

Effect of a Novel Combination of RIAA, Berberine, and Vitamins D and K on Bone Remodeling in Postmenopausal Women with Low Estrogen

The Role of Bone Remodeling in Osteoporosis

The development of osteoporosis is strongly influenced by a dynamic process called bone remodeling that is essential to bone's structural integrity. Bone is constantly being broken down by osteoclast cells that remove old and damaged bone tissue (resorption). Osteoblast and osteocyte cells then deposit a mesh-like, micro-architecture known as the organic bone matrix (formation) into which minerals are incorporated, giving bone its density and strength. This necessary physiological process removes weak bone and repairs microfractures to help ensure bone's structural integrity, thereby reducing the risk of osteoporosis and fractures that contribute to disability and morbidity.

The rate of resorption gradually begins to exceed that of formation as a woman reaches her mid-thirties to early forties; this results in a corresponding gradual net loss of bone. This acceleration of resorption and accompanying decline in formation creates the potential for the construction of a weaker bone matrix. And even the mineralization and hardening of partially formed or incomplete bone matrix leaves it more vulnerable to fracture. When women enter the postmenopause phase, resorption accelerates further due to loss of estrogen and may result in a more rapid loss of bone.

Remodeling is influenced by genetics, aging, diet, medications (e.g., corticosteroids), and lifestyle habits (e.g., smoking, alcohol consumption, exercise), as well as by the function of other body systems and health status (e.g., inflammatory disorders, insulin-related diseases, excess body fat, parathyroid dysfunction).

Bone Remodeling: Inflammation in Balance

While estrogen is recognized as the key sex hormone governing bone homeodynamics in women, new research focuses on the tight interaction between **bone and the immune system** as crucial for understanding the vulnerability of the skeletal architecture to chronic inflammation. Bone marrow houses mesenchymal stem cells (MSCs) and haematopoietic stem cells (HSCs) that differentiate into multiple cell lineages that perpetuate the interplay of these two systems. MSCs are the source of osteoblasts to form bone, adipocytes (fat cells), cartilage cells, immunosuppressive cells, and cytokines that influence osteoclasts. HSCs are the source of immune cells that can interact with bones, and further differentiate into osteoclasts to resorb bone. Osteoclasts and osteoblasts are sensitive to inflammatory and anti-inflammatory messages that originate locally within bone and systemically from distant organs. The nature of these regulatory messages via hormones and cytokines influence remodeling dynamics. Heightened cytokine expression can decrease activation of osteoblasts and increase osteoclasts, resulting in accelerated bone resorption.¹¹

The RANK/RANKL/OPG System

Osteoclast cell formation is stimulated by strong inflammatory signals, specifically, macrophage colony-stimulating factor (M-CSF), interleukin-6 (IL-6), receptor activator of nuclear factor-kappa beta (NF- κ B) ligand (RANKL), and its receptor (RANK). RANKL activates NF- κ B, a transcription factor also vital in the body's response to infection, has been associated with bone degradation.^{3,11}

Osteoblasts, which secrete RANKL to stimulate healthy resorption, also produce an anti-inflammatory protein called osteoprotegerin (OPG, literally "bone protect") to help keep osteoclast formation in check.¹¹ Estrogen has also been shown to interfere with RANK signaling. A decline in estrogen and/or osteoblasts, therefore, further favors

activation of osteoclasts for resorption. The discovery of the role of the RANK/RANKL/OPG system in bone remodeling has created a new arena of research and the identification of agents that may influence this system or its precursors.³

Adiposity, Inflammation & Bone

Aging also creates a gradual shift in favor of resorption as MSCs begin to favor production of adipocytes over osteoblasts. Adipocytes secrete inflammatory cytokines that increase osteoclast function (stimulating bone resorption) and foster the decline of osteoblast function (suppressing bone formation), which ultimately can result in osteoporosis.¹¹

Conditions or lifestyle habits that foster inflammation—or increased fat deposition—beyond that of natural aging, therefore, also accelerate bone turnover. Accelerated turnover rates in inflammatory and autoimmune disorders such as rheumatoid arthritis and inflammatory bowel disease are well established. Research has also demonstrated the association of accelerated bone turnover in obesity and insulin resistance disorders (e.g., type 2 diabetes, metabolic syndrome) that evolve from inflammatory processes and increased cytokine production.^{3,11-13}

Menopause Accelerates Bone Turnover Rate

In women, inflammatory cytokines that influence bone remodeling are normally held in check through protective effects of estrogen. Estrogen decline after menopause, therefore, further accelerates bone turnover by fostering an increase in production of these cytokines, which not only increase resorption but also suppress bone formation.^{2,3,10,14} The difference between early and late postmenopausal bone loss has been attributed to inflammatory cytokine levels.⁵ Generally, women are at the greatest risk of bone loss within the first five to seven years after the onset of menopause, where they may lose up to 20% of their bone mass.¹⁵

