Remodeling is essential for bone health. It begins with resorption of old bone by osteoclasts, followed by the formation of new bone by osteoblasts. Remodeling is coupled (formation is linked to resorption). After middle age or perhaps beginning earlier, bone loss occurs because resorption exceeds formation. This imbalance is accentuated by estrogen deficiency as well as by many diseases and conditions. Biochemical markers that reflect remodeling and can be measured in blood or urine include resorption markers (e.g., collagen cross-links) and formation markers (e.g., alkaline phosphatase). Bone markers exhibit substantial short-term and long-term fluctuations related to time of day, phase of the menstrual cycle, and season of the year, as well as diet, exercise, and anything else that alters bone remodeling. These biological factors, in addition to assay imprecision, produce significant intra- and interindividual variability in markers.

Bone marker measurements are noninvasive, inexpensive, and can be repeated often. Unfortunately, most of the studies that provided insight on clinical situations did not focus on markers as a primary endpoint. Bone markers have been useful in clinical practice and have been helpful in understanding the pathogenesis of osteoporosis and the mechanism of action of therapies. In clinical trials, markers aid in selecting optimal dose and in understanding the time course of onset and resolution of treatment effect. Clinical questions that might be answered by bone markers include diagnosing osteoporosis, identifying “fast bone losers” and patients at high risk of fracture, selecting the best treatment for osteoporosis, and providing an early indication of the response to treatment. Additional information is needed to define specific situations and cut points to allow marker results to be used with confidence in making decisions about individual patients.

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Biochemical markers that reflect the remodeling process and can be measured in blood or urine fall into three categories: (a) enzymes or proteins that are secreted by cells involved in the remodeling process, (b) breakdown products generated in the resorption of old bone, and (c) byproducts produced during the synthesis of new bone. Because of the phenomenon of coupling, these markers reflect the general process of bone turnover when bone is in a steady state; however, markers are usually classified by the part of the remodeling process that they mainly reflect in acute situations (i.e., resorption or formation). Because the process of resorption is shorter than the process of formation, resorption markers respond faster to changes in remodeling than do formation markers.

**Bone Resorption Markers**

Bone resorption markers include an enzyme, tartrate-resistant acid phosphatase (TRAP), and products of bone breakdown, which include calcium and bone matrix degradation products such as hydroxyproline, pyridinium cross-links, and telopeptides (Table 1).

Urinary calcium is affected by diet and renal function and is not sufficiently sensitive or specific for assessment of bone remodeling.

**TRAP**

Acid phosphatase is a lysosomal enzyme found in bone, prostate, platelets, erythrocytes, and spleen. Of the five isoenzymes of acid phosphatase, the bone isozyme is tartrate resistant (TRAP) but unstable. TRAP can be measured in serum or plasma by electrophoresis (after treatment with tartrate) or by immunoassay. Serum acid phosphatase concentrations are typically higher than those in plasma because of the release of acid phosphatase from erythrocytes during clotting.

**Collagen Breakdown Products**

Type 1 collagen, rich in the amino acid hydroxyproline, has a triple helix structure, with strands connected by cross-links between lysine or hydroxylysine residues that join the nonhelical amino- and carboxy-terminal ends of one collagen molecule to the helical portion of an adjacent molecule (1). The cross-links are pyridinolines and deoxypyridinolines (Fig. 2). During bone resorption, hydroxyproline and the pyridinium cross-links may be released either free or with fragments of the collagen molecule attached. They are not reutilized. Although some type 1 collagen is present in nonskeletal tissues,
bone has a much higher proportion and a much higher turnover.

**Hydroxyproline.** Collagen is rich in the amino acid proline, which undergoes posttranslational hydroxylation to hydroxyproline. Most of the free hydroxyproline liberated from bone is catabolized in the liver; ~10% is released in small polypeptide chains that are excreted in the urine. Hydroxyproline is also liberated by the breakdown of complement and nonskeletal collagen, including dietary collagen, and by the breakdown of procollagen extension peptides, which are products of bone formation. Approximately 50% of urinary hydroxyproline is derived from bone collagen breakdown (2). Hydroxyproline is usually measured in urine by colorimetry or HPLC after hydrolysis to convert peptide and polypeptide forms to the free form.

