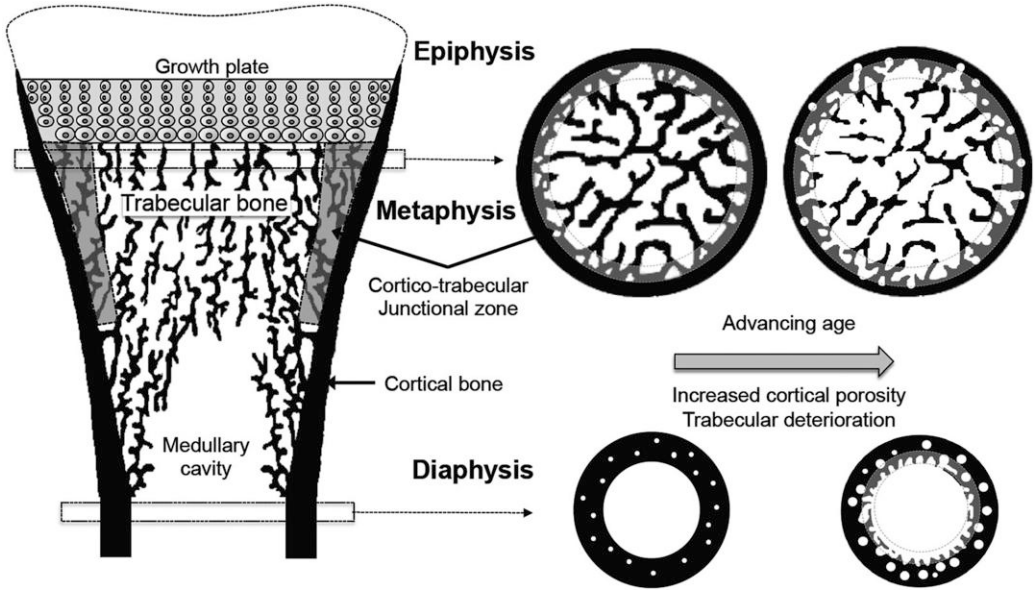


Figure 3. (Left) schematic cross section of the metaphysis of a long bone from a child showing the growth plate forming trabecular bone and the cortical bone that expands by modeling. (Right) sections through the bone in younger and older adults at the metaphysis (upper) and diaphysis (lower) showing thinning and loss of trabecular bone and thinning and greater porosity of the cortical bone. Reprinted from Bala et al. J Bone Miner Res, John Wiley and Sons, Inc. © 2014 American Society for Bone and Mineral Research (26).

Bone growth, modeling, and remodeling in childhood

Children have a remodeling rate that is about 3-times higher than adults (24). They also have modeling as the bone changes shape as the child grows. However, a major contribution to bone turnover markers is from the growth plate (Fig. 3). Here, cartilage is first produced; this is mainly formed of type II collagen and so there is little contribution to bone turnover markers. However, the cartilage is resorbed and replaced by type I collagen that mineralizes to form bone tissue. Thus, there is a large contribution from the growth plate to bone turnover markers. As a result, children have bone turnover marker levels 5 to 20 times higher than adults (25).

The onset of puberty is another period of high bone turnover and is associated with the closure of the growth plates, mediated by estrogen, and linear growth stops at approximately age 14 in girls and 16 in boys (27). However, the growth plates close at different ages, and some are open up to age 30 years. This is why the high levels of bone turnover markers during childhood do not reach those of the mature adults until after the age of 30 years (28). In the clinical interpretation of bone turnover marker results in children and adolescents, consideration needs to be given to remodeling, modeling, growth, and pubertal status.

Assessment of Bone Turnover

Bone turnover markers

History. Bone turnover markers (BTMs) allow us to study the activity of osteoblasts and osteoclasts at a whole-body level and have the advantages that they are noninvasive, inexpensive (£12.50 per test in the UK) and as a result allow for multiple measurements over time (29). The first bone turnover marker to be developed in the 1920s was total alkaline phosphatase, although this marker is not specific to bone. However, it is still widely used clinically for the diagnosis and monitoring of metabolic bone diseases such as Paget's disease and osteomalacia. However, for diseases with smaller changes of bone turnover such as osteoporosis, the BTMs that are more specific to bone have proven more useful.

The next major development in assays for bone turnover was the introduction of hydroxyproline assays in the 1960s (30). In contrast to total alkaline phosphatase, which is a bone formation marker, hydroxyproline reflects bone resorption. Hydroxyproline excretion in the urine is influenced by hydroxyproline in the diet and is not specific to bone. Total deoxypyridinoline is a more bone-specific marker, which is not influenced strongly by diet. The assay was introduced in the 1980s (31). A marker of bone formation that was specific for bone was introduced around 1980, namely osteocalcin, initially called *bone Gla-protein* (32). In the 1990s, immunoassays became available for other bone formation markers such as the C- and N-propeptides of type I procollagen and bone ALP (33). Immunoassays also became available for markers of bone resorption such as CTX and NTX (34, 35) and the enzyme TRACP5b (36).

The final major development was in assay technology; the immunoassays used radioimmunoassay (RIA) and enzyme-linked immunoassay (ELISA) techniques and these were mostly replaced by the more precise and accurate automated immunoassay analyzers that use chemiluminescence or electrochemiluminescence (37). Currently, assays for bone ALP, OC, PINP, CTX, urinary NTX (uNTX), and TRACP5b are widely available, and these will now be described in detail.

Bone ALP. Alkaline phosphatases (ALP) are membrane-bound glycoproteins that hydrolyze phosphate monoesters at basic pH. There are 4 isozymes in man; placental, germ cell, intestinal and tissue-nonspecific (liver, bone, and kidney) (38).

The gene for the tissue-nonspecific ALP (TNSALP) (*ALPL*) is located on chromosome 1. The isoforms for liver, bone, and

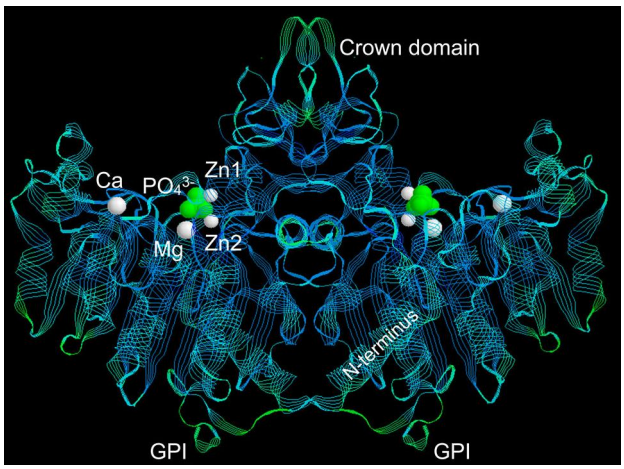


Figure 4. Ribbon representation of the 3D structure of alkaline phosphatase. Also indicated are the flexible exposed sequence known as the "crown domain"; the N-terminal helix of one subunit that reaches close to the active site of the contralateral subunit; and the location where the glycosylphosphatidylinositol (GPI) anchor is attached to the C-terminus of the mature enzyme. © 2015 Millan and Whyte (42).

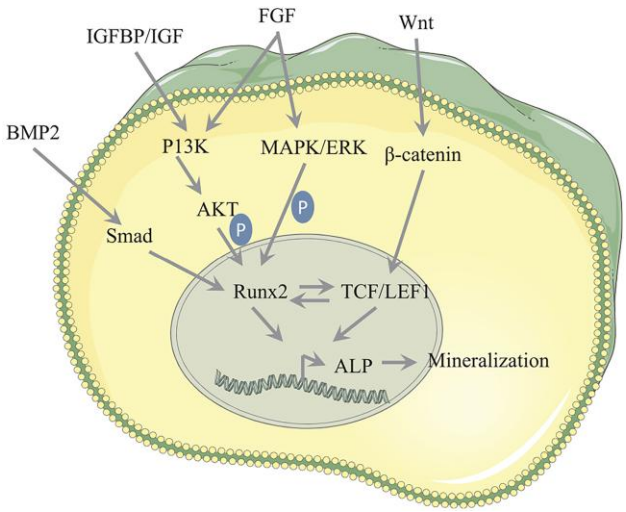


Figure 5. Major signaling mechanism for bone mineralization inducing ALP expression in osteoblasts. BMP2 tends to increase Runx2 expression through downstream signaling like smad, as shown in the diagram. IGFBP/IGF/FGF increases Runx2 via MAPK/ERK/P13 K downstream singling and increases Runx2/TCF/LEF1 via β- catenin through Wnt signaling. Transcription factor, Runx2/TCF/LEF1 promotes bone mineralization by activating ALP expression. Reprinted from Gene 754;144855 Vimalraj et al. © 2020 Elsevier, with permission from Elsevier (48).

kidney all have the same amino acid sequence but differ in their posttranslational modification (Fig. 4). Bone ALP is rich in sialic acid residues, unlike the liver isoform (39) (a property that can be used in separation using wheat germ lectin which only binds bone ALP). Bone ALP is a homodimer anchored to the membrane of matrix vesicles and osteoblasts. It is cleaved from the membrane by a phospholipase and then circulates as a soluble homodimer and measured as a marker of bone formation (40). There are roughly equal amounts of bone and liver ALP in the circulation. Liver ALP shows approximately 20% cross-reactivity in the assay for bone ALP (41).

The principal substrates for ALP are pyrophosphate (PPi) and pyridoxal-5′-phosphate (PLP). Pyrophosphate is an inhibitor of mineralization. When bone ALP is anchored to the membrane, it hydrolyzes pyrophosphate into phosphate which is the substrate for the formation of hydroxyapatite crystals (43). Therefore, bone ALP reflects the mineralization phase of bone formation.

Bone ALP has been shown to inactivate osteopontin by dephosphorylation. Osteopontin is a mineralization inhibitor like pyrophosphate and inhibits hydroxyapatite crystal formation and growth, cell adhesion, and migration (44, 45). The enzyme can also dephosphorylate ATP and lipopolysaccharide (46), although the significance of these effects is unknown.

The production of bone ALP is regulated by 2 main pathways, Runx2 (mediating the effects of bone morphogenic protein 2, IGF-I, and fibroblast growth factor 23 [FGF23]) and beta-catenin (mediating the effects of Wnt) (Fig. 5). The effect of FGF23 is to suppress *ALPL* expression, leading to reduced bone ALP activity and hence increased pyrophosphate and decreased phosphate levels (47). FGF23 is believed to be important in several forms of hypophosphatemic osteomalacia (tumor-induced osteomalacia and X-linked hypophosphatemia [XLH]). Please refer to section on BTM in "Osteomalacia."

In humans, pathogenic mutations of *ALPL* lead to hypophosphatasia (HPP) (42). This is a disorder associated with inhibition of mineralization, likely due to the accumulation of pyrophosphate. The TNSALP activity is low and the pyrophosphate and PLP levels are high. There is now enzyme therapy available as bone-targeted recombinant TNSALP (Asfotase Alfa) (49). Please refer to section on BTM in HPP.

Osteocalcin. Osteocalcin (OC) is a 49–amino acid protein (6 kD) secreted by osteoblasts, odontoblasts, and hypertrophic chondrocytes. It was isolated and sequenced in 1970s (50, 51). It is present in higher concentration in cortical than in trabecular bone. In chick osteoblasts, the order of production of bone matrix proteins is collagen, followed by alkaline phosphatase, and then OC; the latter is produced as the bone mineralizes (52).

In humans, the gene for OC is on chromosome 1. Its synthesis is regulated by 1,25-dihydroxyvitamin D (positively in human and rat, negatively in the mouse) (53) and glucocorticoids through response elements. The effects of glucocorticoids are to cause osteoblast apoptosis (54) but also to reduce the rate of transcription of the OC gene (53), both resulting in lower circulating levels of OC.

OC contains glutamate residues that can be carboxylated under the influence of vitamin K and are inhibited by warfarin. The carboxylated glutamate molecule is *gamma-carboxy glutamic acid* (Gla). Gla proteins in bone have important roles for bone strength as indicated by the harmful effects of warfarin on the fetal skeleton. Other Gla proteins include Protein S, Periostin, Gla-rich protein and Matrix Gla-protein. The critical nature of the Gla residues in binding to hydroxyapatite (by adsorption) is shown in the high homology (up to 95%) of the first helical region of OC that contains the 3 Gla residues (53). The Gla residues are at positions 17, 21, and 24.

Only a proportion of newly synthesized OC appears in the circulation, and it reflects osteoblast activity. In humans, levels of OC correlate with bone formation rates assessed by radiotracer kinetics (20) and bone histomorphometry (20, 55).

In rats, circulating OC originates from new bone synthesis rather than its breakdown (56).

OC circulates as the intact molecule (1-49) and major fragments. It was originally thought that the major fragment was OC (1-43) but detailed analysis using matrix-assisted laser desorption/ionization mass spectroscopic immunoassay show there to be over 12 major fragments (57). Thus, assays that measure both the intact and major fragments are better called *total OC*.

Smaller fragments of OC are released during bone resorption due to the action of matrix metalloproteinases (MMPs) and cathepsin K, but these are in low concentration in the serum and so are best measured in the urine (mid-molecule OC).

OC with fewer than 3 Gla residues is referred to as "undercarboxylated osteocalcin." Undercarboxylated OC can be measured by an immunoassay or by OC assay after hydroxyapatite precipitation of carboxylated OC. It can form up to 50% of total OC and the levels relate to nutritional vitamin K intake. Vitamin K status can be evaluated by calculating the ratio of carboxylated to undercarboxylated OC and the average value is around 1.2 (58). The percent of OC that is carboxylated is dependent upon vitamin K status and only correlates poorly with the total OC (59).

The OC knockout mouse was described 24 years ago and had high bone mass of improved functional quality (60) and it was proposed that OC also acted on the pancreas, liver, fat cells, muscle, male gonads, and brain (61). Thus, some authors claimed that OC may be a hormone with pleiotropic effects. In this theory, it is proposed that the hormonal form of OC is the undercarboxylated form and that this is released during bone resorption, and it affects body weight, adiposity, glucose and energy metabolism, male fertility, brain development, and cognition (62).

However, there is also evidence against this theory (62). Diegel et al (63) deleted the 2 osteocalcin-encoding genes in mice using gene editing. The animals had no OC, but they had normal bone mass, glucose, and male fertility. There were abnormalities of crystal size and maturation of hydroxyapatite.

This study thus differs in the effects of the first described OC knockout mice (60) due to differences in genetic background, modifier genes, and the different approaches to knocking out the *Bglap1* and *Bglap2* (Bone Gamma-Carboxyglutamate Protein) genes. Further experiments are needed.

Procollagen I N-propeptide. Procollagen I N-propeptide (PINP) is a 35-kDa protein that is produced by cleavage from type I procollagen (Fig. 6) (64). The cleavage occurs by a specific endopeptidase that releases the PINP, a trimeric molecule. A small amount may be incorporated into bone (65) but the rest of the PINP is released into the circulation and is degraded by the Kuppfer cells in the liver, its uptake being mediated by the scavenger receptor (33). There may be release of a monomeric form of PINP (a single chain), a 10-kDa fragment that is excreted by the kidney (64). Assays are available for the trimeric form (intact PINP) and both the trimeric and monomeric forms (total PINP). The total PINP assay shows a false elevation in chronic kidney disease (CKD) stages 4 to 5 and dialysis (66), otherwise the assays correlate well with each other (67).

There are 2 genes for type I collagen, as it is made up of the alpha-1 (I) and alpha-2 (I) molecules. The first is the *COL1A1* gene on chromosome 17 and the second is the *COL1A2* gene on chromosome 7 (64).

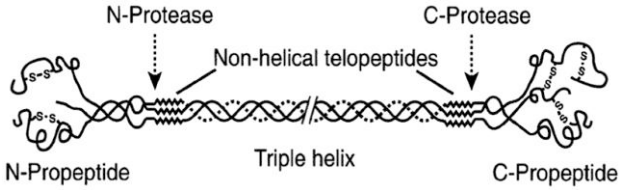


Figure 6. The processing of type I procollagen to PINP (N-propeptide) and PICP (C-propeptide). Reprinted from Clin Biochem 45; Koivula et al, Measurement of aminoterminal propeptide of type I procollagen (PINP) in serum, 920-27. © 2012 Elsevier, with permission from Elsevier (64).

Type I procollagen is formed mostly of triplets, Gly-X-Y, where Gly is glycine and often X is proline and Y is hydroxyproline. Within the endoplasmic reticulum, there are several posttranslational modifications (68). The proline and lysine residues are hydroxylated to hydroxyproline and hydroxylysine. The hydroxylysine may be glycosylated to galactosyl hydroxylysine and disulfide bridges are formed at the C-terminal end. The secreted procollagen molecule winds up as a helix starting at the C-terminal end. The lysine and hydroxylysine molecules are converted to aldehydes by lysyl oxidase and then form pyridinium crosslinks (pyridinoline and deoxypyridinoline) and these stabilize the triple helix. Once the triple helix is formed, the N-propeptide and then the C-propeptide are released by specific endopeptidases. The C-propeptide is PICP and it is released in equimolar amounts to PINP but in a single molecular form, and it is cleared by the mannose receptor in the endothelial cells of the liver (69). This contrasts with the clearance of the PINP, which is not under endocrine control and is cleared by the scavenger receptor in the liver (70). The clearance of PICP is under endocrine control and is accelerated by thyroid hormones and IGF-I; thus, the elevations of PICP are lower than those of PINP in thyrotoxicosis and during growth, times when thyroid hormones and IGF-I, respectively, are elevated.

The circulating level of intact PINP was studied in 371 women with postmenopausal osteoporosis who underwent bone biopsy. It is believed that bone formation rate/bone surface (BFR/BS) is the appropriate comparator to bone formation markers as its estimate requires the administration of tetracycline and so it is a "dynamic" measure. The correlation in the endocortical bone ($r = 0.39$) and the cancellous bone ($r = 0.26$) was modest (71).

PINP is released from synthesis of all type I collagen and many tissues contain this protein (eg, skin, tendon). PINP can be used as a bone formation marker as more than 90% of the protein in bone is type I collagen and about 70% of type I collagen is to be found in bone tissue (64). Furthermore, the turnover of type I collagen in bone is higher than in soft tissues. Thus, most PINP in the circulation is derived from bone; the exceptions include surgery when the healing tissues release excess PINP (72), skin disease (73) and liver fibrosis (74).

CTX and NTX. There is crosslinking between adjacent collagen molecules during the synthesis of type I procollagen, as noted above (under PINP). These crosslinks are usually the pyridinium crosslinks, pyridinoline and deoxypyridinoline. The crosslinks form between 2 chains of the helix and 2 chains of the telopeptide region (Fig. 7). The telopeptide region is the end of the

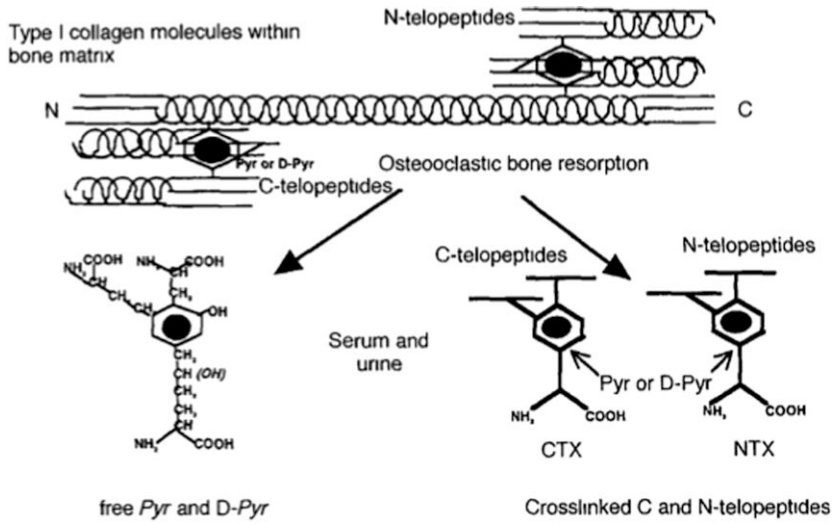


Figure 7. Type I collagen breakdown products as markers of bone resorption. Type I collagen molecules in the bone matrix are linked by pyridinoline crosslinks (pyridinoline [Pyr] or deoxypyridinoline [D-Pyr]) in the region of N- and C-telopeptides, Pyr differs from D-Pyr by the presence of an hydroxyl residue shown in italics. During osteoclastic bone resorption, pyridinoline crosslinks are released the circulation and then excreted in urine as a free form or linked to C- (CTX) or N-telopeptides (NTX) of type I collagen Free Pyr, free D-Pyr, CTX, and NTX can be measured in urine and serum using specific immunoassays. Reprinted from Ballieres Clin Rheumatol, vol 11, Bone Markers, pages 517-37, © 1997 Elsevier, with permission from Elsevier (75).

collagen molecule and there are a N-telopeptide and a C-telopeptide regions. There are 2 sites on the helix which undergo such crosslinking. At position 87 on the helix, the crosslink joins to the C-telopeptide region. At position 930 on the helix, the crosslink joins to the N-telopeptide region (68).

When CTX and NTX are first formed, the amino acid sequence is linear and in the usual alpha configuration. After a few months, the CTX undergoes beta-isomerization which means peptide backbone originally linked to the alpha carbon of the aspartic acid residue moves (non-enzymatically) to the beta-carbon (76). This isomerization does not happen with NTX. During bone resorption by the osteoclast, the type I collagen is digested by enzymes such as cathepsin K and CTX and NTX are released and can be measured in the circulation or the urine. The alpha and beta forms can both be measured in the urine by ELISA (77). The alpha reflects newly synthesized collagen, as is found in high turnover states such as Paget's disease (78) or malignant bone disease (79) or in children. The beta form reflects more mature collagen and is more abundant in disorders such as osteoporosis or in healthy adults.

CTX is usually measured (in the beta form) in plasma (or serum) as the form in urine shows very large day-to-day variability (80). NTX is usually measured in urine and expressed as a ratio to creatinine to adjust for urinary dilution. The assay for serum NTX shows smaller changes in response to antiresorptive therapy given for osteoporosis and so it has not been widely adopted (81). Plasma (or serum) CTX has been recommended by the International Osteoporosis Foundation (IOF) and International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) as a reference marker (82). It correlates modestly with osteoclast surface by bone histomorphometry in the endocortical bone ($r = 0.35$) and the cancellous bone ($r = 0.24$) (71).

The urinary excretion of the urinary pyridinium crosslinks have been widely used for the study of bone resorption. Deoxypyridinoline is more specific to bone than pyridinoline, although it is less abundant. The total deoxypyridinoline correlates well with bone resorption measured using radiotracer techniques (83). It is less used today as the assay is cumbersome; it requires acid hydrolysis of urine followed by high-performance liquid chromatography (HPLC) and so it is a difficult and time-consuming assay. The free form of deoxypyridinoline and pyridinoline can be measured in the urine by ELISA. However, this free form does not respond as expected to antiresorptive treatment of osteoporosis (84), perhaps because bisphosphonates affect renal handling of peptide-bound crosslinks and their conversion to the free crosslink forms (85).

There are other analytes that have been used to assess bone resorption that are based on the degradation of type I collagen. Hydroxyproline assays were the first to be introduced but are no longer used (see above). Galactosyl hydroxylysine can be measured in the urine (86) or serum (87) but it is not widely used as it requires HPLC. Cross-linked carboxyterminal telopeptide of type I collagen (ICTP) is also called CTX-MMP; it is a cross-linked fragment of collagen like CTX but it is generated by metalloproteinases rather than by cathepsin K, as is CTX. ICTP has not become widely used as it does not respond to most antiresorptive therapies (84) and the assays are no longer available.

Tartrate-resistant acid phosphatase type 5b. Tartrate-resistant acid phosphatase type 5b (TRACP5b) is the enzyme produced by the osteoclast. Acid phosphatase is a non-specific hydrolase that hydrolyzes phosphomonoesters at low pH (88). Tartrate-resistant acid phosphatase (TRAP) can be separated into 6 isoenzymes using electrophoresis (36). Type 5b is produced by the osteoclast and 5a is produced by tissues such as macrophages, dendritic cells and the placenta and types 5a and 5b can be separated by immunoassay (89). Type 5b is specific to osteoclasts and macrophages and differs from 5a by having a higher optimal pH (6 as compared to 5) and not containing sialic acid (90).

TRACP5b is synthesized as an inactive proenzyme and then cleaved in the osteoclast (88). Its major activity is as a protein tyrosine phosphatase (88). The gene for TRAP (*Acp5*) is located on chromosome 19 in man (88). TRAP is secreted as a

35 kDa protein of 323 to 325 amino acids (89). Inactivation is associated with loss of the iron component; in the circulation about 90% of TRACP5b circulates as fragments and is removed mostly by the liver (91).

In vitro, the addition of anti-TRAP antibodies to osteoclasts cultured on cortical bone slices inhibits bone resorption and the addition of bisphosphonate to such cultures reduces TRAP activity and bone resorption. TRAP knockout mice have mild osteopetrosis (92).

TRAP is a phosphoprotein phosphatase; it degrades osteopontin, a protein that binds to integrins on the osteoclast surface and promotes adhesion. By preventing adhesion, TRAP may promote osteoclast migration. TRAP also degrades osteonectin. TRAP may also catalyze the generation of reactive oxygen species (89).

TRAP is believed to reflect osteoclast cell number. Such a conclusion is based on human and cell culture models. In cell-rich osteopetrosis due to mutations in the chloride channel 7 (Albers-Schoenberg disease), the TRACP5b activity is high as the cell number is high (91). In cell culture (RAW 264.7 cells) cultured with RANKL, the TRACP5b activity was strongly correlated with osteoclast number and volume (93).

Bone biopsy

Bone biopsy is recommended clinically in several clinical settings such as in early onset osteoporosis, renal osteodystrophy (ROD) before commencing an antiresorptive agent, unexplained bone pain in CKD patients, unexplained hypercalcemia, unexplained pathological fracture, suspicion of abnormal mineralization and bone malignancy.

Bone histomorphometry. Bone biopsy is usually taken from the iliac crest and can be assessed quantitatively and reported using histomorphometry standardized nomenclature, symbols and units as published by the American Society of Bone Mineral Research (ASBMR) Histomorphometry Nomenclature Committee (94, 95).

