


# Osteoporosis: Integrating Biomarkers and Other Diagnostic Correlates into the Management of Bone Fragility

**R. Keith McCormick, DC, CCSP**

Abstract

Bone health, characterized by its mass, density, and micro-architectural qualities, is maintained by a balanced system of remodeling. The lack of these qualities, caused by an uncoupling of the remodeling process, leads to bone fragility and an increased risk for fracture. The prime regulator of bone remodeling is the RANK/RANKL/OPG system. The common origin of both bone and immune stem cells is the key to understanding this system and its relationship to the transcription factor nuclear factor kappaB (NFκB) in bone loss and inflammation. Via this coupled osteo-immune relationship, a catabolic environment from heightened proinflammatory cytokine expression and/or a chronic antigen-induced activation of the immune system can initiate a "switch-like" diversion of osteoprogenitor-cell differentiation away from monocyte-macrophage and osteoblast cell formation and toward osteoclast and adipocyte formation. This disruption in bone homeostasis leads to increased fragility. Dietary and specific nutrient interventions can reduce inflammation and limit this diversion. Common laboratory biomarkers can be used to assess changes in body metabolism that affect bone health. This literature review offers practical information for applying effective strategic nutrition to fracture-risk individuals while monitoring metabolic change through serial testing of biomarkers. As examples, the clinician may recommend vitamin K and potassium to reduce hypercalciuria, α-lipoic acid and N-acetylcysteine to reduce the bone resorption marker N-telopeptide (N-Tx), and dehydroepiandrosterone (DHEA), whey, and milk basic protein (the basic protein fraction of whey) to increase insulin-like growth factor-1 (IGF-1) and create a more anabolic profile. (*Altern Med Rev* 2007;12(2):113-145)

## Introduction

Over 50 percent of women and 13 percent of men over age 50 will sustain an osteoporotic-related fracture[1] and over 10 million Americans have been diagnosed with osteoporosis,[2] at a direct medical cost of 17 billion dollars.[3,4] In addition to improving awareness of bone health and achieving peak bone mass, it is important to use targeted nutrition. Although it has been shown that calcium supplementation slows postmenopausal bone loss[5] and may prevent fragility fractures,[6,7] findings from the Women's Health Initiative clinical trial demonstrate the shortcomings of a limited nutritional approach to bone health. This study shows that giving calcium and vitamin D supplements did not reduce hip fractures and only minimally increased bone mineral density (BMD) in postmenopausal women.[8,9] At the same time, pharmacological intervention has not proven particularly successful in treating bone loss.[10]

Osteoporosis prevention should begin long before menopause. Failure to achieve optimal nutrition from birth (or before) and through the years of adolescence and early adulthood when peak bone mass is attained can result in increased fracture risk later in life.[10] Bone fragility may already have been determined at conception[11] and been modulated *in utero* via genetics and the negative influences of excessive oxidative stress,[12] low levels of maternal 25-hydroxyvitamin D,[13] or other contributing factors.

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Bone density often begins to decline prior to mid-adulthood,[14] before a woman's estrogen levels begin to recede.[15,16] A decline in skeletal integrity may stem from adverse environmental conditions such as smoking, inactivity, or gastrointestinal inflammation and malabsorption; however, for a patient at risk for fragility fracture, strategic nutritional therapy can have a major impact in improving bone health.[17] Although estimates suggest 50 percent of the variance in peak bone mass is due to genetics,[18] it is also estimated that 30-50 percent of the genetic factors that influence bone strength can be affected by the environment in which bone is immersed. The use of biomarkers (laboratory measures of biological processes) facilitates targeted nutritional intervention and is a valuable, underutilized clinical tool. For example, serial testing of urine organic acids can assess the efficacy of carnitine supplementation for improving fatty acid metabolism, while efficacy of α-lipoic acid can be assessed by observing reduction of bone resorption markers.

To be effective, analysis of bone health and treatment of bone fragility must be sufficiently sophisticated to take all these factors into account. Other than histological examination of trans-ilial bone biopsy specimens, there is no direct way to assess bone quality in the clinic. Physicians therefore rely mainly on BMD for diagnosis and treatment efficacy and fail to recognize the benefits of using common biomarkers in the management of patients with bone fragility.

## Bone Fragility: A Term for Defining Increased Fracture Risk Based on the Quantity and Quality Components of Bone

In 1994, a World Health Organization (WHO) study group defined osteoporosis as "a systemic skeletal disease characterized by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fractures."[19] This definition was further characterized as having a BMD T score of at least 2.5 standard deviations below a healthy, young, white female. Table 1 outlines WHO T-score classifications. The measurement of BMD (the amount of mineralized tissue in a scanned area) is most commonly attained through dual-energy x-ray absorptiometry (DXA) and is an areal assessment of bone density designated in $g/cm^2$. Instead of using BMD for evaluating a patient's bone loss, a T score is used to convert $g/cm^2$ from different scanners to a common scale and also to assess the prevalence of osteoporosis within a population. "This value captured 30% of the postmenopausal population with a T score of -2.5 or below at the hip (femoral neck), anterior-posterior lumbar spine, or forearm that matched the lifetime risk for fracture at any of these three skeletal sites in these populations."[20] "Osteopenia" refers only to a loss of bone mass (T score -1.0 to -2.4) and, unlike the term "osteoporosis," does not refer to any aspects of bone quality.

The current emphasis on BMD in the diagnosis and treatment of osteoporosis limits awareness of the importance of bone quality. Although collagen matrix mineralization contributes substantially to bone

**Table 1. World Health Organization Classification of T Score**

| | |
|---|---|
| Normal | BMD ≥ -1.0 |
| Low bone mass (osteopenia) | BMD > -2.5 and < -1.0 |
| Osteoporosis | BMD ≤ - 2.5 |
| Severe (established) osteoporosis | BMD ≤ - 2.5 with history of fragility fracture |

strength (stiffness and resistance to structural failure) and low bone mass is associated with increased risk for fracture,[21,22] BMD by itself is not an accurate predictor of strength,[23] and the terms cannot be used interchangeably. Quality aspects of bone, such as size, shape, integrity of collagen fibers, thickness and connectedness of trabeculae, and the rate of bone turnover also affect the overall strength of bone.[24] For this reason, the term "bone fragility" is used in this article to emphasize the importance of both the quantity and quality aspects of bone in the determination of fracture risk. The health of bone, and therefore its strength and fracture risk, is determined by both density and quality components.

It has become evident that the increase in BMD seen with bisphosphonate therapy for osteoporosis is only weakly associated to overall fracture-risk reduction[25-27] and only slightly improves bone strength.[28] Early reduction in fracture risk by bisphosphonates is achieved through stabilization of only one bone quality component – a reduced number of resorption sites.[29] Despite these findings, physicians have been slow to grasp the importance of bone quality. A lack of non-invasive tools for assessing the compositional quality of bone in the clinical setting is the root of this failure. In addition, because bisphosphonates improve BMD, practitioners believe the problem has been addressed and other factors contributing to bone fragility are ignored.

Practitioners, as well as the general public, have adopted the erroneous belief that the numbers seen on DXA reports equate to an assessment of bone strength and overall bone health. Over-emphasis on BMD is further complicated by questions concerning potential sources of error in serial DXA interpretation[30] and, therefore, in the ability of DXA to help assess efficacy of therapy.[31] Despite concerns of accuracy and inability to assess bone quality, DXA technology remains the primary diagnostic tool in osteoporosis management because it is inexpensive and readily accessible.

Currently the WHO is designing an absolute fracture-risk model that will help clinicians determine who is at risk and when drug therapy should be initiated. Although this may be an improvement to current fracture risk assessment, it is essential to keep in mind the pitfalls of predicting bone loss in a particular patient on the basis of overt characteristics such as sex, age, and lifestyle.

Secondary findings from the Improving Measurements of Persistence of Actonel Treatment (IMPACT) trial showed 38 percent of subjects, ages 65-80, with a diagnosis of osteoporosis had no risk factors.[32] This statistic is important to consider when evaluating the individual patient. Optimal fracture-risk assessment in the individual patient can be achieved by using diagnostic correlates from DXA and currently available biotechnology in a translational medical approach. Using bone-related biomarkers and other laboratory tests not traditionally associated with bone health can improve therapeutic management and identify individuals with elevated fracture risk independent of reduced BMD. Once identified, these patients will benefit from diet and nutritional intervention to improve bone health and reduce fracture risk.

## Pathophysiology of Osteoporosis and Bone Fragility

Bone health is maintained by a balanced remodeling process that ensures the continual replacement of old bone, weakened by microfractures, with new bone. This is a coupled process involving bone resorption by osteoclasts and new bone formation by osteoblasts. Failure to reach peak bone mass or the uncoupling of remodeling can result in bone fragility.

### *The Role of RANK/RANKL/OPG and T Cells in Bone Remodeling*

Although estrogen is the key sex hormone governing bone homeostasis, the primary regulator of bone remodeling is now being recognized as the RANK/RANKL/OPG system (defined below).[33] This osteoimmunological system determines the success or failure of bone homeostasis. The common origin of bone and immune stem cell is the key to understanding this system and the physiology of bone loss. It is also the key to applying effective nutritional therapy for the inflammatory, catabolic-based increase in bone fragility.

Bone-resorbing cells (osteoclasts) and cells of the immune system both originate in the bone marrow from hematopoietic cells. Osteoclasts develop from precursors of the mononuclear monocyte-macrophage cell line after stimulation by macrophage colony-stimulating factor (M-CSF) and receptor for activated nuclear-factor kappa B (RANK) ligand (RANKL) (Figure 1).[34]

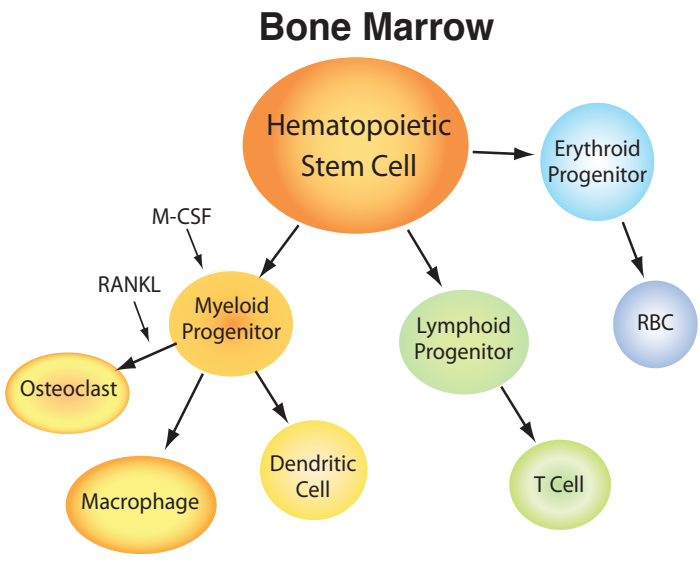
Bone-forming cells (osteoblasts) are of mesenchymal origin and share a common precursor cell with adipocytes. During normal bone remodeling, marrow stromal cells and osteoblasts produce RANKL, which binds to the transmembrane receptor RANK on osteoclast precursors and induces differentiation and activation (Figure 2).[35] This occurs through the transcription factor, nuclear-factor kappa B (NFκB), which is responsible not only for activating osteoclastogenesis but also the body's inflammatory response. Both osteoclast differentiation and the inflammatory process occur via regulation of interleukin-6 (IL-6).