Anti-Resorptive Therapy Shortfalls

There are a number of medications that inhibit resorption, including bisphosphonates, calcitonin, hormone replacement therapy (HRT), estrogen replacement therapy, selective estrogen response modulators, cathepsin K inhibitors, anti-RANKL antibody denosumab, and recombinant parathyroid hormone. While these approaches show merit in many specific applications in clinical use, some serious side effects have been reported, including increased risk of breast cancer, heart attack, blood clots, stroke, serious gastrointestinal issues, musculoskeletal pain, atrial fibrillation, and osteonecrosis.¹⁶⁻¹⁹ Up to 50% of patients discontinue osteoporosis treatment in less than one year due in part to adverse effects.²⁰

Natural Agents of Change

To date, calcium and vitamin D supplementation (recommended as an adjunct to anti-resorptive approaches such as bisphosphonates to facilitate mineralization) have been the only alternative approach for those who cannot tolerate or benefit from—or simply elect not to participate in—more aggressive, side effect-prone treatments. Although they have been shown to reduce osteoporosis and fracture risk, the primary benefit of these natural agents is mineralization for bone density. Calcium supplementation does not influence remodeling of the organic bone matrix (nor do adequate calcium stores). In other words, fortifying a weak matrix with calcium doesn't change the architecture of the matrix itself.

Emerging research on natural agents that may positively influence bone remodeling include studies on *rho* iso-alpha acids (RIAA) derived from hops (*Humulus lupulus*), berberine (*Phellodendron amurense*), vitamin D, and vitamin K.

- **RIAA.** RIAA has been shown to function as a selective kinase response modulator (SKRM) to favorably modulate key kinases involved in bone degradation—glycogen synthase kinase 3 (GSK-3) and spleen tyrosine kinase (Syk).^{4,9} Inhibition of GSK-3 has been shown to promote bone formation, and inhibition of Syk has been identified as a therapeutic target in inflammatory disorders that accelerate bone loss.^{4,6-8} In vitro testing demonstrated that RIAA inhibited RANKL-mediated activity in bone resorption (e.g., NF-κβ abundance). RIAA has also been demonstrated to upregulate IGF-1 activity to promote formation.
- **Berberine.** Similar to RIAA, in vitro studies have demonstrated that berberine inhibited RANKL-mediated activity in bone resorption.²¹ An in vivo study showed similar inhibition of osteoclastic activity.²² It has also been suggested that berberine may positively influence osteoblast formation.²³
- **Vitamin D.** In addition to facilitating calcium absorption for mineralization, vitamin D plays a natural role in regulating bone turnover.^{24,25} Deficiency, which is common in the elderly and postmenopausal women, has not only been associated with increased risk to osteoporosis and fracture but also with inflammatory, autoimmune, and insulin resistance conditions (e.g., metabolic syndrome).^{24,26} Low vitamin D intake has also been linked to increased fracture risk and increased rates of bone loss.²⁷
- **Vitamin K.** Vitamin K is an important nutritional factor in the metabolism of bone proteins (e.g., osteocalcin) crucial to bone quality, integrity, and the support of overall bone mass in both men and women. Regular intake of vitamin K has been linked with increased bone mineral density (BMD).²⁸⁻³¹ Positive clinical outcomes in BMD and bone remodeling have been associated with supplementation of 1 mg daily.²⁷

Bone Matrix Quality vs. Mineral Density

The strength of bone lies not only in its density but also in the integrity of its architecture—the organic bone matrix. Bone turnover affects both, but not necessarily equally. A high rate of bone turnover can increase fracture risk through a reduction in bone matrix quality without substantial impact on BMD.³²⁻³⁴ It has been suggested that in a comparison of two women with identical BMD, a 75-year-old would carry four to seven times greater potential for fracture than a 45-year-old; this can be attributed to the integrity of the bone matrix and rate of remodeling. Thus, bone quality components may be useful in determining long-term bone strength.³³⁻³⁵

The same effect on bone strength must also be considered in treatments that protect against bone loss. Because osteoporosis is usually defined by low bone mineral content (lack of density), drug treatments have traditionally been developed to preserve mineral content—not to ensure the stability of bone architecture. A concern with anti-resorptive therapies is that they have been shown to increase BMD by increasing the mean age of bone tissue; this is accomplished through inhibition of resorption that would normally replace old bone tissue with new, creating sub-optimal foundations for future growth.³⁵

But while bone density may increase, overall bone quality—and protection from fracture—may not.

Re-establishing a rate of remodeling that improves overall bone matrix integrity—rather than fosters inhibition that may be deleterious—is the optimal therapeutic goal in emerging treatments. To date, however, there is a noticeable lack of research demonstrating the ability of natural agents to positively influence the bone remodeling rate or bone matrix integrity.