Quantitative bone histomorphometry reporting consists of dynamic and static parameters. Dynamic parameters are reported per unit time and therefore, require double tetracycline labeling with known time interval, eg, mineral apposition rate (MAR). The bone formation rate/bone surface (BFR/BS) is the widely accepted dynamic histomorphometry parameter for reporting bone turnover, although bone formation rate has also been reported using its other possible referent for mineralizing surface (95). Double bands of tetracycline fluorescence circumscribing the amount of new bone formed during the labeling interval (Fig. 8) are used to measure mineral apposition rate (MAR) and mineralizing surface (MS/BS) which are both used to calculate BFR/BS. MS/BS is the extent of tetracycline fluorescent label present on the sites of active bone formation, and MAR is the distance between double fluorescent labels divided by the number of days between administrations of the 2 labels.

There is no unifying consensus on normal reference range for BFR/BS (97). Reference range studies of BFR/BS have been small, for example, 48 men (98) or 10 children (99). These reported ranges of 18 to 38 and 2 to 30 $\mu m^3/\mu m^2$/year in adults (97, 100), and 97 to 613 $\mu m^3/mm^2$/day in children (99), with no consistent unit measurement for BFR/BS. The ASBMR currently recommended BFR/BS unit measurement is $\mu m^3/\mu m^2$/year (95).

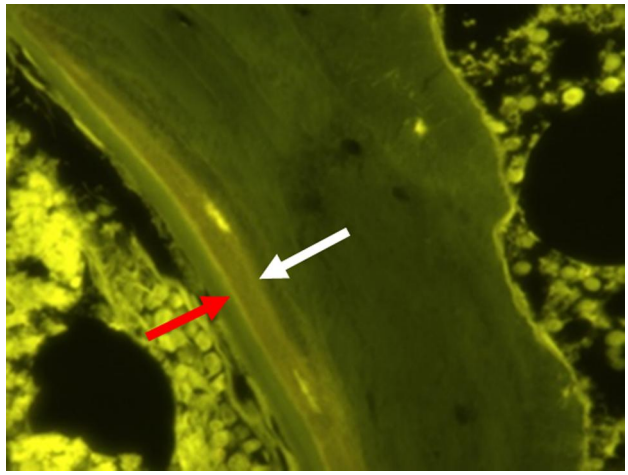


Figure 8. Double label tetracycline circumscribing newly formed bone. The width between the first band (white arrow) and the second band (red arrow) is used to calculate the mineral apposition rate (MAR). Image courtesy of Salam S, University of Sheffield 2022.

There is inherent limitation to using BFR/BS as a measure of bone turnover, as it purely reflects bone formation rate. In healthy bone physiology, BFR/BS is assumed to reflect the overall bone turnover rate due to the coupling of bone resorption and formation. In several bone diseases where the coupling mechanism is disrupted, this assumption no longer applies. However, dynamic assessment of bone resorption is not possible. Thus, static parameters of bone resorption such as osteoclast number, osteoclast surface, and erosion surface need to be included in histomorphometry reporting (94, 95).

There are some limitations to transiliac bone biopsy, which is mostly performed unilaterally. Bone sample from this site may not be representative of the whole skeleton (101, 102). Transiliac bone biopsy also has poor reproducibility given the nature of bone biopsy technique, which needs to avoid immediate repeat sampling near the previous biopsy site. If repeat sampling is performed years later, the exact position and angle of the previous biopsy is impossible to ascertain even by the same operator. Furthermore, pelvic bone fracture is not as common as other fracture sites such as the hip, ankle, wrist, and lumbar spine (103). However, bone biopsy of common fracture sites, such as the lumbar spine, is not possible and has high risk of complications. It is important that interpretation of bone biopsy results takes these limitations into consideration, especially when deciding treatment options. In contrast, BTMs are released by the whole skeleton and may be more representative of the overall bone turnover state.

Relationship between bone turnover on histomorphometry and BTMs. BFR has been related to BTMs in women (20) and in men (104) and both noted a modest to good correlation with OC, less with bone ALP. In osteoporosis, BFR/BS was modestly correlated with PINP and CTX in osteoporosis (see above). BFR showed good correlation with BTM in CKD (105, 106). Overall, the positive relationships between BTMs and bone turnover on histomorphometry are significant but modest.

Isotope methods for estimating bone turnover

Calcium (and strontium) radiotracer kinetics. The accretion and resorption rates of bone can be estimated following the intravenous administration of a radioisotope of calcium (such

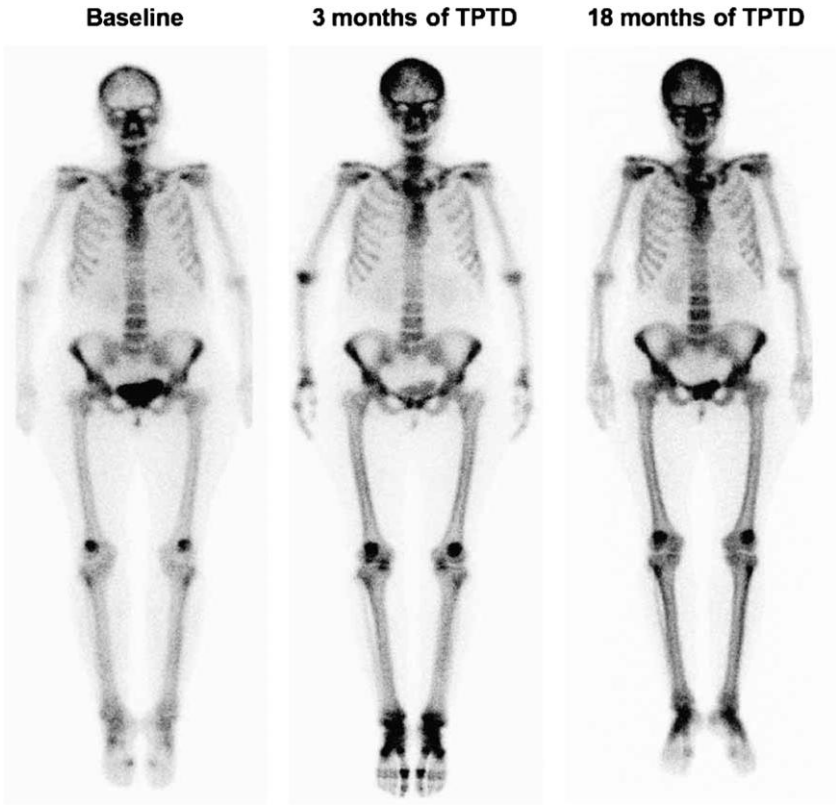


Figure 9. MDP-bisphosphonate labeled with technetium-99 at baseline and after 3 and 18 months of treatment with teriparatide for osteoporosis. Abbreviation, MDP, methylene diphosphonate; TPTD, teriparatide. Reprinted from Moore et al. J Bone Miner Res, John Wiley and Sons, Inc. © 2010 American Society for Bone and Mineral Research (96).

as Ca-47) and strontium (such as Sr-85). The calcium kinetics approach to estimating accretion rate correlated well (r = 0.71-0.83) with serum osteocalcin but not so well with bone ALP (r = 0.55-0.58) in healthy women (20) and people with metabolic bone diseases (107). The strontium kinetics approach to estimating resorption rate (and change in resorption rate with treatment) correlated well with deoxypyridinoline (r = 0.71) but not with hydroxyproline (r = 0.15) in postmenopausal osteoporosis (83).

Radiolabeled bisphosphonate and fluorine 18. Bisphosphonates may be labeled with technetium 99 m and the gamma rays produced may be measured by a whole-body counter 24 hours after intravenous administration (Fig. 9). This tracer retention is believed to reflect bone blood flow and mineralization. Such measurements show a nadir around age 35 years in men and women and a subsequent increase with age, results subsequently reported for BTMs (as discussed later) (108).

Bisphosphonate clearance was compared to fluorine 18 (^{18}F) clearance, and they were found to correlate well (r = 0.76) and to be lower in women taking hormone replacement therapy (109). ^{18}F can be measured by positron emission tomography (PET) which allows the study of regional bone turnover (and bone blood flow) and this can be combined with computed tomography (CT) (110). Fluoride activity in 26 dialysis patients correlated with the bone formation rate assessed by bone biopsy (111).

Stable isotopes of calcium. The major isotope of calcium is calcium 40, but there are several other stable calcium isotopes that are abundant in nature including ^{42}Ca (0.7% of all calcium) and ^{44}Ca (2.1% of all calcium). Such isotopes of calcium have a higher atomic weight than ^{40}Ca and so are slightly slower at crossing the cell membrane, a process referred to as isotope fractionation (112). As a result, the ratio of ^{44}Ca to ^{42}Ca is lower in osteoporosis (a state of lower net bone formation). In children and young adults, there is a positive correlation between the ratio of ^{44}Ca to ^{42}Ca in the serum with bone ALP, but it is weak (113).

Methods for Measuring BTMs

BTMs can be detected in the circulation and quantified using different methods. The most commonly used methods are ELISAs and automated immunoassays.

It is critical to validate an assay before it is used widely. For each analyte, the following needs to be determined: 1) the precision, as represented by the inter- (between runs) and intra-assay (within a run) coefficient of variation (CV); 2) the limits of detection of a blank sample, or the limit of detection (the lowest measurable concentration) or the limit of quantification (the lowest concentration with adequate precision); 3) the linearity (based on serial dilutions); 4) the cross-reactivity with similar analytes; 5) the effect of freeze-thaw cycles; and 6) the effects of short- and long-term storage. Two methods for the same analyte should be compared using method comparison analyses such as Bland and Altman and Passing and Bablock or Deeming regression. Inappropriately used correlation analysis were used by earlier studies (2 methods measuring the same thing are bound to show a significant association).

Table 1. Technical validation of the bone ALP assays

Assay name/manufacturer	Assay method	Limit of detection/ recovery (%)	Measuring range	Precision	References
Beckman Coulter Ostase	Manual IRMA, mAbs, mass (units µg/L)	0.2 µg/L/88-113%	0.2-120 µg/L	Inter CV: 2.6-8.2% Intra CV: 3.6-12%	(121, 125, 126)
Quidel MicroVue	Manual ELISA, mAb, activity (units U/L)	1.0 U/L/-	0.0-140 U/L	Inter CV: 6.2-7.9% Intra CV: 4.0-8.3%	(120, 121)
Access OSTASE, Beckman Coulter, Inc)	Automated CLIA, mAb, activity (units µg/L)	0.1 µg/L/-	0.1-120 µg/L	_	(121)
IDS-iSYS Ostase	Automated spectrophotometry, mAb, activity (units µg/L)	0.1 µg/L	1-75 µg/L	_	(123)
DiaSorin Liaison Ostase	Automated chemiluminescence, mAb, mass (units µg/L)	0.74 µg/L/98.9% ± 4.2%	_	Inter CV: 4.9-5.8% Intra CV: 2.6-4.8%	(124)

Abbreviations: CLIA, chemiluminescent immunoassay; CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay; IDS-iSYS, Immunodiagnostic Systems; IRMA, immunoradiometric; mAb, monoclonal antibody.

Table 2. Technical validation of the OC assays

Assay name/ manufacturer	Assay method	Limit of detection/ recovery (%)	Measuring range	Precision	References
N-MID OC	Manual ELISA, mAb	2.0 µg/L/97 ± 8%	0.0-75.0 µg/L	Inter CV: 3.9-6.7% Intra CV: 3.7-4.5%	(130)
N-MID OC Roche Diagnostics	Automated, ECLIA, mAbs	500 ng/L/81-124%	500-300 000 ng/L	Inter CV: 2.6-8.2% Intra CV: 1.0–6.9%	(131)
N-MID OC iSYS-IDS	Automated, CLIA, mAb	2000 ng/L/-	2000–200 000 ng/L	_	https://www.idsplc.com/products/n-mid-osteocalcin/
Undercarboxylated OC	Manual ELISA, mAbs	200 ng/L/77-103%	_	Inter CV: 11.4% Intra CV: 9.0%	(133)

Abbreviations: CLIA, chemiluminescent immunoassay; CV, coefficient of variation; ECLIA, electrochemiluminescent immunoassay; ELISA, enzyme-linked immunosorbent assay; IDS-iSYS, Immunodiagnostic Systems; IRMA, immunoradiometric; mAb, monoclonal antibody; N-MID, N-terminal mid-fragment; OC, osteocalcin.

Bone ALP

There are several methods for measuring bone ALP mass and activity, such as heat inactivation, electrophoresis, wheat germ lectin precipitation, HPLC, and immunoassays (114-118). The immunoassays are more suitable for use in a clinical laboratory because they use monoclonal antibodies specific to bone and are rapid, easier to use, and reproducible.

Table 1 shows 5 assays currently available for measuring bone ALP using immunoassay methods. Two of the assays are manual assays (119-121) and 3 are automated (121-124). Two of them (Beckman Coulter and Diasorin Liaison) measure the mass of the enzyme and 3 measure enzyme activity. However, of the 3 assays that measure enzyme activity, 2 of them (Access and iSYS) report the mass of the enzyme by cross-calibration with the mass assays. Only 1 assay (Quidel MicroVue renamed from Alkphase B) reports enzyme activity as such. The Diasorin Liaison and Beckman Access were compared by Bland and Altman plots and gave equivalent results (124). However, the Quidel MicroVue was compared with both methods and was found by Deeming regression to give regression lines whose slopes were not equal to 1 (121).

Bone ALP remains stable following long-term storage at −20 °C and up to 3 freeze-thaw cycles (127).

Osteocalcin

Serum OC. OC assays have been available commercially in many formats: RIA, immunoradiometric assay (IRMA), and ELISA, using both polyclonal and monoclonal antibodies and different standards. They are used to detect the intact, mid-molecule and undercarboxylated OC in serum, plasma, and urine (128). However, circulating OC is not a single amino acid peptide but rather several fragments (129). Rehder et al in 2015 identified more than 12 forms of OC in circulation, (57). Therefore, the correct terminology for the assays that measure intact and N-terminal MID fragment (N-MID) OC is *total OC*.

Assays for OC are mainly based on Total OC or N-MID OC and they may be manual ELISA (130) or automated immunoassay analyzers (131) (Table 2). These assays have superseded the earlier methods (129, 132).

The stability of OC has been assessed (134). There was little or no loss of reactivity in serum stored immediately after collection at −70 °C, over the long term. Short-term storage (1 month) at −20 °C is acceptable (135). OC is affected by repeated freeze-thaw cycles. OC measured in hemolyzed samples produced lower values than nonhemolyzed. Up to 90% of the immunoreactivity of a sample is removed by hemolysis, possibly caused by an enzymatic alteration in OC or by interference by hemoglobin binding (134).

Table 3. Technical validation of the PINP assays

Assay name/manufacturer	Assay method	Limit of detection/ recovery (%)	Measuring range	Precision	References
Intact PINP Orion Diagnostica UniQ™	Manual RIA, polyclonal Ab	2.3 µg/L/95.5-100.3%	5-250 µg/L	Inter CV: 6.0-9.8% Intra CV: 9.8-10.2%	(33, 141)
Intact PINP iSYS-IDS	Automated sandwich CLIA, mAb	1.0 µg/L/93%	2-230 µg/L	Inter CV: 4.2-5.3% Intra CV: 3%	(136)
Total PINP Roche Diagnostics	Automated ECLIA, mAb, 2-step sandwich ELISA	5.0 µg/L/94-103%	5-1200 µg/L	Inter CV: ≤ 1.7% Intra CV: 4.4%	(137)
Total PINP Uscn Life Science Inc.	Manual sandwich ELISA	0.04 µg/L/93-105%	-	Inter CV: 4.6-5.3% Intra CV: 2.9-4.9%	(138)

Abbreviations: CLIA, chemiluminescent immunoassay; CV, coefficient of variation; ECLIA, electrochemiluminescent immunoassay; ELISA, enzyme-linked immunosorbent assay; IDS-iSYS, Immunodiagnostic Systems; mAb, monoclonal antibody; PINP, procollagen type I N-propeptide; RIA, radioimmunoassay.

Undercarboxylated OC. Undercarboxylated OC can also be measured indirectly using the hydroxyapatite (HAP) binding assay. Estimated cross-reactivity with carboxylated OC was 5% (Table 2) (133).

PINP

There are 4 commercially available assays for measuring circulating PINP (Table 3). The Uniq PINP RIA (Orion Diagnostic, Oulunsalo, Finland) and the automated iSYS-IDS (Immunodiagnostics System) measure intact PINP (33, 136). The Uscn ELISA (Uscn Life Science Inc., China) and automated electrochemiluminescent Immunoassay (ECLIA; Roche Diagnostics) measure total PINP (137, 138). PINP was first isolated from amniotic fluid and amino acid sequencing identified the high-molecular weight form as a homodimer of the α1 chains of PINP. The assays use different antibodies against the α1 chain of PINP (139, 140).

The IOF and IFCC Bone Marker Standard Working Groups have recognized the need for standardization and harmonization of these commercially available PINP assays, so that the results obtained by different systems/methods can be comparable. Method comparison studies have been conducted (67, 136, 137, 142, 143).

The Roche and IDS assays show higher results than the Orion assay (67, 136, 137). The Roche and IDS assays given similar values (Fig. 10) (67, 142, 144).

The effects of storage on PINP levels have been investigated (137, 143). No significant difference was observed between PINP levels in serum and plasma stored at −20 °C immediately for up to 133 days. In samples stored at −20 °C for 2.5 years, there was a significant increase in PINP of 41.3% (34.8%, $P < 0.0001$) and there was no effect on levels after 5 freeze/thaw cycles (137, 143).

CTX

The available assays for CTX are shown in Table 4.

Urine CTX assays. It is possible to detect the alterations of type I collagen isomerization by measuring the native α and isomerized β CTX fragments in urine using ELISAs (78, 145). The α CTX ELISA uses a monoclonal antibody (mAb) raised against the EKAHDGGR peptide, a sequence specific for a part of the C-telopeptide of the a1 chain of human type I collagen (150). This assay specifically recognizes αCTX with < 2% cross-reactivity with βCTX (151). The βCTX ELISA uses a polyclonal antibody raised against the isomerized βEKAHβDGGR sequence (146, 150, 152). This assay specifically recognizes isomerized βCTX with < 1% cross- reactivity with αCTX (76).The results from all urine CTX assays require correction for creatinine and expressed as a ratio to creatinine.

Serum and plasma CTX assays. The Serum CrossLaps One Step ELISA was developed (147), which uses 2 highly specific mAbs raised against the amino acid sequence AHDβGGR. The assay measured the molecules consisting of 2 chains of EKAHDβGGR (βCTX) that are cross-linked covalently at the lysine residues. Therefore, only the degradation fragments that are derived from matured bone tissue were measured.

The stability of the antigens measured in this assay was also assessed. The serum was stored within 2 hours of blood collection at 4 °C and 20 °C for different time periods and then stored at −20 °C. After 7 days the mean (SD) recovery was 93% (±11%) at 4 °C and 60% (±17) at 20 °C, indicating that the antigens are more stable and for longer in the fridge compared with at room temperature (147).

The first automated analyzers that measured CTX were the Elecsys 2010 and E170 immunoassays (Roche Diagnostics, Penzberg, Germany). This automated CTX assay and the Serum CrossLaps One Step ELISA were shown to significantly correlate in 728 healthy women, $r = 0.82$, $P < 0.0001$ (37).

The stability of CTX stored at room temperature, 4 °C and after freeze/thaw cycles was investigated (Fig. 11) (148). CTX measured in serum and plasma and stored at 4 °C was stable for up to 24 hours but decreased by 14% in serum when stored at room temperature. CTX measured in serum and stored at −30 °C was not affected by 12 weeks of storage or repeated freeze/thaw of up to 9 cycles.

The CTX assay is also available on the IDS-iSYS automated immunoassay analyzer (Immunodiagnostic Systems plc, Boldon, UK). It uses the same monoclonal antibodies and chemiluminescent methodology as the Roche analyzer.

Comparison of the CTX assays. The IOF and IFCC Bone Marker Standard Working Groups have recognized the need for standardization and harmonization of the commercially available CTX assays, so that the results obtained by different systems/methods can be comparable (153). Method comparison studies have been conducted (37, 142, 149, 154). Chubb et al measured CTX in 161 fasting plasma samples from males and females using the Serum CrossLaps ELISA, the Roche CrossLaps, and the IDS-iSYS CTX-I (CrossLaps) (149). Method comparison analyses using Passing and Bablok and

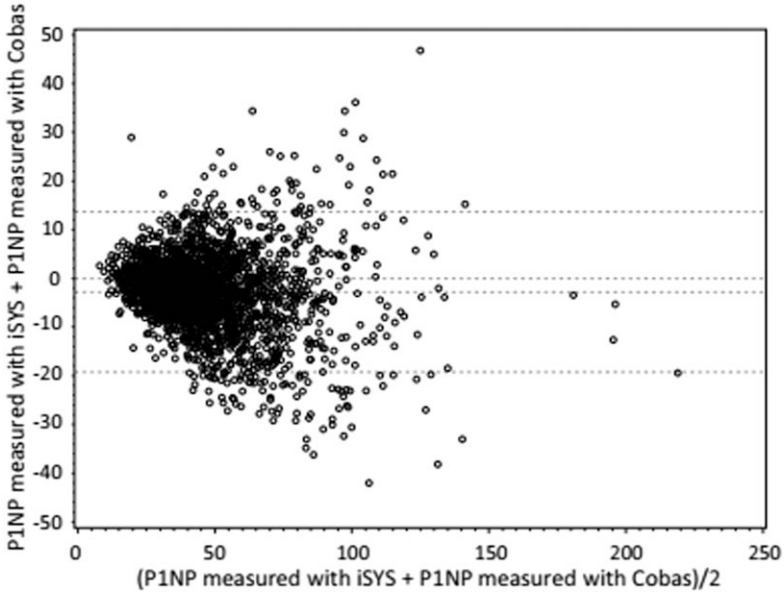


Figure 10. Bland-Altman plots for PINP measured using the iSYS-IDS and the Roche Cobas e411. Reprinted by permission from Springer Nature: Oxford Academic, Osteoporosis International, Comparison of two automated assays of BTM (CTX and P1NP) and reference intervals in a Danish population, Jørgensen NR, Møllehave LT, Hansen YBL, Quardon N, Lylloff L, Linneberg A, ©2017 (142). Abbreviation: P1NP, procollagen type I N-propeptide.

Table 4. Technical validation of the CTX assays

Assay name/manufacturer	Assay method	Limit of detection/recovery (%)	Measuring range	Precision	References
Urine α CTX	Manual ELISA, mAb	0.20 ng/mL/112%		Inter CV: 5.2% Intra CV: 2.7%	(145)
Urine βCTX CrossLaps	Manual ELISA, Polyclonal Ab	0.2 ug/mL/92-115%		Inter CV: < 13% Intra CV: < 10%	(146)
Serum CrossLaps™ One Step ELISA	Manual ELISA, mAb	80 ± 2SD pmol/L/101 ± 4%	500-16 000 pmol/L	Inter CV: 5.4-7.9% Intra CV: 4.7-4.9%	(147)
CTX Roche Diagnostics	Automated, ECLIA, mAbs	10 ng/L/91-101%	10-6000 ng/L	Inter CV: 5.7% Intra CV: 1.2-4.1%	(37, 148)
CTX IDS-iSYS	Automated, CLIA, mAbs	33 ng/L/91-101%	33-6000 ng/mL	Inter CV: 3.4% Intra CV: 1.2-4.1	(149)

Abbreviations: Ab, antibody; CTX, C-telopeptide of type I collagen; CV, coefficient of variation; ECLIA, electrochemiluminescent immunoassay; ELISA, enzyme-linked immunosorbent assay; IDS-iSYS, Immunodiagnostic Systems; mAb, monoclonal antibody.

Bland-Altman graphs showed that these CTX assays gave different results, with proportional and constant bias across the different measuring ranges (Fig. 12). In addition, the iSYS assay gave some results that were below its detection limit of 33 ng/L in some of the samples that were quantifiable by the ELISA and the Roche CTX assay (149).

Cavalier et al conducted a multicenter study to evaluate the harmonization of the CTX assays (154). The IDS-iSYS gave lower CTX values than the Roche and the agreement was better in plasma than in serum. Thus, care needs to be taken moving from one assay to another and further work is necessary.

CTX can be measured in either serum or EDTA and lithium heparin plasma, but it is more stable in EDTA plasma after long term storage at > 4 °C (37, 123, 155-158).

NTX

Urinary NTX. Urinary NTX can be quantified using the Osteomark ELISA (Ostex International, Inc., Seattle, WA, USA) (35, 159). Generally, a peptide fraction from urine was selected using molecular sieve chromatography that was enriched in the N-telopeptide-to-helix intermolecular crosslinking domain of type I collagen. A mAb was generated that specifically bound to an epitope embedded in the α2-chain of the N-telopeptide fragment. This peptide has the sequence QYDGKGVG, where K (lysine) is involved in a trivalent crosslinking site (35).

The concentration of uNTX is expressed as nanomolar bone collagen equivalents (nM BCE). Values are corrected for dilution by urinary creatinine analysis and results are expressed in nM BCE per millimolar creatinine (nM BCE/mM creatinine) (81, 159, 160).

Urinary NTX can also be measured using the Vitros ECi automated immunoassay, using the same mAb (Ortho Clinical Diagnostics) (161).