The major role cytokines play in bone remodeling is demonstrated by the fact that receptors for the proinflammatory cytokines interleukin-1 (IL-1), IL-6, and tumor necrosis factor-alpha (TNF-α) are present on both osteoclast precursor cells and mature osteoclasts. Estrogen exhibits its nuclear regulatory effects by inhibiting IL-6 activation of NFκB during bone remodeling.[36] Osteoblasts also produce osteoprotegerin (OPG), a soluble decoy receptor that blocks RANKL and maintains control of the remodeling process. OPG is vital to the success of the RANK/RANKL/OPG system of bone homeostasis.

## *Chronic Immune Activation and the Uncoupling of Remodeling*

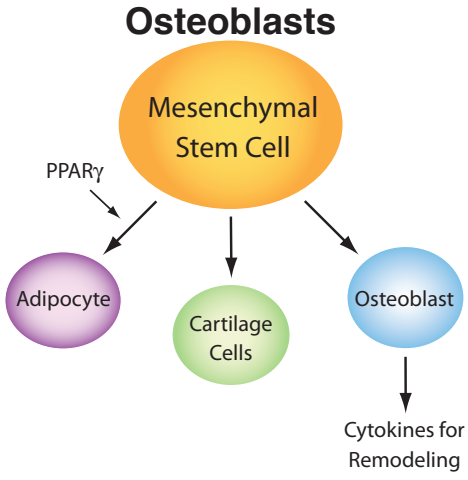
RANKL is also produced by activated T cells. With reduced estrogen levels and/or chronic or recurrent immune activation from either systemic or gastrointestinal origin, there may be a reduction in the body's natural ability to limit the production of RANKL.[37] This results in increased osteoclast activation through a "switch-like" diversion of osteoprogenitor-cell differentiation away from monocyte-macrophage-cell development and toward osteoclastogenesis. Osteoclastic activity, induced by proinflammatory cytokines and activated T cell-induced RANKL, is thought to be modulated by the action of interferon gamma (IFN-γ) on tumor necrosis factor receptor-associated factor 6 (TRAF-6).[38] TRAF-6 is a RANK adaptor protein that mediates NFκB activation (Figure 3).[39,40]

**Figure 1. Osteoclasts, Immune Cells, and RBCs are Derived from Marrow Hematopoietic Stem Cells**



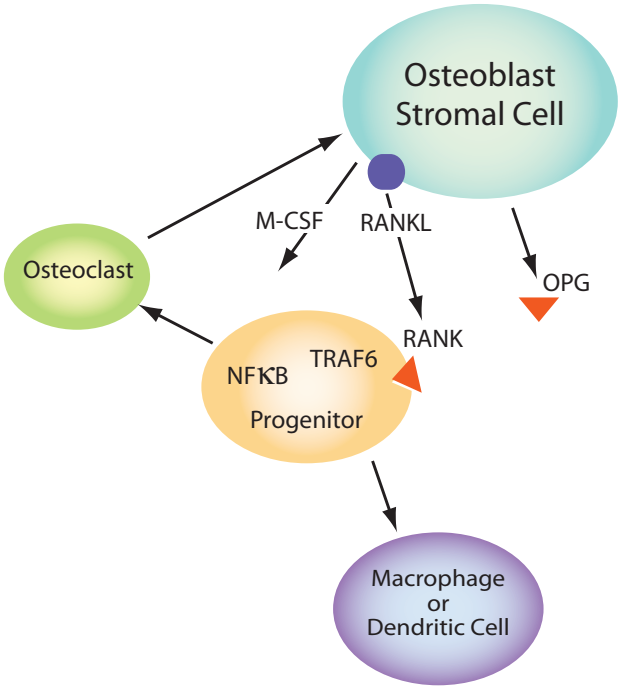

The pluripotent hematopoietic stem cell differentiates into myeloid, lymphoid, and erythroid progenitor cells. The commonality of origin and the factors that govern their differentiation are keys to understanding the relationship between immune regulation and accelerated osteoclastic bone resorption.

**Figure 2. Osteoblasts, Cartilage, and Adipocytes are Derived from Marrow Mesenchymal Stem Cells**




Dysregulation of common precursor-cell differentiation is the link between obesity and low bone density, the two most common disorders in the United States.

**Figure 3. RANK/RANKL/OPG Osteo-immunological System of Bone Homeostasis**




This modulating capacity of IFNγ over RANKL is influenced by both vitamin D and estrogen.

Aging leads not only to a reduction in sex-hormone production, but also to an increase in the general level of proinflammatory cytokines and diminution of immune system function. *In vivo*, free radicals have been shown to increase bone resorption,[41] and oxidative stress reduces BMD in humans.[42] These environmental and/or age-related catabolic stressors contribute to normal bone loss. But when there is chronic, elevated antigenic load or excessive oxidative stress, which increases pro-inflammatory cytokine-induced RANKL, the activation of this "switch" in osteoprogenitor-cell differentiation may, independent of age, adversely affect the balance of bone remodeling. It is in this abnormal state that chronic immune activation may alter IFN-γ modulating capacity. When estrogen is deficient, causing RANKL levels to increase, the body's natural ability to limit the transcription factors TRAF-6 and NFκB may be reduced and IFN-γ may exert a pro-osteoclastogenic effect.[43]

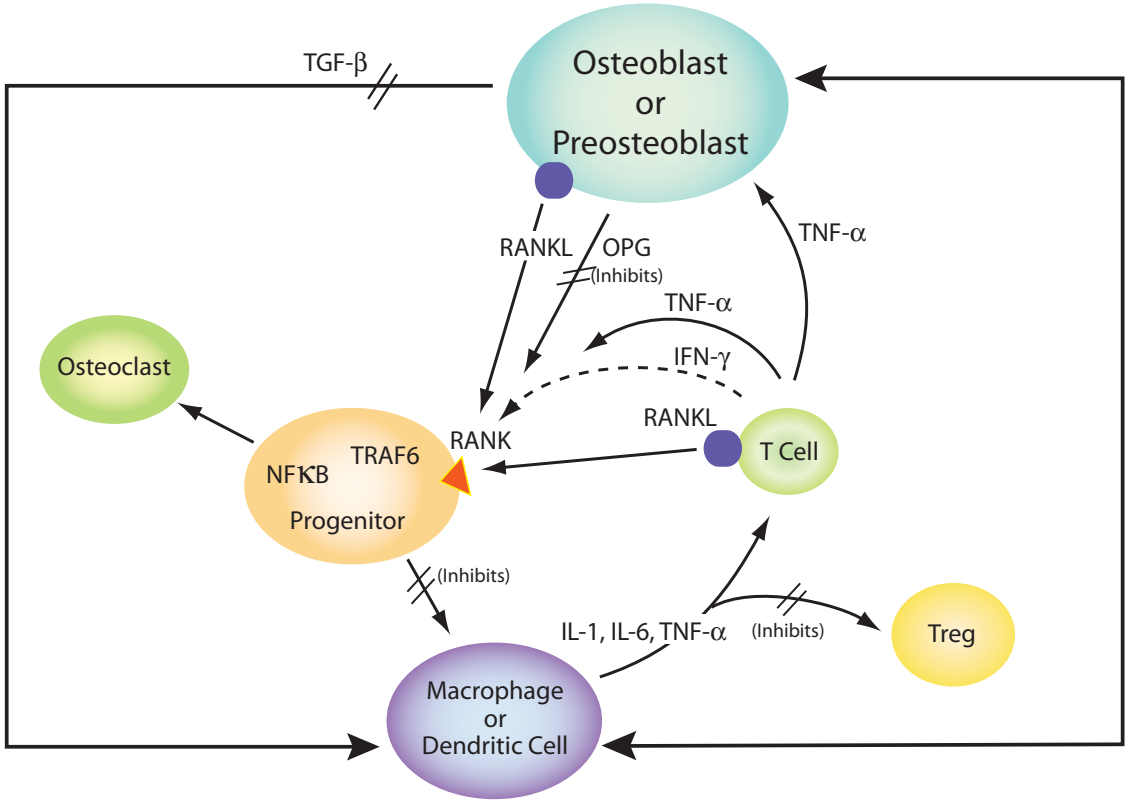
This uncoupling of the remodeling process results in bone loss. In studies using mice, chronic antigenic load with T-cell activation and production of reactive oxygen species (ROS) must be present for low estrogen levels to cause bone loss.[44] It appears that reducing antigenic load and oxidative stress may be equally as important as estrogen in maintaining bone health.

## *Oral Tolerance and Bone Health*

Oral tolerance, the muted immunological response to harmless gut antigens, depends on the presence of commensal microorganisms and an intact healthy gut wall. Epithelial cell integrity is maintained by the presence of beneficial organisms such as Lactobacillus and Bifidobacteria that do not elicit an inflammatory response. When normal gastrointestinal flora are maintained, immunological self-tolerance through the activation of T-regulatory cells (Tregs) favors a non-inflammatory T-helper 2 (Th2) dominant response to gut microbes.[45] Pathogenic bacterial or fungal overgrowth causes inflammation and increased gut permeability that reduces oral tolerance. Focus on the traditional osteo-endocrine explanation for bone homeostasis fails to acknowledge the important role of the immune system in remodeling and the possible role of oral tolerance in maintaining bone health. It is now understood that a high systemic antigen load of bacterial or viral origin and/or a loss of oral tolerance due to pathogenic microbial overgrowth (long suspected as major contributing factors in other chronic degenerative diseases) may also contribute to the pathogenesis of bone loss.

Estrogen normally helps preserve bone by enhancing macrophage production of transforming growth factor β (TGF-β) and limiting CD4+ T-cell activation. Reduced levels of estrogen result in an increase in antigen-presenting cells and a reduction in TGF-β and Tregs. This leads to T-cell activation and production of proinflammatory cytokines and RANKL, which stimulates osteoclastogenesis (Figure 4). By improving gut health and oral tolerance, antigen presentation to T cells is reduced, TGF-β production is maintained, Tregs are enhanced, and RANKL-induced osteoclastogenesis is limited, even with reduced levels of estrogen.