New Perspectives on Biochemical Markers for Evaluating Postmenopausal Bone Remodeling

BMD measurements via central dual energy absorptiometry (DEXA) are the accepted standard for diagnosing the risk to osteoporosis. But it may take up to two years for noticeable fluctuations in BMD measurements that could not be attributed to inherent method imprecision. BMD, which typically measures only 20% of the skeleton, also fails to capture all the risk factors for fracture. Consequently, for comprehensive risk assessment non-invasive serum and urinary biochemical markers of bone remodeling are also utilized.^{33,36}

These biochemical markers—which have steadily been refined over the past 30 years and provide quantitative changes in bone remodeling—reflect activity of the *entire* skeleton and can be evaluated quickly and frequently due to the dynamic nature of the physiological factors they represent. This is of particular use in the face of accelerated bone loss where timely and effective intervention is paramount. Research suggests that the correlation between BMD and bone biochemical markers in postmenopausal women increases with age, further indicating their value in monitoring this large, at-risk population.³³

Table 1. Primary Biochemical Markers for Evaluating Postmenopausal Bone Remodeling		
Marker/ Interpretation	Specimen/ Reference Range	Remarks
Osteocalcin Turnover	Serum 3.7–10.0 ng/ml	Hormone produced by osteoblasts
P1NP Formation	Serum 16–96 µg/L	Product of proliferating osteoblasts and fibroblasts that is partly incorporated in the extracellular matrix of bone
NTx Resorption	Urine 3–65 nM BCE/mM Cr	Type 1 collagen with the highest contribution from bone
IGF-1 Formation	Serum 40–258 ng/ml	Decreases collagen degradation; increases bone matrix deposition and osteoblast recruitment

Within the past decade, a growing body of scientific research suggests that bone remodeling markers—such as **osteocalcin (OC)**—may have special interpretations in postmenopausal women (See *Table 1*). Osteocalcin is a hormone that regulates the deposit of new bone

matrix and is regarded as a marker of formation when levels are within the normal range (3.7–10.0 ng/ml). As osteoclasts destroy old bone, however, OC is released into the bloodstream. Thus, an elevated OC level becomes a more valuable indicator of the bone remodeling rate (turnover), and has been suggested as a primary marker for postmenopausal women.³⁷⁻⁴¹ Elevated OC has also been associated with greater risk of fracture.³⁷ As serum OC decreases after menopause, clinical interpretation is that the remodeling rate is moving toward equilibrium.

N-terminal propeptides of type 1 collagen (**P1NP**) is another serum marker that has been suggested as a sensitive indicator of remodeling status in women after menopause. These circulating type 1 collagen propeptides can be attributed to bone due to its faster turnover rate in comparison to other tissues.^{33,37,41-43} It is often helpful to review OC and P1NP in combination with other established markers—such as crosslinked N-telopeptides of type 1 procollagen (**NTx**) and insulin growth factor-1 (**IGF-1**)—for a more complete picture of bone remodeling.

Urinary NTx is considered a sensitive marker of bone resorption at any age and has been validated as an indicator of osteoporosis risk. Elevated levels in women indicate a greater risk to rapid bone loss and subsequent fracture. Levels above 65 nM BCE/mM Cr have been associated with increased risk of osteoporosis of the hip and spine.⁴⁴

IGF-1, which naturally declines with age, directly influences bone remodeling through osteoblasts, osteoclasts, and osteocytes. It influences as many as one-third of factors involved in skeletal growth, and bone tissue responds to even slight changes in levels. Low IGF-1 has been associated with reduced estrogen in postmenopausal women and increased risk of fracture, while higher levels have been positively associated with BMD in older women.^{14,24,45-48} Low levels of IGF-1 have been associated with metabolic syndrome and identified as a potential indicator of fracture risk in women with type 2 diabetes.^{24,46}

The value of monitoring the status of serum **25(OH) Vitamin D₃** to help evaluate bone health status and measure treatment effectiveness in postmenopausal women has also been recognized and widely accepted in clinical practice. This steroid hormone modulates OC synthesis and IGF-1 for bone formation and RANKL for balanced remodeling.^{24,25}

Recent Clinical Study

Researchers at the Functional Medicine Research CenterSM (FMRC), the clinical research arm of Metagenics, Inc., sought to determine if a lifestyle intervention featuring a supplement combining RIAA, berberine, and vitamins D and K could positively influence the remodeling rate in postmenopausal women with low estrogen more effectively than an intervention of diet and exercise alone.⁴⁹ (Researchers from Boston University assisted in post-trial data analysis.)

Methodology. A randomized, single blind, placebo-controlled study of 77 postmenopausal women with low estrogen levels was conducted over 14 weeks (a 2-week run-in phase included). Two subgroups were also included in the trial: subjects with metabolic syndrome (n=45) and subjects who were generally healthy (n=32). The metabolic syndrome subjects were included due to their secondary risks to osteoporosis beyond that of natural estrogen decline.

Subjects were divided into 4 arms:

- Arm 1: Control. Postmenopausal women with low estrogen and metabolic syndrome (n=23).
- Arm 2: RIAA/berberine/vitamins D & K (RBDK). Postmenopausal women with low estrogen and metabolic syndrome (n=22).
- Arm 3: Control. Healthy postmenopausal women with low estrogen (n=17).
- Arm 4: RBDK. Healthy postmenopausal women with low estrogen (n=15).