The effects of storage at −20 °C on uNTX and creatinine have been investigated (162). Levels of uNTX and creatinine were significantly decreased by 18% and 22% after 4 months,

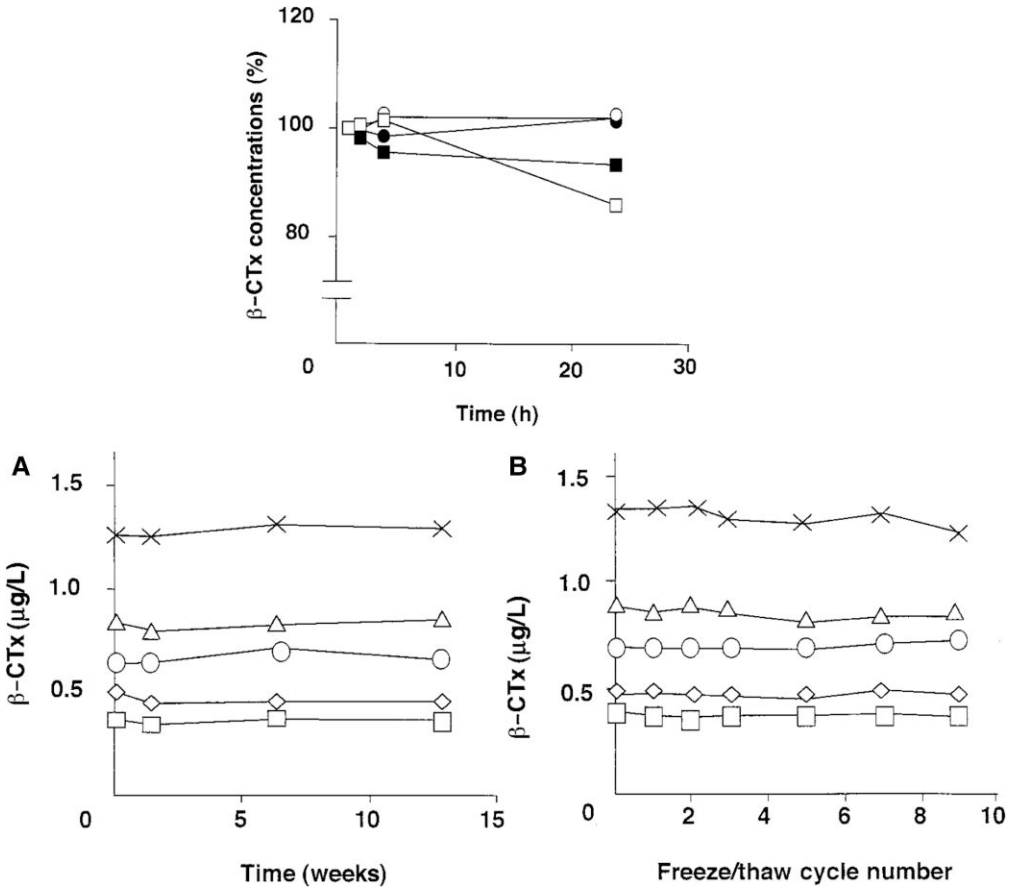


Figure 11. (Top) the stability of βCTX in serum at 4 °C (filled square) and at room temperature (open square) and plasma at 4 °C (black square) and at room temperature (white square). A) The stability of βCTX in serum samples stored at −30 °C and B) after cycles of freezing and thawing in 5 subjects. Okabe, Reiko; Nakatsuka, Kiyoshi, Clinical Evaluation of the Elecsys β-CrossLaps Serum Assay, a New Assay for Degradation Products of Type I Collagen C–Telopeptides, Clinical Chemistry, 2001, 47 (8), 1410-4, by permission of Oxford University Press (148). Abbreviations: CTx (C-telopeptide of type I collagen), µg/L (microgram per liter).

respectively. Levels of uNTX did not significantly change after 5 freeze/thaw cycles (160).

Table 5 shows the validation data of the uNTX assays.

TRACP5b

Levels of TRACP can be determined using kinetic assays developed for the specific measurement of band 5b in serum (163). However, kinetic TRACP assays are sensitive to hemolysis and may lack specificity because they do not distinguish between the 5a and 5b isoforms (164). Therefore, several immunoassays have been developed to quantify the isoforms and have specificity to TRACP5b and are insensitive to hemolysis. In these assays, the pH is 6.1, where the activities of TRACP5b are close to optimal and TRACP5a is minimal (36, 165).

The assays that are available for TRACP5b are shown in Table 6. Three of these are manual assays (Finland, IDS, Nittobo) that are claimed to measure the 5b isoenzyme (36, 166-168), and 1 is an automated assay (IDS-iSYS) (168). The assay from Nittobo has an antibody against inactive TRACP5b to try and make it more bone-specific.

The Nittobo and the IDS-iSYS TRACP5b assays were compared using samples from different clinical populations. Method comparison results showed that the harmonization of the results obtained is possible by using a common commutable calibrator (168).

Stability of TRACP5b. The stability of TRACP5b has been studied in detail and it has been shown that it is stable for routine laboratory measurements if the samples are stored appropriately (36). It is stable for 2 days at room temperature (25 °C) and 3 days at 4 °C. For long-term storage, serum collected should be stored immediately at −70 °C (or below), conditions which allow TRACP5b to be stable for several years as long as there are no episodes of thawing (169) (Fig. 13) (36). Freeze/thawing of the sample is not recommended because the activity of TRACP5b may decrease as a result (169). However, others have stated that repeated freeze/thawing had no effect on TRACP5b activity (36, 166).

Reference Intervals

The clinical interpretation of the BTMs should be based on a comparison with a BTM reference interval, measured with the same assay and in the same population (170). For females, these reference intervals are usually based on BTMs in young healthy premenopausal women who are assumed to have the lowest bone turnover and the lowest rates of bone loss (170). These levels are thought to reflect "optimal bone health"; therefore, when treating older women with osteoporosis this range is the target. For males, the reference intervals are based on samples from healthy males from

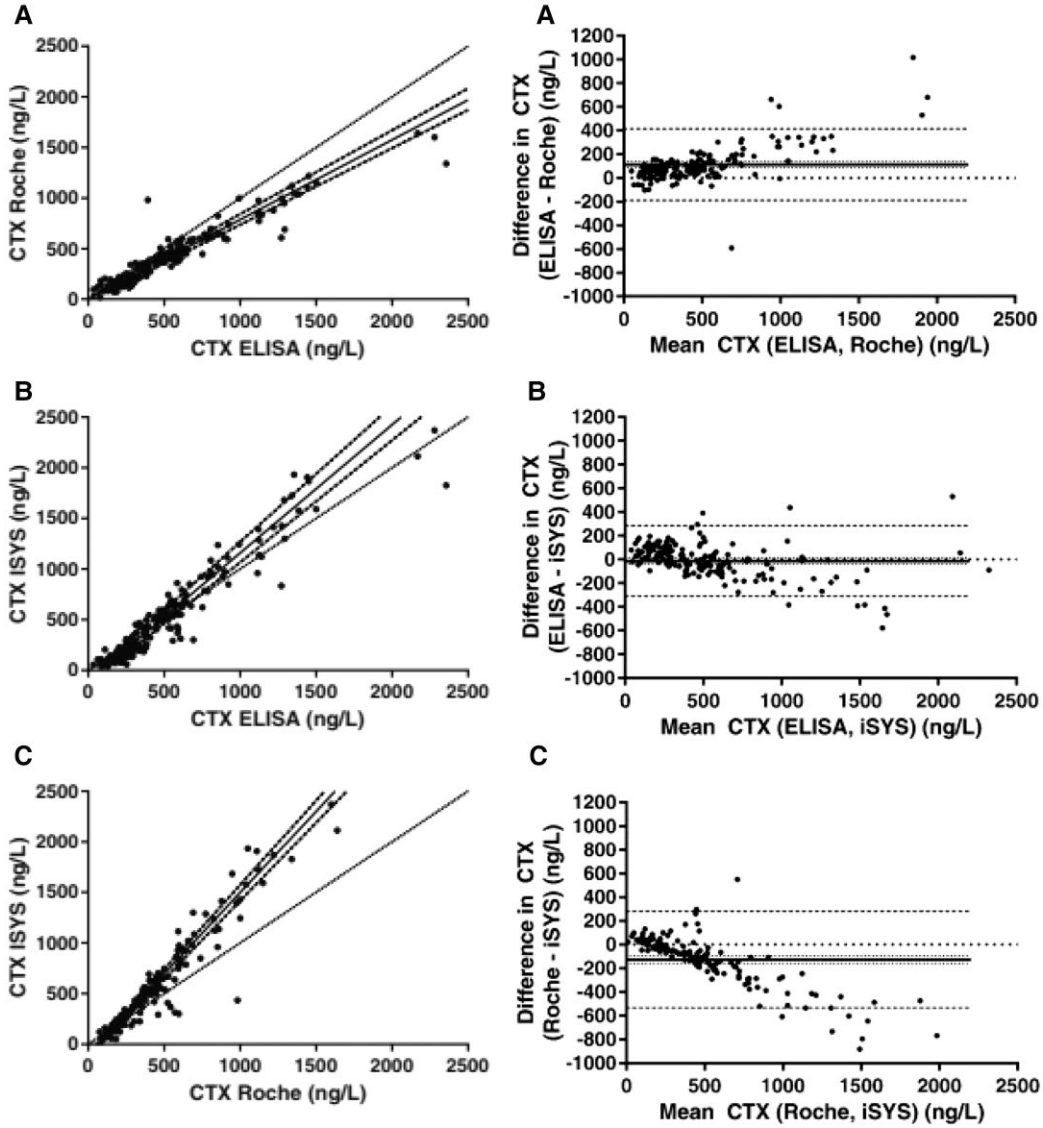


Figure 12. Passing and Bablok and Bland-Altman plots for CTX measured using the iSYS-IDS and the Roche Cobas e411. Reprinted from Clin Biochem, 48(7-8), Chubb S A, Mandelt C D, Vasikaran S D, Comparison of results from commercial assays for plasma CTX: The need for harmonization, 519-524, ©2015, with permission from Elsevier (149). Abbreviations: CTX, C-telopeptide of type I collagen; IDS-iSYS, Immunodiagnostic Systems.

Table 5. Technical validation of the uNTX assays

Assay name/manufacturer	Assay method	Limit of detection/ recovery (%)	Measuring range	Precision	References
Osteomark ELISA NTX (Ostex International, Inc)	Urine, manual ELISA, mAb	20 nmol BCE/L/100% ±12%.	_	Inter CV: ≤10.0% Intra CV: 4.3-26.6%	(81, 159, 160)
Vitros ECi NTX (Ortho Clinical Diagnostics)	Urine, automated, mAb	_	0-3000 nmol BCE/L	Inter CV: 3.8-6.1% Intra CV: 1.1-6.7%	(161)

Abbreviations: BCE, bone collagen equivalent; CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay; mAb, monoclonal antibody; TRACP5b, tartrate-resistant acid phosphatase type 5b; uNTX, N-telopeptide of type I collagen.

between 40 to 60 years (171).There are regional differences that might be associated with sources of variability, such as ethnicity (172), smoking, exercise, menstrual cycle, vitamin D status (170). Tables 7 and 8 show reference ranges for CTX and PINP for females and Tables 9 and 10 for males. Studies performed in different populations, using different assays, showed similar reference values for PINP. In contrast, the results for CTX were less consistent, highlighting the need for specific reference ranges.

Sometimes, it is clinically useful to compare the BTM results from an older woman with women of the same age. For example, the clinical question may be "is the bone turnover

Table 6. Technical validation of the TRACP5b assays

Assay name/manufacturer	Assay method	Limit of detection/recovery (%)	Measuring range	Precision	References
Bone TRAP Finland	Manual ELISA, mAb, pH 6.1	0.06 U/L/96.6 ± 2.7%	-	Inter CV: 6.9% Intra CV: 3.2%	(36, 169)
BoneTRAP® IDS	Manual ELISA, mAb, pH 6.1	1.3 U/L	1.0-10.0 U/L	Inter CV: 6.9% Intra CV: 3.2%	(168)
BoneTRAP® iSYS-IDS	Automated, mAb, pH 6.1	≤0.6 U/L	0.9-14.0 U/L	Inter CV: 5.0-13.6%	(168)
TRACP5b Nittobo Medical Co., Ltd	Manual ELISA, mAb, pH 6.4-6.6	0.02 U/L/91.5%	0.1-15.0 U/L	Inter CV: 4.3-8.3% Intra CV: 3.4-5.0%	(168)

Abbreviations: CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay; IDS-iSYS, Immunodiagnostic Systems; mAb, monoclonal antibody; TRACP5b, tartrate-resistant acid phosphatase type 5b; TRAP, tartrate-resistant acid phosphatase.

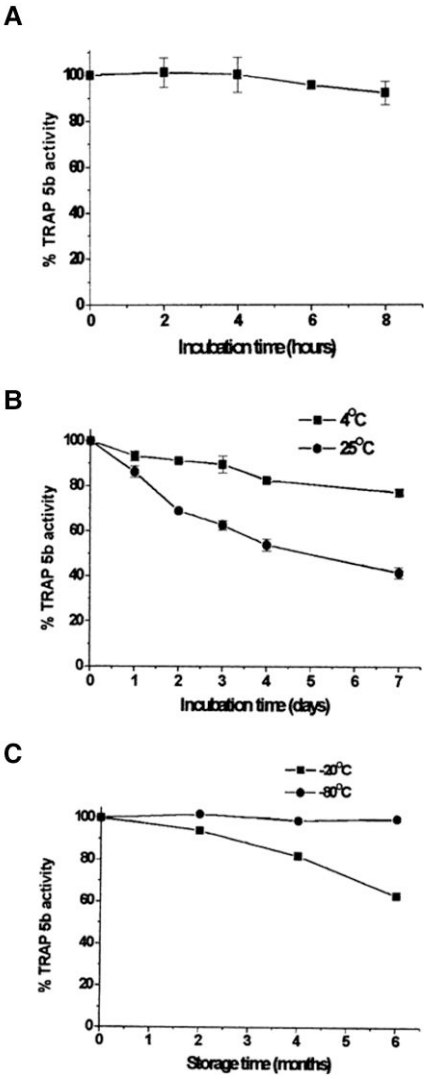


Figure 13. The stability of TRACP5b. A) 8 hours of incubation at 25 °C, B) 7 days incubation at 4 °C and 25 °C and C) 6 months incubation at −20 °C and −80 °C. Reprinted by permission from John Wiley and Sons, Journal of Bone and Mineral Research, Bone Turnover Markers – basic biology to clinical application, Halleen J M,. Alatalo S L,, Suominen H, Cheng S, Janckila J A, H. Väänänen H K, ©2000 (36). Abbreviation: TRAP 5b, tartrate-resistant acid phosphatase 5b.

high for her age?" Reference ranges for postmenopausal women are reported in Table 11.

Variability

Age

BTMs reflect both bone formation and resorption. The magnitude of these processes varies during the lifespan, making age an important source of variability.

Children and adolescents. Most of the BTMs peak at Tanner stage II (182, 183) or III (25). Although all BTMs increase during puberty, the amount of increase varies between several BTMs, ranging from 2 times higher than the mean level for adults for TRACP5b, to 10 times higher for bone ALP in mid-puberty girls (184). This mid-puberty peak is followed by a decrease to adult levels in late puberty. The decrease is later in boys than in girls, reflecting the later male pubertal spurt (182, 183). Although BTMs reflect skeletal growth, they cannot predict bone mineral density (BMD) or bone mineral content in growing children and adolescents (183, 185). Recently, reference intervals of CTX, PINP, OC and bone ALP have been proposed for children and adolescent from 8 to 18 years stratified by age (2-years age spans) and Tanner stage, based in a study of 762 participants (25). All these sources of variability should be considered while interpreting BTM results in the developing skeleton.

Adulthood and Aging. Upon the completion of linear growth, BTMs decrease (186) (Fig. 14). Most of the bone mass is attained by the middle of the third decade and the small increases observed thereafter are associated with remaining modeling (periosteal apposition). BTMs reflect the peak of bone mass achievement and cortical consolidation (187) and the nadir is only reached in the fourth decade both in men and women (186, 188). BTMs remain stable during adulthood, following normal sex steroid levels (189). The decrease in sex steroids leads to an increase in BTMs. A sharp increase is observed in women following the menopause; a two-fold increase has been reported to CTX and uNTX (188, 190, 191) and a 50% increase was reported to PINP (188). In men, data are less consistent. While some studies report stable levels of BTMs in

Table 7. Median, reference intervals, and intra-assay coefficient of variation for CTX on fasting blood samples

Region	Median (ng/L)	Reference interval (ng/L)	*n*	Age range	CV	Reference
Roche assay						
England	270[a]	100-620	153	35-45	1.2-4.1%	Glover et al (28)
France[b]	NR	105-589	157	35-45	1.7-4.1%	Claudon et al (173)
Italy	250	70-610	82	45-50	NR	Adami et al (174)
Spain	255	137-484	164	35-45	NR	Guanabens et al (175)
Denmark	308	150-635	111	30-39	NR	Jorgensen et al (142)
France/Denmark	297	111-791	188	35-39	1.2-4.9%	Eastell et al (176)
France/Belgium/UK/USA	317	114-628	637	30-39	1.6-3.0%	Glover et al (170)
USA	280	94-659	237	28-45	4.1-5.3%	De Papp et al (177)
Australia	264	100-700	215	30-39	NR	Jenkins et al (178)
	246	100-600	209	40-49	NR	Jenkins et al (178)
Korea	261	108-607	70	30-39	<5%	Cho et al (179)
China	210	112-497	406	35-45	NR	Hu et al (180)
Saudi Arabia	217	163-274	765	35-45	NR	Ardawi et al (181)
IDS						
Spain	230	109-544	164	35-45		Guanabens et al (175)
Denmark	273	83-895	110	30-39	NR	Jorgensen et al (142)

Abbreviations: CV, coefficient of variation; CTX, C-telopeptide of type I collagen; n, number of participants.
[a]Geometric mean.
[b]Multiplex assay.

Table 8. Median, reference intervals, and intra-assay coefficient of variation for PINP on fasting blood samples for females

Region	Mean/median (μg/L)	Reference interval (μg/L)	*n*	Age range	CV	Reference
Roche assay						
England	31.4[a]	16.2-60.9	153	35-45	1.0-2.1%	Glover et al (28)
France[b]	NR	17.9-60.4	157	35-45	6.0-6.8%	Claudon et al (173)
Italy	34.7 (trimmed mean)	14.6-63.5		45-50	NR	Adami et al (174)
Denmark	43[a]	19-99	111	30-39	NR	Jorgensen et al (142)
Spain	35.9	22.7-63.1	164	35-45	NR	Guanabens et al (175)
France/Denmark	38.0	17.3-83.4	188	35-39	1.2-4.9%	Eastell et al (176)
France, Belgium, USA, and UK	38.7	16.3-78.2	637	30-39	1.6-3.0%	Glover et al (170)
Australia	31	15-80.0	215	30-39	NR	Jenkins et al (178)
Saudi Arabia	32.5	22.3-42.9	765	35-45	NR	Ardawi et al (181)
China	32.9	13.72-58.67	406	35-45	NR	Hu et al (180)
IDS						
Spain	36.6	21.8-65.5	164	35-45	NR	Guanabens et al (175)
Denmark	41[a]	18-93	111	30-39	NR	Jorgensen et al (142)

Abbreviations: CV, coefficient of variation; PINP, procollagen type 1 N-propeptide; n, number of participants.
[a]Geometric mean.
[b]Multiplex assay.

elderly men (192, 193), mild increases in BTMs were reported in men, associated with a decrease in bioavailable estrogens (189).

Gender

During childhood, BTMs are similar in boys and girls (182, 183). During puberty, there is a sharp increase in BTMs (182, 183, 194). This peak of BTMs levels is around 2.5 years later in boys (183, 194) and is followed by a decrease to adult levels in both males and females (182, 183, 194). Adult males have higher BTMs than females, up to the menopause in women (187, 194, 195). The increase in bone turnover observed after the menopause leads to an increase in BTMs in women (188, 193), which is not observed in men (192-194). Thus, postmenopausal women have higher BTMs than same-age men, due to higher bone turnover.

Table 9. Median, reference intervals, and intra-assay coefficient of variation for CTX on fasting blood samples for males

Region	Mean/median (ng/L)	Reference interval (ng/L)	*n*	Age range	CV	Reference
Roche						
Denmark	382[a]	182–801	234	40-49	NR	Jorgensen et al (142)
Denmark	345[a]	161–737	248	50-59	NR	Jorgensen et al (142)
France[b]	234	144–400	33	40-59	1.7-4.1%	Claudon et al (173)
Australia	328	130–600	332	40-60	NR	Jenkins et al (178)
China	400[a]	100–612	226	35-45	NR	Hu et al (180)
	340[a]	252–428	45	45-49	NR	Hu et al (180)
IDS/iSYS						
Denmark	374[a]	125–1116	235	40-49	NR	Jorgensen et al (142)
Denmark	312[a]	93–1060	249	50-59	NR	Jorgensen et al (142)

Abbreviations: CV, coefficient of variation; CTX, C-telopeptide of type I collagen; n, number of participants.
[a]Geometric mean.
[b]Multiplex assay.

Table 10. Median, reference intervals, and intra-assay coefficient of variation for PINP in fasting blood samples for males

Region	Mean/median (μg/L)	Reference interval (μg/L)	*n*	Age range	CV	Reference
Roche						
France[b]	47.2	27.9-79.6	33	40-59	6.0-6.8%	Claudon et al (173)
China	44.0[a]	16.9-65.5	226	35-45	NR	Hu et al (180)
	36.62[a]	29.4-43.9	45	45-49	NR	Hu et al (180)
Denmark	49	24-98	234	40-49	NR	Jørgensen et al (142)
Denmark	41	20-84	248	50-59	NR	Jørgensen et al (142)
IDS						
Denmark	46	24-89	234	40-49	NR	Jørgensen et al (142)
Denmark	40	20-79	249	50-59	NR	Jørgensen et al (142)

Abbreviations: CV, coefficient of variation; PINP, procollagen type 1 N-propeptide; n, number of participants.
[a]Geometric mean.
[b]Multiplex assay.

Table 11. Median, reference intervals, and age ranges for PINP and CTX in fasting blood samples for postmenopausal women

Region	BTM	Mean/median	Reference interval	*n*	Age range	Reference
Roche						
France[b]	PINP (μg/L)	63	20.2-162.0	56	48-80	Claudon et al (173)
	CTX (ng/L)	559	154-1,140	56	48-80	Claudon et al (173)
Denmark	PINP (μg/L)	53[a]	23-125	579	NR	Jørgensen et al (142)
	CTX (ng/L)	424[a]	177-1015	578	NR	Jørgensen et al (142)
China	PINP (μg/L)	47[a]	34.78-59.36		60-64	Hu et al (180)
	CTX (ng/L)	441[a]	307-584		60-64	Hu et al (180)
IDS						
Denmark	PINP (μg/L)	50[a]	22-114	579	NR	Jørgensen et al (142)
	CTX (ng/L)	430[a]	125-1477	572	NR	Jørgensen et al (142)

Abbreviations: BTM, bone turnover marker; CTX, C-telopeptide of type I collagen; n, number of participants; PINP, procollagen type 1 N-propeptide.
[a]Geometric mean.
[b]Multiplex assay.

Menstrual cycle. Small variations (less than 20%) on BTMs during the menstrual cycle have been reported. Several studies did not report variation for OC (194, 196-198) or bone ALP (196-198). However, a 10% to 20% increase for bone ALP (199, 200), CTX (198, 200, 201), and uNTX (198, 202) were reported in the follicular phase. Therefore, some variation has been reported on BTMs in the menstrual cycle, but it does not seem to be clinically significant.

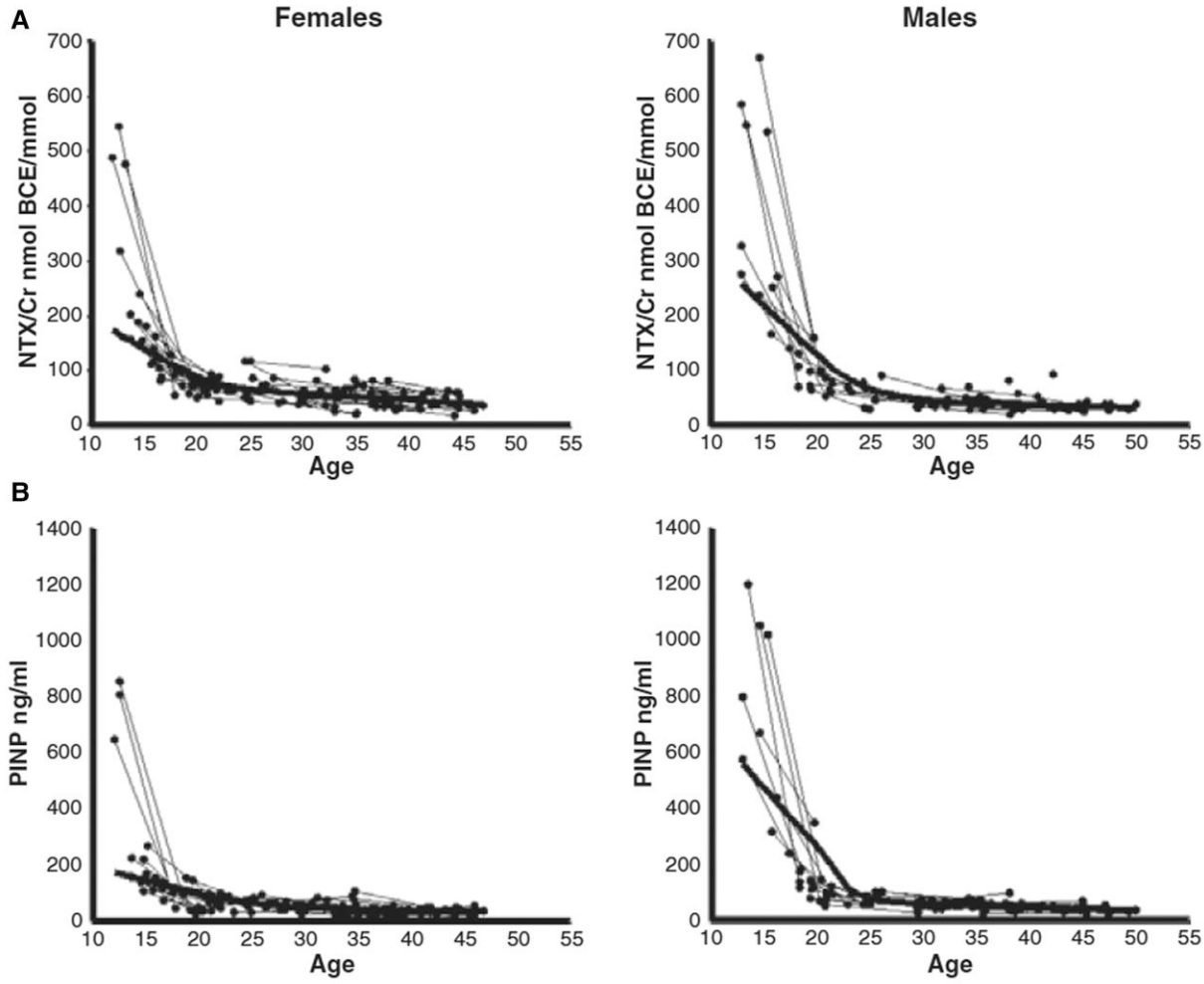


Figure 14. Bone turnover markers with age. Individual changes with Lowess fitted curves. Women (left) and men (right). uNTX/Cr (A) and aminoterminal propeptide of type I procollagen (B). Single points represent subjects with only one measurement. Reprinted with permission from Walsh et al in Clinical Endocrinology Wiley (186). Abbreviations: Cr creatinine; uNTX urinary N-telopeptide of type I collagen.