## Figure 4. Chronic Immune Activation Leads to Bone Loss




Activation of T cells is necessary for osteoclast differentiation. RANKL activation of NFκB through the RANK adaptor protein, TRAF6, increases osteoclastogenesis from progenitor cells. IFN-γ can either increase or limit bone resorption through modulation of this cascade. This "fail safe" mechanism, under normal circumstances, limits bone resorption. But with chronic T-cell activation and a predominate Th1 response, IFN-γ no longer limits osteoclast activation and bone resorption increases.

Estrogen increases vitamin D receptor activation and calcitonin release. It also increases osteoblast release of TGF-β, IGF-1 and OPG, which limits M-CSF and RANKL and increases osteoclast apoptosis. With reduced estrogen levels, TGF-β decreases and antigen presentation to T cells increases the release of RANKL and TNF-α, diverting progenitor cell differentiation toward osteoclastogenesis. Vitamin D and normal gut flora help preserve tolerogenic dendritic cells and reduce activation of RANKL-induced osteoclastogenesis.

### T-Helper 1 (Th1) Dominance

Imbalance in the Th1/Th2 adaptive immune response initiated by antigenic stress may play a part in specific cases of osteoporosis.[43] With T-cell activation now known to have a major role in RANKL-induced osteoclastogenesis, more research is needed to determine whether early maturational and/or chronic immunological stressors contribute to excessive bone loss in later years. In addition to nutrient malabsorption, high antigen load from food allergies or intestinal microbial overgrowth may contribute to bone loss.

Mature osteoclasts gain access to bone surfaces only after mononucleated preosteoclasts have traveled from the circulatory system to the bone, possibly through mechanisms involving transendothelial migration.[46] The gut-associated lymphoid tissue normally provides an immunological barrier against disease. When this barrier becomes compromised by endothelial hyperpermeability secondary to food allergy or bacterial overgrowth, nutrient absorption is reduced, and a loss of oral tolerance can initiate a gastrointestinal-immunological stressor of the bone remodeling process.

RANKL regulates not only the function of osteoclasts but also that of dendritic cells (professional antigen-presenting cells). In chronic inflammation, RANKL promotes dendritic cell survival and the expression of proinflammatory cytokines.[47] As the gut is overrun by pathogens, professional antigen-presenting cells, through the activation of toll-like receptors and C-type lectin receptors, are no longer able to silence immune activation[48] and release proinflammatory cytokines that activate T cells and reduce Tregs. This antigenic stress leads to a Th1-dominant, cell-mediated immune system[49,50] with increased RANKL, reduced IFN-γ, and a possible uncoupling of bone remodeling.

## Toll-like Receptors

The production of gut-related proinflammatory cytokines is reduced by the maintenance of a healthy gut flora. Toll-like receptors are transmembrane receptors found on macrophages, dendritic cells, and some epithelial cells, and play an integral role in maintaining oral tolerance. These receptors recognize the molecular patterns of bacteria and elicit an inflammatory, destructive response to pathogenic microbes and a tolerogenic response to commensal bacteria.

An example of how a disease-related genetic polymorphism can be influenced through the reduction of metabolic stressors can be seen in the case of toll-like and IL-1 receptors. Because the cytoplasmic portion of the toll-like receptor is similar to that of the IL-1 receptor, an individual suffering from chronic dysbiosis and also carrying the polymorphism for the IL-1 receptor antagonist gene could, in theory, be susceptible to an increased diversion or "switch" of cells from the monocyte-macrophage cell line to form osteoclasts. A reduction of antigen load and oxidative stress, no matter the cause (e.g., insulin/glucose imbalance, toxicity, or gut pathogenic microflora), could reduce proinflammatory cytokine-induced chronic inflammation and T-cell activation.

## Involution of Thymus Gland and the Beginning of Bone Loss

Reduced oral tolerance may be a factor in the apparent coincidence between thymus gland involution (and subsequent reduction of naive T-cells) and the onset of bone loss that begins in humans in their mid-30s. Although BMD does not usually decrease significantly until menopause, accelerated bone loss can commence at an earlier age for some individuals. Reduced numbers of naive T cells from chronic systemic inflammation or antigen overload from the gut leads to oligoclonal T-cell expansion and increased T-cell senescence.[51] Senescence reduces a T cell's ability to produce IFN-γ[33] and is a sign of immune aging.

The primordial thymus developed as a bud on the immature digestive tract, providing embryological evidence of the uniquely co-dependent and interrelated functions of the thymus gland and gastrointestinal tract.[52] As an infant grows, the function of the thymus is to relieve the gut of its primordial function of lymphopoiesis.[52] With involution of the thymus, the adult gastrointestinal tract remains the source of at least 75 percent of the body's immune cells;[53] therefore, it is in the gut that an adult's immune health is maintained or lost. As an individual ages, antigen load often increases and oral tolerance decreases, leading to reduced levels of IL-2 (necessary for T-cell proliferation and differentiation into activated [effector] cells) and IFN-γ, and ultimately to a greater cache of RANKL-expressing (and thus osteoclast-activating) memory cells harbored in the bone marrow.

# Patient Evaluation

Osteoporosis is a polygenic, multifaceted, metabolic disorder necessitating a complete understanding of its etiopathology. Even after the initial thorough consultation, physical examination, DXA scan, vertebral fracture assessment (VFA) or other spinal imaging (if appropriate), and laboratory evaluation, therapeutic intervention for a patient with increased fracture risk should entail follow-up serial testing and ongo-

**Table 2. Risk Factors for Fragility Fracture**

| |
|---|
| Advanced age |
| Personal history of fracture related to mild-to-moderate trauma as an adult |
| Family history of hip fracture |
| Low body weight |
| Weight loss |
| Loss of height |
| Late onset of sexual development |
| Poor health |
| Gonadal hormone deficiency |
| Poor nutrition |
| Use of certain medications |
| Smoking |
| Alcoholism |
| Inadequate physical activity |
| Frequent falls |

ing decision making to fine-tune treatment. Given the complexity of the disease, relying solely on biannual DXA exams to monitor treatment efficacy may not be sufficient.

## *Consultation and Physical Examination*

Bone fragility is associated with multiple risk factors (Table 2), among the most important being advancing age, female gender, low body weight, low BMD, prior fragility fracture, early menopause, eating disorders, and maternal history of osteoporosis.[54] Patient history should include assessment of these risk factors as well as looking for secondary factors that could potentially contribute to bone loss, such as malabsorption syndromes (Table 3), disease processes, or the previous or current use of certain medications. Celiac disease and lactose intolerance are common conditions causing reduced calcium absorption and increased bone loss.[55] Inflammatory diseases, endocrinopathies, primary biliary cirrhosis, eating disorders, environmental toxicity,[56,57] cancer, and the loss of estrogen are all implicated in the development of osteoporosis.

**Table 3. Biomarkers for Malabsorption**

| | | |
|---|---|---|
| Cholesterol | —— | low |
| Total protein | —— | low |
| Albumin | —— | low |
| Calcium | —— | low |
| Vitamin D | —— | low |
| Anemia (hypochromic/microcytic or macrocytic) | | |

Many common medications can increase bone loss. Glucocorticosteroids (e.g., prednisone), even in doses as low as 2.5 mg/day, are known to increase fracture risk.[58] As observed in A Diabetes Outcome Progression Trial (ADOPT), thiazolidinediones (e.g., Avandia® and Actos®) for type 2 diabetes, in addition to their potential for liver toxicity, can suppress osteoblast cell differentiation in favor of adipocytes from mesenchymal

precursor cells, and were linked to increased fracture risk in women.[59] Aromatase inhibitors for breast cancer (e.g., Arimidex®) and luteinizing-hormone releasing hormone agonist therapy for prostate cancer (e.g., Lupron®) increase bone loss. Depot medroxyprogesterone acetate for birth control[60] and heparin therapy during pregnancy[61] both reduce bone density. The CaMos study found daily use of cyclooxygenase-2 (COX-2) inhibitors decreased load-induced bone formation in men. On the other hand, women in the study gained bone density with COX-2 inhibitor use; however, the bone protective effect was lost when COX-2 inhibitors were combined with exogenous estrogen therapy.[62] Proton-pump inhibitors have recently been shown to increase hip fractures.[63] Anticonvulsants such as phenobarbitone, phenytoin, and carbamazepine are known to interfere with vitamin D metabolism leading to hypocalcemia, low 25-hydroxyvitamin D (25(OH)D), and bone loss.[64]

In addition to gaining important information on risk factors and a subjective account of nutritional history from the patient, a comprehensive physical examination may reveal clues directly relevant to the patient's bone health status. A patient may complain of muscle pain or they may have sensitive shins and sternum seen with osteomalacia – both associated with a vitamin D deficiency. Magnesium deficiency can cause muscle cramping, constipation, or depression. Steatorrhea may indicate intestinal microbial overgrowth or liver dysfunction and a reduction in vitamin D and calcium absorption. Fingernail changes may indicate a mineral deficiency. Examination of the oral cavity may reveal a white coated tongue indicative of Candida infection, angular cheilitis or tooth discoloration of celiac disease, increased caries from low oral pH and poor dental mineralization, or the receding, red, swollen, and boggy gums of periodontal disease that can be associated with osteoporosis.

## *Laboratory Evaluation*

Theranostics, the use of serial laboratory studies for diagnosing and tailoring individual treatment, can help define the etiology of bone loss and also guide a clinician's specific nutritional intervention program. The term theranostics was first used by the pharmaceutical industry to describe specific diagnostic tests, either laboratory based or point-of-care tests, which could be linked to drug therapy. The N-telopeptide (N-Tx) test for assessing bone resorption activity is a good example of a theranostic development for bisphosphonate use in the treatment of osteoporosis. Common laboratory biomarkers such as urine calcium or salivary cortisol can also be used as theranostic indicators for nutritional intervention and its therapeutic efficacy. The 2004 *Bone Health and Osteoporosis: A Report of the Surgeon General* supports the use of laboratory bone-turnover markers to assess treatment effectiveness.[65] The use of these and other biomarkers as foundational tools in caring for fracture-risk patients has potential for optimizing bone health and reducing fracture morbidity.