Subjects in the control arms were asked to follow a Mediterranean-style, low-glycemic-load (LGL) diet and exercise aerobically 150 minutes per week. A number of clinical studies have established a positive link between fruit and vegetable consumption and bone mass and metabolism, and unhealthy eating patterns have been associated with accelerated bone loss.⁵⁰⁻⁵³

A typical Western diet (high in animal proteins, caffeine, and processed foods) is characterized by a higher acid-base load that increases bone resorption and calcium loss from bone—particularly in women—and contributes to osteoporosis.⁵¹⁻⁵³

The LGL diet utilized in this study for all arms is a modified Mediterranean diet. This food plan produces a lower acid load by reducing red meat content and increasing recommendations for fish and vegetables. Further, it has been shown effective in other FMRC clinical trials and case management studies in subjects with metabolic syndrome and other inflammatory conditions. Other clinical studies with similar diets have demonstrated specific benefits related to bone health. One study of Greek women compared a typical Mediterranean diet to one with reduced red meat consumption and higher olive oil and fish intake. The modified Mediterranean diet was positively related to bone mass, while the standard Mediterranean diet was found to have no association with bone mass indices.⁵⁴ In a study of premenopausal women, a diet high in fish, fruit, and vegetables that was low in meat was suggested to have a positive effect on BMD.⁵⁵

Arms 2 and 4 received the same diet and exercise recommendations as the control arms. They also were instructed to take a nutraceutical featuring a combination of RIAA, berberine, and vitamins D and K twice daily (after a 2-week run-in phase on placebo, diet, and exercise).

Note: Subjects did not take calcium supplements or a multivitamin (that might have contained calcium and/or vitamin D) during the study, per instruction. Control subjects had no vitamin D supplementation whatsoever. This also influenced the length of the study to help prevent any unnecessary risk to long-term bone health.

Results. The RBDK intervention collectively showed more positive effects on markers of bone remodeling and formation in postmenopausal women with low estrogen levels (with or without metabolic syndrome) than the control intervention (diet and exercise alone), especially in those with higher risk of fracture. (Results summarized in *Table 2*.)

Efficacy endpoints evaluated during the trial included serum OC, serum P1NP, urinary NTx, serum IGF-1, and serum 25(OH) Vitamin D₃. Estradiol levels were also measured.

- **Osteocalcin.** At baseline, subjects in both the control and supplement arms had elevated OC levels indicating increased bone turnover and an increased risk to osteoporosis. The therapeutic target in postmenopausal women is a decrease in serum OC, interpreted as a positive indicator of a reduced bone remodeling rate. Results for all subjects (with or without metabolic syndrome):
 - Control: Negative effect with significant increases (median +16.40%).
 - RBDK: *Positive* effect with significant decreases (median -31.07%).
- **Osteocalcin (elevated NTx subset).** A closer look at OC levels in those subjects with urinary NTx > 65, indicating increased bone turnover and higher risk to osteoporosis, yielded similar results (with or without metabolic syndrome).
 - Control: Negative effect with significant increases (median 22.64%).
 - RBDK: *Positive* effect with significant decreases (median -31.69%).
- **Osteocalcin (low baseline IGF-1 subset).** Researchers also analyzed OC levels in conjunction with lower IGF-1 levels (the lowest tertile), indicating an increased risk to fracture. Results for subjects with or without metabolic syndrome:
 - Control: Negative effect with a significant mean increase of 3.44 nmol/L (or 20.12 ng/ml).
 - RBDK: *Positive* effect with a significant mean decrease of 6.03 nmol/L (or 35.26 ng/ml).

Note: On average, subjects lost weight during the program, which is associated with increased urinary excretion of calcium. Because control subjects were not consuming any supplemental nutrients to support mineralization (calcium, vitamin D), this may explain the increase in OC (suggesting an increased rate of bone remodeling) despite otherwise healthy dietary and lifestyle changes that have been positively associated with slowing the rate of bone turnover.

- **P1NP (high baseline IGF-1 subset).** P1NP, a marker of bone formation, was analyzed in a subset of subjects in the highest tertile of baseline IGF-1 levels. Researchers took note of the metabolic syndrome group, which achieved the greatest results. A decline in IGF-1 levels are associated with metabolic syndrome; higher levels are associated with increased potential for bone formation.
 - Control: Negative effect with significant decrease (mean -9.93 µg/L).
 - RBDK: *Positive* effect with significant increase (mean +9.73 µg/L).
- **IGF-1.** IGF-1 is an emerging biomarker for vertebral fracture risk on postmenopausal women, independent of BMD testing. Here both groups were found to have experienced positive results. An increase in IGF-1 in women with low estrogen is noteworthy.
 - Control: *Positive* effect with significant increase (median 13.10%).
 - RBDK: *Positive* effect with significant increase (median 21.19%).
- **Estrogen.** Estradiol levels of 57 subjects were measured (30 RBDK; 27 Control). At baseline, 84.2% of subjects had estradiol levels under the detection limit (<20 pg/ml) and none had estradiol levels >90 pg/ml. No observable change was found during the trial. At end of the trial, 85.7% of patients had an estradiol level <20 pg/ml and none had estradiol >90 pg/ml. These results suggest that the positive effect on markers of remodeling could not be attributed to non-targeted modulation of estrogen that would influence bone turnover.