Pregnancy. BTMs vary with pregnancy and lactation. Bone resorption markers (CTX, uNTX) increase gradually with pregnancy (203). The increase is significant from 14 weeks of gestation, with a marked increase in the last trimester (204) (Fig. 15). In contrast, no significant change in bone ALP was observed before 36 weeks (203, 204).

Ethnicity

Several studies have compared BTMs between Black and White people and the results are inconsistent. Higher levels of OC have been reported in pre- and perimenopausal White women (172) and White men aged 30 to 79 years (205) compared with African Americans, while no differences were reported in studies including both male and female adults in the United Kingdom (195). As regards to resorption markers, no difference was reported for uNTX (172, 195) while CTX was reported to be higher in White men (205).

Conflicting results were also observed in the assessment of the Chinese population living in different countries; data collected from a Chinese village showed similar patterns of OC between Chinese and White British men and women. In contrast, a cohort enrolling Chinese people living in the United States reported lower levels of OC and uNTX compared to White and Black pre- and perimenopausal women (172).

Therefore, despite ethical and geographical variation on BTMs reported, there is no consistent pattern of variation.

Fracture

Fracture healing leads to an increase in BTMs. This increase reflects the formation of a callus and the modeling and remodeling involved in bone repair. Ivaska et al have shown that a few hours after a fracture, no change is observed in PINP, OC, CTX, and TRACP5b and therefore immediate postfracture sampling may provide baseline information on these BTMs (206). Both formation and resorption markers increase after a fracture; however, the timing of the peak differs between BTMs, reflecting the specific phases of bone modeling and remodeling reflected by each BTM (Fig. 16). For example, after a distal forearm fracture, bone ALP peaks after 2 to 4 weeks, PINP peaks at 6 weeks and OC at 26 weeks. Bone resorption markers seem to peak a bit earlier, with CTX peak reported as early as 2 weeks (207), uNTX peak at 6 weeks, and TRACP5b peak at 12 weeks (208). However, no increase in uNTX was observed after an ankle fracture (209). BTMs have been reported to return to baseline after 1 year following

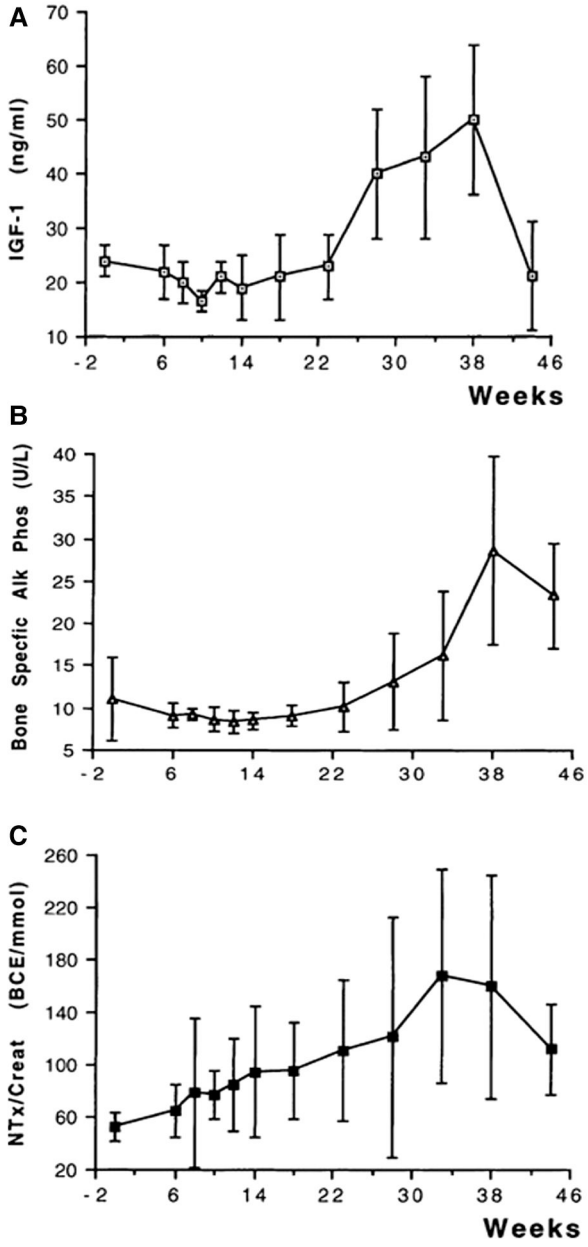


Figure 15. (A) IGF-1 during pregnancy (B) Serum bone ALP during pregnancy (C) Urinary NTx during pregnancy. Adapted with permission from Black et al Journal of Bone and Mineral Research Wiley (204). Abbreviations: ALP, alkaline phosphatase; IGF-1, insulin-like growth factor 1; NTX, N-telopeptide of type I collagen.

a wrist or ankle fracture (208, 209) but some of them might remain high (206, 208, 210). These findings suggest that increased remodeling and mineralization continue after fracture healing, probably as a response to the fracture and immobilization (206). After a fracture, the amount of increase in BTMs varies over time between the different BTMs and depends on the size of the bone that was fractured. Some studies suggest that the size of the fractured bone is the main determinant of the increase (208, 209), but other features such as BMD of the fractured bone, the fracture's bone surface, the need for surgery, and the degree of immobilization also play a role (206). Therefore, an increase in most BTMs is observed up to 1 year after a fracture.

Seasonality

Data on BTM variability across the seasons are conflicting. Several studies report no seasonal variation for OC (211-213), PINP (211), bone ALP and CTX (211). In contrast, other studies reported seasonal variation and suggested that this could be driven by variations in vitamin D, and consequently, PTH. Some studies reported wintertime increase in CTX associated with decreased vitamin D (212, 214), suggesting that secondary hyperparathyroidism could play a role in increasing bone turnover. Conversely, Woitge et al reported increased bone ALP and OC in men and women (50-81 years of age) and decreased vitamin D in women in the winter despite no variation in PTH (215). Variation seems to be higher in women than in men (216). Despite conflicting results, there is some agreement that seasonal variation is not clinically significant (216, 217).

Exercise

Several studies have shown changes in BTMs with exercise. However, the response to exercise is not uniform and varies according to age, sex, and exercise mode, intensity, and duration. In addition, different BTMs respond differently to exercise. A systematic review evaluated the effect of an acute exercise intervention (aerobic, resistance, or impact) in people older than 50 years (218). The analysis of 13 studies included showed no change in OC and bone ALP in older adults (>65 years) following acute exercise but some increase in middle-aged adults (50-65 years). In addition, OC and CTX responses to acute exercise appear to be more sensitive in middle-aged men than women, suggesting a gender-specific response. Most of the studies reported no change in OC following acute exercise in this age group, regardless of the intensity, except for a study involving jogging (218-220). In contrast, bone ALP increased after cycling and walking (218-222). Markers of resorption, such as CTX, seem to be more responsive to longer exercise protocols (>60 minutes) (218-222). The effect of exercise in BTMs is difficult to quantify, therefore it is advisable that subjects should refrain from exercise for at least 24 hours before sample collection (171, 223).

Immobilization and weightlessness

Immobilization increases bone turnover, especially bone resorption. Acute immobilization (14 days) in men led to an increase in CTX both in young (mean age 23 years) and old men (mean age 60 years), while PINP decreased only in young men (224). In another study, 12-week immobilization of young men and women led to a 50% increase in uNTX, while bone ALP and OC did not change significantly (225). Chronic immobilization (>6 months) due to stroke in postmenopausal women lead to an increase in both CTX and bone ALP and while CTX correlated positively with sclerostin, bone ALP correlated negatively (226). Similarly, data from space flight missions showed an increase in uNTX as early as the first week of flight and return to baseline levels shortly after landing (227). However, uNTX doubled during space flight, while the increase observed with bedrest was around 50% (227). These data suggest that the human skeleton seems to respond to unloading by a rapid and sustained increase in bone resorption and a smaller decrease in bone formation.

Circadian rhythm

BTMs show circadian variation. CTX showed the greatest diurnal fluctuation. Peak levels were observed between 0130 and

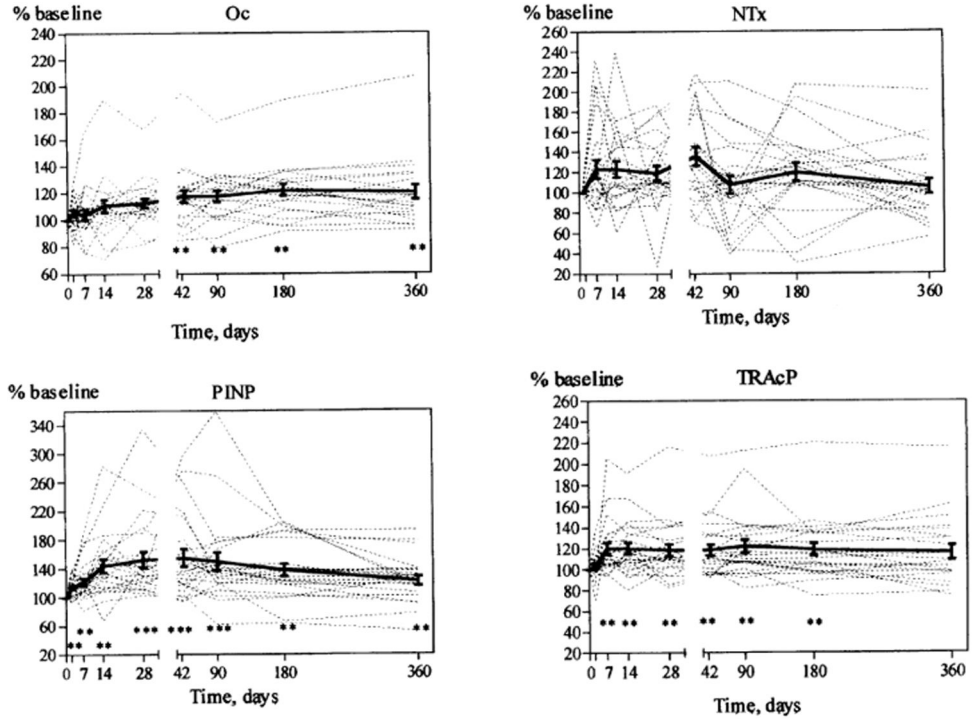


Figure 16. Changes in biochemical markers of bone turnover following distal forearm fracture, showing markers of bone formation (left) and bone resorption (right). The thick continuous line and error bars represent the mean ± SEM for all subjects. The thin broken lines represent individual subjects. The scale on the y-axis (percentage of baseline) is the same for all regions (**$P < 0.01$; ***$P < 0.001$). Abbreviations: iFDpd, immunoreactive free pyridinoline; NTx, N-telopeptides of type I collagen; Oc, osteocalcin; PINP, procollagen type I N-terminal propeptide; TRAcP, tartrate-resistant acid phosphatase. Adapted by permission from Springer Nature Osteoporosis International Osteoporos Int (1999) 10:399–407 Changes in bone mass and bone turnover following distal forearm fracture. Ingle, B. M., Hay, S. M., Bottjer, H. M. & Eastell, R. 1999 © International Osteoporosis Foundation and National Osteoporosis Foundation (208).

0430 hours and were 40% to 60% higher than the 24-hour mean, while the nadir was observed between 1100 and 1500 hours. The lowest value was 40% to 60% below the 24-hour mean (228-230) (Fig. 17). This fluctuation was independent of age, gender, postmenopausal status, and ethnicity (229, 230).

uNTX also follows a circadian rhythm, with peak excretion between 0300 and 0700 hours and a nadir between 1500 and 1900 hours (184). The amplitude of this variation (peak to through) was 60% in premenopausal women (184) and around 40% in a sample including elderly men and women, with men showing half the mean values of women (231).

OC rhythm follows a similar pattern, with high levels overnight and lower levels in the afternoon; however, the amount of variation is smaller (20%) (229, 231, 232). For OC, the peak and trough did not exceed a 10% change compared with the 24-hour mean (Fig. 17). This variability was not affected by ethnicity (229) or gender (231, 232), but it was abolished in the absence of cortisol circadian rhythm l(233, 234).

Bone ALP also showed diurnal variation, but the pattern was rather discordant (171). Both 1 and 2 peaks have been reported (199, 231). In healthy men and women aged 23 to 36 years, 2 peaks were observed, at 1430 and 2330 hours, and a nadir at 0630 hours. These variations were within a 30% amplitude (199). Conversely, in elderly adults, a single peak between 1100 and 1300 hours and a nadir between 0200 and 0600 hours were reported, and the variation did not exceed a 10% amplitude (231).

Finally, PINP showed the smallest variation, with most studies reporting no rhythm in men and women (229, 232, 235, 236). A slight increase at night in men with a peak at 0200 hours has also been reported (232). Furthermore, sleep restriction and circadian disruption were reported to decrease PINP both in men and women (237, 238).

Therefore, circadian variation is important for bone resorption markers. To reduce variability, samples should be collected at the same time of the day, ideally before 1000 hours.

Food intake/fasting

Feeding/fasting state has an impact on BTMs. Morning feeding decreased PINP (3.8%), OC (4.1%), uNTX (7.9%), and CTX (17.8%) while bone ALP did not vary significantly (235). Recently, Gossiel et al have shown no effect on TRACP5b, while CTX was decreased by 29% and PINP by 10% (239). Feeding also impacts the circadian variation of CTX. Fasting reduced CTX diurnal variation from 35% to 40% to 9% to 16% (230, 240) but no impact was observed in OC (240-243) or PINP (243) (Fig. 17). The intake of food, glucose, fat, and protein reduced CTX and this seems to be independent of age and gender (241). Part of this variation seems to be mediated by the nutrient-induced release of the gastrointestinal hormone glucagon-like peptide-2 (GLP-2) (242, 243). Experimental studies have shown that food intake was followed by an increase in GLP-2 and a decrease in CTX (242). Octreotide abolished the decrease in BTMs (244). Furthermore, a decrease in CTX was observed with the administration of GLP-2 (243). The clinical impact

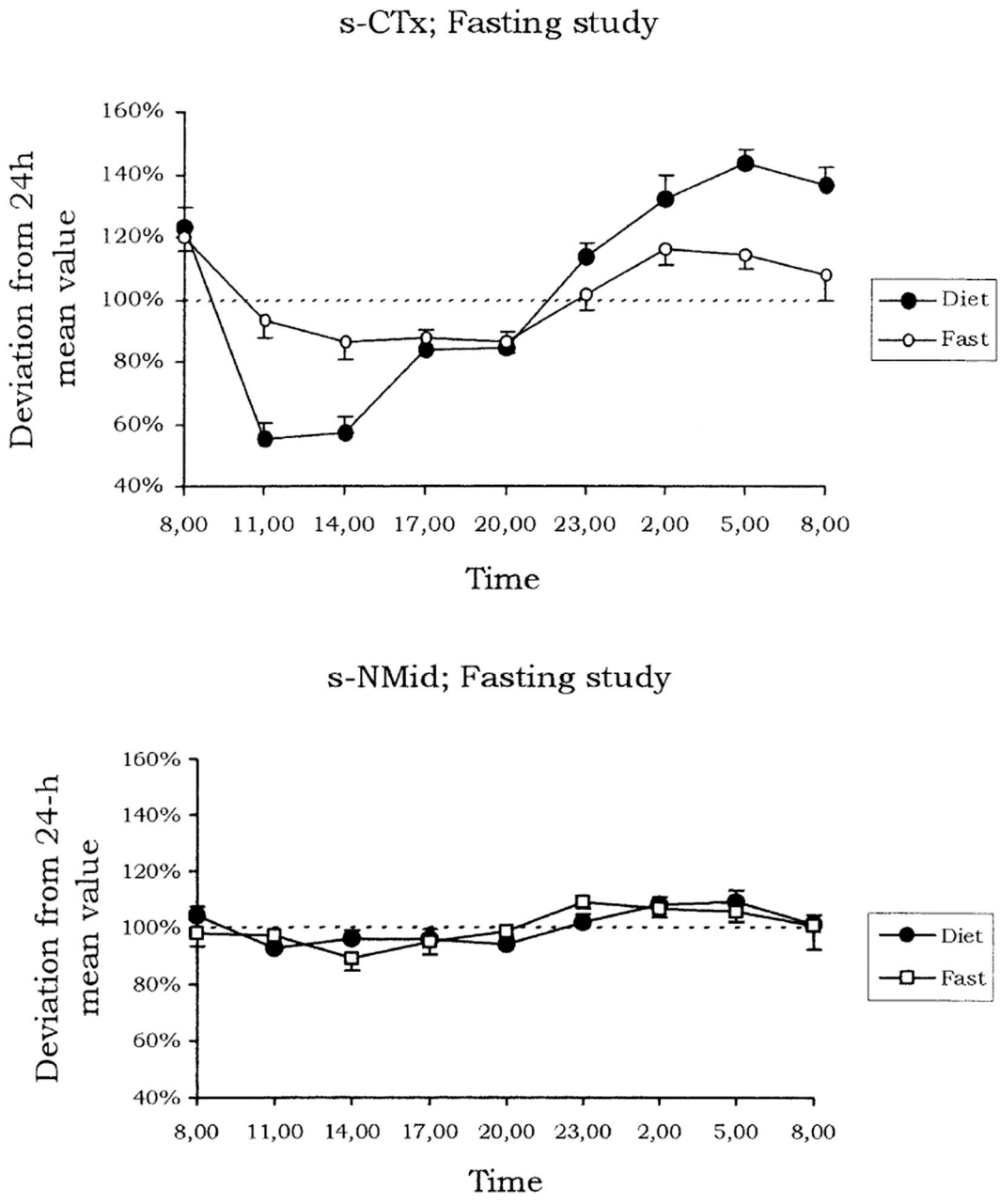


Figure 17. Upper panel: s-CTx in premenopausal women on a normal diet and during fasting. The cyclical variation was statistically significant both on normal diet ($P = 0.0004$) and during fasting ($P = 3.7 \cdot 10^{5}$). Lower panel: s-NMid (osteocalcin) in the same women on a normal diet and during fasting. Both exerted a significant cyclical variation ($P = 0.002$ and $P = 0.016$, respectively). Reprinted from Bone, 31(1), Circadian variation in the serum concentration of C-terminal telopeptide of type I collagen (serum CTx): effects of gender, age, menopausal status, posture, daylight, serum cortisol, and fasting. Qvist, P., Christgau, S., Pedersen, B. J., Schlemmer, A. & Christiansen, C. 57-61 © 2002 with permission from Elsevier (230). Abbreviations: s-CTx, serum C-telopeptides of type I collagen; s-NMid osteocalcin.

of feeding/ fasting on most BTMs interpretation is small, except for CTX. These data support the preference for fasting sample collection (235).

Diet

Calcium ingestion has significant impact in BTMs. In young women, calcium-fortified ice cream led to a decrease in CTX. This decrease was up to 20% within the first hour and persisted after 28 days (245). In contrast, PINP increased by 10% in 7 days following calcium supplementation, but this difference was not persistent after 28 days (245). In 17 studies assessing calcium-fortified foods, there was a decrease in BTMs (mainly CTX) both in young and old volunteers (246). In contrast, BTMs were not affected by mineral supplementation with copper (247), magnesium, (248) or zinc (246).

Vitamin D supplementation decreased BTMs (mainly CTX) both in young and old volunteers. Vitamin K supplementation reduces the undercarboxylated osteocalcin levels. For the other BTMs, no consistent effects were reported in studies of BTMs involving fortification with vitamin K, folic acid, or isoflavone (246).

The increase in the consumption of fruit and vegetables from 2 to 5 or more portions a day had no impact on BTMs (249).

Vegan diet has been associated with increase in bone ALP and PINP compared with omnivores, among adults (250). In children, ovo-lacto-vegetarian diet was associated with increase in CTX (13%) and bone ALP (20%) (251). In both, the variation was small and not clinically significant. Data in low carbohydrate diet are conflicting, with a study showing no variation (252), while another study reported an increase in CTX (25%) and uNTX (11%) (253).

Day-to-day variation

Because there are several sources of variability, samples collected from the same healthy individual, under steady-state conditions, show physiological fluctuations. This is called the intra-individual coefficient of variation (CV) and it is different from the assay CV reported in Tables 7 to 10. To make sure BTMs have varied (for example after an intervention), the change observed needs to be greater than this physiological intra-individual variation. The least significant change (LSC) can be used for this purpose. A variation greater than the LSC gives 95% confidence that the change observed is greater than the physiological variation for that marker. LSC is calculated as $LSC = 2.77 \times CV$ (82).

Drugs

Hormonal contraception. Several cross-sectional and longitudinal studies have reported the effects of hormonal contraception on BTMs. Overall, the combined oral contraception pill (COC) is associated with a decrease in BTMs (254). For example, in a study that assessed women aged 35 to 49 years, BTMs were lower in COC users compared to nonusers, the reduction for OC was 24% for bone ALP was 17% and for uNTX was 28% (255). In a prospective study in women aged 24 to 31 years, using COC with ethinylestradiol and drospirenone for 6 months, bone ALP was decreased by 17% and OC by 26% in COC users compared to nonusers (256). The decrease in BTMs seems to be more pronounced in women with high turnover (254).

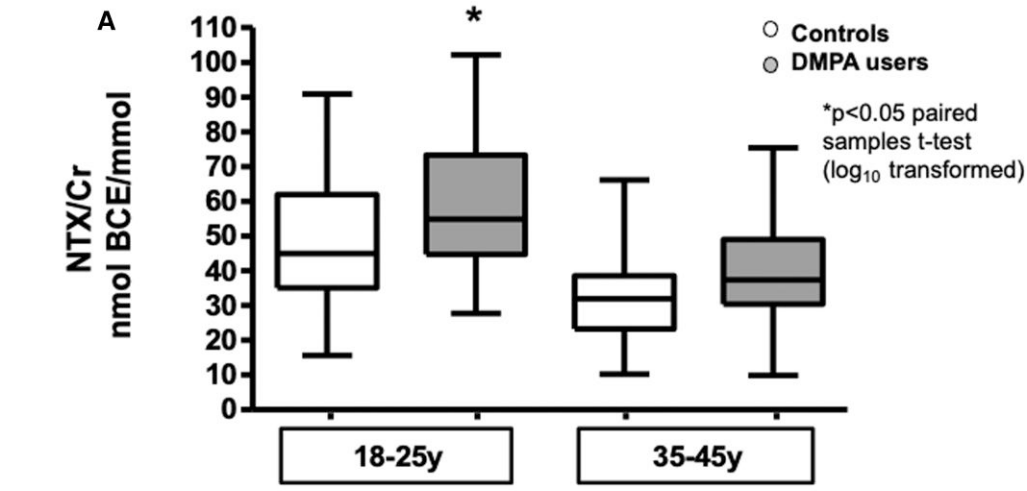
In contrast, progestin-only hormonal contraceptives are associated with an increase in BTMs. In women 18 to 39 years using depot medroxyprogesterone acetate (DMPA), uNTX was 20% higher than in controls. Bone ALP followed the same pattern but did not reach statistical significance (257). In another study, the participants were categorized according to the length of DMPA use and longer use was associated with a greater increase in OC; there was a 70% increase in participants who used it for less than 1 year and a 2-fold increase in participants who used it for more than 5 years (258). In a case-control study including 100 participants aged 18 to 25 and 100 aged 35 to 45 years, uNTX was 27% and 23% higher in the DMPA group compared with nonusers and PINP were 40% and 22% higher, respectively (259) (Fig. 18). Multiple regression models suggested that DMPA effects on the BTMs are associated with estrogen deficiency (259).

Anti-epileptic drugs. Anti-epileptic drugs (AED) are widely used in clinical practice, and their indications go beyond epilepsy treatment. Epilepsy is a common chronic neurological disorder associated with a 2-fold increase in the risk of fractures (260). Several factors are involved such as reduced BMD, impaired bone quality (due to osteoporosis and/or osteomalacia), increased risk of falls, and the occurrence of traumatic fractures associated with seizures or loss of consciousness (261). The impact of the trauma during a seizure is important; when seizure-related fractures are excluded, the risk of fractures in epilepsy is only increased by 30% (260). Epilepsy usually requires long-term treatment with AED. AED use is associated with a decrease in BMD and an increased risk of fractures (260).

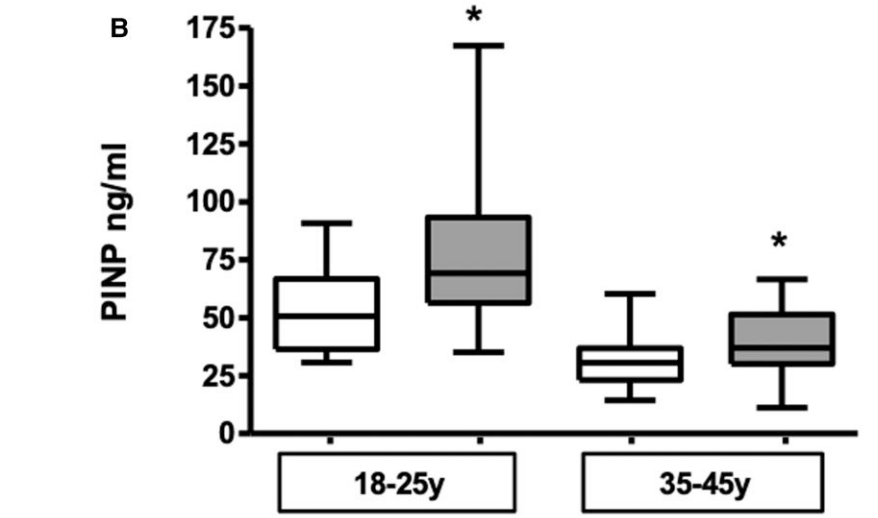
AEDs can be classified according to their ability to induce cytochrome P450 (CYP450). The inductors of CYP450 enzymes are phenytoin, phenobarbital, carbamazepine, and primidone. They accelerate vitamin D metabolism and therefore decrease plasma levels of both 25-hydroxyvitamin D and 1,25 dihydroxyvitamin D (262, 263). This effect might lead to a secondary increase in PTH. Most studies with carbamazepine have reported normal levels of serum calcium. In contrast, phenytoin and phenobarbital have been associated with hypocalcemia and in vitro studies suggest an inhibition of cellular response to PTH by both anticonvulsants (262, 264). Oxcarbazepine has a limited effect in inducing CYP450. Conversely, other AEDs do not induce CYP450 enzymes, such as valproate, lamotrigine, clonazepam, gabapentin, and topiramate. Despite no enzymatic induction, valproate has been associated with an increase in bone turnover, but the mechanism is unclear (262, 263). Lamotrigine has not been associated with changes in BMD or BTMs (263). Benzodiazepines have been associated with an increased risk of fractures, but there are no data on BTMs (260, 265). Gabapentin has been associated with a decrease in BMD but changes in BTMs have not been reported (262). Topiramate has been associated with mild hypocalcemia, lower bicarbonate concentration and an increase in bone turnover (266). Table 12 summarizes the effect of AED on BTMs.