Minimal laboratory screening for patients with either low bone density (T score < -1.0) or risk factors that arouse concern would include complete blood count (CBC), chemistry profile, functional metabolic profile of urine organic acids, urine pH, urine calcium/creatinine ratio, serum 25-hydroxyvitamin D, serum calcium and phosphorus, tissue transglutaminase antibody, N-Tx, thyroid-stimulating hormone (TSH), estrogen, testosterone, and sex hormone-binding globulin (SHBG). An extended assessment may include immunoelectrophoresis, insulin-like growth factor-1 (IGF-1), homocysteine, dehydroepiandrosterone (DHEA), follicle-stimulating hormone (FSH), parathyroid hormone (PTH), vitamin B12, cortisol, food-allergy testing, stool analysis, salivary secretory IgA, and others.

The following summary of laboratory tests is intended only as a brief guide to the use of theranostics in the management of patients with low bone density or high fracture risk. It is not intended as a diagnostic outline but as a way to introduce how biomarkers can be used to assess the need for, and efficacy of, nutritional care in patients with bone fragility. Other than the bone resorption and formation tests, the biomarkers discussed here are not specific to bone and can be used effectively in managing bone fragility only when employed in the broader context of obtaining overall health. There are many conditions that lead to bone loss, some extremely serious and life threatening such as multiple myeloma. If the physician has any reason to suspect the diagnosis of osteoporosis is secondary to another disease process, the patient should be referred to an endocrinologist for further evaluation.

## Specific Laboratory Tests

### *CBC and Chemistry Profile*

A complete blood count (CBC) and chemistry profile provide the clinician with a general survey of multiple organ systems. These tests often contain a wealth of clues that may be overlooked as borderline-low or -high results. For example, a mild decrease in albumin coupled with hypocalciuria may indicate malabsorption;[66] mild hypocalcemia can indicate magnesium deficiency;[67] anemia may be related to celiac disease and resulting malabsorption of bone-building nutrients;[68] and a low red blood cell (RBC) count may be secondary to the effects of elevated proinflammatory cytokines[69] or the reduced hematopoietic capability of the osteoporotic patient's fat-infiltrated bone marrow.[70,71]

Alkaline phosphatase (ALP) is an enzyme found in bone, liver, intestine, kidneys, and placenta. Although it is an indicator of osteoblastic activity, ALP is not specific to bone tissue and is therefore not typically used in the management of osteoporosis. ALP may be normal or increased in postmenopausal women[72] and may be reduced in celiac disease, hypothyroidism, pernicious anemia, or zinc deficiency.[73] Elevated ALP levels may also be an indication of cancer metastasis to the liver or bone.

### *Bone-Turnover Biomarkers*

#### Resorption Markers

Bone resorption markers (e.g., urinary N-Tx and deoxypyridinoline [Dpd]) reflect the level of osteoclastic activity in the bone-remodeling process. Accelerated osteoclastic activity increases bone turnover and is associated with low bone mass in both pre- and postmenopausal women.[74] Elevated levels of resorption markers indicate increased osteoclastic activity and a higher risk for osteoporotic hip fracture, independent of BMD.[75,76] Even when BMD is not in the osteoporotic range, increases in urine N-Tx (cross-links of N-terminal telopeptide of type-1 collagen) and/or Dpd indicate increased osteoclastic-bone resorption and risk for fracture.[66] A decrease in N-Tx, especially when monitored serially, can be used as an early predictor of reduced bone resorption with stabilization or increase of bone mass in response to treatment.[77]

Dpd levels are influenced by muscle-collagen breakdown.[10] Because N-Tx is more sensitive to change in bone metabolism than is Dpd,[78] using serial testing of Dpd to evaluate for therapeutic efficacy may not provide as useful an indicator as N-Tx. The resorption test C-telopeptide (C-Tx) is a serum marker for C-terminal telopeptide of type-1 collagen used predominately in Europe.

#### Formation Markers

Currently, osteoblastic bone formation can be measured clinically using three different tests – serum osteocalcin, serum bone-specific ALP, and serum intact N-terminal propeptide of type-1 procollagen (P1NP). Elevated levels of osteocalcin, bone ALP,[79,80] and P1NP are seen with increased bone remodeling and bone loss. Bone ALP and P1NP are considered early markers of formation, while osteocalcin, which is greatly influenced by genetics,[81] is a later marker of osteoblastic activity; osteocalcin, although related to fracture risk,[82] is a less responsive indicator. Although bone ALP is influenced by genetics, it remains an excellent formation marker for determining osteoclastic over-suppression in patients using bisphosphonate therapy.[83] Osteocalcin and bone ALP have been shown to increase with vitamin K supplementation.[84]

Serum concentration of P1NP is directly proportional to the amount of new collagen produced by osteoblasts.[85] P1NP is useful for assessing bone turnover in postmenopausal women[86] and is the best marker for monitoring patients on teriparatide (recombinant human PTH) therapy.[87]

### *Metabolic Functional Assessment*

Metabolic function assessment, through the use of urine organic acids, can help identify nutrient-related inadequacies in the metabolism of fats, carbohydrates, and amino acids, and can be useful for the nutritional management of degenerative catabolic diseases such as osteoporosis. Biomarkers for oxidative damage and intestinal dysbiosis can also illuminate potential underlying causes of osteoporosis.

## Urine Organic Acids

Osteoporosis is not just a disease of deficiency; it is a catabolic disease with high correlation to diabetes, Alzheimer's disease, and cardiovascular disease. Similarities among these degenerative diseases include chronic low-level inflammation and reduced mitochondrial bioenergetics. Testing with urine organic acids can signal the presence of an inflammatory catabolic physiology. For example, elevated levels of urine lactate or the ketone body, β-hydroxybutyrate, may indicate the catabolic profile of chronic metabolic acidosis from poor glucose utilization.[88]

Another indication of chronic inflammation and immune system activation is demonstrated by altered levels of organic acid intermediates from the kynurenine pathway of tryptophan metabolism. The intermediate, xanthurenic acid (XA), is used to identify pyridoxine deficiency (vitamin B6 is a cofactor for several enzymes in the kynurenine pathway and a deficiency raises XA levels).[88] Recently, other intermediates have been identified as contributors to various disease processes, including osteoporosis.[89] Stone and Darlington review the involvement of pathway intermediates – kynurenine, kynurenic acid (KynA), anthranilic acid (AA), 3-hydroxyanthranilic acid (3HAA), XA, and quinolinic acid (QUIN) – in modulating glutamate receptors, activating NFκB, regulating cell proliferation, and controlling microbial invasion and modulation of the T-cell response by professional antigen-presenting cells. When macrophages are stimulated by IFNγ, the initiating enzyme indoleamine-2,3-dioxygenase (IDO) for the kynurenine pathway is activated. IDO reduces T-cell activation and is modulated by estrogen, TGF-β, and proinflammatory cytokines.[89] Forrest et al observed reduced blood levels of 3HAA and increased AA in patients with osteoporosis. Because both XA and 3HAA are metabolites of AA, the levels of these two biomarkers correspond. Patients with osteoporosis demonstrate a shift from 3HAA and XA toward AA. Reduced levels of 3HAA and XA lead to reduced QUIN and, therefore, a reduced QUIN/KynA ratio. Bone cells have glutamate receptors sensitive to kinurenines, and altered levels appear to have direct effects on bone homeostasis.[90] The urine organic acids KynA, XA, and QUIN are readily observable biomarkers.

Organic acid testing can also help identify reduced oxidative phosphorylation of mitochondrial bioenergetics, which is "the unifying concept" of chronic age-related disease.[91] Uncoupling of mitochondrial function leads to decreased energy for daily activity and reduced muscle protein synthesis[17] of sarcopenia often seen in patients with osteoporosis. The loss of muscle mass and strength due to reduced mitochondrial function is not only intimately correlated to bone loss but also contributes to an increased risk of falling, the major risk factor for fragility fractures.[21]

In the borderline-anemic osteoporotic patient, reduced oxygen supply (hypoxia) secondary to poorly vascularized fatty bone marrow leads to hypoxia, local acidosis,[92] and may stress mitochondrial energy production. Hypoxia not only reduces an individual's strength and energy level (leading to incoordination and falls), but it has also been shown to increase osteoclastic bone resorption *in vitro*.[93]

Testing for cellular energy function can identify inefficiencies in the processing of food for the production of adenosine triphosphate (ATP). The urine markers citrate, cis-aconitate, isocitrate, α-ketoglutarate, succinate, fumarate, and malate are intermediates of the oxygen-requiring, mitochondrial citric acid cycle. Abnormal levels of these intermediates may indicate energy production inefficiencies as a result of polymorphism-related enzymatic dysfunction or deficiencies in B-complex vitamins, coenzyme Q10, or α-lipoic acid – cofactors necessary for metabolism.[88] In either case, urine organic acid testing can identify the need for specific nutritional supplementation and help improve energy production.

## Markers of Oxidative Damage

Oxidative damage from free radicals is a major contributing cause of degenerative diseases and, specific to osteoporosis, to the increase in osteoclastogenesis and subsequent bone loss. ROS, among the most damaging free radicals, are constantly produced during mitochondrial respiration. Grassi et al demonstrated *in vivo* that ROS are necessary for bone loss to occur in estrogen-deficient mice.[44] Thus, the use of biomarkers to identify patients with oxidative stress may be helpful in managing osteoporosis. Urine or serum lipid peroxides and urine 8-hydroxy-2-deoxyguanosine (a product

of oxidative damage to DNA) are biomarkers that indicate increased oxidative stress.[88] By reducing their levels in the low bone-mass patient, the physician may also be limiting mechanisms that lead to RANKL-induced osteoclastogenesis and Peroxisome Proliferator-Activated Receptor-gamma- (PPARγ-) induced reduction of osteoblastogenesis. When the bone resorption marker N-Tx is elevated along with these oxidative stress biomarkers, antioxidants such as vitamin C, α-lipoic acid, and N-acetylcysteine could theoretically reduce all three. Vitamin C has been shown to be markedly decreased in aged osteoporotic women.[94] Tobacco smoking, a pro-oxidant stressor, has been linked to osteoporosis.[95]

### Additional Metabolic Function Biomarkers

Metabolic function testing can provide a wealth of information relative to detoxification efficiency, adrenal stress, and the presence of intestinal pathogenic microbial compounds. Intestinal dysbiosis biomarkers are indirect evidence of microbial overgrowth and increased toxic load. Microbial overgrowth can cause steatorrhea, a sign of reduced vitamins D and K and calcium absorption. Overgrowth may also cause generalized nutritional deficiency and increases in specific microbial biomarkers may offer evidence of specific deficiencies. For example, elevated levels of the biomarker tricarballylate, a byproduct of a certain strain of aerobic bacteria, can lead to reduced magnesium, calcium, and zinc levels.[88] In this author's experience, correcting major dysfunctions identified by these tests can lead to parallel improvements in bone-formation markers or the reduction in resorption marker N-Tx.

