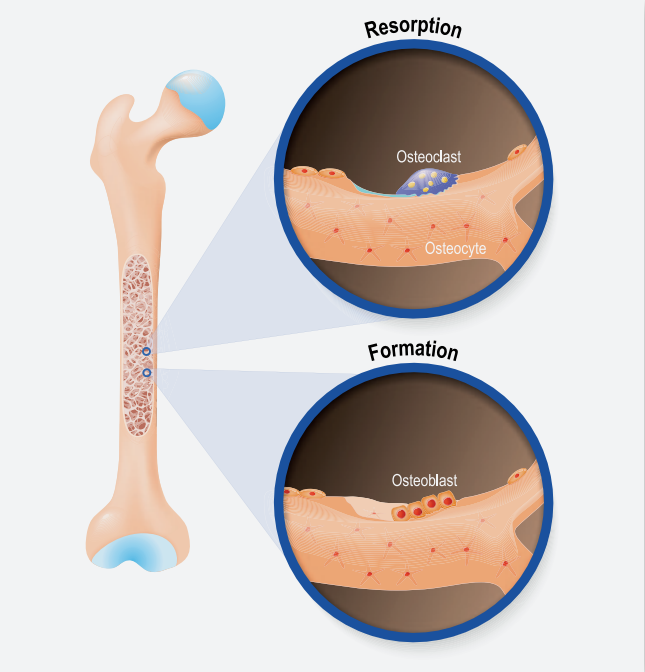
# Bone turnover markers for diagnostics and therapy monitoring

by Dr Jacqueline Gosink

Bone turnover markers (BTMs) provide a non-invasive method to assess bone remodelling – the process by which old bone tissue is removed and replaced. Measurement of BTMs supports diagnosis of metabolic bone diseases, such as Paget's disease, and is a useful aid for monitoring therapy responses in osteoporosis. Panels of peptide and enzyme biomarkers of bone turnover can be analysed using automated ChLIA or ELISA systems.




**Bone remodelling**
Osteoclasts and osteoblasts are responsible for bone resorption and bone formation, respectively (AdobeStock)

## Bone structure

Bone is composed of collagen fibres and an inorganic component comprising crystals of hydroxyapatite, which contain calcium and phosphate. There are two types of bones. Up to 80% of the skeletal system is made up of cortical (compact) bone, which has a high resistance to bending and torsion and provides strength and protection. Trabecular (spongy) bone, which accounts for approximately 20% of bone, is found at the ends of long bones, near joints and in the interior of vertebrae. It is more porous than compact bone, with the larger surface area facilitating a higher metabolic activity. Trabecular bone, therefore, has a higher turnover rate than cortical bone [1].

## Bone turnover

Bone turnover, also known as remodelling, is the biological cycle by which skeletal integrity is maintained. It is a dynamic, life-long process, which allows the body to respond to injuries, microdamage and mechanical stress. There are two tightly coupled processes that contribute to bone turnover: bone resorption refers to the removal of old bone, whereas bone formation is the laying down of new bone. Osteoclasts and osteoblasts are the specialized cells responsible for bone remodelling. Osteoclasts degrade old and damaged bone tissue and osteoblasts synthesize new bone matrix and promote bone mineralization. The balance between the two processes is regulated through the action of various hormone systems, including parathyroid hormone (PTH), vitamin D and other steroid hormones, and local mediators such as cytokines and growth factors. The bone resorption process takes about 3 weeks, while bone formation takes typically 3 months [1].

During the first year of life, an infant's skeleton is completely resorbed and replaced. Bone remodelling then continues at a rate of 10 to 20% per year until the age of about 30 when peak bone mass is reached. In a mature skeleton, the rate of bone turnover is about 5%. In older adults, bone mass declines due to an increase in bone resorptive activity combined with a reduction in bone formation. Age-related bone loss is greater in women than men owing to post-menopausal estrogen deficiency [1].

## Diseases of bone turnover

Imbalances in bone remodelling can be caused by aging, metabolic bone disorders, states of increased or decreased mobility, therapeutic interventions and other conditions. Osteoporosis is the most common disease affecting bone remodelling and leads

to deterioration of bone structures and loss of bone mass. The second most common metabolic bone disorder is Paget's disease, a chronic, slowly progressing condition with rapid bone resorption and disorganized bone formation [2]. Hypophosphatasia is a rare inherited condition which is characterized by impaired mineralization of bones and teeth due to an enzyme deficiency. Osteomalacia is caused predominantly by vitamin D deficiency and results in altered skeletal mineralization and weakened bones. Tumour diseases can also affect the bones, with metastases common in cancers such as breast, prostate and thyroid cancer. Further metabolic bone disorders include fibrous dysplasia, primary hyperparathyroidism and chronic kidney disease-mineral bone disorder (CKD-MBD) [1].

## Bone turnover markers

During bone remodelling, compounds are released from the bone or from the osteoblasts and osteoclasts into the blood and/or urine. Measurement of such bone turnover markers (BTMs) is used to support the diagnosis of certain metabolic bone disorders, for example Paget's disease, hypophosphatasia and osteomalacia. Moreover, BTMs are commonly used to assess therapy effectiveness, especially in osteoporosis. Since bone turnover is a dynamic process, the levels of BTMs change very quickly following onset of therapy, allowing rapid assessment of the suitability of the treatment and confirmation of therapy compliance. In contrast, bone mineral density measurements only show changes around 1 year after start of therapy. BTM tests also offer the advantage that they are non-invasive, inexpensive and, therefore, allow for multiple measurements over time [1]. BTM parameters are classified into markers of bone resorption and markers of bone formation.