Anti-estrogens. Anti-estrogen therapy (aromatase inhibitors and tamoxifen) is used in breast cancer treatment. Despite their common anti-estrogen activity, aromatase inhibitors and tamoxifen have opposite effects on bone turnover. Aromatization is a key step in estrogen production and aromatase inhibitors reduce estrogen levels by 90%. This results in bone loss and an increase in bone turnover (267). Data from clinical trials have shown that aromatase inhibitors are effective in the secondary prevention of breast cancer, but this effect is associated with an increase in the risk of fractures, especially at the spine (268). In a trial with nonosteoporotic postmenopausal women, 1 year of anastrozole use resulted in an increase in CTX (26%), uNTX (15%), PINP (18%), and bone ALP (20%) (269). In contrast, tamoxifen use was associated with a decrease in BTMs. Tamoxifen is a selective estrogen receptor modulator that has anti-estrogen effects on the breast tissue but estrogen-like effects on bone. Therefore, tamoxifen leads to a decrease in bone turnover and favorable effects in BMD. In the same trial, treatment with tamoxifen was associated with a decrease in BTMs; CTX was decreased by 56%, uNTX by 52%, PINP by 72%, and bone ALP by 16%. The decrease in BTMs was also observed in the group that received the combined therapy (anastrozole and tamoxifen) (269) (Fig. 19).

Anti-androgens. Men with prostate cancer are often treated with androgen deprivation therapy (ADT). Sustained reduction of androgens (and estrogens) is achieved with the use of gonadotrophin releasing hormone agonists (eg, goserelin and leuprorelin) or antagonists (eg, degarelix). ADT leads to an increase in bone turnover, a decrease in BMD and an increase in the risk of



Geometric mean (95% CI)	44.5 (34.7-57.2)	56.8 (51.8-62.3)	29.9 (27.7-33.7)	36.8 (32.9-41.1)
%Difference	27.6		23.0	



Geometric mean (95% CI)	51.1 (41.8-62.6)	71.8 (64.9-79.3)	30.4 (27.4-33.8)	37.3 (33.5-41.6)
%Difference	40.5		22.7	

Figure 18. Bone turnover in DMPA users and controls: urine NTX (A) and serum PINP (B). Box and whisker plots show median, 25th to 75th centile, and range. 18 to 25 yr DMPA, n 50; controls, n 14; 35 to 45 year DMPA, n 50, controls; n 44). Abbreviations: Cr, creatinine; DMPA, depot medroxyprogesterone acetate; NTX, N-telopeptide of type I collagen; PINP, procollagen type 1 N-propeptide; Walsh, J. S., Eastell, R. & Peel, N. F. Effects of Depot medroxyprogesterone acetate on bone density and bone metabolism before and after peak bone mass: a case-control study. J Clin Endocrinol Metab, 2008 93(4), 1317-23 by permission of Oxford University Press (259).

fractures (270). In men with prostate cancer, the introduction of ADT resulted in a progressive increase of PINP, uNTX, bone ALP, and OC after 6 and 12 months (270). In a cross-sectional study, ADT was associated with a 30% increase in uNTX, compared with healthy controls (271). In another study comparing men with prostate cancer with and without bone metastases taking ADT and prostate cancer patients without ADT, bone ALP and uNTX were higher in men taking ADT compared to those not taking ADT (272).

In contrast, a study that investigated the effect of cyproterone acetate in 17 sex offenders reported no change in PINP, TRACP5b, or CTX but a 40% decrease in bone ALP and 12% decrease in OC after 2 to 4 months (273).

Part 2. The Use of BTM in Clinical Practice: Potential, Problems, and Pitfalls

BTM are a valuable tool to investigate bone turnover in clinical practice. BTMs can be useful in the diagnosis and management of both bone-related diseases and systemic disease that affect the skeleton.

Osteoporosis

BTMs and the prediction of bone loss

The menopause is associated with rapid bone loss and an increase in the risk of fractures. Studies in both premenopausal

Table 12. Effect of AED in BTMs

Drug	25OHD	BTM
EI-AED		
Phenytoin	↓	↑Bone ALP, ↑uNTX (262, 263)
Phenobarbital	↓	↑Bone ALP ↑CTX (262, 265)
Carbamazepine	↓	↑Bone ALP, ↑OC, ↑uNTX (262)
NEI-AED		
Valproic acid	↔	↑ALP, ↑OC (262)
Lamotrigine	↔	↔ (262, 263)
Topiramate	↔	↑Bone ALP, ↑OC (266)

Abbreviations: 25OHD, 25-hydroxy vitamin D; BTM, bone turnover marker; AED anti-epileptic drugs; ALP, alkaline phosphatase; CTX, C-telopeptide of type I collagen; EI-AEDs enzyme inductor anti-epileptic drugs; NEI-AED, non-enzyme inductor anti-epileptic drugs; OC, osteocalcin; uNTX, urinary N-telopeptide of type I collagen.

and postmenopausal women have shown that higher BTMs have been associated with faster bone loss in different skeletal sites (eg, spine (274), total hip (275), femoral neck (276), radius (277)) and BTM use has been proposed in order to decide which women might benefit from treatment (278).

When examining the relationship between BTM and bone loss, several factors need to be considered that may affect the BMD. Thus, the accuracy on measurement of BMD change depends on the skeletal site, the duration of the follow-up period, the precision error, and the number of measurements (279). Higher BTMs seem to correlate more to cortical than trabecular bone loss (276). This might be due to progressive degeneration of the spine with age, which, in turn, affects the rate of change. Overall, BTMs seem to be better correlated with BMD loss at the hip rather than the spine (278). Using serial measurements of BTMs has been proposed as a means to improving the precision and increasing this correlation (280). Longer follow-up periods can also improve the accuracy (278); however, other factors can influence the analysis when having longer follow-up.

One approach that was proposed when evaluating this relationship was the use of thresholds to identify fast bone losers; one example was the use of more than 3% annual bone loss (281), or use of tertiles. There have been studies showing that women above the defined BTM thresholds had a higher risk of bone loss (277, 282).

Apart from using individual BTMs, the use of bone balance index was assessed; this was defined as the relationship between resorption (uNTX) and formation (OC). Each SD decrease in bone balance index was associated with faster loss at the spine BMD but not at the femoral neck, consistent with the fact that in early menopause bone loss mainly occurs at the spine (283). Another approach evaluated in the TRIO study, was the T-score approach. This was calculated as follows (188).

T-score = (lg10 BTM − mean lg10 BTM)/lg10 SD)

Bone balance = (T-score bone formation − T-score bone resorption)

The markers evaluated were PINP and CTX. Bone balance was weakly correlated with bone loss at the total hip while bone turnover was associated with change in the lumbar spine BMD in women up to 10 years from menopause; higher bone turnover was associate with rapid bone loss (188).

In general, all these associations have been moderate and their use in individuals is quite limited (275, 276, 278). Bone loss can vary through time. Some women might experience rapid bone loss in the early postmenopausal period; however, this might not be maintained. One BTM value is associated to a range of individual annual BMD changes. BTMs had a poor predictive value in categorizing women into fast and slow losers (274); increased BTMs could only identify 40% to 55% of the fast losers (279).

As expected, limited data are available for men. The MrOS study evaluated men older than 65 years and found a positive association between BTMs and hip bone loss but, as in women, this is of insufficient strength to predict bone loss in an individual (284).

Overall, BTMs have limited value in clinical practice in predicting bone loss.

BTMs and prediction of fracture

Various prospective studies in women have shown an association between at least one marker of bone turnover and subsequent fracture risk (285-288). The associations were more consistent with bone resorption markers rather than bone formation markers. These associations would be useful if the risk was independent of BMD. However, not all studies have shown this (82).

There are fewer studies available for older men, and these suggest the same association. A study in Finland (men and women) showed an association of low carboxylated s-OC/total s-OC ratio to the increase of osteoporosis fractures, but not total osteocalcin; the predictive value only lasted 3 years (288). The MrOS study also suggested an association between PINP and CTX (not TRACP5b) and hip and non-spine fractures, but this association did not remain evident after adjusting for BMD (284).

There are several challenges with these studies, making it difficult to interpret the findings. The fracture classifications varied a lot and most studies evaluated hip fractures, with only a few assessing vertebral or nonvertebral fractures (289). In one study there could be up to 10 different BTMs evaluated and the results for 1 marker varied tremendously between studies, from nonsignificant to strong prediction. The analytic methods used and the timing of the tests also were heterogenous. Moreover, there is heterogeneity in the way they express risks (82).

The International Osteoporosis Foundation and the International Federation of Clinical Chemistry and Laboratory Medicine recommended the use of reference BTMs (PINP and CTX) for future studies (82). Therefore, a meta-analysis was performed using standardized ways of expressing the risks and evaluating the proposed BTMs. The meta-analysis evaluated 6 prospective studies from men and women and estimated the gradient of risk (GR) which is the hazard ratio (HR) for fracture per SD difference in BTMs. The study showed a significant but modest association. The gradient of risk (GR) for major osteoporotic fracture was similar for CTX (HR 1.18; 95% CI, 1.05-1.34) and PINP (1.23; 95% CI, 1.09-1.39). The association between CTX and hip fracture was slightly higher (HR 1.23; 95% CI, 1.04-1.47). Three papers evaluated CTX when adjusted for BMD, but the association was not significant (289). A more recent meta-analysis also showed a positive association between PINP, CTX, and the risk of fractures. This study adjusted for BMD. After adjusting for confounders such as

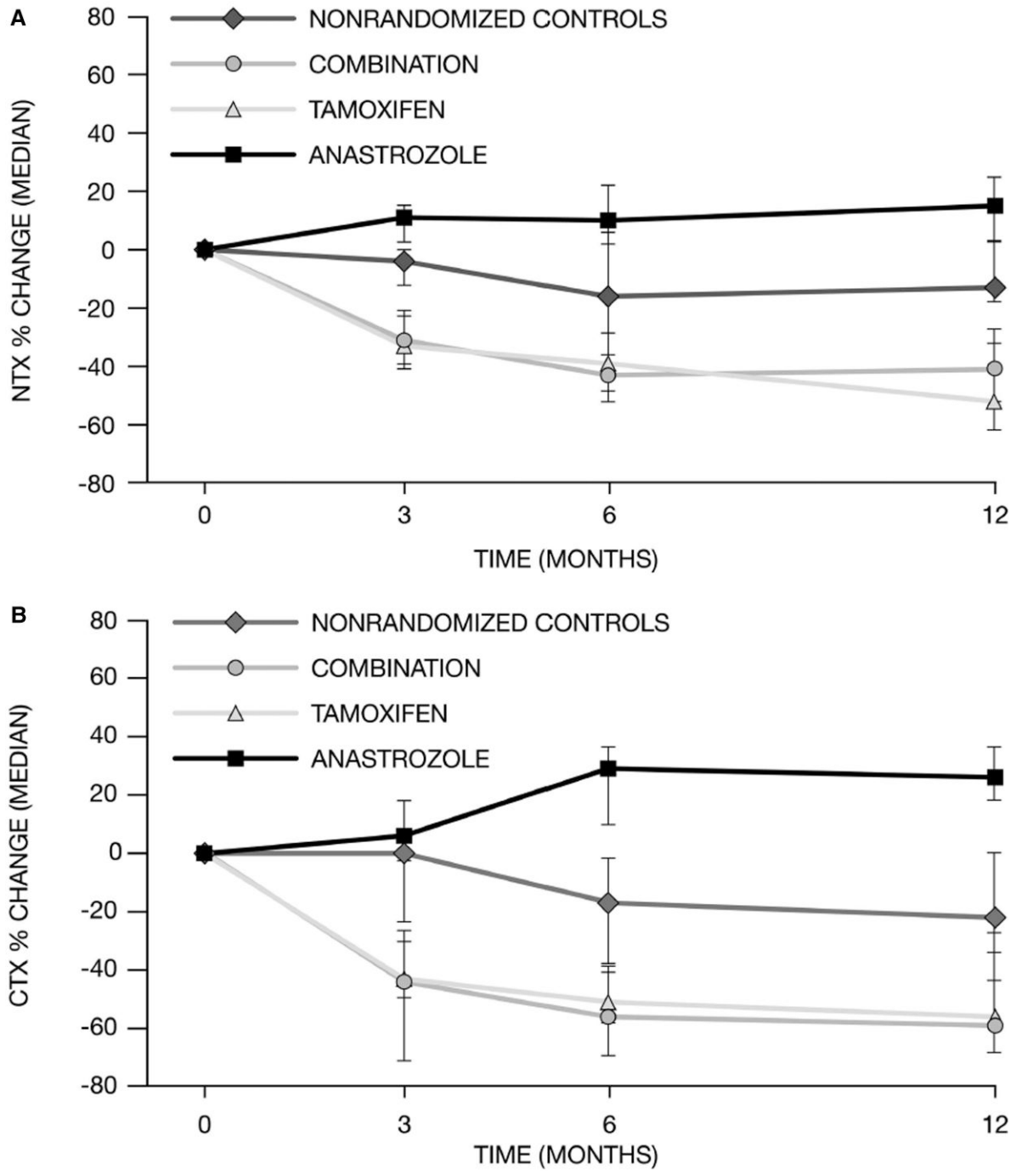


Figure 19. Unadjusted median percentage change in the bone resorption markers (A) NTX and (B) CTX after 3-, 6-, and 12-month treatment. Bars represent 95% CI. Reprinted with permission Eastell, R. J Bone Miner Res, Willey (269). Abbreviations: CTX, C-telopeptide of type I collagen; NTX, N-telopeptide of type I collagen.

age, body mass index, mobility score, past fractures, and hip BMD, PINP demonstrated a significant association with fracture (adjusted GR, 1.28; 95% CI, 1.15-1.42). CTX also showed a significant association (adjusted GR 1.20; 95% CI, 1.05-1.37). The subgroup analysis showed that PINP was associated with fractures in women. CTX was associated with fractures in the elderly, females, and hip fracture patients. The study concludes that the associations are modest and further studies are required to validate these results (290).

For all the reasons mentioned above, plus the fact that studies are not representative of the whole population internationally and they mainly focus on women, BTMs are not currently included in prediction tools like the Fracture Risk Assessment tool (FRAX) (291).

Overall, BTMs have limited value to predict fractures in individuals. More studies are needed that use the recommended BTMs and standardized ways of evaluating the BTMs and estimating the risk.

Baseline BTMs at the initiation of treatment: selection of treatment and prediction of response

Treatments for osteoporosis mainly work in either inhibiting bone resorption (antiresorptive) or stimulating bone formation (anabolic). An issue under discussion is whether higher baseline BTMs are associated with treatment efficacy, either expressed in BMD gain or fracture risk reduction. Intuitively, one would expect patients with high baseline BTMs to respond better to the former group of treatment and patients with low baseline BTMs to respond better to the latter.

Results are conflicting about the effects of antiresorptive medication on BMD change. In the Fracture Intervention Trial (FIT), higher baseline PINP levels were associated with greater increases in BMD at the spine in osteoporotic women. In nonosteoporotic women, higher levels of PINP, CTX, and bone ALP were associated with increases in hip BMD, whereas PINP with increases in spine BMD (292).

In a study of community-dwelling elderly women, randomized to either alendronate, hormone replacement therapy, or combination of the two, baseline uNTX was weakly associated with positive changes in BMD at 3 years ($r = 0.187$ hip, $r = 0.176$ spine, $r = 0.153$ femoral head). The baseline values of the BTMs measured (NTX, OC, and bone ALP) were also negatively associated with the percent change in the BTMs at 6 months. When the study used both the baseline values and the 6-month percent change as predictors of the change in BMD, the baseline values did not come up as significant predictors. According to the researchers, it is the association with the percent change that matters and that baseline BTM values are not independent predictors of BMD change (293). The problem with this analysis is the fallacy of the common variable effect; the baseline BTM affects the change at 6 months, thus the 2 variables are not independent and should not have been used in the same analysis.

In terms of antifracture efficacy, postmenopausal osteoporotic women with higher levels of PINP at baseline had greater reductions in nonvertebral fractures after being treated with alendronate. This was not evident when other BTMs were evaluated. There was no relationship between the baseline BTMs and the vertebral fracture efficacy. In osteopenic women, the nonvertebral fracture efficacy was also greater the higher the baseline levels of PINP; vertebral fracture efficacy was greater the higher the levels of bone ALP (292).

When risedronate was given to postmenopausal women, higher BTMs were associated with higher spine BMD at 12 months. However, even in women whose BTMs were in the normal range or in the lower tertile, BMD still showed significant gain. There was not enough statistical power to assess antifracture efficacy in this study (294). Low baseline BTMs were found to be an independent predictor of inadequate response to risedronate (295).

In postmenopausal women treated with zoledronate, the response in BMD was found to be greater in women with greater bone loss before treatment; this was thought to be as a result of higher PINP levels (296).

Data are also conflicting about trials evaluating teriparatide. In a study of postmenopausal women with a median treatment duration of 19 months, baseline BTMs were significantly correlated with changes in lumbar spine BMD at 18 months (297). In another trial of postmenopausal women with glucocorticoid-induced osteoporosis (prednisolone 5-20 mg/day), no correlation was found between baseline levels of OC and 18- and 24-month changes in BMD (298). A more recent study showed that elderly patients (93% female) with higher baseline levels of PINP or CTX had greater increases in their lumbar BMD. However, clinically significant changes in BMD were independent of baseline BTMs (299). There is no evidence that baseline BTMs affect teriparatide's antifracture efficacy (300).

Overall, there are insufficient data to suggest the use of baseline BTMs in the process of deciding for osteoporosis treatment and in the prediction of the response.

BTMs and osteoporosis medications: monitoring the treatment and the offset

Antiresorptive medications cause a decrease in the bone resorption markers at first, and then, due to the coupling with bone formation, decreases in bone formation markers. In a recent meta-regression, it was found that changes in the bone formation markers bone ALP and PINP predicted the vertebral fracture efficacy of these medications, but not the hip or nonvertebral fracture efficacy. Surprisingly, the changes in the bone resorption markers CTX and uNTX/Cr did not predict fracture efficacy, but this was likely due to the smaller number of trials that used these BTMs (Fig. 20) (301).

Bisphosphonates. Oral bisphosphonates, such as alendronate, ibandronate, and risedronate are usually the first-line option for the treatment of osteoporosis. They all have similar efficacy, although ibandronate is not as successful in reducing the risk of hip or nonvertebral fractures (302, 303).

Different strategies to monitor treatment have been proposed. These aim to assess response and encourage continued compliance. Serial BMD measurements have been in use for years and they are usually repeated every 1 to 3 years (302). The problem with BMD measurement is that changes take time to occur. As a result, the effect of treatment might be compromised. Thus, BTMs have been proposed as a better way of assessing treatment, as they respond more rapidly (82). The IOF and European Calcified Tissue Society (ECTS) Working Group recommended the use of BTMs to assess the adherence to oral bisphosphonates and they proposed the use of CTX and PINP (82, 304). They suggested the measurement of one of these 2 BTMs (or both), before starting oral bisphosphonates. The measurement should be repeated at 3 months to assess whether the decrease exceeds the LSC (see in Part 1, "Variability"). Patients whose BTMs exceed the LSC during treatment are considered as responders. The problem with the LSC approach is that 2 measurements are needed, which is not always possible in clinical practice. For that reason, a second approach was proposed that uses the average value for young premenopausal women (the "reference mean" approach). These concepts have also been used in other diseases (eg, monitoring patients with diabetes mellitus using hemoglobin A_{1C}) (305).

These approaches were evaluated in the TRIO study, where the effect of 3 oral bisphosphonates (ibandronate, alendronate, and risedronate) on postmenopausal osteoporotic women was studied (16). In this clinical trial, at least 70% of women achieved a good response for CTX and PINP. The magnitude of response was greater for alendronate and ibandronate than for risedronate. The LSC used was 56% for CTX and 38% for PINP (16) (Fig. 21).

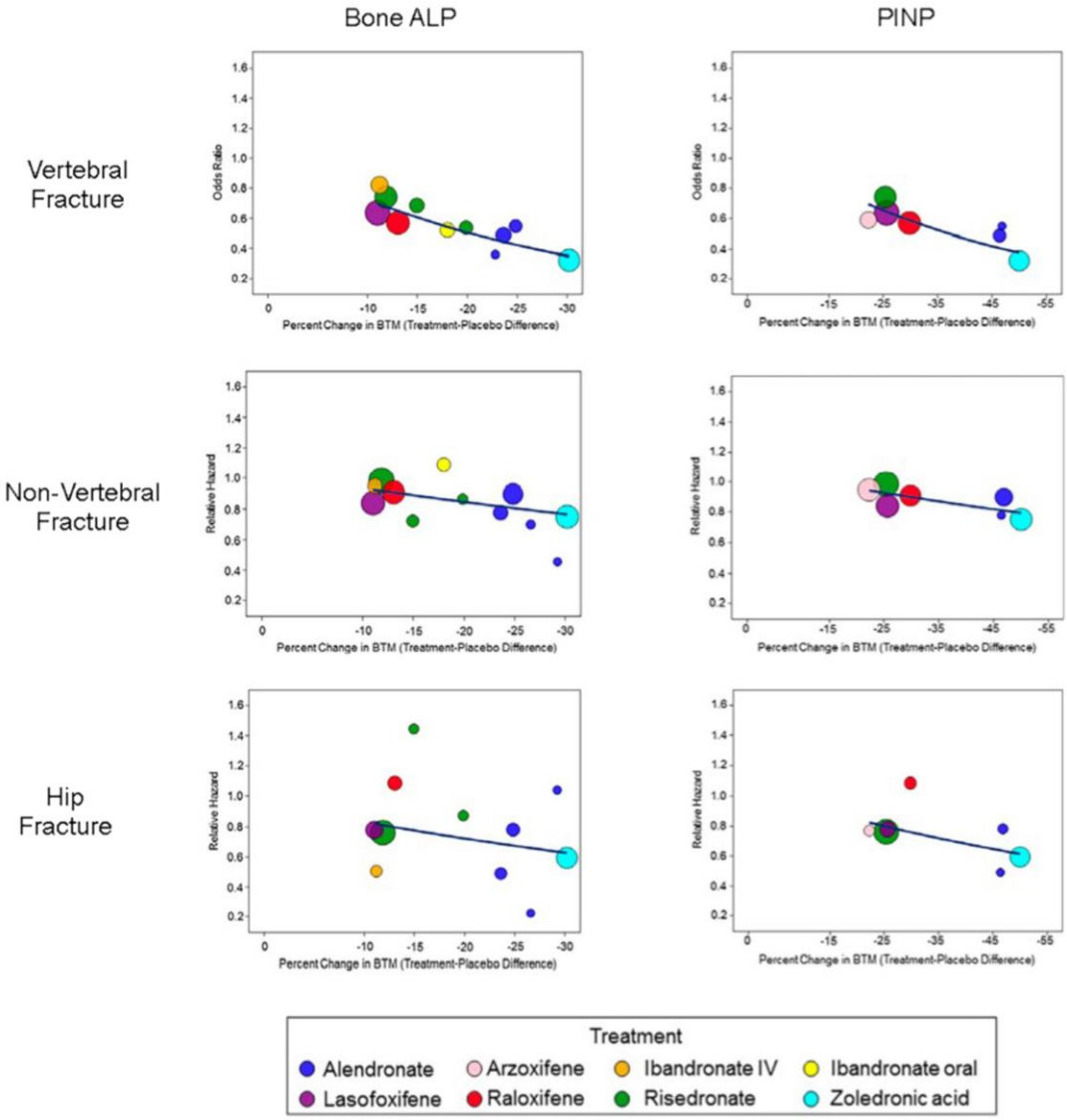


Figure 20. The relationship between the fracture risk and the percentage change in bone turnover markers (bone ALP and PINP) between the treatment and the placebo group. The relationship is expressed as odds ratio for vertebral fracture and as relative hazard for nonvertebral and hip fracture. The larger circles indicate studies with more fractures. Abbreviations: ALP, Alkaline phosphatase; PINP, procollagen type 1 N-propeptide (301). © 2017 American Society for Bone and Mineral Research.

In everyday clinical practice, it has been suggested that patients having a decrease in PINP values of more than 10 μg/L (LSC) or to below 35 μg/L (geometrical mean) are considered as responders. As regards to CTX, the respective values should be 100 ng/L or 280 ng/L (306). The benefit of using PINP, as mentioned above, is that the sample can be taken at any point in the day and fasting is not required. On the other hand, CTX changes seem to occur earlier during the treatment course (16). When using these thresholds, patients can be divided into responders and nonresponders. In the case of nonresponders, the clinician should check the adherence to treatment, carry out investigations for secondary osteoporosis, and consider conditions of poor drug absorption (304). In some cases, change to an intravenous medication like zoledronate or ibandronate might be more appropriate. An algorithm suggested was to measure BTMs at baseline, check compliance at month 1, and repeat the BTM measurement at month 6. If there is a response (as defined above), then further monitoring is advised at year 5 (BMD measurement), to consider a pause in treatment (306).

A recent study evaluated patients treated with bisphosphonates in primary care. The subjects monitored with PINP were more likely to start oral bisphosphonate treatment, switch to zoledronate and have follow-up BMD measurements. They also had greater increases in hip BMD. A cost-effectiveness analysis was also performed, which showed that PINP monitoring has the potential to be cost-effective (29).