## *Acid-Base Balance: Metabolic Acidosis*

Bone has three general functions – structural support, vital organ protection, and storage for mineral reserves. This last function is intimately connected to the pathophysiology of osteoporosis and, in particular, to the maintenance of blood pH.

Arterial blood pH must be maintained at approximately 7.4; even small changes can be life threatening. Acidic hydrogen ions, continuously produced during biological activity, are buffered by the dietary intake of alkaline minerals. When the net endogenous production of acid (NEAP) is higher than the dietary base load during brief periods, sodium and potassium from the metabolically active membrane surrounding bone are tapped to maintain normal pH.[96] When NEAP becomes chronically higher than the dietary base load, ion exchange at the bone membrane becomes insufficient and calcium salts from bone matrix are tapped.

Acidosis is caused by poor diet, excessive protein intake, prolonged intense exercise, aging, airway disease, and menopause (from reduced hormone-induced respiratory acidosis that causes an increase in serum bicarbonate).[97] The phosphate-rich Western diet, high in sulfur-containing animal and grain protein and low in alkaline fruits and vegetables, results over time in low-grade metabolic acidosis.[98] Metabolic acidosis can be reduced by promoting renal calcium retention. In a study using oral potassium bicarbonate (60 mEq/day), Frassetto et al demonstrated a complete neutralization of net acid excretion in healthy older men and women by reducing urine calcium losses by just 28 mg/day.[99]

It has been known for over 80 years that metabolic acidosis leads to bone loss[100] and *in vitro* research has illuminated the exact mechanisms for this loss. Long-term acidosis increases osteoclastic activity,[101] reduces osteoblastic function,[102] increases urine calcium loss, reduces IGF-1,[103] and increases prostaglandin $E_2$ ($PGE_2$), RANKL, and M-CSF[104] – all of which increase protein catabolism, muscle wasting,[105] and bone resorption.[106] Mild acidosis also increases activity of cathepsin K, a metallo-protease secreted by osteoclasts for bone-matrix resorption.[102] Even very small pH changes (as little as 0.05) result in a doubling or halving of resorption-pit formation in cultured osteoclasts.[92] PTH release is also affected by acidosis. It has been demonstrated in dogs that acute metabolic acidosis stimulates PTH secretion and prolongs its half-life, thereby increasing calcium resorption.[107] In a study using growing rats, a high phosphate diet, even with adequate calcium intake, was shown to increase PTH and reduce BMD and bone strength.[108]

Hypoxia from increased anaerobic metabolism reduces energy production and contributes to metabolic acidosis. The source of hypoxia does not matter; its detrimental effects will be the same whether it is produced from oxygen debt in the body of a high-intensity, well-trained, endurance athlete or from reduced hematopoietic and oxygen-carrying capability of the fat-infiltrated marrow of an 80-year-old patient's severely osteoporotic bones.[93]

Although it is generally agreed that diets high in fruits and vegetables reduce acid load and protect against bone loss,[109] it appears more than alkalinity is responsible for their protective effects. Muhlbauer et al found reduced bone resorption in rats from a diet high in alkaline fruits and vegetables may be a reflection of pharmacologically active compounds rather than the alkalinity of the diet and concluded that the acid hypothesis of bone loss may only be applicable to older adults.[110]

Patients with mild metabolic acidosis often have low urine pH (<6.0). Although normal kidneys produce urine with a wide range of pH, serial pH assessment of first-morning urine averaging <6.0 is a good indicator of mild metabolic acidosis and can provide a useful tool for improving patient compliance. In a study of the catabolic effects of chronic metabolic acidosis on muscle protein in postmenopausal women, 60-120 mmol/day potassium bicarbonate resulted in a reduction in pretreatment levels of metabolic acidosis and the rate of muscle proteolysis.[111] In a similar study of postmenopausal women, 60-120 mmol/day potassium bicarbonate resulted in reduced urinary calcium excretion and bone resorption and increased bone formation.[98]

In addition to low urine pH, hypercalciuria, low-normal blood bicarbonate, elevated $1,25(OH)_2D$, low IGF-1, mild hypothyroidism, and mild hypophosphatemia may be indicators of low-grade metabolic acidosis.[112] When assessing a patient for mild metabolic acidosis, a more global view of the patient is preferable to relying solely on urine pH. As a cautionary note, elevated urine alkalinity of pH >8.0 can be a sign of urinary tract infection or renal disease.

## *Vitamin D Status*

Receptors for vitamin D have been found on cells in many organ systems throughout the body. In addition to its role in mineral metabolism and maintenance of intracellular calcium levels, it is important for cell differentiation and proliferation, muscle growth, and strength. Vitamin D deficiency not only causes bone fragility but has also been linked to increased cancer risk, type 1 diabetes, and heart disease.[113] Vitamin D is essential for normal insulin secretion and is thought to act as an immunomodulator since vitamin D receptors (VDRs) are seen on macrophages, dendritic cells, and activated T cells. In the presence of vitamin D, dendritic cells mature as more tolerogenic, thus modulating T-cell response, increasing Tregs, and reducing interleukin-12 (IL-12) and Th1 dominance, all of which result in a moderating effect on osteoclastic bone resorption.[33]

To maintain normal bone cell function, serum 25(OH)D level should be considered the functional indicator for vitamin D status[114] and serum levels maintained between 30 and 80 ng/mL. Vitamin D deficiency (<15 ng/mL)[113] leads to secondary hyperparathyroidism and, when severe, can present as severe bone pain, muscle weakness, and increased body sway that can lead to falls and hip fractures.[115] Improved levels of vitamin D increase muscle strength and balance and can reduce fracture risk independent of BMD.[116,117] 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) is the biologically active form of vitamin D and is considered a hormone. Although reduced serum levels are seen with impaired renal function,[118] $1,25(OH)_2D$ is usually normal even when 25(OH)D levels are extremely low.

## *Vitamin K Status*

While vitamin D is important for calcium absorption from the gut, vitamin K is needed to reduce renal calcium loss,[119] and a deficiency is associated with reduced BMD and increased fracture risk.[120,121] Vitamin K acts as a cofactor in the gamma-carboxylation of glutamic acid residues of many calcium-binding proteins. This post-translational carboxylation is important for normal blood coagulation and bone formation.

There are three known bone-matrix vitamin K-dependent proteins important for bone formation: osteocalcin, matrix Gla protein (MGP), and protein S. Osteocalcin production by osteoblasts is induced by $1,25(OH)_2D$,[122] and vitamin K is responsible for the carboxylation activation of osteocalcin that appears to be necessary for nucleation of the hydroxyapatite crystal. Vitamin K deficiency leads to the under-carboxylation of osteocalcin.[123] Oral anticoagulants, which are antagonists to vitamin K, have been shown to cause an under-carboxylation of both MGP and osteocalcin.[124] Low levels of carboxylated osteocalcin or high levels of under-carboxylated osteocalcin (ucOC) carry an increased risk for femoral neck fracture.[124] MGP is both a bone-matrix protein and a chondrocyte protein that inhibits calcification. Vitamin K deficiency can reduce MGP and lead to excess soft-tissue calcification. Kiel et al identified a correlation between arterial calcification and osteoporosis.[125]

Estrogen is thought to play a part in vitamin K metabolism. Serum phylloquinone levels are not linearly related to the levels of carboxylated osteocalcin and estrogen may be an influencing factor in this discrepancy.[126] Yasui et al demonstrated serum ucOC has a negative correlation with estradiol and a positive correlation with FSH levels in perimenopausal women.[127] Therefore, even with adequate serum phylloquinone, reduced levels of estrogen may impair carboxylation of osteocalcin and limit bone mineralization.

The body stores minimal vitamin K and, although severe deficiency leading to impaired blood clotting time is uncommon, mild levels of vitamin K deficiency are common. Although prothrombin time (PT) is a test for deficiencies in vitamin K-dependent clotting factors, it is not a sensitive biomarker for mild vitamin K deficiencies. Although ucOC is a very sensitive marker for vitamin K, especially when used as a ratio in proportion to osteocalcin levels, it has not been approved for clinical use.

Although serum phylloquinone is a clinically available test for vitamin K status, it is of limited use because it is influenced by estrogen, triglycerides,[128] and recent dietary vitamin K intake. In addition, serum phylloquinone levels do not determine the status of vitamin K-dependent bone-matrix protein carboxylation. Therefore, currently there are no clinically available and reliable tests for assessing vitamin K or ucOC status.

Martini et al showed a significant reduction of serum N-Tx (an indicator of bone resorption) in postmenopausal women treated with 450 mcg phylloquinone daily.[129] Very high doses (45 mg/day) of vitamin K2 have been used to treat postmenopausal osteoporosis in Japan with no reported adverse effects.[130-132]

## *Urine Calcium Loss*

Excess urine calcium loss is a common finding in osteoporosis (and kidney stones). Hypercalciuria causes renal-magnesium wasting[133] and may be caused by excess calcium intake, increased intestinal calcium absorption, hyperparathyroidism, or a benign renal calcium leak. Although influenced by sodium intake and urine volume,[134] urine calcium/creatinine ratio (uCa/Cr) can be used as a general screening tool for calcium levels in the urine[135] – normal ratio is 0.1-0.2. In the case of a ratio >0.2, 24-hour urine calcium should be obtained (upper limits of 250 mg/day for females and 300 mg/day for males). A high urine volume is often seen in hypercalciuric patients because urine calcium stimulates calcium receptors in the renal collecting ducts and inhibits antidiuretic hormone. These patients tend to have frequent clear urination of a pH< 6.0. If the uCa/Cr is <0.1, ensure that the patient has adequate calcium intake and if the ratio remains <0.1, malabsorption should be ruled out. The uCa/Cr ratio should only be used as a screening test for hypercalciuria and not as a diagnostic screen for osteoporosis.[136] Hypocalciuria is seen in celiac patients and can be confirmed with a serum anti-tissue transglutaminase assay.

Hypercalciuria is a common contributing factor in osteoporosis and therefore, in addition to bisphosphonate therapy, thiazide diuretics are often prescribed. Although thiazides reduce urine calcium excretion and lower serum PTH levels, they do not affect gastrointestinal calcium absorption. Studies are mixed as to the effectiveness of thiazide diuretics for the treatment of osteoporosis. Although some studies show an increase in BMD with thiazide use,[137,138] others fail to show a statistically significant difference in reduced fracture rate between thiazide users and nonusers.[139,140]

Because thiazide diuretics have been linked to reduced glucose tolerance, the possibility for increased falls,[141] and a reduced hypocalciuric effect over time, it makes therapeutic sense to approach hypercalciuria nutritionally with a trial of supplemental vitamin K and potassium. Vitamin K is not only important for carboxylation of osteocalcin but also for calcium reabsorption.[142] Potassium (citrate and bicarbonate) has been shown to reduce urine calcium loss[98,143] and improve BMD.[144,145] Counseling the patient to increase dietary intake of fruits and vegetables, while restricting acidic foods and high-phosphoric-acid cola drinks (shown to reduce BMD),[146] will also reduce urine calcium losses.

