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Mineral Changes in Osteoporosis A Review

Dan Faibish, DMD^{*}, Susan M. Ott, MD[†], and Adele L. Boskey, PhD^{*}

^{*}*Musculoskeletal Integrity Program, Hospital for Special Surgery, New York, NY*

[†]*Department of Medicine, University of Washington, Seattle, WA.*

Abstract

Bone mineral composition, crystallinity, and bone mineral content of osteoporotic patients are different from those of normal subjects. We review the evidence that these mineralization parameters contribute to the strength (fracture resistance) of bone and the methods that have been used to examine them. A specific example is provided from analysis of biopsies from the Multiple Outcomes in Raloxifene Evaluation trial. For the analyses, randomly selected biopsies from placebo, low-dose, and high-dose groups (n = 5 per group) obtained at time zero and 2 years after treatment were examined by infrared imaging spectroscopy. In all cases, comparable increases in mineral content were found, but there were no significant variations in mineral crystallinity.

Osteoporosis is a devastating disease that affects more than 10 million people in the United States, with annual costs in excess of 13.5 billion dollars.¹²³ According to the US Surgeon General's Report,¹²³ by the year 2020 1/2 of all Americans older than 50 years will be at risk of an osteoporotic fragility fracture. Osteoporosis is characterized by low bone mass and structural deterioration of bone, leading to bone fragility and an increased tendency to fracture. Fracture resistance is determined by the strength of the bone, which in turn depends on its geometric properties (size, shape, and connectivity), the activities of the cells in the tissue, and the material properties of the tissue.^{36,73,109} The material properties of bone include the mineral content,⁷³ mineral composition and mineral crystal size,²⁷ and matrix content and composition.³⁵ The most frequently used clinical indication of osteoporosis and fracture risk, bone mineral density (BMD), is also the most readily accessible non-invasive measure of bone mineral content.⁸⁵ The purpose of this review is to describe the additional properties that may be predictive of mechanical strength obtained by analyses of bone tissue specimens. Methods of analysis and recent data obtained by these methods also are reviewed to show how material properties are altered in osteoporosis.

Specific questions addressed are how the composition of bone is altered in osteoporosis; how mineral crystal composition and size vary in osteoporosis; how spectroscopic analyses can be used to characterize these alterations in properties with high spatial resolution; and how therapies currently in clinical use affect these properties.

The Composition of Bone

Bone is a composite consisting, in decreasing order, of mineral (an analogue of geologic hydroxyapatite [HA]), an organic matrix, cells, and water.¹⁴ The organic matrix predominately

Correspondence to: Adele L Boskey, PhD, Hospital for Special Surgery, 535 East 70th Street, New York, NY 10021. Phone: 212-606-1453; Fax: 212-472-5331; E-mail: boskeya@hss.edu..

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is Type I collagen but includes a small percentage of noncollagenous proteins. These constituents are distributed in different patterns in various types of bone. Classical chemical analyses of ash content (percent mineral after the water and organic components are burned-off)^{14,71,88,99,124,128}; mineral ion composition;^{13,21,22,56,71,88,83,120} electron microscopic and xray diffraction analysis of bone mineral crystal size^{6,8,11,23,55,56,57,107,118,122}; and vibrational spectroscopic analysis of mineral content (mineral to matrix ratio), carbonate content, and acid phosphate content^{15,16,23,24,30,77} have been used to analyze homogenized biopsy and cadaver tissues and bones from animal models of osteoporosis.^{13,71,88,99,125} Newer techniques, such as backscatter electron imaging,^{8,18} atomic force microscopy,^{58,119} small angle neutron or xray scattering,^{108,109,110} nuclear magnetic imaging,^{20,28,128} Fourier transform infrared (FTIR) imaging, and Raman microscopic imaging,^{16,24,75} more recently have been used or have the potential to be used in the analyses of mineral properties in osteoporotic tissues. These chemical analyses show an age-dependent and site-dependent variation in mineral properties in healthy individuals, which are not apparent in osteoporotic tissues.¹⁵ They also have shown alteration in collagen composition in osteoporotic patients.⁵ Each of these parameters can have substantial effects on the mechanical performance of bone. We focus on the mineral changes in osteoporosis.

How Mineral Properties Affect Mechanical Strength

Mineral content in vertebrae and long bones is correlated with a variety of whole bone mechanical properties (stiffness, strain, ultimate load, etc.).^{60,82} Currey^{34,35} showed that the observed torsional strength is proportional to and most dependent on mineral content. More recent analyses using microcomputerized tomography show that correlations improve when microarchitecture and mineral content are included in the regression.⁶³ However, even when mineral content and microarchitecture are considered, only about 80% of the variance is accounted for, indicating there are additional factors that must contribute to bone strength.