Figure 1. Significant Decrease in Serum Osteocalcin Suggesting a Reduced Rate of Bone Turnover

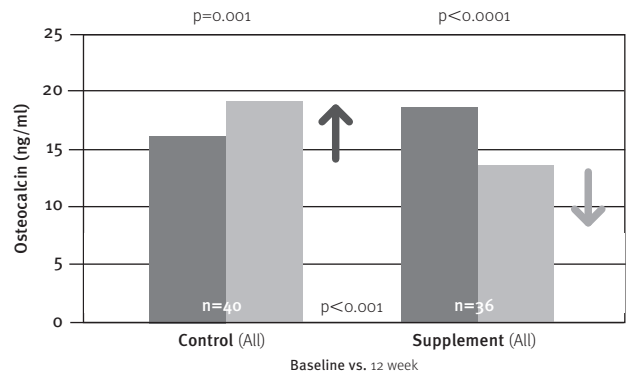


Figure 2. Significant Decrease in Serum Osteocalcin in Subjects with Elevated Urinary NTx

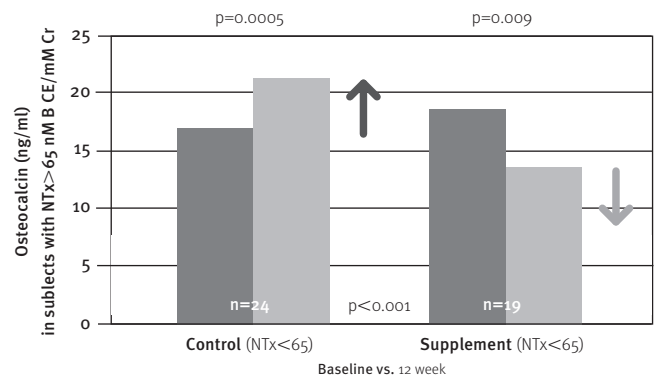


Figure 3. Significant Increase in P1NP in Subjects with Metabolic Syndrome (MS)

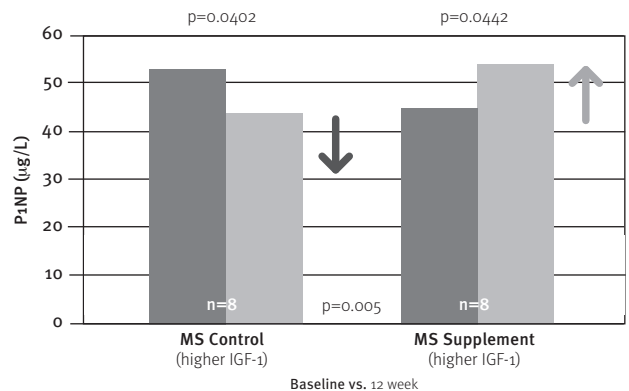
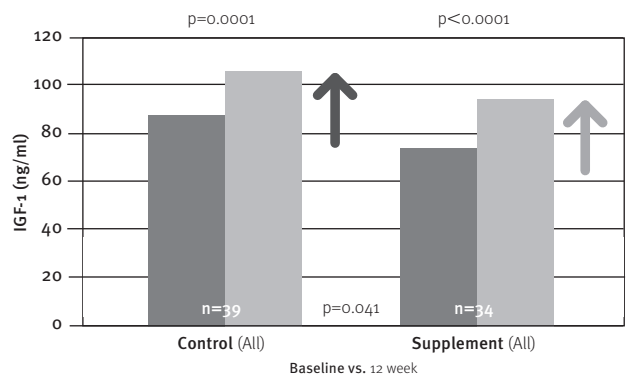
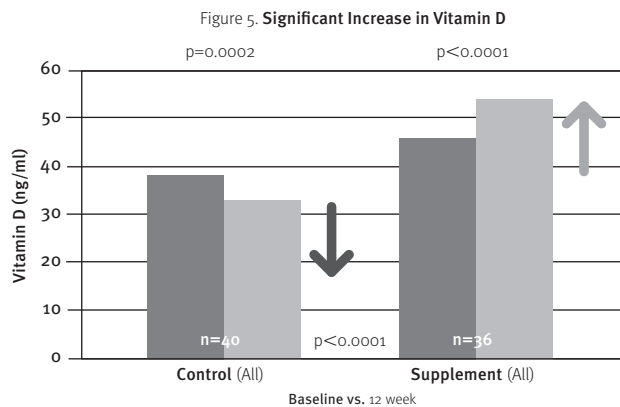


Figure 4. Significant Increase in IGF-1 Suggesting Increased Potential for Bone Formation



○ **Vitamin D Status.** Contrary to expectations, all subjects were found to be sufficient at baseline (≥ 30 ng/ml). Researchers interpret this as a suggestion that other findings cannot be attributed to the correction of a vitamin D deficiency with supplementation. Results for all subjects (with or without metabolic syndrome):

- Control: Negative effect on serum vitamin D with a significant decrease (median -14.63%).
- RBDK: *Positive* effect on serum vitamin D with a significant increase (median 16.21%).