## *Serum Calcium, Phosphorus, and Parathyroid Hormone*

### Hypercalcemia

Hypercalcemia is the hallmark of hyperparathyroidism (malignancy is the cause of hypercalcemia in approximately 50 percent of hospitalized patients),[147] and when calcium levels are >10.5 mg/dL, serum PTH will confirm the diagnosis. In this hypercalcemic condition, PTH stimulates 1-alpha hydroxylase in the kidney, elevating $1,25(OH)_2D$ and increasing urine calcium losses.

### Hypocalcemia

Hypocalcemia may be from malabsorption, magnesium deficiency,[148] or reduced levels of PTH. Even borderline-low serum calcium signals a possible magnesium deficiency. Commonly deficient, magnesium is often low due to diabetes, alcoholism, malabsorption, steatorrhea, poor diet, or genetically-based reduced intestinal uptake. Magnesium is an intracellular cation, and its depletion reduces renal conservation of potassium vital for cellular water balance and pH homeostasis. Because only one percent of magnesium is found in the extracellular fluid, serum magnesium testing does not accurately reflect overall levels.[133]

### Hyperphosphatemia

Phosphorus is one of the most abundant minerals in the body and along with calcium constitutes a major portion of the hydroxyapatite crystal in bone mineralization. Phosphorus is found in most foods and therefore dietary intake is usually sufficient for bone health, if not excessive in the Western culture. Phosphorus and calcium levels are maintained through PTH, vitamin D, and changes in renal tubular reabsorption rates.

High dietary intake of phosphorus inhibits renal reabsorption, thus maintaining normal serum levels. But in severe renal failure or in the osteoporotic patient with chronic metabolic acidosis, high dietary intake of phosphorus can lead to hyperphosphatemia. In these situations the elevation in serum phosphorus leads to reduced $1,25(OH)_2D$ production, limiting intestinal calcium absorption and resulting in hypocalcemia and secondary hyperparathyroidism.[149] Hyperphosphatemia is also observed in several genetic disorders and may be present in patients using bisphosphonates.[149]

### Hypophosphatemia

$1,25(OH)_2D$ increases intestinal absorption of phosphorus and, in vitamin D deficiency, serum and urine phosphorus levels may be reduced,[112] which can lead to osteomalacia. If serum phosphorus is low from a nutrient-deficient diet or intestinal malabsorption, $1,25(OH)_2D$ production increases and improves intestinal absorption, and PTH will decrease thereby reducing renal losses of phosphorus. Because PTH increases renal clearance of phosphorus, hypophosphatemia may be seen in hyperparathyroidism. Hypophosphatemia can therefore be seen with either reduced $1,25(OH)_2D$ production in the hypocalcemic patient with renal disease or in the hypercalcemic primary hyperparathyroid patient.[149] Hypophosphatemia is also present in other disease processes and several genetic disorders.

### Hypoparathyroidism

Functional hypoparathyroidism is characterized by low $1,25(OH)_2D$, hypocalcemia, and low-to-normal PTH, and appears to be associated with magnesium deficiency.[150] Typically, low vitamin D levels lead to an elevation of PTH to maintain calcium homeostasis; however, a failure of PTH to increase is termed functional hypoparathyroidism. Although PTH production depends on at least one magnesium-dependent enzyme,[151] magnesium's full role in PTH production is still unclear. PTH secretion may be dependent on the magnesium-dependent enzymes adenylate cyclase or guanine nucleotide.[152] In functional hypoparathyroidism, magnesium supplementation will increase PTH levels to normal, even in the face of vitamin D deficiency, underscoring the importance of checking both $1,25(OH)_2D$ and 25(OH)D.

### Hyperparathyroidism

Serum IL-6 levels, known to increase with estrogen deficiency, are elevated with both symptomatic and mild asymptomatic primary hyperparathyroidism.[153] With IL-6 testing not easily available, elevated PTH can be reduced with vitamin D and calcium supplementation, but also by reducing IL-6-related chronic inflammation and improving acid/base balance. Depending on estrogen levels, the use of exogenous estrogen replacement may also be considered.

Secondary hyperparathyroidism is seen with aging and the loss of the calcitrophic effects of estrogen. Mild increases in PTH are often observed in vitamin D deficiency, hypercalciuria, or insufficient intake or reduced absorption of calcium.

## *Anti-Tissue Transglutaminase and Celiac Disease*

Celiac disease (CD) is caused by an autoimmune reaction to the protein gluten that results in inflammation and villous atrophy of the small-intestinal mucosa. Common symptoms include diarrhea, flatulence, steatorrhea, musculoskeletal complaints, abdominal distention, and dermatitis herpetiformis;[154] however, in its atypical form there may be no overt symptoms. Associated malabsorption of iron, folic acid, calcium, zinc, and the fat-soluble vitamins A, D, E, and K can lead to anemia and bone loss, often present in celiac patients.

Serological tests aid in the diagnosis of gluten sensitivity and should be considered in a patient with reduced BMD, gastrointestinal symptoms, unidentified neuro-musculoskeletal complaints, or if the patient has a first-degree relative with CD. Serum tissue transglutaminase antibody (tTGA) is a highly sensitive test for identifying both classical and atypical CD.[155] Further testing for CD includes anti-gliadin antibodies and anti-endomysial antibodies. If necessary, histological confirmation of CD is made through small bowel biopsy or a gliadin rectal challenge. Treatment of CD is through strict avoidance of gluten protein found in wheat, barley, rye, and oats.

## *Thyroid Effects on Bone*

### Hyperthyroidism

Thyrotoxicosis can cause hypercalcemia with secondary suppression of PTH and increased urine calcium losses, leading to bone loss. Elevated thyroid hormone increases bone remodeling by directly stimulating both osteoblast and osteoclast activity and increasing production of IGF-1. While triiodothyronine (T3) increases bone resorption, thyroid-stimulating hormone (TSH) from the anterior pituitary gland directly suppresses osteoclastic resorption.[156] Serum TSH of <0.5 μU/mL appears to inhibit the formation, function, and survival of osteoblasts, independent of T3 and T4, and is associated with bone loss and increased fracture risk.[157] In a study of 959 healthy postmenopausal women, those with low-normal TSH levels (0.5-1.1 μU/mL) had lower BMD of the lumbar spine and femoral neck than those with high-normal levels (2.8-5.0 μU/mL).[158]

### Hypothyroidism

Reduced thyroid function has been linked to low femoral neck BMD,[159] decreased IGF-1 production,[160,161] and increased fracture risk.[162] Suboptimal zinc levels in patients with hypothyroidism may also contribute to bone loss. Zinc is important for energy metabolism and also for bone growth and production of thyroid hormones. Zinc deficiency retards bone growth and can be a contributing factor in the development of osteoporosis.[163] The enzyme 1,5′-deiodinase that converts thyroxine (T4) to T3 is zinc dependent. Baltaci et al demonstrated in rats that T4, T3, and TSH levels decrease with zinc deficiency.[164] Zinc deficiency[165] and reduced thyroid function are common in high-intensity athletes. Chronic metabolic acidosis has also been linked to reduced thyroid function.[166]

## *Homocysteine*

Homocysteine (HCY), a metabolite of the amino acid L-methionine, interferes with collagen cross-linking and is related to increased hip-fracture rate,[167] independent of BMD.[168] Osteoporosis is a common symptom of homocysteinuria, a rare autosomal recessive disease caused by mutation of the gene for methylenetetrahydrofolate reductase (MTHFR). The enzyme MTHFR is necessary for HCY metabolism. Milder elevations of HCY occur with a T homozygous polymorphism for MTHFR and may be linked to an increase in fracture risk. This common polymorphism creates a less-active enzyme, causing serum HCY to increase and was shown in a study with postmenopausal Japanese women to be associated with lower BMD.[169]

Serum HCY levels >15 μmol/L are associated with increased bone turnover markers (osteocalcin and Dpd) and increased fracture risk.[170] Gjesdal et al observed a statistically significant 2.5-fold increase in fracture risk with HCY levels >15 μmol/L. The study demonstrated a positive linear relationship between HCY levels and fracture risk (p for trend).[171] In a review, Saito

describes that even mild hyperhomocysteinemia reduces bone quality and increases bone fragility.[172] HCY levels increase with age and, because 5-15 percent of Caucasian women in North America are T homozygous for MTHFR, serum testing for homocysteine is important when assessing a patient with bone loss.

In addition to increased bone loss and fracture risk,[168] elevated HCY has been linked to chronic inflammation. In cultured cells, HCY has also been shown to increase apoptosis of osteoblasts by increasing intracellular ROS.[173] Homocysteine is also correlated with increased lipoprotein oxidation and the formation of PPARγ ligands, associated with reduced BMD in mice.

Vitamins B6 and B12, folate, and riboflavin are necessary cofactors for the metabolism of HCY.[174] Because a defect in MTHFR may cause a deficiency of the active form of folate, 5-methyltetrahydrofolate would be the form of choice for managing homocysteinemia. Testing of urine organic acid helps identify vitamin B insufficiencies. Elevated methylmalonate, a metabolic intermediate of the amino acid valine, indicates the need for vitamin B12.[88]

The conditionally essential amino acid taurine is an end-product of methionine metabolism and oral supplementation can help counteract the negative actions of elevated HCY levels.[175] Taurine and N-acetylcysteine are also known to inhibit hydrogen peroxide and superoxide anion secretion[176] and to help increase superoxide dismutase levels,[177] all of which are important for reducing the catabolic state of chronic degenerative disease.

## *Insulin-like Growth Factor-1*

The anabolic actions of growth hormone are, to a great extent, due to the effects of IGF-1 from the liver. IGF-1 is a potent anabolic agent estimated to directly influence approximately one-third of the factors involved in skeletal growth, including calcium and phosphorus metabolism.[178] Because IGF-1 is homologous to human pro-insulin, it is affected by glucose metabolism and insulin levels.