There have been a few studies that have evaluated whether BTM monitoring can improve adherence to treatment. One study found that monitoring patients increased adherence by 57% at 1 year, but there was no difference between nurse-led monitoring and BTM monitoring (307). In the Improving Measurements of Persistence on Actonel Treatment (IMPACT) study, it was found that reinforcement (information given to patients regarding their BTMs) resulted in improved persistence, but just in the cases where the result was

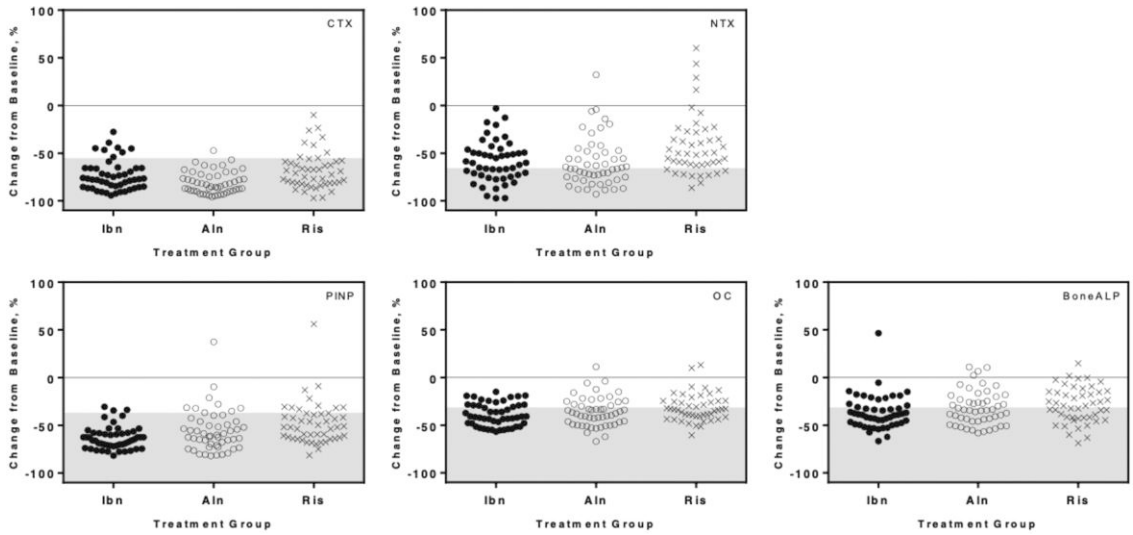


Figure 21. The percentage change of different bone markers in response to treatment with oral bisphosphonates [filled circles for ibandronate (ibn), open circles for alendronate (ald), multiplication symbols for risedronate (ris)]. Least significant change (LSC) is represented in the shaded area. Abbreviations: bone ALP, bone alkaline phosphatase; CTX, C-telopeptide of type I collagen; NTX, N-telopeptide of type I collagen; OC, osteocalcin; PINP, procollagen type 1 N-propeptide. Reprinted by permission from Springer Nature, Response of bone turnover markers to three oral bisphosphonate therapies in p-ostmenopausal osteoporosis: the TRIO study, Naylor et al, Osteoporosis International, copyright 2016 (16).

positive (ie, decrease in BTMs). This was also associated with a 60% lower incidence of new radiologically determined vertebral fractures (308).

In a study of postmenopausal women with low BMD, treated with either zoledronate or alendronate, there was a greater and faster reduction of BTMs in the zoledronate group (309). The suppression of BTMs after annual zoledronate infusions for 6 years is maintained over the whole duration of treatment (310). Similar results were observed in a study of 3-monthly intravenous ibandronate (311).

More recently, TRACP5b has also been proposed for the monitoring of patients on oral bisphosphonates and zoledronate, since it has similar diagnostic accuracy to PINP and CTX (239).

The current recommendation is that treatment with oral bisphosphonates should continue for 5 years and intravenous bisphosphonate for 3 years. After this period, a pause in treatment should be considered. It has been shown that bisphosphonates have this distinct characteristic which other anti-osteoporosis medications do not have; they can accumulate in the skeleton and can still provide benefit to the bones even when treatment is stopped (312, 313). Moreover, after discontinuing treatment, the antifracture efficacy persists (314). Long-term treatment with bisphosphonates has been related to rare side effects like atypical femoral fractures and osteonecrosis of the jaw and their risk may be minimized by discontinuing treatment (302). The discontinuation of bisphosphonates has been associated with rapid decreases in the risk of atypical femoral fractures (315). Continuation for a duration greater than the one described above (ie, 10 years for oral bisphosphonates and 6 years for intravenous ones) should be considered in high-risk patients (vertebral or hip fracture, T-score below −2.5) (316).

When a pause in treatment has been decided, it is important to monitor the patients to decide whether future treatment would be needed. Similar to monitoring patients on treatment, 2 methods have been proposed for monitoring the offset effect. BMD has been used; only 29% of patients had a decrease of total hip BMD of more than 5% at 5 years after stopping alendronate. This percentage was only 1% when using lumbar spine BMD (317). For this reason, and after the results of the TRIO extension trial, the use of BTMs for monitoring has been proposed. An increase of greater than the LSC was observed in 66% of women when CTX was evaluated and 72% of women when PINP was evaluated (48 weeks after stopping the bisphosphonate). When the reference mean was used, the numbers were 64% for CTX and 42% for PINP. Women with the largest increases in BTMs had the greatest decreases in total hip BMD (318).

There was a recent study that compared changes in BMD and PINP levels after a pause in alendronate (Fracture Intervention Trial Long-term Extension [FLEX trial]) and zoledronate (Health Outcomes and Reduced Incidence with Zoledronic Acid Once Yearly-Pivotal Fracture Trial Extension I [HORIZON-PFT E1 trial]). It showed that a greater proportion of patients from the FLEX trial rather than the HORIZON-PFT E1 group had BMD decreases and PINP increases greater than the LSC. The researchers evaluated whether there was a BMD loss greater than the LSC 3 years after stopping; 25.2% of the FLEX group and 18.7% of the HORIZON-PFT E1 met that criterion at the total hip. The numbers were similar for femoral neck (28.4% and 19.8% respectively) but smaller for lumbar spine BMD (7.9% and 4.8% respectively). In contrast, 3 years after stopping treatment, 42% in the FLEX trial and 24.6% in the HORIZON-PFT E1 trial had an increase in PINP that was above the median for premenopausal levels, thus showing greater sensitivity of BTMs rather than BMD (319).

Zoledronate is usually given annually, but the timing of the dose could be individualized by the measurement of BTMs. Even 1 dose of zoledronate can suppress BTMs for at least 5 years (320), while women who had treatment every 18 months still had antifracture benefits, suggesting that longer intervals can be considered, especially if one takes into account the long-term adverse events (321). More studies are needed to address this issue.

Denosumab. Denosumab, also an antiresorptive treatment, is a monoclonal antibody against RANKL. The Fracture Reduction Evaluation of Denosumab in Osteoporosis Every 6 Months (FREEDOM) trial was a multicenter, randomized, double-blind, placebo-controlled trial with a 3-year duration period (7808 postmenopausal women, mean age 72.3 years). Denosumab rapidly decreased CTX by 86% at 1 month and by 72% at 3 years. PINP responded more slowly with a nadir at about 6 months. The respective percentages for PINP were 18% and 76% (322).

In the FREEDOM Extension trial, all patients received denosumab for 7 years. The women who received denosumab in the first 3 years in the FREEDOM trial comprised the long-term arm and had treatment for a total of 10 years. The women who received placebo for the first 3 years comprised the crossover arm and received denosumab for 7 years. There was no control group. Both CTX and PINP remained well suppressed in the long-term arm, for the duration of the study (323).

When comparing denosumab to alendronate, the CTX decrease was more rapid in the denosumab arm by one month (89% vs 61%). By month 6, the decreases were similar and still statistically significant. By month 12, there were no significant differences (77% vs 73%). PINP also showed greater decreases with denosumab throughout the whole period, with a maximal reduction at month 3 for denosumab (−76%) vs month 9 for alendronate (−65%) (324). Similar results with CTX responses were observed when comparing denosumab and risedronate (325). When comparing denosumab to zoledronate, the decreases in BTMs were greater in the denosumab group at all time points after day 10 for CTX and after month 3 for PINP (326). TRACP5b has also been studied in patients treated with denosumab, using the reference mean approach, and was found to have similar diagnostic accuracy to PINP and CTX (239).

As mentioned previously, denosumab is an antibody and thus it circulates in the bloodstream, unlike bisphosphonates which are incorporated into the bone (327). Therefore, when treatment is discontinued, a rapid increase in bone turnover is observed. This overshoot can result in bone loss and vertebral fractures (328). Factors associated with increased incidence of fractures are prevalent vertebral fractures, greater hip BMD increase while on treatment, longer duration of treatment but also longer discontinuation (327). There is inconsistency as to whether bisphosphonates prior to treatment with denosumab can attenuate the effects after denosumab discontinuation (329, 330).

BTM levels after the discontinuation were described to be higher than the premenopausal reference range (331). CTX peaked at 6 months after discontinuation (peak median change 63%) and PINP at 12 months (peak median change 47%); the levels were higher than the pretreatment levels. The levels returned to the baseline 2 years after discontinuation (332). The mechanism behind this is not yet understood.

Different strategies have been proposed to prevent this rapid bone loss after discontinuation. Selective estrogen receptor modulators (SERMs) have been used but with no effect on BTMs and vertebral fractures (333). Oral bisphosphonates were also suggested. Alendronate was given to postmenopausal women for 1 year after 1 year of denosumab. Although this was a short duration study, it showed promising results in terms of maintaining BTM suppression for 2 years (334). Risedronate (for 3 months), did not prevent bone loss (335).

Infusions of zoledronate have also been studied. After a mean duration of 2.2 years on denosumab, women who received one infusion of zoledronate after 6 months of discontinuation, maintained BMD for 1 year and, in the majority, for the whole 2-year follow-up period. BTMs (CTX and PINP) had a small but significant increase during the first year and stabilized after that. PINP at 24 months was above the upper limit of normal for the postmenopausal range in 7.4% of the women, and above the premenopausal reference range in 18.5%. CTX was above the premenopausal range in 7.4% women (336). One infusion of zoledronate failed to fully suppress bone turnover (PINP) after a 7-year treatment duration with denosumab (337). After a mean period of 4.6 years on denosumab, treatment with zoledronate at either 6 or 9 months after discontinuation did not fully prevent bone loss. In the same study and in one-third of the patients, the infusion was given when an increase in CTX above 1.26 μg/L (50% above the normal range for postmenopausal women and elderly men) was observed (observation group). This approach also failed to fully prevent bone loss (338). The study was recently extended to a 24-month follow-up. CTX remained within the reference range during the second year and no patient required further treatment with zoledronate because of an increase of CTX more than 1.26 μg/L (339). It seems that the duration of treatment with denosumab affects the outcome in terms of BMD. In women treated for less than 3 years (≤ 6 injections), zoledronate maintained the BMD gains; this was not the case with women treated for a period longer than that (> 6 injections). BTMs on the other hand, increased in all the included patients 6 months following the infusion; there was no difference between the 2 groups (340).

The current recommendations around denosumab and its discontinuation involve the assessment of BTMs. In low fracture risk patients, where denosumab is only continued for a short duration of time, oral bisphosphonates for 12 to 24 months can be considered. According to the ECTS working group, BTMs should be measured at 3 months after initiation of the oral bisphosphonate and should be below 280 ng/L for CTX and 35 μg/L for PINP. After stopping oral bisphosphonates, monitoring with BTMs should continue, initially at 3 months and, if stable, every 6 months. In patients where treatment with denosumab was longer, or in patients that cannot tolerate oral bisphosphonates, zoledronate should be considered. The ECTS working group suggested a pragmatic approach of giving zoledronate 6 months after the last denosumab injection. The effect should be monitored with BTMs (3 and 6 months after the infusion). If these are increased, then another infusion of zoledronate should be considered. When BTMs are not available, a second infusion of zoledronate 6 months after the first infusion was suggested. The duration of zoledronate should be tailored to the duration of increased BTMs until more data is available (328).

Selective estrogen receptor modulators. Selective estrogen receptor modulators (SERMs) are antiresorptive medications that act on estrogen receptors and their action varies depending on the tissue. Raloxifene (60 mg daily) is the most commonly used medication. Bazedoxifene is available in Europe and Japan. It is only licensed in the United States and Canada in combination with conjugated estrogen.

In the Multiple Outcomes of Raloxifene Evaluation (MORE) study, OC at 36 months decreased by 26.3% in the raloxifene group vs 8.6% in the placebo group, while

the respective percentages for urinary CTX were 34% vs 8.1% (341). Similar results were observed after 5 years of bazedoxifene (20 mg) with greater reductions in CTX and OC in the treated group (28.8% and 25.2% respectively) (342).

BTMs have been proposed for monitoring treatment response, using similar methods as above (LSC, mean of reference interval). Greater reductions in BTMs (bone ALP and uNTX) were observed in the alendronate group when compared to the raloxifene group (343). In one study, 60% had decreases in CTX greater than the LSC at 48 weeks; the respective percentage for PINP was 65%. When using the reference interval approach, the percentage of women responding to treatment at 48 weeks was 40% for CTX and 45% for PINP (344). A greater decrease in BTMs was correlated with better adherence to raloxifene (345).

Hormone replacement treatment. In selected cases, hormone replacement treatment (HRT) can be considered. HRT also causes a decrease in BTMs, but the response is slower than other antiresorptives, taking about 6 to 9 months for a complete response (346, 347). Both oral and transdermal HRT regimens are effective in doing so (347, 348). Tibolone, has similar effects with reduction of bone turnover marker at about 6 months (349).

In terms of monitoring, the approach described above for bisphosphonates can be used with HRT.

Teriparatide. Teriparatide is a recombinant PTH molecule (1-34) that works by increasing bone formation. PINP was found to have the highest signal-to-noise ratio of all the BTMs (350). PINP increases in the early days of treatment have been associated with an increase in lumbar BMD (351).

The challenge with patients taking teriparatide is that often they have been on treatment with bisphosphonates before. An algorithm using BTM for monitoring patients on teriparatide has been proposed (350, 352). PINP should be checked at baseline and then 1 and 3 months after starting treatment. An increase in PINP of more than 10 µg/L and an increase to above the reference interval of 69 µg/L is considered as a good response to treatment. At month 6, BMD should be assessed. After 2 years of treatment, PINP and BMD measurements should be used as baseline before switching to antiresorptive treatment (306).

Romosozumab. Romosozumab is a monoclonal antibody that binds sclerostin; it has a unique way of action, in that it stimulates bone formation and inhibits bone resorption as shown by BTMs. The PINP increase is temporary as it returns to baseline 4 to 8 weeks after starting treatment. OC and bone ALP had similar behavior; CTX returned to baseline 3 to 6 weeks after the last dose (353). In a more recent study, PINP increased rapidly (peak on day 14) and returned to baseline levels by 9 months of treatment. On the other hand, βCTX decreased early (nadir day 14) and remained at levels below those of the placebo group at 12 months (354). When patients were transitioned to alendronate during the second year, the levels of PINP and β-CTX decreased and remained below the baseline levels at 36 months (355).

Romosozumab has recently been approved in some countries, but no official recommendations are available for monitoring treatment.

Conclusions. BTMs can be very useful when managing patients with osteoporosis and these should include PINP or CTX. They can be used in monitoring patients on antiresorptive medications, mainly bisphosphonates. Two approaches can be used, which include the least significant change and the reference mean approach. Patients can be divided into responders and nonresponders. Following the discontinuation of bisphosphonates, these 2 approaches can also be used to monitor the offset of effect and decide whether treatment needs to be restarted. When using teriparatide, BTMs can be used to monitor the response in treatment. When stopping treatment with denosumab, there is a risk of an overshoot in BTMs. Current recommendations suggest the use of BTMs for deciding the introduction of a bisphosphonate to prevent bone loss, but the timing has not been optimized.

Currently, BTMs have limited value in clinical practice when predicting bone loss and in predicting fractures. There is also insufficient data to suggest their use in deciding about osteoporosis treatment and in the prediction of response.

Other Bone Diseases

Primary hyperparathyroidism

Primary hyperparathyroidism (PHPT) is an endocrine disorder characterized by elevated PTH and calcium concentrations. It has been linked to complications such as osteoporosis and kidney stones. The definitive treatment is parathyroid surgery (356). A milder form has been described, referred to as *normocalcemic hyperparathyroidism* (NPHPT). This is usually defined as persistent normal calcium, with increased PTH, although intermittent hypercalcemia has also been described; other causes of elevated PTH have to be excluded before the diagnosis is made (eg, vitamin D deficiency, idiopathic hypercalciuria, etc.) (357).

PHPT is characterized by high bone turnover (358). BTM levels are significantly increased in patients with PHPT compared with controls (359). This is true irrespective of whether they have skeletal symptoms (360) or whether they have mild disease (361). Although increased, not all are above the reference range (362). These BTMs do not seem to increase when following up patients for 5 years (363). It is impossible to draw conclusions in the setting of NPHPT due to the different definitions used in publications. Some studies found that PHPT and NPHPT patients had similar BTM levels (364), while others showed lower levels in NPHPT (365-367). When comparing NPHPT to controls, results are still inconsistent with one study showing higher levels (368), while another study showed similar levels (369).

Parathyroid surgery in PHPT can lower BTMs within the first 6 to 12 months (61% for PINP and 78% for CTX) (370). This reduction starts within the first few days after surgery (371). It has been found that bone resorption markers decrease first, slowly followed by bone formation markers (372). This is believed to be the cause of the rapid improvement in BMD, especially at trabecular sites (373). Higher preoperative BTMs were found to be associated with greater BMD responses (374, 375). BTMs decreased after surgery, independent of the presurgical use of bisphosphonates. A further decrease was observed in patients who received zoledronate after parathyroidectomy (376).

Treatments for osteoporosis have also been used in managing patients with PHPT. These reduce the BTMs to a similar extent as in osteoporotic patients. Treatments used are

raloxifene (377), alendronate (378-381), and HRT (382). In a study of alendronate, uNTX was reduced by 66% at 3 months and bone ALP by 54% at 9 months (379). In men, the decrease was 47% for bone ALP and 61% for uNTX after 1 year (383). Stopping treatment has been followed by a rapid increase in the BTMs; within 18 to 24 weeks for alendronate (378, 381) and 4 weeks for raloxifene (377). In NPHPT patients, alendronate also decreased the BTMs (384).

Cinacalcet has also been used in PHPT in order to lower the serum calcium levels. Its effect on BTMs is not consistent (385-387).

Conclusion. BTMs can be increased in some but not all patients with PHPT and can decrease as a result of parathyroidectomy. They can also be used when monitoring patients treated for osteoporosis. There is insufficient data on BTMs in NPHPT.

Osteomalacia

Osteomalacia is a metabolic bone disease characterized by incomplete mineralization of the underlying organic bone matrix (osteoid) in adults(388). Looser's zones or pseudofractures are the characteristic findings on plain radiographs. These are lucent zones perpendicular to the cortex, the result of unmineralized osteoid deposition at sites of stress or along nutrient vessels (388). Other main features are hypocalcemia and muscle weakness.

Vitamin D deficiency. The most common cause of osteomalacia is vitamin D deficiency. Both low vitamin D and osteomalacia are associated with an increase in ALP and OC (389, 390) and treatment with vitamin D leads to a decrease in ALP levels (391). Previous studies have shown an inverse relationship between 25-hydroxyvitamin D and ALP (390, 392). The typical histomorphometric features of osteomalacia are associated with 25-hydroxyvitamin D levels below 12.5 nmol/L, but less severe vitamin D deficiency might cause secondary hyperparathyroidism (SHPT) (388) and an increase in bone turnover (388). In patients with SHPT, an increase in CTX and PINP has been observed (393, 394).

Hypophosphatemia. Inherited hypophosphatemia can also lead to osteomalacia. BTMs were reported in a cohort of adults with inherited hypophosphatemia: 21 had XLH, 2 had autosomal dominant hypophosphatemic rickets (ADHR), and 2 had none of the known mutations. Abnormal elevation of bone ALP was observed in 96% of patients, CTX in 72%, PINP in 52%, uNTX in 48%, and OC in 28%. Those patients receiving phosphate supplements and alfacalcidol ($n = 13$) had significant elevation in CTX compared to those not receiving this treatment; however, it is possible that patients with baseline increases in BTMs were more likely to receive treatment. Several patients had SHPT (395). These results agree with previous histomorphometric studies which showed osteomalacia with increased bone volume and increased bone turnover, associated with SHPT (396). In patients with XLH, 24 weeks of treatment with burosumab (an anti-FGF23 antibody) resulted in 81% increase in PINP and 38% in CTX (397).

Osteomalacia can also be caused by phosphatonin-producing tumors, the so-called tumor-induced osteomalacia. These tumors can secrete FGF23. This phosphatonin inhibits phosphate reabsorption in the proximal renal tubule, leading to hyperphosphaturia. FGF23 also decreases 1α-hydroxylation of 25-hydroxyvitamin D, thereby reducing intestinal phosphate absorption. The excess of FGF23 results in marked low phosphate and osteomalacia. In 2 recent series including 5 and 10 patients, all of them had elevated levels of ALP (398, 399). In 2 case reports, both had increased serum CTX, uNTX, bone ALP, and PINP, but not OC. All BTMs increased sharply shortly after the curative surgery of these tumors, suggesting high bone turnover during acute mineralization of osteoid tissue, and then normalized after several months (400). The same pattern was observed for bone ALP in 2 other patients after surgery (398). In patients with tumor-induced osteomalacia who were treated with burosumab, bone ALP, CTX, PINP, and osteocalcin showed rapid increases followed by slow decrease to baseline levels in 3 years, which were within the normal limits for the study population (401).

Conclusion. BTMs are high in osteomalacia, whatever the cause. High ALP is classically associated with osteomalacia, but high CTX, PINP, uNTX, and OC are also observed. Osteomalacia healing is also associated with increase in BTMs due to accelerated bone mineralization followed by normalization.

Paget's disease

Paget's disease is the second most common metabolic bone disorder after osteoporosis. It is characterized by increased bone activity (increased bone resorption by abnormal osteoclasts followed by disorganized bone formation) and can be monostotic or polyostotic. It most commonly affects men after the age of 40 and the bones usually affected are the femora, spine, skull, sternum, and pelvis (402). The pathogenesis of the disease involves abnormal bone turnover and so BTMs have been used for the diagnosis, with the most common one being ALP. Bisphosphonates have been used for the management of symptomatic patients with Paget's disease, with 61.7% showing improvement of their bone pain. As a result, BTMs have also been used in the monitoring of the treatment response (403).

There are several studies which assessed the utility of BTMs in Paget's. Among these is a recent systematic review and meta-analysis (404). In general, BTMs were higher in patients with polyostotic than monostotic disease. The sensitivity of the bone formation markers was 77% to 100% for PINP, 69% to 100% for total ALP, and 82% to 100% for bone ALP. In terms of bone resorption markers, uNTX had the highest sensitivity (94%-100%) and was the one that had the greatest sensitivity for low levels of disease activity (404). However, this conclusion was only drawn from one study (405) which did not evaluate newer BTMs like PINP. The correlation between marker concentrations and scintigraphic activity at baseline was moderate to strong: 0.750 (95% CI, 0.621-0.839) for bone ALP, 0.756 (95% CI, 0.692-0.809) for PINP, 0.617 (95% CI, 0.518-0.700) for total ALP, 0.583 (95% CI, 0.324-0.761) for sCTX, 0.589 (95% CI, 0.332-0.765) for uCTX, and 0.796 (95% CI, 0.702-0.862) for uNTX (404). Although BTMs correlate with the extent of the disease, this correlation was not obvious when there was skull involvement (406).

The current recommendation for the biochemical evaluation is that total ALP should be used as the first-line screening test. This should be combined with liver function tests. If total

ALP is normal and there is a strong clinical suspicion, then other BTMs like bone ALP, PINP, and uNTX may be used (407). Bone ALP is especially useful in cases of abnormal liver function, which could affect both the total ALP and PINP. Intact PINP is useful in cases of CKD (408).

As mentioned before, antiresorptive treatments have been used for the treatment of Paget's disease and they result in a decrease of the BTMs. In terms of bone formation markers, PINP and bone ALP are the ones with the most marked decrease, while uNTX is the resorption marker with the greatest decrease. PINP has the highest correlation with bone scintigraphy after treatment ($r = 0.704$ [95% CI, 0.559-0.808]), making it a more attractive option for monitoring. However, it is not widely available and is more expensive when compared to total ALP (404).

OC does not respond well to treatment and possible explanations include the increase in 1,25-dihydroxyvitamin D because of the secondary hyperparathyroidism that follows the bisphosphonate administration; this active form of vitamin D regulates the gene expression of OC. Moreover, the OC distribution in bone is altered in Paget's disease (409, 410).

The CTX fragments have also been studied in Paget's and the α-α CTX isoform was found to be elevated when compared to the β-β isoform. This reflects the fact that the first one is more abundant in newly synthesized bone, while the levels of the second form increase with the age of the bone. This ratio (α-α/β-β CTX) is affected in Paget's disease, because of the increase in bone formation. The ratio normalizes after bisphosphonate treatment. The α-α marker was found to be significantly associated with the disease activity and had the best response to treatment compared to other BTMs. These BTMs were measured by ELISA (411). Thus, the most usual assay for CTX (the β-β form) is not useful in Paget's disease.

BTMs (total ALP and bone ALP) have been proposed to monitor the effect of treatment; a decrease of 25% or more after treatment is considered significant. On the other hand, an increase of more than 25% from the nadir during the off-treatment period, should prompt physicians to check for symptoms and consider treatment (412). A recent study used this approach to define treatment responders to risedronate (413).

There have been debates as to whether treatment should be aimed at treating symptoms or whether a treat-to-target strategy should be followed, ie, aiming to normalize ALP. The current guidance is to aim for the relief of symptoms. Bisphosphonates are the drugs of choice, while medications like denosumab and calcitonin have limited use (407).

Conclusion. BTMs are very useful in diagnosing Paget's disease and the recommended marker is ALP. PINP and bone ALP can also be used depending on the clinical situation. The current practice is to treat patients according to their symptoms and not aim to normalize BTMs.

Fibrous dysplasia

Fibrous dysplasia (FD) is a rare disorder with a very broad clinical spectrum. It is caused by the gain-of-function mutations of the G_s alpha subunit (Ga_s), one of the proteins that stimulate the cAMP-dependent pathway by binding and activating the enzyme adenylyl cyclase. Following this mutation, skeletal stem cells fail to differentiate. As a result, the normal bone marrow is replaced with a fibro-osseous tissue. FD is characterized by expandable skeletal lesions prone to fractures; these can be either monostotic or polyostotic. The disorder is characterized by pain, functional impairment, and disability (414).