**Pyridinium cross-links (pyridinoline and deoxypyridinoline).** Posttranslational modification of lysine and hydroxylsine produces the nonreducible pyridinium cross-links, pyridinoline (Pyr) and deoxypyridinoline (Dpd), that stabilise mature collagen. Both Pyr and Dpd are released from bone in a ratio of approximately 3:1. Dpd is relatively specific for bone; Pyr is also found in articular cartilage and in soft tissues (ligaments and tendons). Approximately 60% of the cross-links released during resorption are bound to protein, with the remaining 40% being free (not protein bound). Pyridinium cross-links are not metabolized or absorbed from the diet (3). Pyr and Dpd can be measured in urine by HPLC or immunoassay (4–8) either before or after hydrolysis.

**Cross-linked telopeptides.** In the process of bone resorption, amino- and carboxy-terminal fragments of collagen are released with cross-links attached. These fragments with attached cross-links are called telopeptides. N-telopeptides (NTx) and C-telopeptides (CTx) are excreted in the urine. NTx is measured by immunoassay using an antibody to the α-2 chain of the NTx fragment (which contains the pyridinium cross-links, but the assay does not recognize the cross-link itself) (9). CTx is measured by immunoassay (10). Urine has been the most convenient sample for assay, but efforts have been directed at developing serum assays (11–14).

### Bone Formation Markers

Bone formation markers include an enzyme (alkaline phosphatase) and three byproducts of bone matrix synthesis (osteocalcin and amino- and carboxy-terminal procollagen I extension peptides; Table 2).

#### Alkaline Phosphatase

Osteoblasts are rich in alkaline phosphatase; however, alkaline phosphatase, an enzyme associated with the plasma membrane of cells, is also found in liver, intestine, and placenta (15), all of which may contribute to the total amount of alkaline phosphatase found in blood. The bone isoenzyme predominates in childhood and particularly during puberty; however, in adults the bone and liver isoenzymes contribute approximately equally to the total, with the intestinal fraction accounting for <10%. The function of alkaline phosphatase is unknown. The condition hypophosphatasia, in which the enzyme is lacking, is characterized by osteomalacia, suggesting that alkaline phosphatase has a role in the mineralization of newly formed bone. Measurement of total serum alkaline phosphatase is useful when the amount from bone is exce...
tionally high (such as in Paget disease of bone) and concentrations from other sources are not increased and are stable. Because of the multiple sources of origin and the fact that the bone isoform is usually not increased in osteoporosis and other metabolic bone diseases, total alkaline phosphatase has not enjoyed widespread use as a bone remodeling marker.

Bone, liver, and intestinal isoforms of alkaline phosphatase are posttranslational modifications of the same gene product and can be identified by their unique carbohydrate content (16). Measurement of “fractionated” alkaline phosphatase recognizes that heating destroys the skeletal fraction, which can be determined by subtraction of the stable fraction from the total. This procedure is not sufficiently reproducible to be used clinically. Assays for bone alkaline phosphatase [BAP; also known as bone-specific alkaline phosphatase, or skeletal alkaline phosphatase (SAP)] have been developed using electrophoresis, isoelectric focusing, lectin precipitation, and immunoassay techniques. Immunoassay is the method of choice because of high specificity and satisfactory precision. Commercially available immunoassays have been developed that measure either enzyme activity or mass (17–19). Because BAP is cleared by the liver, the skeletal fraction may be increased in patients with liver diseases. There may also be some cross-reaction of BAP antibodies with liver alkaline phosphatase.

OSTEOCALCIN
Osteocalcin, the major noncollagen protein of bone matrix, is a small 49-amino acid protein that is rich in glutamic acid (GLA) (20). Osteocalcin is also known as bone GLA protein and BGP. In addition to bone, it is also found in dentin. The function of osteocalcin is not clear; it may serve as a site for hydroxyapatite crystals. In the process of matrix synthesis, some osteocalcin is released and circulates in blood with a short half-life determined mainly by renal clearance. Although no intact osteocalcin is released during bone resorption, fragments are released in vitro and also during resorption and formation (Fig. 3) (21–23). Osteocalcin can be measured by immunoassay in plasma or serum. Osteocalcin is labile in blood. It is reduced in lipemic serum because of binding of osteocalcin to lipids, and osteocalcin may be degraded in vitro by proteolytic enzymes liberated from erythrocytes. Assays for osteocalcin are not standardized (24), and different antibodies clearly recognize different fragments (25, 26). Antibodies that recognize both the intact molecule and the large N-terminal midmolecule fragment appear to provide the best clinical information (27).