Receptors for IGF-1 are found on osteoblasts, osteoclasts, and osteocytes,[179] and even small changes in IGF-1 levels in mice have been shown to affect bone mass.[178] Oxidative stress and IL-6 increase with aging[180-182] and have been shown to inhibit IGF-1 secretion, which leads to sarcopenia and bone loss. A decline in IGF-1 is seen with waning estrogen in postmenopausal women[183] and higher IGF-1 levels are associated with greater BMD in older women.[184] Although reductions in IGF-1, insulin, and growth hormone are associated with longevity,[185] low serum IGF-1 in the presence of bone fragility strongly indicates the need for a protein-enriched, anabolic-promoting diet and a reduction in catabolic influences such as hyperglycemia. Although hyperinsulinemia is correlated with increased IGF-1 levels, in a study by Martin et al, IGF-1 was only weakly positively correlated to insulin resistance.[186]

Dietary protein supplementation[187] with whey and milk basic protein (whey's basic protein fraction),[188,189] colostrum,[190] and the amino acids L-arginine, glutamine, ornithine, lysine, and glycine raise serum IGF-1 and may reduce fractures. Milk basic protein has been shown to reduce bone resorption and increase bone formation in women[191] and men.[192] Supplementation with DHEA and zinc, improving sleep patterns, and engaging in moderate exercise can help increase IGF-1 levels.[193,194] Osteoporotic patients are known to have lower zinc levels,[195] and both zinc and copper are recommended[163] for reducing bone loss in patients with osteoporosis.[196,197] In studies with rodents, zinc and IGF-1 stimulated DNA synthesis for bone growth,[198] increased osteoblastic bone formation, and reduced osteoclastic resorption.[199]

Abnormally low levels of IGF-1 may indicate a catabolic physiology. However, because genetics substantially influence IGF-1, low serum levels may be found even with normal endocrine function. For this reason, and the fact that a majority of IGF-1 in bone is derived from osteoblasts and not from the circulation,[200] it is important to look at a patient's overall presentation and take into consideration all the biomarkers used in the evaluation. For example, when retesting for efficacy of treatment, improvements in one or two biomarkers (e.g., reduction in N-Tx and urine calcium losses) may indicate improved catabolic physiology even with no improvement in IGF-1. Testing of IGF-1 can be through serum or saliva (active, free form).

## C-Reactive Protein

C-reactive protein (CRP) is an abnormal glycoprotein produced in the liver in response to inflammation. Although several studies have failed to demonstrate a direct association between serum CRP and bone density,[201-203] others show that higher CRP levels are associated with reduced BMD.[204,205] Pasco et al found a 23-percent increase in fracture risk, independent of BMD, with each standard deviation increase in high-sensitivity CRP (hs-CRP).[206] The inflammatory cytokine IL-6 has been shown to stimulate the production of CRP.[182] In addition to the IL-6 involvement in osteoclastogenesis and bone loss, it is also catabolic to muscle tissue by reducing IGF-1 levels[207,208] and contributes to the sarcopenic phenotype commonly seen in the osteoporotic patient.

Because clinical testing for proinflammatory cytokines is not commonly available, testing for hs-CRP can reflect increased proinflammatory cytokine levels. Lifestyle changes and dietary supplementation such as α-lipoic acid, N-acetylcysteine, taurine,[209] and curcumin[210,211] to relieve oxidative stress may help reduce hs-CRP levels. Omega-3 fatty acids DHA and EPA are known to reduce proinflammatory cytokines, and the omega-6 α-linolenic acid has been shown to reduce CRP.[212] CRP concentrations were reduced in smokers (or individuals passively exposed to cigarette smoke) with 515 mg/day supplemental vitamin C.[213]

## Cortisol, Neurotransmitters, and DHEA

Both cortisol elevation and reduced levels of DHEA are linked to osteoporosis. Cortisol is released from the adrenal cortex when stimulated by adrenocorticotropic hormone (ACTH) from the pituitary gland in response to stress. Prolonged exposure to cortisol is associated with adipocyte bone marrow infiltration, reduced BMD, and increased fracture risk.[214,215] In a study with sleep-deprived women, Specker et al demonstrated that elevated cortisol levels were associated with reduced volumetric BMD.[216] Volumetric density, a measurement that includes bone size, is obtained through quantitative computer tomography and designated in $g/cm^3$, compared to projected areal density of DXA, which is designated in $g/cm^2$. Increased fracture risk in older adults has been linked to mild increases in endogenous cortisol levels.[217] And even the subtle stress of dietary restraint in normal-weight females increases urine and salivary cortisol concentrations[218,219] and has been shown to reduce bone mineral content. In a study of 65 healthy women with high cognitive eating restraint scores, reduced levels of serum osteocalcin indicated lower bone turnover (although BMD changes were not apparent).[220]

Mild chronic stress causes bone loss in mice via activation of the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal (HPA) axis.[221] Stimulation of the SNS increases RANKL in osteoblasts and progenitor cells,[222] which increases osteoclastic activity. Stress and chronic activation of the HPA axis raise serum cortisol and reduce levels of another adrenal steroid, DHEA. The effects of chronically elevated cortisol or reduced DHEA can be similar to those of exogenous glucocorticoids – a decrease in IGF-1 with a concomitant increase in bone marrow infiltration by adipocytes.[215]

Cortisol can be measured in the blood, urine (24-hour urine sample), or saliva. Cortisol levels have a circadian rhythm and normally peak in the morning (0700 hours) and are at their lowest in the late evening (2300 hours). With multiple saliva samples a 24-hour cortisol circadian rhythm can be determined.[223] Salivary assays are useful in the identification of adrenal insufficiency[224] and late-night salivary cortisol measurement is a useful screen for Cushing's Syndrome.[225] Elevated night-time salivary cortisol has been linked to reduced lumbar spine BMD in men and women (not on HRT).[226] In a study to compare the accuracy of two different methods – radioimmunoassay (RIA) and enzyme immunoassay (EIA) – for measuring salivary cortisol, RIA was the more accurate method, while EIA overestimated cortisol concentration.[227] This underscores the importance of comparing values from similar methods when doing serial testing.

DHEA is metabolized to androstenedione, which is the precursor to androgens and estrogen. DHEA levels are inversely correlated with N-Tx in anorexic girls[228] and oral supplementation can reduce resorption markers and increase bone formation markers

in anorexic women.[229] Kahn et al, however, did not find a reduction in bone turnover markers in men supplemented with 90 mg/day oral DHEA for six months.[230] Oral DHEA supplementation also increases estradiol levels in men and postmenopausal women[231] and can increase osteocalcin and IGF-1.[232] When levels of DHEA/DHEA-S are abnormally low, oral supplementation of 50-100 mg/day can increase BMD.[229] Moderate-to-intense exercise helps increase endogenous DHEA.

DHEA and DHEA-S levels may be assessed through serum[233] or saliva. Salivary assays measure unbound, free-circulating hormones. Evaluation using cotton-absorbing, sample-collecting materials have been shown to artificially elevate DHEA levels.[234]

Urine organic acids are helpful in defining and monitoring stress and therapeutic efficacy in the fracture-risk patient. Urinary breakdown products of adrenal medullary neurotransmitters can help assess a patient's stress level and adrenal health. These breakdown products include vanillylmandelic acid (VNA; end-product of epinephrine and norepinephrine metabolism), homovanillate acid (HVA; metabolite of dopamine), and 5-hydroxyindoleacetic acid (5-HIAA; metabolite of serotonin).

## *Low-Density and High-Density Lipoproteins*

The biomarkers, low-density lipoprotein (LDL) and high-density lipoprotein (HDL), most relevant to cardiovascular disease (CVD), may also be indicators of reduced bone quality and increased fracture risk. HDL, long understood as protective in CVD, has recently been identified as a possible regulator of osteoblast cell differentiation.[235,236] Parhami et al demonstrated that products of lipid and lipoprotein oxidation from a high-fat diet are the source of PPARγ-activating ligands and can lead to a loss of bone density in mice.[237] It is the commonality of osteoblast and adipocyte stem cells that links osteoporosis and obesity (Figure 2),[238] two of the most common conditions in the United States, and an over-expression of PPARγ interferes with normal differentiation of their common precursor cell.

Bone marrow mesenchymal stem cells (mMSC) differentiate into either osteoblasts or adipocytes, depending on their environment. Elevated triglycerides, reduced control of blood glucose, abnormally low serum IGF-1, and/or loss of estrogen can lead to an increase in PPARγ production and adipocyte development at the expense of osteoblastogenesis. Both an increase in bone marrow adiposity and a suppression of osteoblastic activity are seen with the activation of the adipocyte-specific transcription factor PPARγ2 in aging mice.[239] Magnetic resonance imaging reveals that postmenopausal women have twice the level of bone marrow fat as premenopausal women, and the lower the bone density the greater the saturated-fat content.[240] Parhami has hypothesized that accumulation of bone marrow fat in an environment of increased oxidative stress increases lipoprotein oxidation. With oxidation, ligands for the expression of PPARγ increase and mMSC differentiation is diverted from osteoblasts to adipocytes. This oxidation-driven diversion creates an increase in RANKL-dependent osteoclastic activity.[237] Therefore, hyperlipidemia and markers for free radical oxidative stress (such as urine lipid peroxides) may be risk markers relevant to increased bone fragility. Many nutrients and plant extracts can decrease lipid peroxidation. For example, lycopene has been shown to decrease both lipid peroxidation[241,242] and the bone-resorption marker N-Tx in postmenopausal women.[243]

## *Glucose*

Abnormal blood glucose control is also a risk factor for bone loss. Reduced bone mass and increased fracture rate are common in type 1 diabetes and are linked to PPARγ and preferential adipogenesis over osteoblast-cell development.[244] Diabetic mice demonstrate reduced IGF-1 levels, increased bone marrow adipocyte-infiltration that leads to reduced RBCs, and reduced osteoblast formation and maturation (reflected by a reduction in osteocalcin).[244] Diabetic patients have reduced late-stage differentiation of osteoblasts and a decrease in osteoblast function.[245]

Advanced glycated end products (AGEs) have been linked to abnormal development of osteoblasts and are thought to enhance bone resorption and induce apoptosis of mMSCs.[246] Enzymatic cross-linkage of collagen fibers gives strength to bone, but AGE-induced, non-enzymatic collagen cross-linkage leads to increased fracture risk.[247] AGE accumulation from increased oxidative stress occurs more readily in highly mineralized bone,[247] such as that which occurs in long-term bisphosphonate use, and may contribute to increased fracture

risk. For this reason, glucose control of the patient on bisphosphonate therapy is essential.

Thiazolidinediones (Avandia; Actos), artificial PPARγ agonists used for the treatment of type 2 diabetes, inhibit osteoblastogenesis and have been shown to reduce BMD in mice.[248] Therefore, these medications should be avoided if possible in patients with low BMD.