Bones are known to become more brittle when the mineral content exceeds a critical value⁵⁰ and to be less able to bear load when the mineral content is too low.¹¹⁴ Bone mineral density is related directly to mechanical strength, and the decreased bone mineral density associated with fracture risk in patients with osteoporosis^{25,42,67,69,76} is confirmed by decreases in the distribution of mineral density determined by density fractionation in tissues from animal models of osteoporosis^{23,55,66} and spectroscopically determined decreases in mineral to matrix ratio in osteoporotic tissues.^{9,11,48,61,62,81,91,93} Variation in mineral content in osteoporosis is important, but there are other mineral properties that also contribute to the loss of mechanical strength in osteoporotic bones.

The HA crystals found in bone are nanocrystalline and contain a large number of imperfections and impurities.¹⁴ Hydroxyapatite crystal size and perfection first were suggested to contribute to the mechanical strength of bones in the early 1980s.²⁷ Increased bone mineral particle size is associated with increased bone fragility²⁷; crystals that are too small do not reinforce the bone composite, suggesting there also is an optimal size range for bone mineral crystals.⁵⁰ Compositional changes may be caused by alterations in crystal size (smaller crystals have more surface area to adsorb foreign ions) or may be indicative of the size changes. In osteoporosis and in aging, there are reports of increased magnesium content and decreased fluoride, acid phosphate, boron, strontium, and carbonate contents.¹ The significance of these impurities to the mechanical competence of the bones is not yet known.

Spectroscopic Analysis of Bone Material Properties

Vibrational spectroscopy has been used extensively for the analysis of mineral properties in bones, teeth, and other mineralized tissues. The wavelength of many infrared and Raman absorption bands are characteristic of specific types of chemical bonds, and vibrational

spectroscopy has been used to confirm the identity of particular compounds in mineralized tissues. In infrared bands, asymmetric vibrations are most intense; in the Raman spectrum the strongest (and sharper) bands are the symmetric vibrations. In both, band shapes and positions are sensitive to the molecular environment; examining spectral details at discrete sites in the tissue provides insight into environmental changes at well-defined anatomic locations. Raman bands are sharper and better defined, while the infrared bands are more intense (Fig 1). Vibrations that seem strong in Raman generally are weak in the infrared and vice versa, making these two techniques complimentary. For bone, similar mineral parameters generally are evaluated for both; however, these are based on different vibrations (Table 1). Classically, these spectroscopic techniques were applied to homogenized bones; Cohen and Kitzes³⁰ were among the first to document changes in bone mineral content and composition in osteoporosis. The introduction of multichannel array detectors allowed the development of infrared imaging and Raman imaging microspectroscopy in which multiple spectra are recorded simultaneously.⁷⁴ An image cube is generated in which the x-axis and y-axis correspond to the x-position and y-position in the tissue sample, and the z-axis is the intensity of a particular vibration (Fig 2A), a ratio of two peak areas (Fig 2B), or a ratio of two intensities (Figs 2C, D). Pixel histograms provide statistical information on the distribution of values in any given sample (Figs 2E-G).

These spectroscopic imaging techniques (Table 2) have been applied to bone biopsies from patients and animals with osteoporosis and osteomalacia and treated versus with nontreated subjects.^{9,15,16,75,89,91,92,93,94,95} In general, in the osteoporotic tissues the mineral content (degree of mineralization) is decreased,¹⁰ the HA crystal size and perfection is increased, the carbonate content is increased, and the acid phosphate content is decreased.¹⁵ Treatment with fluoride increases crystal length and decreases width,^{7,47,121} and treatment with several of the bisphosphonates has been reported to have no effect^{23,53} or to increase crystal width slightly.¹²² Strontium decreases bone mineral crystal size,⁵⁶ but strontium ralenate increases BMD and mineral distribution.¹¹ Parathyroid hormone restores the mineral content and crystallinity to normal values in a monkey model of osteoporosis⁹³ whereas hormone replacement therapy in perimenopausal women increases mineral content and decreases crystal size.⁹²

Spectroscopic parameters that are usually evaluated in the infrared spectra of bone include the mineral to matrix peak area ratio (which is correlated with ash weight),^{44,101} the 1660/1690 cm^{-1} peak intensity ratio (which is related to variations in collagen cross-linking),^{95,96} the 1030 cm^{-1} /1020 cm^{-1} peak intensity ratio (which is correlated with mineral crystal size and perfection as determined by xray diffraction line broadening),⁴⁹ the carbonate/phosphate or carbonate/amide I ratio (which indicates the amount of carbonate substitution for phosphate or hydroxide in the mineral crystals),^{15,98} and the acid phosphate content (a parameter that decreases as the crystals become more mature).¹⁵