Although vitamin K status does not affect the total osteocalcin concentration, it does affect the amount of carboxylation. Undercarboxylated osteocalcin may be a better predictor of certain outcomes such as fracture (28, 29).

### Problems with Markers

The ideal marker would have no short-term biologic variability (i.e., stable over at least several days or weeks). The assay would be simple and automatable. There would be a reliable synthetic standard. There would be little or no assay imprecision or interference. The marker could be measured in a convenient nonfasting blood sample or random urine. It would respond rapidly and dramatically to relevant diseases and treatments.

Unfortunately, the ideal marker does not exist. Although changes in remodeling can be extreme, as in Paget disease or renal osteodystrophy, the changes are usually rather subtle, as in osteoporosis.

### PROCOLLAGEN EXTENSION PEPTIDES

Osteoblasts secrete large procollagen molecules that undergo extracellular cleavage at the amino and carboxy termini. Byproducts of type 1 collagen synthesis are the amino- and carboxy-terminal procollagen 1 extension peptides (PINP and PICP) (14, 30–33). PINP is an elongated protein of 35 kDa. PICP is a globular protein of 1000 kDa and contains disulfide bonds. Both extension peptides are cleared by the liver. Both may be incorporated into bone matrix. Both can be measured by immunoassay. The concentrations of both increase with increased turnover of nonskeletal collagen (e.g., skin and muscle).

### FACTORS RESPONSIBLE FOR VARIABILITY AND FLUCTUATIONS IN BONE MARKERS

Bone remodeling varies in a diurnal rhythm; changes with the phase of the menstrual cycle and the season of the year; is altered by bed rest, exercise, and extremes of diet; and basically is affected by anything that alters bone remodeling. Neither baseline nor posttreatment values for bone markers in the “normal” population follow a gaussian distribution. An individual’s rate of remodeling may vary over time.

Urinary excretion of Dpd is 50–70% higher at night.
than in the morning (34, 35). Similar fluctuations are seen for other resorption markers. Diurnal variation is less of a factor for alkaline phosphatase (36) and osteocalcin (37) because they have longer half-lives. Diurnal change is not influenced by posture, age, menopause, or osteoporosis (38). The day-to-day variation is ~10% for formation markers and 20% for resorption markers. During the menstrual cycle, marker concentrations are slightly higher in the luteal phase (39). There can be a seasonal change of up to 12%, with values higher in winter than summer (40). Marker concentrations increase during puberty and again after menopause. They are low in late pregnancy (41). After fracture, marker concentrations go up 20–60% and remain high for 6 months or more. With weightlessness or prolonged bed rest, markers increase by 40–50% (42), but the patterns of recovery vary depending on the marker (43).

Markers are only relatively specific for bone. Alkaline phosphatase is derived from nonskeletal sources, and osteocalcin fragments may reflect both resorption and formation. Osteocalcin and BAP give discordant results in patients with osteoporosis or imbalances in bone remodeling, such as Paget disease and renal osteodystrophy (44).

Metabolism and the clearance of markers influence their concentrations. For example, the proportions of different fragments of osteocalcin depend on renal function. Liver clearance affects BAP; renal clearance affects NTx, CTx, and pyridinium cross-links (45). Another factor affecting urinary bone markers, which are usually normalized to creatinine, is the variability of creatinine excretion (46).

When there is a change in the rate of remodeling, resorption markers fall faster than formation markers (2–12 weeks for resorption markers, 3–6 months for formation markers) because of the shorter time of resorption than formation.

**General Uses of Bone Markers**

Markers can be used in both generalized disorders of bone remodeling, such as osteoporosis or osteogenesis imperfecta, or in localized disorders of bone turnover, such as Paget disease and cancer metastases (47). Because of coupling, a single marker gives useful information. When remodeling rates are changing, a combination of markers, such as one resorption marker and one formation marker, might give more information than a single marker. Eastell et al. (48) have suggested normalizing resorption and formation markers as z-scores and expressing the ratio of a resorption and a formation marker as a "coupling index".

Multiple or duplicate measurements can be used to minimize the effect of intra-individual variation. Another approach is calculation of the "least significant change" or "critical difference", which incorporates the biological and analytical variation (49). At \( P < 0.05 \), using a one-tailed approach, the least significant change is 2.33 times the individual CV. It is in the range of 15% for BAP (50) and osteocalcin (51), and 25–40% for Pyr (49, 51, 52), Dpd (49, 51, 52), and NTx (53, 54).