## *Sex Hormones*

### Estrogen

Since publication of the findings of the Women's Health Initiative (WHI) program, the role of hormone replacement therapy (HRT) in the management of osteoporosis has been questioned, even in light of a 33-percent reduction in hip-fracture rate.[249] Although HRT (estrogen plus progestin) slows bone loss, increases bone mineral density, and reduces fracture rate,[250] the risks of hormone replacement may outweigh the benefits. Although HRT is still used by many physicians, there are concerns that it should no longer be the first choice in treatment for osteoporosis.[25] The use of bioidentical hormones, thought to be safer than conventional synthetic hormones due to their differences in metabolism,[251] have become more popular since the WHI. However, as yet, there have been no large-scale trials to assess their efficacy or risk.

Estrogen is the major hormone regulator of bone remodeling and is important for men as well as women. Estrogen's role in maintaining bone health is far reaching – from maintaining calcium homeostasis by stimulating the release of calcitonin and activating $1,25(OH)_2D$ receptors in the gut to its function within the osteo-endocrine-immunological axis.

Estrogen limits bone loss through its effects on osteoblast and osteoclast activity. Estrogen-receptor activation of osteoblasts stimulates release of the growth factors TGF-β and IGF-1, and OPG. This in turn limits M-CSF and RANKL, which reduces osteoclastogenesis and increases osteoclast apoptosis.

Reduction in estrogen leads to increased osteoclastic activity from the reduced hormonal control over proinflammatory cytokines, IL-1, IL-6, and TNF-α (which increase when there is a loss of estrogen).[252] Elevated serum IL-6 levels are considered the strongest determinant of femoral bone loss in women within the first decade after menopause.[253] Proinflammatory cytokines increase T-cell activation and the release of RANKL, which promotes NFκB, the key transcription factor in osteoclastogenesis. The rise in proinflammatory cytokines from reduced estrogen also stimulates mMSC production of IL-7, which has been shown to suppress bone formation and increase RANKL stimulation of osteoclasts in mice.[254]

As mentioned previously, oxidative stress plays a major role in chronic degenerative diseases including osteoporosis. Estrogen suppresses ROS, which are known to stimulate osteoclast activity[255] through the activation of NFκB; with a loss of estrogen, ROS increase. Antioxidant supplementation to down-regulate IL-6 gene expression and TNF-α-induced NFκB is recommended, especially in women with low estrogen levels.[255] It has been shown in mice that NAC protects against bone loss, possibly through the reduction of ROS.[44] The use of α-lipoic acid and NAC have been shown to reduce ROS-mediated apoptosis of cultured human stromal cells (the precursors of osteoblasts).[256]

Estrogen improves oral tolerance by suppressing the release of TGF-β by dendritic cells and macrophages, which then limits their antigen presentation.[257] With modulation of antigen presentation, RANKL and TNF-α production from T-cell activation is reduced. When estrogen is lost dendritic-cell-antigen presentation and T-cell activation increases. Because dendritic cells are activated through redox signaling, the rise in ROS associated with reduced estrogen in osteoporotic patients could potentially be offset by the use of probiotics and antioxidants when appropriate.

Estrogen levels fluctuate during the menstrual cycle and are stable after menopause. A substantial loss of bone occurs when endogenous serum estrogen falls below a critical level in women (approximately 10 pg/mL).[258] By understanding estrogen's connection with the immune system as it relates to bone remodeling, the nutritionally-oriented clinician can guide patients in making choices with regard to hormone replacement to reduce bone loss.

### Follicle Stimulating Hormone

High serum follicle stimulating hormone (FSH) >40 mIU/mL in perimenopausal women is a reflection of low estrogen. The effect of FSH on BMD appears, however, to be independent of estrogen, and a gradual rise in the follicular phase FSH to >70 mIU/mL is correlated with reduced BMD.[259] FSH receptors are found on osteoclasts and, as the hormone increases, it heightens osteoclastic activity and stimulates TNF-α release from macrophages (which is also osteoclastogenic). Starting premenopause, the use of serial serum FSH testing may provide an early indication of accelerated bone loss.

### Progesterone

Although amenorrhea and oligomenorrhea are obvious signs of hormonal dysfunction, anovulatory and short-luteal-phase cycles may not be as evident; all scenarios may be associated with reduced BMD.[260] Activation of the HPA axis, even in mild chronic stress, reduces gonadotropin-releasing hormone (GnRH) and could result in reduced hormone levels and subclinical ovulatory disturbances. These abnormal (yet normal length) cycles can be detected through blood or salivary progesterone measurements. For example, suppressed progesterone during the second half of the cycle would indicate an anovulatory cycle. A short luteal phase would be indicated if progesterone elevation lasted less than 10 days.[218]

Topical progesterone creams made from diosgenin, extracted from Mexican yams, are frequently used for osteoporosis, but their ability to increase BMD remains controversial. Much of the attention for the use of progesterone has been due to the conclusions of Lee on the efficacy of transdermal progesterone cream to increase BMD in postmenopausal women.[261] In a study by Leonetti et al there was no increase in BMD in menopausal women treated daily for one year with 20 mg progesterone cream.[262]

### Testosterone and Sex Hormone-Binding Globulin

Although reduced estradiol has a clear association with increased fracture incidence,[263] the effects of low testosterone on bone in men and women have been less definitive. As men age, there is a rise in the proinflammatory cytokine marker soluble IL-6 receptor secondary to reduced sex steroid levels.[264] This is an indication that androgens may play a role in both the inflammatory changes and increased bone loss related to aging.

Although hypogonadal men demonstrate reduced BMD,[265] the relationship of testosterone to fracture risk has remained unclear. In a recent study, Mellstrom et al showed low-normal levels of serum free testosterone were an independent predictor of BMD and increase in fractures.[266] Lorentzon et al showed the effects of sex hormone-binding globulin may be independent of estrogen and testosterone and that its effects on bone may be age dependent.[267] In another study of 609 men over the age of 60, low serum testosterone and high SHBG levels were associated with increased risk of osteoporotic fractures independent of BMD.[268] Elevated SHBG in young men may be beneficial for improved bone mass, whereas in elderly men it appears to be a negative predictor of BMD.[267]

Testosterone replacement therapy in men with severe subnormal serum testosterone levels has been shown to reduce bone resorption and reverse deterioration of trabecular architecture.[269] Serum testosterone can be measured as free testosterone or bound to SHBG; only free testosterone can be measured through saliva.

## Effect of Exercise on Bone Health

Exercise has an important influence on bone health and is essential for reducing fracture risk. Exercise in adolescence and into adulthood increases BMD and preserves bone health.[270,271]

In addition to improving mitochondrial bioenergetics, exercise also increases the anti-inflammatory cytokine IL-10 and the inflammatory modulator IL-1 receptor antagonist molecule.[272,273] Encouraging exercise in patients increases IGF-1[193] and is vital for increasing muscle strength and coordination. Exercise reduces the risk for falls, the number-one risk factor for fracture.[274,275]

Although exercise has many benefits for bone and general health, chronic, high-intensity, excessive exercise subjects the athlete to hypoxia, metabolic acidosis, excessive oxidative stress, and elevated levels of proinflammatory cytokines;[276] instead of being anabolic, exercise becomes catabolic when taken to excess.

Athletes participating in an ultra-distance running race experienced an 8,000-fold elevation in IL-6 levels,[277] and in another study of male long-distance runners, bone turnover markers were increased and bone mass was reduced compared to controls.[278] Elevated levels of IL-6 result in increased lipid oxidation and PPARγ production; T-cell activation with increased RANKL production can also result.[236] The sleepiness and fatigue of "sickness syndrome" produced by IL-6 during severe exercise helps prevent athletes from inflicting excess tissue damage on their bodies.

## The Need for a Different Approach to Bone Health

Hormone replacement therapy remained the first choice in osteoporosis prevention and treatment for postmenopausal women until July 9, 2002, when the Women's Health Initiative study[250] was abruptly halted. The study was discontinued with the early observation that conjugated equine estrogen plus synthetic progestin (medroxyprogesterone acetate) increased the risk of breast cancer, cardiovascular disease, stroke, and venous thromboembolism.[279] Although the study also showed a decreased risk of hip fracture with the use of HRT, the risks outweighed the benefits.[248]

In the last decade the use of bisphosphonate therapy for osteoporosis has gradually increased. And, although it has been well documented that bisphosphonates limit osteoclastic bone resorption,[280] increase BMD, and reduce fractures,[281-283] there have been safety concerns.

Bisphosphonates work by reducing resorption which, when excessive or in the absence of equivalent bone formation, leads to bone loss. But osteoclastic resorption is essential for bone health, and over-suppression may lead to increased accumulation of microdamage,[284-286] impaired mineralization,[287] reduced toughness, increased brittleness,[285,288] and a general reduction in the biomechanical competence of bone.[289]

One of the first bisphosphonates used for the treatment of osteoporosis was etidronate (Didronel®), which suppressed bone turnover but, when used in higher doses, resulted in osteomalacia by impairing osteoid mineralization.[290] Current bisphosphonates, such as alendronate (Fosamax®) and risedronate (Actonel®), for the treatment of osteoporosis have also raised safety concerns, including a possible increase in fracture rate after long-term use.[291] However, a multicenter study of 994 postmenopausal women concluded there was no increase in fracture rate after 10 years of alendronate therapy.[292]

Most recently, it has been demonstrated that bisphosphonates can cause severe suppression of bone turnover[293] and possibly lead to osteonecrosis of the jaw (ONJ). Pamidronate (Aredia®) and zoledronic acid (Zometa®), both intravenous bisphosphonates for cancer chemotherapy,[294] and the oral bisphosphonates alendronate and risedronate for the treatment of osteoporosis,[295] have all been linked to ONJ.

## Conclusion

When assessing treatment options for patients with increased bone fragility, fracture risk needs to be weighed against the potential for iatrogenic effects of bisphosphonates. Because bisphosphonates have not proven cost-effective in treating women with femoral neck T scores better than -2.5 with no history of fracture,[296] the use of biomarkers to monitor bone health and the use of nutritional therapy to prevent or treat osteoporosis seems prudent. But despite mounting scientific evidence pointing to the efficacy of nutritional interventions for bone preservation, conventional physicians have been slow to embrace such interventions. This was demonstrated in a study that found, even though hypovitaminosis D is common among physicians, they still do not check serum 25(OH)D levels in patients.[297]

Biomarker testing allows detection of metabolic change long before alterations in BMD, underscoring the need to refocus attention away from reliance solely on BMD testing. A comprehensive approach would employ biomarkers to assess risk and identify underlying disease mechanisms, including inflammation, oxidative stress, hormone imbalances, nutrient deficiencies, and malabsorption. Once the risk factors have been identified, a comprehensive, individualized plan, including diet, exercise, and targeted nutrients, can create a metabolic environment conducive to balanced remodeling and healthy bone.

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