### Markers of bone resorption

Bone resorption markers are measures of osteoclastic activity. During degradation of the protein matrix, amino- and carboxy-terminal fragments of collagen type I are released with cross-links attached. These are known as N-telopeptides (NTX-1) and C-telopeptides (CTX-1 or CrossLaps). The telopeptides enter the circulation and are excreted in urine. CTX-1 is preferred over NTX-1 as a marker, as it shows greater changes in response to therapy. There are two isoforms of CTX-1: alpha- and beta-CTX-1. The alpha form is isomerized into the beta form during the process of bone maturation. Alpha-CTX-1 thus reflects young, immature bone and is found in high turnover states such as Paget's disease or malignant bone disease. Measurement of alpha-CTX-1 supports diagnosis of these diseases as well as monitoring of antiresorptive therapy in Paget's disease patients. Beta-CTX-1 is more abundant in conditions such as osteoporosis in which old bone tissue is broken down. The concentration of beta-CTX-1 in serum can be used as an indicator to assess the course and/or response to therapy in osteoporosis.

A further bone resorption marker is the enzyme tartrate-resistant acid phosphatase 5b (TRAcP 5b), which is produced by osteoclasts and plays a role in dissolution of the mineral matrix [1]. TRAcP 5b is not cleared by the kidneys and is, therefore, not affected by renal dysfunction, making it a suitable marker for assessing bone turnover in patients with kidney diseases [1].

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## >> Markers of bone formation

Bone formation markers are measures of osteoblastic activity. Procollagen 1 N-propeptide (P1NP) is released into the circulating blood during collagen synthesis. P1NP together with beta-CTX-1 are the recommended reference markers to support the management of patients with osteoporosis [3, 4]. Osteocalcin represents the most abundant non-collagenous protein in the bone matrix and is secreted by osteoblasts during bone formation. Bone-specific alkaline phosphatase (BAP) is synthesized by osteoblasts and reflects the mineralization phase of bone formation. BAP and P1NP are, like TRAcP 5b, suitable markers for patients with impaired kidney function [1].

### Markers of calcium metabolism

Bone acts as a reservoir for calcium, and bone resorption and formation play an essential role in calcium homeostasis. Disruption of calcium metabolism leads to hypocalcemia or hypercalcemia with harmful health effects.

The calcium level in the blood is regulated by the hormones PTH and calcitonin. PTH is secreted in response to a low blood calcium level and acts on the kidneys, intestine and bone to increase circulating calcium. The absorption of calcium from the intestine is itself regulated by 1,25-dihydroxy vitamin D, which is produced from the inactive form 25-OH vitamin D in response to PTH. When blood calcium is elevated, calcitonin is released and acts to decrease it [5].

Elevated levels of PTH will stimulate bone resorption to release calcium, increasing the risk of fractures, osteopenia and osteoporosis. Excessive PTH can also affect bone mineralization, causing osteomalacia. Measurement of PTH and vitamin D can aid in the diagnosis of calcium metabolism disorders [5].

## Laboratory analysis

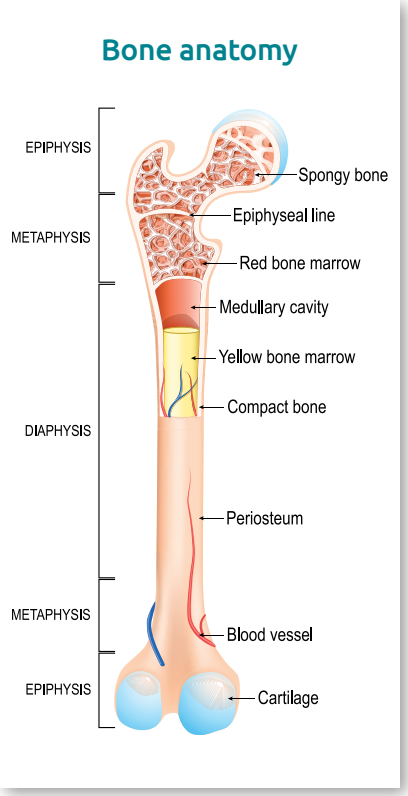
Chemiluminescence immunoassay (ChLIA) and enzyme-linked immunoassay (ELISA) technologies are the main laboratory methods used for the quantitative measurement of BTMs. A comprehensive portfolio of ChLIAs and ELISAs for markers of bone-related diseases is offered by EUROIMMUN and Immunodiagnostic Systems (IDS).

The ChLIA range for BTM measurement in serum or plasma samples encompasses the parameters CTX-1, TRAcP 5b, intact P1NP, osteocalcin and BAP, while ELISAs are available for measurement of CTX-1, TRAcP 5b, osteocalcin and BAP. CTX-1, alpha-CTX-1 and beta-CTX-1 can also be measured in urine samples using specialized ELISAs. Assays for assessment of calcium homeostasis include ChLIAs and ELISAs for the analytes intact PTH, 25-OH vitamin D, and 1,25 dihydroxy vitamin D. All IDS and EUROIMMUN assays can be automated on established instruments, which offer flexibility to accommodate different laboratory throughput requirements.

Since the bone resorption marker CTX-1 is subject to circadian variability, the timing of blood withdrawal must be carefully controlled. Fasting morning samples are recommended for optimal results. Follow-up samples should be collected at the same time of day as the baseline sample [1].


## Summary

BTMs enable assessment of the rate of bone resorption versus bone formation and thus support diagnosis of a range of bone remodelling disorders. In osteoporosis, BTMs play an important role in monitoring adherence to and effectiveness of therapy. Due to aging populations, the prevalence of osteoporosis is predicted to rise in the future, increasing the need for reliable and cost-effective testing systems to support treatment decisions. Immunoassays enable fast and minimally invasive measurement of a range BTMs and can be automated for increased efficiency.


**Structure of a long bone**
(AdobeStock)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