Patients with FD tend to have elevated BTMs (approximately 75%) and their levels have been associated with disease activity (415). The natural history of BTMs in FD has only been recently studied in a retrospective study of 178 patients with FD, of which 73 were treated with bisphosphonates (416). The natural history of BTMs in these patients is quite different than in the general population. As described above, BTMs peak in the general population at puberty and then decline toward adult levels. They then remain stable during adult life and increase again in postmenopausal women. On the other hand, patients with FD show a persistent and progressive decline with age. The difference is possibly related to the abnormal bone resorption and formation and the creation of dysplastic bone. The highest mean values for ALP and uNTX have been described in the age group from 0 to 9 years. The highest mean value of OC was described in the group from 10 to 17 years of age. Although decreasing with age, BTMs remain higher than the age-specific values in these patients. ALP was described to be high in most of the patients studied aged 18 to 29, with the values being 60% above the upper limit of normal for 30-year-old adults. BTMs were similar in patients (both children and adults) having pain to those that did not. This suggests that pain does not seem to correlate to the disease activity (416).

This pattern of age-related decrease in BTMs described above is probably related to the fact that mutated FD cells seem to be decreasing with age and substituted by normal ones. However, this takes place progressively over decades (417, 418). This observation makes things complicated when considering treatment options in patients with FD. Due to the high turnover described in this disorder, antiresorptive medications like bisphosphonates and denosumab have been used in these patients, and BTMs have been used as endpoints in studies evaluating these. The results from these studies have been conflicting in terms of pain and BTM levels (414). The problem with most of these studies is that there was no control group. There was a randomized clinical trial of alendronate in patients with FD; uNTX decreased in the treated group but OC did not show a significant change. The latter may be due to the fact that OC is released by mature osteoblasts, but FD cells are less mature. There was no difference in pain scores or the skeletal burden of the disease (419).

BTMs decreased in a similar way when studying patients previously exposed to bisphosphonates and comparing them with nonexposed individuals; ALP and OC had similar values in the 2 groups. The only exception was total ALP in the over-30 group; treated patients had higher values (416) but this could be because people with higher BTMs are more likely to get treatment. In patients <18 years of age, uNTX values were lower in the treated group. Meanwhile, uNTX values were similar in patients older than 18 years. Moreover, after 2 years of treatment, 83% patients still had elevated ALP. All these findings suggest that bisphosphonates might have a limited activity in FD lesions. Thus, the decrease in BTMs observed could be the age-related decrease described above, plus the effect of bisphosphonates on normal bone (416). This is also consistent with the previous finding that pamidronate treatment did not alter the histomorphometric findings of dysplastic lesions (420). In one described case, despite treatment,

the FD lesions continued to grow, and there was evidence of bisphosphonate action only in the normal bone (421).

Overall, the latest data suggest that treatment with bisphosphonates in these patients should be considered carefully and that more research is needed in this field to assess its benefit. Moreover, one also needs to consider the potential long-term side effects, such as osteonecrosis of the jaw. In a recent study of 76 patients with FD, 5.4% had osteonecrosis of the jaw (422).

Denosumab has also been proposed for the treatment of FD. There was a case of a 9-year-old boy who was treated with denosumab (once monthly, starting dose of 1 mg/kg with dose increases planned every 3 months) and had reduction in pain and size of lesions. BTMs (PINP and CTX) decreased significantly after the first dose and remained suppressed while on treatment (210 days). However, after discontinuation there was a dramatic outcome, with BTMs rapidly increasing, peaking approximately at 90 days post treatment and returning to pretreatment levels about 5 months after. This was more intense with CTX, with levels being 2.5 times more than the pretreatment levels. Other case series have been reported, with similar decreases of BTMs while on treatment. In the case of a 20-year-old man, the pain increased between denosumab injections while BTMs remained suppressed, suggesting that the mechanism of pain does not depend on bone turnover (423). Similar biochemical results were described in other case series (424). Moreover, the effect on pain is not always sustained despite additional treatment (425). Currently, there is an ongoing clinical trial with a primary aim of evaluating the effect of denosumab on BTMs (NCT03571191) (426).

Conclusion. BTMs are high in patients with fibrous dysplasia but seem to decrease with age. Clinicians need to take this into account when deciding to treat patients with bisphosphonates, as the decrease observed could be age-related.

Hypophosphatasia

Hypophosphatasia (HPP) is a rare inherited skeletal dysplasia caused by pathogenic variants in *ALPL*, the gene that encodes TNSALP. Disease-causing changes in *ALPL* reduce enzyme activity. Deficient enzyme activity leads to extracellular accumulation of the substrates, such as PLP and pyrophosphate. Pyrophosphate is a potent inhibitor of mineralization and its accumulation results in defective mineralization of bone and/or teeth, leading to musculoskeletal symptoms and dental abnormalities. Low serum ALP and elevated serum PLP suggest the diagnosis. Race-, gender- and age-specific reference intervals for PLP have also been proposed (427). A recent study suggested a threshold of 43 IU/L or less for ALP and 120 nmol/L or more for PLP to distinguish HPP from other metabolic bone diseases (Fig. 22) (428). The diagnosis can be confirmed by the identification of pathogenic variants in *ALPL* by genetic testing (429).

Low ALP is the hallmark of HPP diagnosis and bone ALP is also low (428). However, in some circumstances, such as after a fracture, ALP might not be low. Other causes for low ALP are recent cardiac surgery and cardiopulmonary bypass, malnutrition, magnesium deficiency, hypothyroidism, and severe anemia (430). Data on other BTMs are conflicting; while one study in adults has reported lower PINP and CTX in patients with HPP compared with controls (431), another study has reported no difference in OC and PINP and higher TRACP5b and CTX in HPP patients compared with osteopenic patients (the majority taking bisphosphonates) (428). The treatment of severe forms with asfotase alfa (a human recombinant TNSALP enzyme replacement therapy) was associated with a transient increase in TRACP5b (3 months), osteocalcin, and PINP (both at 3 and 6 months), consistent with an early phase of osteomalacia healing. These findings were followed by an increase in BMD T-score (432).

Conclusion. Low ALP is the hallmark of the diagnosis of HPP, the disease caused by a decrease in *ALP*. There is no robust data on other BTMs.

Other Systemic Diseases/Medications that Affect Bone

Glucocorticoid-induced osteoporosis

There are a variety of guidelines around the pharmacological management of glucocorticoid-induced osteoporosis (GIO). Most recommend supplementation with calcium and vitamin D and treatment with a bisphosphonate for high-risk patients (older, previous fragility fracture, steroids for more than 3 months) (316, 433, 434).

The use of BTMs in monitoring treatment in GIO is not well established (434). One problem is that glucocorticoids can affect the levels of BTMs. OC is rapidly decreased (within 24 hours) after the administration of glucocorticoids. In a study of young men, doses of 10, 15, or 20 mg of prednisone decreased OC to 83%, 78%, and 74% of baseline, respectively (5 mg of prednisolone had no significant effect). In subjects given 60 mg for 5 days, OC reached its nadir between 48 and 96 hours (435). Similar results were observed with intravenous steroids (436). An older study suggested that glucocorticoids could have a direct effect on the OC gene promoter, by antagonizing the active 1,25-dihydroxyvitamin D to induce this gene. Not even high concentrations of the active vitamin D could reverse the inhibition (437). Alkaline phosphatase seems to decrease in some (438, 439) but not all studies (440). PINP levels also decrease (441) but not as much as OC (442). PINP decreased in patients with rheumatoid arthritis taking steroids, but not in patients with polymyalgia rheumatica (PMR) although the doses were similar (443). In another study of patients with PMR, PINP decreased as a result of steroids (444). TRACP5b on the other hand, seems to be unaffected (438).

Older studies on bone resorption markers have been inconsistent (439, 440, 445). Urinary NTX was found to be increased and treatment with alendronate resulted in its decrease (446). CTX was decreased in more recent studies (441, 443, 444).

Conclusion. Overall, the use of BTMs in the setting of glucocorticoids can be complicated, especially during monitoring the treatment for osteoporosis and any drug holiday. If the dose of steroids remains unchanged, then BTMs could be used for monitoring therapy; the problem arises when there is a change in the dose of steroids.

Diabetes mellitus

Fracture risk is increased in diabetes mellitus, both type 1 (T1D) and type 2 (T2D) (447). This increase in the risk of fractures is not explained by reductions in BMD, as BMD is

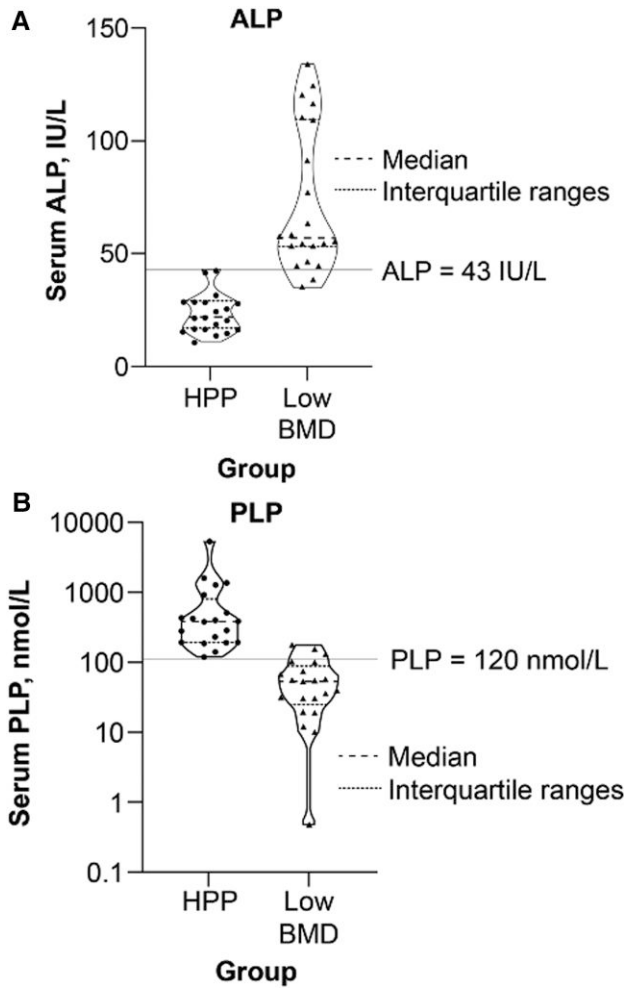


Figure 22. Violin plots of laboratory thresholds in the HPP and low-BMD groups. (A) All the patients with HPP had a total ALP activity of 43 IU/L or lower; 2 patients in the low-BMD group were below this threshold. (B) All patients with HPP had PLP above 120 nmol/L; 18 of 21 patients in the low-BMD group had PLP below 120 nmol/L. ALP, alkaline phosphatase; BMD, bone mineral density; HPP, hypophosphatasia; PLP, pyridoxal-5-phosphate. Reprinted from Bone, 144 Desborough, R., Nicklin, P., Gossiel, F., Balasubramanian, M., Walsh, J. S., Petryk, A., Teynor, M. & Eastell, R. Clinical and biochemical characteristics of adults with hypophosphatasia attending a metabolic bone clinic © 2021with permission from Elsevier (428).

only slightly decreased in T1D and paradoxically increased in T2D (448). Several studies have reported a decrease in BTMs in both T1D and T2D. In T1D, CTX and PINP were lower in patients with and without distal peripheral neuropathy when compared with controls, but not different between the 2 diabetic groups (449). Similar findings were reported for patients with and without microvascular disease (450). In patients with newly diagnosed T2D (treatment-naïve), PINP and OC correlated negatively with hemoglobin A1c. Both BTMs were lower in participants with T2D compared with controls without diabetes, while bone ALP was higher (451). Meta-analyses have summarized data on BTMs in diabetes; OC, PINP, and CTX were lower in patients with diabetes compared with controls (452). The analysis by diabetes subtype has shown lower levels of OC (452, 453) and CTX in T1D compared with controls, but no difference for TRACP5b, uNTX, PINP, or bone ALP (452). In T2D, OC (452, 453), PINP, TRACP5b, CTX, were lower than in controls, but not uNTX or bone ALP (452). A methodological study in vitro has shown no impact of high glucose on BTM measurements (453). Despite the effects of diabetes on bone turnover, the antidiabetic medication thiazolidinediones can increase bone turnover (454).

Conclusion. Overall, both T1D and T2D are associated with a decrease in bone turnover.

Hyperthyroidism

Hyperthyroidism is characterized by the overproduction of thyroid hormones. Symptoms include weight loss, increased heart rate, sweating, and irritability. Hyperthyroidism is a risk factor for osteoporosis and patients often present with low BMD (455). Thyroid hormone excess leads to an increase in osteoclast and osteoblast activity. Previous studies have shown that total ALP, bone ALP, OC, and CTX are increased in hyperthyroidism (455-457) and correlated positively with free thyroid hormones (456, 457). Hyperthyroidism treatment and normalization of thyroid function are followed by an increase in BMD and normalization of the BTMs (455).

The suppression of thyrotropin (TSH) by L-thyroxine (L-T4) for the treatment of differentiated thyroid cancer was associated with an increase in CTX (458, 459), uNTX, and OC (459) in postmenopausal women, but not in estrogen-sufficient women.

Conclusion. Overall, hyperthyroidism is associated with an increase in BTMs.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is an autoimmune inflammatory disease characterized by articular erosions, periarticular bone loss and several degrees of systemic inflammation (460). RA is associated with focal, juxta-articular, and systemic bone loss (460) and an increased risk of fragility fractures (461). Several pathological mechanisms are involved, leading to increased bone resorption and decreased bone formation. This imbalance in bone remodeling leads to bone loss. Bone loss in RA correlates with measurements of inflammation and functional status (460).

Studies in women with RA have shown a decrease in OC (462, 463) and an increase in uNTX (463-465). No difference was found for CTX and PINP between RA and osteoarthritic patients (465, 466) or healthy controls (464). While some studies showed a correlation between uNTX and erythrocyte sedimentation rate and C-reactive protein (467), others did not find correlations between BTMs and inflammation markers or cytokines (462, 463). Besides the effects of RA on bone turnover, it is important to bear in mind that RA is often associated with immobilization and glucocorticoid use, which also impact bone turnover as described above (468).

The effect of RA treatments on BTMs has also been investigated. Methotrexate use was associated with a decrease in uNTX in 6 months (469). In the same period, the use of the tumor necrosis factor (TNF)-blocker infliximab was associated with a 28% reduction on CTX but returned to baseline levels after 1-year follow-up (470). In contrast, no change in PINP was observed (470). Another TNF-blocker, etanercept was associated with an increase in bone ALP (16%) but no difference in uNTX after 6 months (471).

Conclusion. In RA, uNTX is the most investigated BTM; uNTX was higher in patients with RA, correlated with inflammation markers in some but not all studies and decreased with treatment with methotrexate. However, these findings have no clinical application.

Metastatic cancer

BTMs in the diagnosis of bone metastases. The skeleton is the most common site of metastases in breast cancer. Data from cross-sectional studies have shown that TRACP5b, PINP, and CTX were higher in patients with bone metastases compared to patients without metastases (472-474). In a prospective study, bone ALP, CTX, PINP, and TRACP5b were significantly higher in patients with bone metastases. The logistic regression analysis has shown that TRACP5b was the most accurate single marker 0.784 (95% CI, 0.651-0.916) in the early detection of bone metastases, but the combination of bone ALP + PINP + TRACP5b was the most accurate combination of BTMs with area under the receiver operating characteristic curve (AUROC) 0.889 (95% CI, 0.798-0.981) (475).

In another study that assessed ALP, bone ALP, OC, and CTX (serum, urinary nonisomerized [αuCTX]) and β isomerized [βuCTX]), all BTMs were higher in men with metastatic prostate cancer when compared with healthy controls and men with prostate cancer without metastases. The median was increased by 67% for OC, 128% for ALP, 138% for bone ALP, 220% for αuCTX, 149% for βuCTX, and 214% for serum CTX (79). In another study, PINP was elevated in 87% of patients with bone metastases from prostate cancer (476) and the increase in PINP levels in this group was detectable 8 months before the first positive bone scintigraphy (477). ALP was also increased in metastatic prostate cancer. A cutoff level of 100 IU/L showed 79% sensitivity and 88% specificity for the diagnosis of bone metastases and an AUROC of 0.9. Similar results were reported for bone ALP with a cutoff level of 30 IU/L (478). Bone ALP was also higher in other solid tumors with bone metastases compared to patients without metastases, as confirmed by a meta-analysis (479).

Prognostic role of BTMs in bone metastases. In a cross-sectional study that assessed treated patients with bone metastases, TRACP5b was lower in responders and cases of stable disease than those with disease progression. In this study, TRACP performed better than uNTX and ALP for clinical evaluation of bone metastases (474).

Retrospective analyses from trials of antiresorptive therapy suggested that BTMs might also have some prognostic role. Three large randomized trials of patients with bone metastases (breast, $n = 490$; prostate, $n = 411$; myeloma, $n = 210$; non-small cell lung, $n = 183$; other, $n = 168$) which used zoledronate or pamidronate have shown that both bone ALP and uNTX correlated with clinical outcomes. Urinary NTX levels were associated with adverse events (Fig. 23). Patients with high (≥100 nmol BCE/mmol creatinine) and moderate uNTX levels (50-99 nmol BCE/mmol creatinine) had a 2-fold increase in their risk of skeletal complications and disease progression compared with patients with low (<50 nmol BCE/mmol creatinine) uNTX levels ($P < 0.001$ for all). In each solid tumor category, high uNTX levels were associated with a 4- to 6-fold increased risk of death during the study, and moderate uNTX levels with a 2- to 4-fold increased risk compared with low uNTX levels ($P < 0.001$ for all). In addition, normal uNTX was associated with a 40% reduction in the risk of death and a 52% reduction in the risk of pathological fractures (480). High bone ALP also correlated with the occurrence of skeletal-related events (481, 482). In patients with bone metastases from castration-resistant prostate cancer, lung cancer, or other solid tumors who received placebo, both baseline and on-study elevations in bone marker levels were associated with increased risks of skeletal-related events, disease progression, and death (483) (Fig. 23). Exploratory analysis has shown that patients with aggressive skeletal disease and baseline uNTX ≥ 100 nmol BCE/mmol creatinine, significantly benefited from zoledronate treatment; there was a 31% reduction of the risk of death ($P = 0.0028$) which was independent from the prevention of skeletal complications (484) (Fig. 24).

BTM response to bisphosphonate treatment is associated with clinical outcomes. Data from the same 3 large trials have shown that after 3 months of antiresorptive treatment, reductions from baseline uNTX levels correlated with benefits. Moreover, the normalization of uNTX correlated with reduced risks of skeletal complications and death, compared with patients with persistently elevated levels of uNTX (486).

Conclusion. Despite several studies showing higher BTM levels in patients with bone metastases, most studies are characterized by suboptimal specificity, sensitivity, and diagnostic accuracy at an individual patient level. This limits the value of BTM use in the diagnosis and prognosis of bone metastases in clinical practice (487).

Chronic kidney disease–mineral bone disorder

Chronic kidney disease–mineral bone disorder (CKD-MBD) is a common complication of CKD. It is defined as a systemic disorder of bone and mineral metabolism due to CKD manifested by either one or a combination of the following: 1) abnormalities of calcium, phosphorus, PTH, or vitamin D metabolism; 2) abnormalities in bone turnover, mineralization, volume, linear growth, or strength; and 3) vascular or other soft tissue calcification (488).

Clinical importance. CKD-MBD is associated with increased risk of fracture, cardiovascular disease, and mortality. SHPT in CKD-MBD is a physiological change in response to biochemical abnormalities which worsen as CKD progresses, such as low calcitriol and hypocalcemia. However, uncontrolled SHPT may be harmful. A PTH level that is extremely high or extremely low is associated with increased mortality in dialysis patients (489). The lowest mortality risk is associated with full-length PTH (called *intact PTH*; iPTH) level between 100 and 600 ng/L. Hence, the Kidney Disease Improving Global Outcomes (KDIGO) Clinical Practice Guideline for the Diagnosis, Evaluation, Prevention, and Treatment of CKD-MBD recommends an iPTH target of 2 to 9 times the upper limit of normal for the assay in dialysis patients (490). There is no PTH target range for nondialysis CKD patients.

It is well known that PTH has a direct effect on bone where it stimulates bone turnover. This becomes more complex in SHPT due to CKD because there is skeletal resistance to PTH. Except for the extremely high or extremely low levels outside the target range previously mentioned, iPTH levels do not necessarily reflect the bone turnover status in advanced CKD.

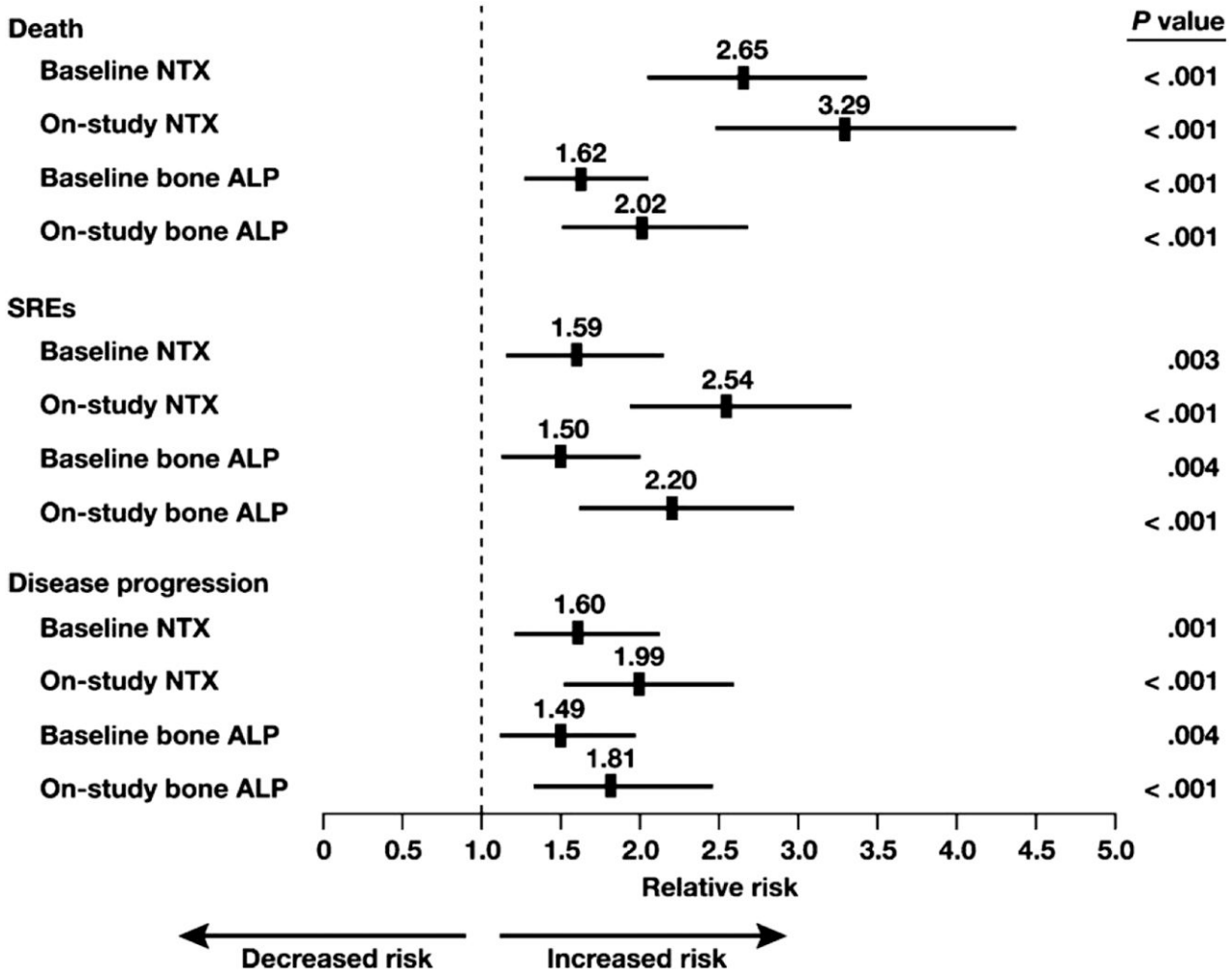


Figure 23. Correlations between elevated bone marker levels (NTX ≥ 100 nmol/mmol creatinine; bone ALP ≥ 146 U/L- upper limit of reference) and clinical outcomes in patients with bone metastases from solid tumors who did not receive bisphosphonate therapy. Abbreviations: bone ALP, bone-specific alkaline phosphatase; NTX, N-terminal cross-linked telopeptide of type I collagen; SRE, skeletal-related event. Reprinted from Crit Rev Oncol Hematol, 80(3) Coleman, R., Costa, L., Saad, F., Cook, R., Hadji, P., Terpos, E., Garnero, P., Brown, J., Body, J. J., Smith, M., Lee, K. A., Major, P., Dimopoulos, M. & Lipton, A. Consensus on the utility of bone markers in the malignant bone disease setting 411-32 © 2011 with permission from Elsevier (485).

Abnormal bone turnover can impact bone strength and increase the risk of fracture. CKD patients have a high risk of fracture, which is associated with increased morbidity and mortality. Several studies suggest that even minimal kidney impairment is associated with increased risk of bone loss and fracture (491, 492). The risks of hip fracture in advanced CKD and dialysis patients are 2.5 and 4 times higher, respectively, compared with those without CKD (493).

Bone biopsy in renal osteodystrophy. The term *renal osteodystrophy* (*ROD*) is used exclusively to define bone abnormalities associated with CKD; these are characterized by bone turnover and mineralization abnormalities seen on bone histomorphometry. The subtypes of ROD are shown in Table 13. Additionally, osteitis fibrosa is often associated with bone marrow fibrosis (Fig. 25a). *Adynamic bone disease* describes bone biopsy which shows absent bone turnover (ie, no tetracycline label uptake and no osteoclast or osteoblast present) (Fig. 25b). However, a spectrum of low to absent bone turnover is often categorized together as adynamic bone disease.

Abnormal bone turnover in CKD may be an important risk factor for fracture, as it may affect bone quality and quantity. However, a large bone biopsy series (N = 2507) showed no difference in fracture history between low and high bone turnover (494). So far, there is no large prospective study assessing the relationship between abnormal bone turnover and fracture incidence in advanced CKD.

ROD moves from one subtype to another under the influence of worsening CKD, worsening SHPT, PTH resistance in bone, and treatment such as phosphate binder, vitamin D, and calcimimetic (495-497). Repeated bone biopsy to monitor these treatment effects on bone turnover is almost impossible, mainly due to patient reluctance.

Parathyroid hormone. PTH measurement is routinely available and the normal reference range is well established. SHPT usually becomes apparent when estimated glomerular filtration rate (eGFR) is less than 45 mL/min/1.73 m^2 but it is now accepted that SHPT is a relatively late change in CKD-MBD compared to FGF23 and calcitriol (498).