Conclusions. The disruption of bone remodeling homeostasis and progression to osteoporosis following menopause-related estrogen decline may be positively affected through a Mediterranean-style/LGL diet, exercise, and supplementation with a specific nutrient combination of RIAA, berberine, and vitamins D and K. A lifestyle intervention that included these natural agents was found to positively modulate factors indicative of healthy bone remodeling, formation, and overall bone matrix quality better than an LGL diet and exercise alone.

While the overall results achieved by healthy lifestyle changes alone were less than what was achieved with the addition of the supplement, there were positive changes seen. The design of this study did not incorporate subjects who made no dietary or exercise changes. Results here should not negatively reflect on the value of lifestyle intervention in clinical practice. Research has demonstrated the value of healthy lifestyle changes in bone metabolism in comparison to the Standard American Diet, which negatively impacts bone remodeling. This study was not intended to replicate that research or to discredit the value of healthy lifestyle changes.

As previously stated, control subjects were advised not to take calcium or vitamin D. This lack of mineralization support—to help offset calcium loss through the associated increase in urinary excretion with initial weight loss—may have negatively affected the short-term results suggested by some of the bone biomarkers in this at-risk population (where remodeling rate may already be more volatile). Previous FMRC research and clinical experience with a modified Mediterranean diet without calorie restrictions has demonstrated 1 lb. per week weight loss.

Participants were also restricted to 150 minutes of aerobic exercise—non-weight-bearing exercise—per week. This may have been a decrease in baseline activity for some participants in the control group and represented relative inactivity, which is associated with a decrease in bone density.

The RBDK intervention group experienced similar weight loss, calcium deprivation, and exercise restrictions, yet showed an *improvement* in biomarkers of bone remodeling and formation within the same short trial period. Researchers conclude that the nutrients and phyto-nutrients in the supplement may have helped compensate for what may have been less-than-optimal lifestyle intervention components (i.e., lack of calcium supplementation and weight-bearing exercise) specifically for postmenopausal women. This suggests the increased potential for positive outcomes in targeted lifestyle intervention programs for postmenopausal bone health with strategic nutritional support for both mineralization and bone remodeling.

If a lifestyle implementation with added nutritional support for bone remodeling were to be implemented in clinical practice, researchers recommend the adjunct of an appropriate source of calcium supplementation for adequate mineralization support.

Table 2. **Effects of RIAA/Berberine/Vitamin D/Vitamin K on Key Biomarkers of Bone Remodeling**

	Biochemical Markers of Bone Remodeling	Nutraceutical Program (Diet & Exercise plus RIAA/Berberine/Vitamins D & K Combination)	Control Group (Diet & Exercise Alone)
Rate of Remodeling	Osteocalcin (all subjects)	Positive effect -31.07%	Negative effect +16.40%
	Osteocalcin (in subjects with elevated NTx)	Positive effect -31.69%	Negative effect +22.64%
	Osteocalcin (in subjects with low baseline IGF-1)	Positive effect -6.03 nmol/L (or 35.26 ng/ml)	Negative effect +3.44 nmol/L (or 20.12 ng/ml)
Formation	P1NP (in metabolic syndrome subjects with relatively high baseline IGF-1)	Positive effect +9.73 µg/L	Negative effect -9.93 µg/L
	IGF-1 (all subjects)	Positive effect +21.19%	Positive effect +13.10%
	Vitamin D (all subjects)	Positive effect +16.21%	Negative effect -14.63%