Changes in Mineral Properties in Osteoporosis before and after Therapeutic Treatment

Using the techniques discussed above, mineral changes have been noted after treatment of osteoporosis in a variety of animal models and in a limited number of studies of patient biopsies from clinical trials. Fluoride was the first therapy reported to have an effect on mineral properties. Given with calcium and vitamin D, fluoride increases bone density⁸³ but also increases the incidence of hip (but not vertebral) fractures.¹⁰⁶ Fluoride therapy increases HA crystal length, decreases crystal width, and increases mineral content.^{6,26,121} The decreased crystal width is correlated with decreased ability of the tissue to withstand load (increased risk of fracture).²⁶

Hormone replacement therapy was used for the treatment and prevention of osteoporosis before fluoride treatment.^{102,115} Estrogen and progesterone have been shown in animal models to improve mechanical strength and create a broader distribution of mineral crystals sizes.^{21, 65,116} Using infrared imaging, we previously examined biopsies from perimenopausal women who were treated with hormone replacement therapy after their initial biopsies. A second biopsy obtained after 2 years showed an apparent increase in mineral content, a decrease in the average crystal size, and a broadening of the population of crystal sizes in each sample.⁹² This is in agreement with the finding that estrogen treatment accelerates fracture healing in ovariectomized rats, while at the same time creating a population of new crystals and newly synthesized collagen.⁹⁰

In related studies, several growth factors and phytoestrogens were evaluated in rodent models of osteoporosis.^{19,32,68,100} These agents increase density but have no effect on mechanical properties in monkeys.¹⁰³ Effects of most of these agents on mineral characteristics have not been reported but effects on collagen have been.⁸⁴ In one study, mineral properties including crystal size in homogenized bones from male rats treated short-term with a synthetic isoflavone derivative did not differ from controls; however, the mechanical strength increased.²⁹ This indicates that information may be lost when homogenized tissues are examined. Similarly, the cytokine insulin like growth factor-1 (IGF-1) has variable effects on bone density, does not increase mechanical strength,^{31,111} however, mice overexpressing IGF-binding proteins show substantial alterations in crystallite size and perfection.³

Rodent models, although widely used, are not thought to be good models of human osteoporosis. With age, human bones become thinner and more brittle while rodent bones become thicker and stronger.⁴ Mineral properties in ovariectomized rodents also are not comparable with those in humans.⁹⁷ However, the FDA accepts small animal models for initial evaluation of osteoporotic therapies; therefore, there are a large number of studies in rats and mice. Lessons about the effects of therapies on bone properties can be approximated from these studies as long as it is recognized that these models do not generally mimic human osteoporosis.

The bisphosphonates used in the treatment of osteoporosis (didronel, ibandronate, pamidronate, alendronate, risedronate, tiludronate, zoledronic acid, etc.) all have nonhydrolyzable P-C-P bonds and in general, high affinities for the HA crystal surface.^{59,64} They have direct inhibitory effects on osteoclast action but as they also bind to HA, they can regulate mineral crystal growth and dissolution.^{45,112} Each of the bisphosphonates increase bone density and sometimes increase mechanical strength or microcrack density,^{41,52,70,72, 81} but their effects on mineral properties have only been evaluated in a limited number of instances. Bohic et al⁹ used 31P NMR, vibrational spectroscopy, and chemical analyses to characterize the effects of the bisphosphonate tiludronate on HA properties. Comparing treated and untreated ovariectomized rats they found no change in the NMR spectra, no change in the acid phosphate, carbonate content, or mineral content, by Raman and infrared spectroscopy. In dogs, high-dose alendronate (5× normal) and high-dose risedronate did not have any effect on mineral crystal properties in the homogenized vertebrae despite large changes in mechanical properties.^{23,123} The authors of one study suggested that the failure to find a difference was because of low sensitivity of the measurement.²³ As noted previously, homogenization of the tissue before analysis could have masked changes at the sites of newly formed or newly remodeled bone. In contrast, intravenous pamidronate given to dogs for a 1-year period caused a dose dependent increase in bone mineral density and a decrease in crystallite size and HA structure.⁵⁷ Very few studies of mineral properties have been reported in biopsies from humans treated with any of the bisphosphonates. These limited studies in patients and baboons treated with alendronate showed that an increase in the degree of mineralization paralleled a rise in the mechanical strength in the cortical and cancellous bone.^{46,57,78}