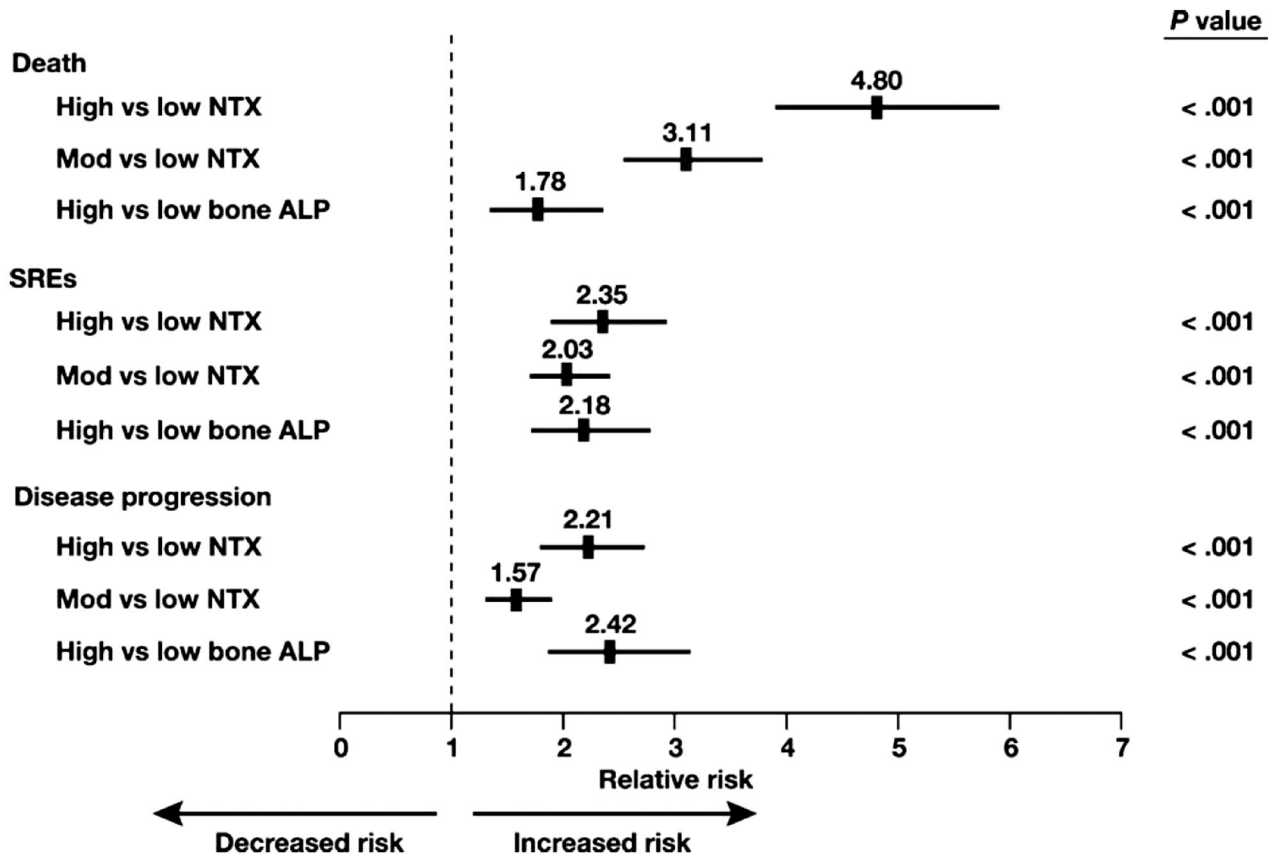


Figure 24. Correlations between bone marker levels and clinical outcomes in patients receiving bisphosphonate therapy (zoledronic acid 4 mg every 3-4 weeks) for bone metastases from solid tumors. High uNTX, ≥ 100 nmol/mmol creatinine; moderate uNTX, 50 to 99 nmol/mmol creatinine; low uNTX, <50 nmol/mmol creatinine. High bone ALP, ≥146 U/L; low bone ALP, <146 U/L (146 was the upper limit of reference range). Abbreviations: bone ALP, bone-specific alkaline phosphatase; Mod, moderate; SRE, skeletal-related event; uNTX, N-telopeptide of type I collagen. Reprinted from Crit Rev Oncol Hematol, 80(3) Coleman, R., Costa, L., Saad, F., Cook, R., Hadji, P., Terpos, E., Garnero, P., Brown, J., Body, J. J., Smith, M., Lee, K. A., Major, P., Dimopoulos, M. & Lipton, A. Consensus on the utility of bone markers in the malignant bone disease setting 411-32 © 2011 with permission from Elsevier (485).

Table 13. Classification of ROD subtypes based on bone turnover and mineralization abnormalities

ROD subtype	Turnover	Mineralization
Osteitis fibrosa	High	Normal
Mixed bone disease	High	Abnormal
Adynamic bone disease (ABD)	Low	Normal
Osteomalacia	Low	Abnormal

Abbreviation: ROD, renal osteodystrophy.

Raised serum PTH in CKD-MBD is due to a combination of increased secretion by parathyroid gland and accumulation of PTH fragments. PTH has 84 amino acids, with a molecular weight of 9500 Daltons. It has a short half-life of a few minutes once it is released from the parathyroid gland into the circulation. It is then metabolized in the liver and kidneys into 2 main fragments: the N-terminal and the C-terminal fragments. The N-terminal fragment is fully metabolized in the liver, while the C-terminal fragments are usually cleared by the kidneys, thus it accumulates in advanced CKD (499).

Various PTH assays have been developed over the last few decades with the aim of improving its sensitivity and specificity to detect the biologically active PTH molecule (Fig. 26). First-generation PTH assays detect either the N-terminal or the C-terminal end of the molecule, which also means that the whole molecule and its inactive fragments (mostly the C-terminal fragments) are measured. Second-generation assays measure the full-length, intact PTH (iPTH). However, the assays detect both the 1-84 PTH molecule and the 7-84 PTH fragment. This large amino-truncated PTH fragment was initially thought to be biologically inactive, but animal studies have shown that 1-84 PTH increases bone resorption when injected into parathyroidectomized rats, while 7-84 PTH fragments antagonize the effect of 1-84 PTH in bone (500, 501). This may explain why CKD patients seem to demonstrate skeletal resistance to PTH where bone turnover is suppressed, despite the high PTH level.

Third-generation PTH assays, also known as *whole PTH* assays, measure mainly 1-84 PTH molecules. The assays also detect a posttranslational modified form of PTH 1-84 in region 15 to 20, created by phosphorylation of a serine residue; this is known as nontruncated amino-terminal PTH (N-PTH) (502). N-PTH represents up to 15% of the PTH detected by the assays in advanced CKD. It is not yet clear whether the whole PTH assays will better predict underlying bone disease in CKD or patient-centered outcomes, such as

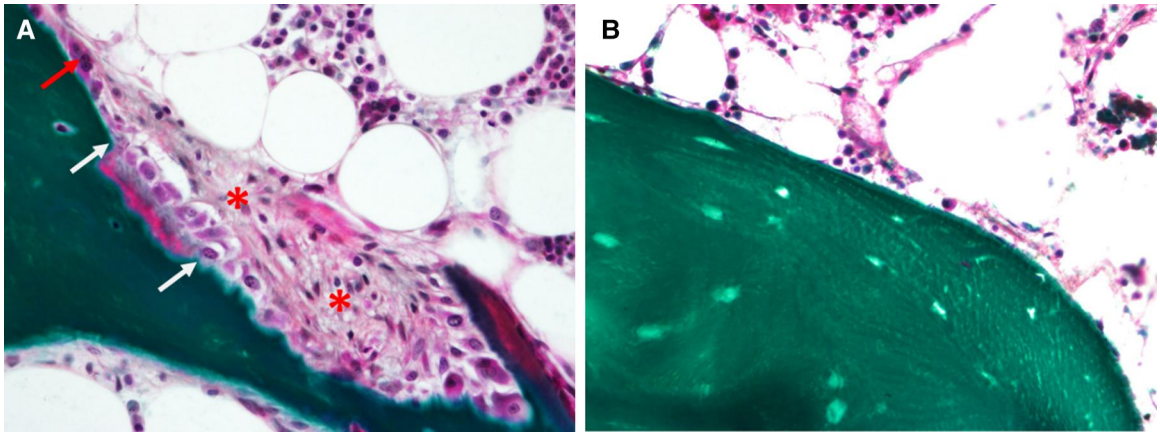


Figure 25. Bone histology sections stained with Masson Goldner trichrome at 20× magnification from (A) high and (B) low bone turnover ROD patients. (A) Osteitis fibrosa with areas of marrow fibrosis*, osteoclast (red arrow) and osteoblasts with underlying osteoid indicating bone formation (between white arrows); (B) Adynamic bone disease with absence of osteoclast and osteoblast. Image courtesy of Salam S, University of Sheffield 2022.

mortality, when compared with iPTH assays (503-505). Therefore, iPTH assays remain the commonly used in clinical practice, although there are clinically significant analytical differences in PTH concentrations between the commercially available assays (506).

BTMs for ROD diagnosis. PTH is a poor predictor of bone turnover in ROD, although extremely high (>600 ng/L) or extremely low (<100 ng/L) iPTH level may predict high or low bone turnover respectively in CKD (490). Most advanced CKD patients have iPTH values between those levels. Tables 2 and 3 show the inconsistency in the diagnostic accuracy of PTH, which ranged from poor to good. Whole PTH has not shown greater accuracy than iPTH when tested in the same study (507).

Salam et al showed that other BTMs such as bone ALP, intact PINP, and TRACP5b have significantly better diagnostic accuracy to diagnose low bone turnover ROD than iPTH, with areas under the curve (AUCs) ≥ 0.8 (105). These are also the BTMs which do not accumulate in advanced CKD, as they do not rely on renal clearance (508, 509). In contrast, the diagnostic accuracy of these BTMs are similarly suboptimal to iPTH (AUCs 0.7-0.8) for diagnosing high bone turnover ROD (105).

A larger study by Sprague et al (N = 450) also showed suboptimal diagnostic accuracy for iPTH, whole PTH, bone ALP, and total PINP for low and high bone turnover ROD, with AUCs <0.8 (507). It is important to note that Sprague et al only recruited dialysis patients whereas Salam et al recruited CKD stages 4 to 5 and dialysis patients. Despite the differences in CKD severity and sample size, it is unlikely that the diagnostic accuracy of these BTMs could be further improved by doing even larger studies. This is due to the measurement noise of these BTMs and the transiliac bone biopsy limitations as previously discussed. Tables 14 and 15 summarize the findings over the last decade for BTM diagnostic accuracy for low and high bone turnover ROD and its respective optimum cutoff values. Earlier studies have been extensively reviewed and summarized in the 2009 KDIGO CKD-MBD Clinical Practice Guideline (510).

Several practical considerations need to be considered when using BTMs in advanced CKD. BTMs such as serum CTX, NTX, and OC are known to accumulate in advanced CKD and decline significantly after a hemodialysis session (514, 515). Urine NTX is not useful in this patient population due to the impaired renal clearance and the test is not feasible to perform in dialysis patients who are often anuric. Although several studies have shown that these BTMs were associated with low BMD or bone loss in advanced CKD, they are not useful as a diagnostic tool for bone turnover status in ROD (516-519).

Intact PINP is a useful diagnostic test, whereas total PINP is not as robust. This is because the intact PINP assay measures the trimeric PINP whereas the total PINP assay measures the trimeric and monomeric fragments of PINP (105, 507, 512). The monomeric fragments accumulate in advanced CKD (520). Therefore, intact PINP is the preferred assay for advanced CKD rather than total PINP.

Care needs to be taken when interpreting the cutoff values for bone ALP. The cutoff values for low and high bone turnover are different between assays that measure the enzyme activity and enzyme quantity (121). Although bone ALP is the most reliable BTM studied in advanced CKD so far, it is also a marker of bone mineralization and its level could fall with vitamin D supplementation (521). Concurrent high bone ALP level and 25-hydroxyvitamin D deficiency is suggestive of mineralization abnormalities. Therefore, assessment of bone turnover status using bone ALP should be avoided in those with 25-hydroxyvitamin D deficiency. In the absence of vitamin D deficiency, bone ALP > 20 µg/L or > 30 U/L are useful cutoff values for ruling out low bone turnover ROD.

Finally, combining BTMs or combining a BTM with PTH (intact or whole) did not show improvement in diagnostic accuracy to diagnose low or high bone turnover ROD (105, 507). Based on the available evidence, we would recommend bone ALP as the BTM of choice in advanced CKD for the diagnosis of low or high bone turnover ROD while intact PINP and TRACP5b are complimentary.

BTMs and fracture. It is well known that higher levels of BTMs such as bone ALP, PINP, TRACP5b, CTX, and OC are associated with low BMD and bone loss in CKD, dialysis, and kidney transplant patients (522, 523). However, the findings

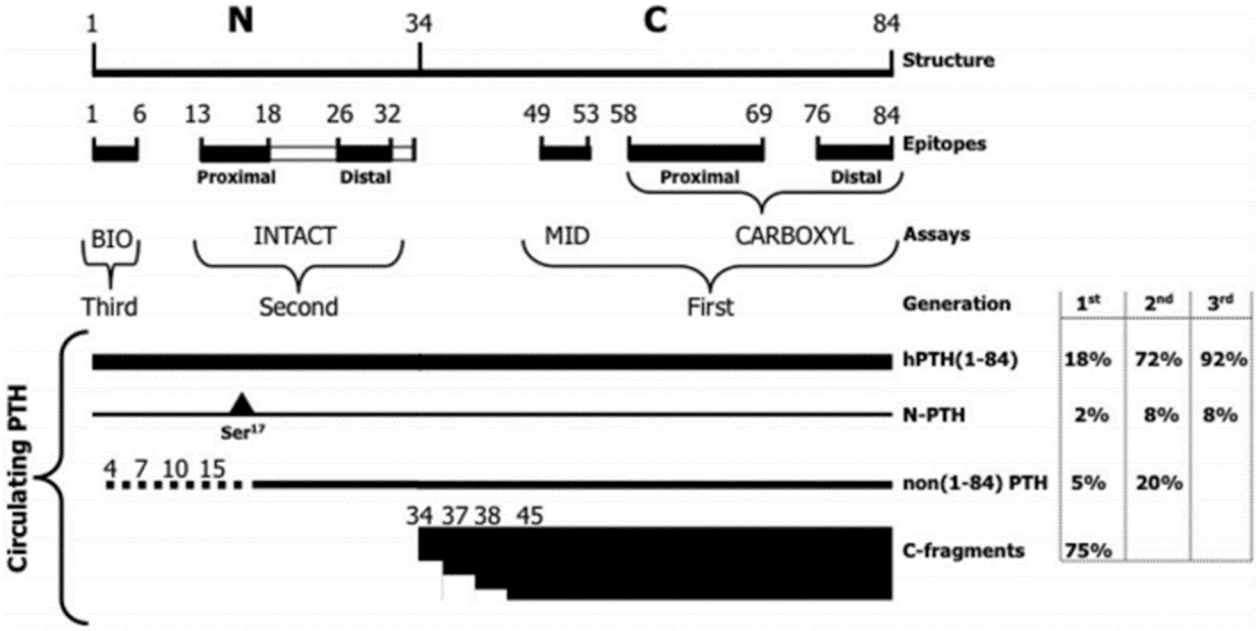


Figure 26. Circulating PTH molecular forms measured by the three generations of PTH assays. R. Eastell, M. L. Brandi, A. G. Costa, P. D'Amour, D.-M. Shoback and R. V. Thakker. Diagnosis of asymptomatic primary hyperparathyroidism: proceedings of the Fourth International Workshop. J Clin Endocrinol Metab 2014 Vol. 99 Issue 10 Pages 3570-9. By permission of Oxford University Press (502). Abbreviation: PTH, parathyroid hormone.

have been inconsistent for the relationship between BTMs and fracture in this population.

BTMs such as bone ALP, intact PINP, OC, TRACP5b, CTX, and NTX are not robust diagnostic tools to discriminate CKD or end-stage kidney disease patients with prevalent fractures (523, 524). These BTMs also failed to predict incident fractures in kidney transplant recipients who were followed up for 5 years (525). This could be because bone disease and fracture risk post kidney transplantation are even more complex and do not just relate to the bone turnover status at the time of kidney transplantation. CKD-MBD complications could last for many years after transplantation in addition to the effects of immunosuppression treatment such as glucocorticoids and calcineurin inhibitors on bone (526-528).

Similarly, BTMs failed to predict incident fracture in hemodialysis patients who were followed for 5 years and had monthly BTM measurements (529). However, the study showed that the bone ALP level just before the fracture event had the highest AUROC (0.77) for predicting incident fracture. This study suggests that bone ALP with a cutoff value of >20 µg/L is a predictor of impending fracture. Coincidentally, this is also the cutoff level for ruling out low bone turnover ROD (105, 530). Whether low bone turnover ROD is protective against fracture remains unknown. Fracture in advanced CKD is a result of complex interactions between abnormal bone turnover, low BMD, poor bone microstructure, and frailty.

BTM and bone-specific treatment. Patients with mild to moderate CKD without obvious CKD-MBD biochemical abnormalities (such as SHPT) are less likely to have overt ROD. The safety and efficacy of osteoporosis treatment to improve BMD and reduce fracture risk in this group are similar to those without CKD (531-534).

In contrast, patients with advanced CKD (ie, eGFR < 30 mL/min/1.73 m^2) or on dialysis, with CKD-MBD biochemical abnormalities, are more likely to have ROD. CKD-MBD management so far has focused on controlling SHPT and administering vitamin D supplementation in those with vitamin D deficiency or insufficiency (490). However, there is no evidence from interventional study to show that these approaches reduce fracture risk (535).

Bone-specific treatment to reduce fracture risk in advanced CKD was previously limited as bisphosphonates are contraindicated in patients with eGFR <30 mL/min/1.73 m^2. This is because bisphosphonates have been associated with worsening kidney function in those with CKD stages 3 to 5 (536). Denosumab, a monoclonal antibody to RANKL, has overcome this limitation as it is not contraindicated in advanced CKD (537). Since it is an antiresorptive treatment which suppresses bone turnover, denosumab should be avoided in patients with preexisting low bone turnover.

The effects of denosumab on BTMs in advanced CKD are as expected from its antiresorptive mechanism. Block et al showed a rapid fall in CTX within 48 hours of giving denosumab in all stages of CKD and dialysis patients (537). Meanwhile, small randomized controlled trials of denosumab in hemodialysis patients and kidney transplant recipients over 12 months also showed that BTM levels fell and remained suppressed throughout (538, 539).

Teriparatide (described above) is an anabolic agent that stimulates bone turnover and thus, should be avoided in those with preexisting high bone turnover ROD. Teriparatide is currently not licensed in advanced CKD but a post hoc analysis of a postmarketing surveillance study of teriparatide over 24 months in 33 Japanese women with high risk of fracture and CKD stages 4 to 5 showed no additional safety concerns (540). Six patients had available PINP measurements, which increased 2.5 times from baseline levels after 3 months.

Table 14. Diagnostic accuracy for PTH and BTMs in diagnosing low vs non–low bone turnover ROD in CKD 4-5D in the last decade

Biomarkers	Study	Population, sample size	AUC*	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Optimum cutoff level
iPTH	Sprague et al 2016 (507)	HD, N = 450	0.70					104 ng/L
	Salam et al 2018 (105)	CKD 4-5D, N = 43	0.56	70	53	32	85	183 ng/L
	Nickolas et al 2020 (511)	CKD 3-5D, N = 23	0.84					
	Ursem et al 2021 (512)	Dialysis, N = 31	0.79					
Whole PTH	Haarhaus et al 2015 (513)	HD, N = 40	0.85					
	Sprague et al 2016 (507)	HD, N = 450	0.71					48 ng/L
	Jorgensen et al 2021 (106)	CKD 4-5D, N = 80	0.82					
Bone ALP	Haarhaus et al 2015 (513)	HD, N = 40	0.89					
	Sprague et al 2016 (507)	HD, N = 450	0.76					33 U/L
	Salam et al 2018 (105)	CKD 4-5D, N = 43	0.82	89	77	53	96	21 µg/L
	Nickolas et al 2020 (511)	CKD 3-5D, N = 23	0.78					
	Ursem et al 2021 (512)	Dialysis, N = 31	0.83					
	Jorgensen et al 2021 (106)	CKD 4-5D, N = 80	0.94					
Intact PINP	Salam et al 2018 (105)	CKD 4-5D, N = 43	0.79	80	75	50	92	57 ng/mL
	Jorgensen et al 2021 (106)	CKD 4-5D, N = 80	0.89					
Total PINP	Sprague et al 2016 (507)	HD, N = 450	0.65					499 ng/mL
	Salam et al 2018 (105)	CKD 4-5D, N = 43	0.72	80	68	44	91	124 ng/mL
	Ursem et al 2021 (512)	Dialysis, N = 31	0.86					
TRACP5b	Salam et al 2018 (105)	CKD 4-5D, N = 43	0.80	89	71	47	96	4.6 U/L
	Ursem et al 2021 (512)	Dialysis, N = 31	0.85					
	Jorgensen et al 2021 (106)	CKD 4-5D, N = 80	0.93					

Abbreviations: ALP, alkaline phosphatase; AUC, area under the receiver operating characteristic curve; BTM, bone turnover marker; CKD, chronic kidney disease; HD, hemodialysis; iPTH, intact parathyroid hormone; NPV, negative predictive value; PINP, procollagen type 1 N-propeptide; PPV, positive predictive value; PTH, parathyroid hormone; ROD, renal osteodystrophy; TRACP5b, tartrate-resistant acid phosphatase type 5b.
*The diagnostic accuracy classification based on AUROC is as follows: 0.6-0.7 is poor, 0.7-0.8 is fair, 0.8-0.9 is good and 0.9-1.0 is excellent.

Several small studies have reported teriparatide use in dialysis patients with low bone turnover (541-544). Cejka et al showed no difference in BTMs between baseline and at 6-month follow-up assessment (541). However, Yamamoto et al showed a 20% increase in bone ALP while the OC level nearly doubled in the first month of treatment (543). This is also supported with a study by Sumida et al, which showed that bone ALP, OC, and PINP increased significantly from baseline and these elevated levels were maintained for at least 24 weeks (544). Meanwhile, a double-blind, placebo-controlled study in kidney transplant recipients over 6 months found no difference in bone ALP, OC, and CTX between the teriparatide and placebo groups (545). This lack of change was also confirmed on bone biopsy histomorphometry.

Although it is now possible to use these treatment options in advanced CKD (licensed or off-licensed use) with BTM evidence supporting the drug mechanism, evidence on their fracture risk reduction in this population is limited (546, 547). Large randomized controlled trials of denosumab and teriparatide have largely excluded advanced CKD patients with SHPT. Large interventional trials using these agents tailored to individuals' bone turnover status to assess fracture outcome remain elusive. In the absence of this crucial evidence, a European consensus statement on the use of these bone-specific treatment in CKD stages 4 to 5D patients has been published by the European Renal Osteodystrophy (EUROD) Initiative and the committee of Scientific Advisors and National Societies of the IOF (548). This document aims to promote a cohesive approach to treatment in this population with high fracture risk and complex metabolic bone disease. Careful risk and benefit assessment in a multidisciplinary approach involving nephrologists and metabolic bone physicians is recommended.

The use of bone-specific treatment tailored to individual bone turnover status seems to be the most sensible approach, consistent with personalized medicine. However, this concept remains divisive, as patients may be denied bone-specific treatment without a bone biopsy. This is where BTMs have a role in ruling out low bone turnover patients before starting an antiresorptive and conversely, ruling out those with high bone turnover before starting an anabolic treatment. Similar to bone biopsy, BTMs should only be used if knowledge of the bone turnover will impact treatment decisions. Routine use is not recommended (490).

Table 15. Diagnostic accuracy for PTH and BTMs in diagnosing high vs non–high bone turnover ROD in CKD 4-5D in the last decade

Biomarkers	Study	Population, sample size	AUC*	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Optimum cutoff level
iPTH	Sprague et al 2016 (507)	HD, N = 450	0.72					323 ng/L
	Salam et al 2018 (105)	CKD 4-5D, N = 43	0.76	53	96	90	75	327 ng/L
	Ursem et al 2021 (512)	Dialysis, N = 31	0.80					
								61 ng/L
Whole PTH	Sprague et al 2016 (507)	HD, N = 450	0.68					
	Jorgensen et al 2021 (106)	CKD 4-5D, N = 80	0.66					
Bone ALP	Sprague et al 2016 (507)	HD, N = 450	0.71					42 U/L
	Salam et al 2018 (105)	CKD 4-5D, N = 43	0.75	56	83	69	74	31 µg/L
	Ursem et al 2021 (512)	Dialysis, N = 31	0.91					
	Jorgensen et al 2021 (106)	CKD 4-5D, N = 80	0.78					
Intact PINP	Salam et al 2018 (105)	CKD 4-5D, N = 43	0.77	53	92	82	74	107 ng/mL
	Jorgensen et al 2021 (106)	CKD 4-5D, N = 80	0.84					
Total PINP	Sprague et al 2016 (507)	HD, N = 450	0.74					621 ng/mL
	Salam et al 2018 (105)	CKD 4-5D, N = 43	0.73	75	68	60	81	142 ng/mL
	Ursem et al 2021 (512)	Dialysis, N = 31	0.86					
TRACP5b	Salam et al 2018 (105)	CKD 4-5D, N = 43	0.71	81	58	57	82	4.6 U/L
	Ursem et al 2021 (512)	Dialysis, N = 31	0.88					
	Jorgensen et al 2021 (106)	CKD 4-5D, N = 80	0.75					

Abbreviations: ALP, alkaline phosphatase; AUC, area under the receiver operating characteristic curve; BTM, bone turnover marker; CKD, chronic kidney disease; HD, hemodialysis; iPTH, intact parathyroid hormone; NPV, negative predictive value; PINP, procollagen type 1 N-propeptide; PPV, positive predictive value; PTH, parathyroid hormone; ROD, renal osteodystrophy; TRACP5b, tartrate-resistant acid phosphatase type 5b.

Conclusion

BTMs have a noninvasive role in diagnosing ROD subtypes, thus they may assist treatment decisions to reduce fracture risk in advanced CKD.

Summary

BTMs have proven useful in several clinical settings. Their use requires robust reference ranges relevant to the patient and knowledge of the sources of variability. Assays are now available that are easily available on automated platforms and inexpensive (€20 in Europe). They have also proven to be very useful in the development of new drugs for diseases such as osteoporosis.

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Conflict of Interest

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