References

- McCormick RK. Osteoporosis: integrating biomarkers and other diagnostic correlates into the management of bone fragility. *Altern Med Rev*. 2007;12:113-145.
- Pfeilschifter J, Köditz R, Pfohl M, Schatz H. Changes in proinflammatory cytokine activity after menopause. *Endocr Rev*. 2002;23(1):90-119.
- Mundy GR. Osteoporosis and Inflammation. *Nutrition Reviews*. 2007;65(12SII):S147-S151.
- Kulkarni NH, Onyia JE, Zeng Q, et al. Orally bioavailable gsk-3alpha/beta dual inhibitor increases markers of cellular differentiation in vitro and bone mass in vivo. *J Bone Miner Res*. 2006;21:910-920.
- Scheidt-Nave C, Bismar H, Leidig-Bruckner G, et al. Serum interleukin 6 is a major predictor of bone loss in women specific to the first decade past menopause. *J Clin Endocrinol Metab*. 2001;86(5):2032-2042.
- Pine PR, Chang B, Schoettler N, et al. Inflammation and bone erosion are suppressed in models of rheumatoid arthritis following treatment with a novel Syk inhibitor. *Clin Immunol*. 2007;124(3):244-257.
- Zou W, Kitaura H, Reeve J, et al. Syk, c-Src, the alphavbeta3 integrin, and ITAM immunoreceptors, in concert, regulate osteoclastic bone resorption. *J Cell Biol*. 2007;176(6):877-888.
- Wong BR, Grossbard EB, Payan DG, Masuda ES. Targeting Syk as a treatment for allergic and autoimmune disorders. *Expert Opin Investig Drugs*. 2004;13(7):743-762.
- Eliopoulos AG, Das S, Tschlis PN. The tyrosine kinase Syk regulates TPL2 activation signals. *J Biol Chem*. 2006;281(3):1371-1380.
- Allori AC, Sailon AM, Warren SM. Biological basis of bone formation, remodeling, and repair-part I: biochemical signaling molecules. *Tissue Eng Part B Rev*. 2008;14(3):259-273.
- Rosen CJ, Bouxsein ML. Mechanisms of disease: is osteoporosis the obesity of bone? *Nat Clin Pract Rheumatol*. 2006;2(1):35-43.
- Schett G. Joint remodeling in inflammatory diseases. *Ann Rheum Dis*. 2007;66 Suppl 3:iii42-44.
- Semenkovich CF, Teitelbaum SL. Bone weighs in on obesity. *Cell*. 2007;130:409-411.
- Forsblad H, Mattsson LA, Ohlsson C, Nordborg E, Carlsten H. Hormone replacement therapy in rheumatoid arthritis is associated with lower serum levels of soluble IL-6 receptor and higher insulin-like growth factor 1. *Arthritis Res Ther*. 2003;5:R202-R209.
- National Osteoporosis Foundation. Fast Facts on Osteoporosis. Available at <http://www.nof.org/osteoporosis/diseasefacts.htm>. Retrieved 11/20/08.
- Nelson HD, Humphrey LL, Nygren P, Teutsch SM, Allan JD. Postmenopausal hormone replacement therapy: scientific review. *JAMA*. 2002;288(7):872-881.
- U.S Food and Drug Administration. Information on bisphosphonates. FDA alert issued 1/7/2008. Available at <http://www.fda.gov/cder/drug/infopage/bisphosphonates/default.htm>. Retrieved 11/26/08.
- Sarin J, DeRossi SS, Akintoye SO. Updates on bisphosphonates and potential pathobiology of bisphosphonate-induced jaw osteonecrosis. *Oral Dis*. 2008;14(3):277-285.
- U.S. Food and Drug Administration. Update of safety review follow-up to the October 1, 2007 early communication about the ongoing safety review of bisphosphonates. Available at http://www.fda.gov/cder/drug/early_comm/bisphosphonates_update_200811.htm. Retrieved 11/26/08.
- Seeman E, Compston J, Adachi J, et al. Non-compliance: the Achilles' heel of anti-fracture efficacy. *Osteoporos Int*. 2007;18(6):711-719.
- Hu JP, Nishishita K, Sakai E, Yoshida H, Kato Y, Tsukuba T, Okamoto K. Berberine inhibits RANKL-induced osteoclast formation and survival through suppressing the NF-kappaB and Akt pathways. *Eur J Pharmacol*. 2008 Feb 2;580(1-2):70-79.
- Li H, Miyahara T, Tezuka Y, et al. The effect of kampo formulae on bone resorption in vitro and in vivo. II. Detailed study of berberine. *Biol Pharm Bull*. 1999;22(4):391-396.
- Lee HW, Suh JH, Kim HN, et al. Berberine promotes osteoblast differentiation by Runx2 activation with p38 MAPK. *J Bone Miner Res*. 2008 Aug;23(8):1227-1237.
- Hyppönen E, Boucher BJ, Berry DJ, Power C. 25-hydroxyvitamin D, IGF-1, and metabolic syndrome at 45 years of age: a cross-sectional study in the 1958 British Birth Cohort. *Diabetes*. 2008;57(2):298-305.
- Kim S, Yamazaki M, Shevde NK, Pike JW. Transcriptional control of receptor activator of nuclear factor-kappaB ligand by the protein kinase A activator forskolin and the transmembrane glycoprotein 130-activating cytokine, oncostatin M, is exerted through multiple distal enhancers. *Mol Endocrinol*. 2007;21(1):197-214.
- Holick MF. The vitamin D epidemic and its health consequences. *J Nutr*. 2005;135(11):2739S-2748S.
- North American Menopause Society. Management of osteoporosis in postmenopausal women: 2006 position statement of The North American Menopause Society. *Menopause*. 2006;13(3):340-367.
- Heiss C, Hoesel LM, Wehr U, et al. Diagnosis of osteoporosis with vitamin K as a new biochemical marker. *Vitam Horm*. 2008;78:417-434.