**Clinical Applications**

Clinical questions that might be answered by the use of bone markers include the following: Which patients have low bone mass? Which patients are likely to be losing bone? Is this patient at a high risk of fracture? If treatment is needed, what treatment would be best? Is the patient responding to treatment?

**Which Patients Have Low Bone Mass?**

Although bone is a dynamic tissue, studies that have examined the relationship between turnover markers and bone density in young individuals have shown either a weak correlation or none at all (55, 56). The relationship is somewhat stronger in elderly women, but not strong enough to allow the use of a bone marker measurement to identify individuals with low bone mass (57, 58).

**Which Patients Are Likely to Lose Bone?**

At least two studies have suggested that change in bone mass over time correlates with the concentrations of markers (59, 60). In both of these studies, markers were measured at the end of the observation period. In a prospective study, Chesnut et al. (61) found a modest correlation between baseline urine NTx and the rate of bone loss during the following year in recently menopausal women (Fig. 4). However, no correlation has been seen between baseline bone markers and future bone loss in large prospective studies such as the Postmenopausal Estrogen-Progestin Intervention (PEPI) (62), the Fracture Intervention Trial (63), the Phase III alendronate study (Fig. 5) (64), and other prospective trials (65, 66).
IS THIS PATIENT AT HIGH RISK OF FRACTURE?
A French study, Epidemiologie de L’osteoporose (EPIDOS), evaluated 7598 elderly women and showed correlations between high concentrations of the resorption markers urine CTx and free Dpd and increased hip fracture risk similar in magnitude to that between low hip bone mineral density (BMD) and increased hip fracture risk (67). For urine CTx more than 2 SD above the premenopausal mean, the sensitivity in predicting hip fracture was 36% and the specificity was 81% (64% false negatives and 19% false positives); however, the positive predictive value was only 3%. The correlation was not seen for all resorption markers and was not seen at all for formation markers. Similar findings for the resorption markers total Pyr, free Pyr, total Dpd, and free Dpd in relation to hip fracture emerged from the Rotterdam Study (68), which involved 10,275 men and women 55 years and older. In EPIDOS (67), the combination of low hip BMD and high resorption marker concentration gave greater predictive value for hip fracture than either risk factor alone. However, the number of patients in EPIDOS who fell into the high-risk categories for both of these variables was small (only 16% of the total sample). A relationship between previous fractures and increased Pyr and osteocalcin was seen in a cross-sectional study of 351 women in Rochester, MN (56). Most of the Rochester women with osteoporosis had high bone turnover.

IS THE PATIENT RESPONDING TO TREATMENT?
Chesnut et al. (61) found a fairly strong relationship \( r = 0.25; P < 0.01 \) between baseline urine NTx and BMD response to 1 year of hormone replacement therapy in recently menopausal women (Fig. 6). Greenspan et al. (70) found a similar relationship between urine NTx and BMD response to alendronate. However, other investigators have failed to find consistent correlations between baseline marker concentrations and changes in BMD after treatment with estrogen (62) or alendronate (Fig. 7) (63, 64, 71).

If baseline markers fail to predict changes in BMD with treatment, perhaps changes in the concentrations of markers soon after initiation of treatment would predict later changes in BMD. Women receiving hormone replacement

**Fig. 5.** Relationship between change in spinal BMD and urine Dpd in women receiving calcium only for 3 years.
Based on data from Hirsch et al. (64).

**Fig. 6.** Relationship between change in spinal BMD and urine NTx in women receiving estrogen for 1 year.
Based on data from Chesnut et al. (61).

**Fig. 7.** Relationship between change in BMD and urine Dpd in women receiving 10 mg of alendronate daily for 3 years.
Based on data from Hirsch et al. (64).
therapy who had the greatest decline in NTx at 6 months had the greatest increase in BMD at 1 year (61). A 30% decrease in NTx at 6 months had 80% sensitivity and 59% specificity, with 80% positive and 42% negative predictive values. However, the range of change in urine NTx from baseline to 6 months in the treated group was +192% to -87%. In the same study, correlations were also seen between 6-month changes in free Dpd and BAP and an increase in BMD at 1 year (72). However, these relationships were not confirmed with hormone replacement therapy in the PEPI trial (62). Changes in marker concentrations have been shown to correlate with increases in BMD after alendronate treatment in some studies (70, 73) and with ibandronate (74). The correlations, however, are too weak to use markers to identify “high gainers” vs “low gainers”. Of interest, at least with bisphosphonate treatment, is that total and bound Pyr and Dpd decrease substantially but free Pyr and Dpd do not (75).