- Lanham-New SA. Importance of calcium, vitamin D and vitamin K for osteoporosis prevention and treatment. *Proc Nutr Soc*. 2008;67(2):163-176.
- Cheung AM, Tile L, Lee Y, et al. Vitamin K supplementation in postmenopausal women with osteopenia (ECKO trial): A randomized controlled trial. *PLoS Med*. 5(10):e196.
- Bugel S. Vitamin K and bone health in adult humans. *Vitam Horm*. 2008;78:393-416.
- Eastell R, Hannon RA. Biomarkers of bone health and osteoporosis risk. *Proc Nutr Soc*. 2008;67:157-162.
- Garnero P, Delmas PD. Contribution of bone mineral density and bone turnover markers to the estimation of risk of osteoporotic fracture in postmenopausal women. *J Musculoskelet Neuronal Interact*. 2004;4(1):50-63.
- Ravn P, Rix M, Andreassen H, Clemmesen B, Bidstrup M, Gunnes M. High bone turnover is associated with low bone mass and spinal fracture in postmenopausal women. *Calcif Tissue Int*. 1997;60(3):255-260.
- Burr DB. Summary—osteoporosis and fracture risk: bone matrix quality session. *J Musculoskelet Neuronal Interact*. 2002;2(6):544-545.
- Vasikaran SD. Utility of biochemical markers of bone turnover and bone mineral density in management of osteoporosis. *Crit Rev Clin Lab Sci*. 2008;45(2):221-258.
- Cheng S, Suominen H, Väänänen K, Käkönen SM, Pettersson K, Heikkinen E. Serum osteocalcin in relation to calcaneal bone mineral density in elderly men and women: a 5-year follow-up. *J Bone Miner Metab*. 2002;20(1):49-56.
- Kim SW, Park DJ, Park KS, et al. Early changes in biochemical markers of bone turnover predict bone mineral density response to antiresorptive therapy in Korean postmenopausal women with osteoporosis. *Endocr J*. 2005;52(6):667-674.
- Nabipour I, Larijani B, Jafari SM, Amiri M, Amiri Z. Reference database of CrossLaps and osteocalcin for a healthy Iranian population. *Arch Iran Med*. 2008;11(2):203-206.
- Ivaska KK, Hentunen TA, Vääräniemi J, Ylipahkala H, Pettersson K, Väänänen HK. Release of intact and fragmented osteocalcin molecules from bone matrix during bone resorption in vitro. *J Biol Chem*. 2004;279(18):18361-18369.
- Garnero P. Biomarkers for osteoporosis management: utility in diagnosis, fracture risk prediction and therapy monitoring. *Mol Diagn Ther*. 2008;12(3):157-170.
- Seibel MJ. Biochemical markers of bone turnover—part 1: biochemistry and variability. *Clin Biochem Rev*. 2005;26:97-122.
- Chen P, Satterwhite JH, Licata AA, et al. Early changes in biochemical markers of bone formation predict BMD response to teriparatide in postmenopausal women with osteoporosis. *J Bone Miner Res*. 2005;20(6):962-970.
- Schneider DL, Barrett CE. Urinary N-telopeptide levels discriminate normal, osteopenic, and osteoporotic bone mineral density. *Arch Intern Med*. 1997;157:1241-1245.
- Yakar S, Rosen CJ. From mouse to man: redefining the role of insulin-like growth factor-I in the acquisition of bone mass. *Exp Biol Med*. 2003;228:245-252.
- Kanazawa I, Yamaguchi T, Yamamoto M, Yamauchi M, Yano S, Sugimoto T. Serum insulin-like growth factor-1 level is associated with presences of vertebral fractures in postmenopausal women with type 2 diabetes mellitus. *Osteoporos Int*. 2007;18:1675-1681.
- Posaci C, Altunyurt S, Islekel H, Onvural A. Effects of HRT on serum levels of IGF-I in postmenopausal women. *Maturitas*. 2001;40(1):69-74.
- Pepene CE, Seck T, Diel I, Minne HW, Ziegler R, Pfeilschifter J. Concentration of insulin-like growth factor (IGF)-I in iliac crest bone matrix in premenopausal women with idiopathic osteoporosis. *Exp Clin Endocrinol Diabetes*. 2004;112(1):38-43.
- Functional Medicine Research Center. Interim report: summary of healthy menopause I trial. September 2008. Gig Harbor, WA.
- New SA. Intake of fruits and vegetables: implications for bone health. *Proc Nutr Soc*. 2003;62(4):889-899.
- Massey LK. Dietary animal and plant protein and human bone health: a whole foods approach. *J Nutr*. 2003;133(3):862S-865S.
- Maurer M, Riesen W, Muser J, Hulter H, Krapf R. Neutralization of Western diet inhibits bone resorption independently of K intake and reduces cortisol secretion in humans. *Am J Physiol Renal Physiol*. 2003;284:F32-F40.
- Welch AA, Bingham SA, Reeve J, Khaw KT. More acidic dietary acid-base load is associated with reduced calcaneal broadband ultrasound attenuation in women but not in men: results from the EPIC-Norfolk cohort study. *Am J Clin Nutr*. 2007;85:1134-1141.
- Kontogianni MD, Melistas L, Yannakoulia M, Malagaris I, Panagiotakos DB, Yiannakouris N. Association between dietary patterns and indices of bone mass in a sample of Mediterranean women. *Nutrition*. 2008 [Epub ahead of print]
- Okubu H, Sasaki S, Horiguchi H, et al. Dietary patterns associated with bone mineral density in premenopausal Japanese farmwomen. *Am J Clin Nutr*. 2006;83(5):1185-1192.

FUNCTIONAL MEDICINE
RESEARCH CENTER

MET1635 0209