Different markers exhibit different degrees of change with bisphosphonate therapy (76). NTx showed the greatest decline (58%), but also had the greatest long-term variability (29.5%). BAP was the marker that showed greater than the minimum significant change in the highest number of patients (74%), compared with 57% of patients using NTx or 48% of patients using free Dpd.

There are no published data on BMD change in treated individuals who do not show a change in markers or about marker change in patients who lose bone despite being on treatment. Finally, there is some information suggesting that the change in BMD may not reflect a change in fracture risk (77). If this is true, it would render moot the search for a marker correlation with BMD change after treatment. On the other hand, if the change in fracture risk is related to both changes in bone mass and changes in bone turnover, as suggested by Riggs et al. (78), markers may become a very important tool for assessing the response to treatment.

The use of bone markers in clinical practice is limited by the lack of studies done with markers as the primary endpoint. Most of the information comes from clinical trials of osteoporosis therapies, in which the endpoints were increases in BMD and markers were measured secondarily. Only a few studies have had a decrease in fracture rates as the main endpoint, and none of them specifically examined the relationship between the occurrence of fractures and baseline marker concentrations or between fractures and the change in marker concentrations with treatment. Almost all of the positive data are from studies of elderly women. Although there are some normative data in young women and in men (79), there are essentially no data to guide the use of markers in men or younger women.

Because of a paucity of data, it is difficult for the clinician to know which marker to measure, when to measure it (i.e., baseline or after treatment), and what cut points to use. For almost all of the clinical questions that might be answered with bone markers, positive and negative predictive values are on the order of 70–80%, with false-negative and false-positive results in 20–30% of patients.

**Current Uses, Future Directions**

Having said that guidelines for specific clinical uses of biochemical markers of bone remodeling are lacking, I regularly use bone markers in my clinical practice. I use them (a) to aid in the decision to treat women with borderline low bone mass who are undecided about treatment (treat if the marker is increased, observe if it is not), (b) to determine whether an adequate antiresorptive effect has been achieved in patients who have been on treatment whose follow-up BMD measurements do not meet expectations (certainly for someone whose BMD has decreased substantially, and sometimes for someone who has failed to gain), and (c) to provide an earlier indication of response than can be obtained with BMD in patients with severe osteoporosis, measuring markers at baseline and after 3–6 months of treatment. For these purposes, I use the collagen cross-links, either NTx or Dpd.

Continued research is needed to identify the best marker or combinations of markers for prediction of treatment response (either a change in BMD or antifracture effect) and for prediction of bone loss or fracture in untreated patients. It is not clear that a measurement today will predict BMD or fracture 10 or 20 years in the future. Certainly, efforts at standardization of methods and reduction of preanalytic and analytic variables are important. The use of assays in serum, sweat, or saliva might minimize some of the variability seen with urine markers.

**Conclusions**

Bone marker measurements are noninvasive, inexpensive, and can be repeated often. Major changes occur in a short time. Markers are derived from both cortical and trabecular bone and reflect the metabolic activity of the entire skeleton. They do not reflect the activity of individual cells or the process of mineralization. There is large intra- and interindividual variability. Marker concentrations may be affected by the rate of clearance and certainly are likely to be altered after fracture. Markers have been very helpful in studies of the pathogenesis of osteoporosis and in understanding the mechanism of action of therapies. In clinical trials, markers may aid in selecting optimal doses and in understanding the time course of onset and resolution of treatment effect. There are several potential clinical applications for markers of bone remodeling; however, there is a need for more data to help the clinician decide which marker to measure, when to measure it, and which cut point to use.

**References**


ence of age, sex, season, and smoking habits. Calcif Tissue Int 1990;47:254–90.


73. Garnero P, Shih WJ, Gineyts E, Karpf DB, Delmas PD. Comparison of new biochemical markers of bone turnover in late postmeno-
78. Riggs BL, Melton LJ III, O’Fallon WM. Drug therapy for vertebral fractures in osteoporosis: evidence that decreases in bone turn-
 over and increases in bone mass both determine antifracture efficacy. Bone 1996;18(Suppl 3):197S–201S.