The dog studies mentioned previously suggest a species-dependent difference in response to therapy. Calcitonin, which blocks osteoclastic resorption, has been reported to improve trabecular bone mechanics in all but one model.^{126,127} In beagles treated with human calcitonin for a 16-week period, comparison with controls showed tibia tested in torsion failed at lower load and had less torsional stiffness.¹⁰¹ Cancellous bone in treated animals was similarly weaker, cortical and cancellous bones had reduced BMD, and mineral/matrix ratio and greater carbonate/phosphate ratio. Patients with osteoarthritis, in contrast, have an improvement in crystallinity (based on xray diffraction) indicative of new bone formation after calcitonin treatment.⁸⁰ In an animal model of osteoarthritis, infrared microspectroscopy failed to show any differences in the mineral crystal properties of the thickened calcified cartilage plate.⁷⁹ Unfortunately, with the exception of one rodent study,⁵¹ there are no reports of mineral properties in patients or animals with osteoporosis treated with calcitonin. In the ovariectomized rats treated with calcitonin, the bone mineral calcium to phosphate ratio and the crystallite size were similar to those in the control untreated rats. The reduced bone formation and mineral content noted in the Calcitonin-treated dogs and their increased carbonate to phosphate ratios are in agreement with the mineral changes noted in animal models of osteoporosis.

Aging monkeys lose bone mass and have fractures when studied in their natural environments.⁵⁴ The changes noted in their trabecular bone during aging and after ovariectomy mimic those observed in humans.^{48,55,65,66} There have been three reports of the effects of antiresorptive and anabolic therapies on mineral properties in ovariectomized monkeys.^{48,61,93} Nandrolone decanoate, a resorption inhibitor, was shown to improve the mechanical properties and increase mineral content; however they have no effect on carbonate, acid phosphate, or collagen cross-links.^{48,61} Parathyroid hormone in low and high doses caused notable changes in the distribution of mineral parameters in treated ovariectomized monkeys after 18 months relative to ovariectomized negative controls.⁹³ The mineral/matrix ratio and the crystallinity shifted to lower values, indicative of the formation of new bone, and mechanical strength was increased in the animals treated with parathyroid hormone.¹¹³

To illustrate how these techniques can be applied to the evaluation of biopsies obtained in therapeutic trials and how such data correlates with other methods, we present data from an evaluation of raloxifene, a selective estrogen receptor modulator. The changes in spectroscopic parameters at 6 μ m spatial resolution in patients treated with raloxifene or placebo controls were evaluated in a randomly selected subset of biopsies from a large trial evaluating raloxifene therapy in postmenopausal women.^{33,43} In this trial, the treatment of osteoporosis with two different doses of raloxifene was examined in a cohort of 7705 postmenopausal women.⁴³ Raloxifene reduced the risk of vertebral fracture in the postmenopausal women and quantitative microradiographic analyses of bone biopsies from this trial showed an increase in mineral content with treatment of 2.1% (60 mg/d group) and 2.4% (120 mg/d group). Increase in mineral density as measured by dual energy xray absorptiometry (DEXA) in the spine was 0.5% in the placebo group, 3.1% in the 60 mg/day group, and 3.2% in the 120 mg/day group. Vertebral fracture risk was reduced in both experimental groups relative to the placebo, 0.7 (60 mg/d) and 0.5 to 0.8 (120 mg/d); the prevalence of fractures at other sites did not change. The purpose of our study was to evaluate the mineral changes in a randomly selected group of iliac crest biopsies from this study.

MATERIALS AND METHODS

We retrospectively analyzed randomly selected iliac crest biopsies (designated by a statistician from Eli Lilly, Co.) from three groups of patients (n = 5 per group) enrolled in the MORE (Multiple Outcomes in Raloxifene Evaluation) trial. In this trial, there were two experiment groups, treated with 60 mg or 120 mg of raloxifene for 2 years, and a placebo group. All patients

were supplemented with calcium and vitamin D. Biopsies obtained at baseline and 2 years after treatment were embedded in polymethylmethacrylate (PMMA), cut in 2 μm -thick sections, and spectra recorded in imaging mode using a Fourier Transform Infrared Imaging (FTIRI) spectrometer (Spotlight, Perkin Elmer, Shelton, CT). For each biopsy, spectra from six $200 \times 200 \mu\text{m}$ regions were collected, three in the cortical bone and three in the trabecular at a spectral resolution of 8 cm^{-1} . The spectra were baselined linearly and the contribution of the embedding media² was subtracted spectrally using commercial software (ISYS, Spectral Dimensions, Olney, MD). Spectroscopic parameters examined were mineral/matrix peak area ratio, $1660/1690 \text{ cm}^{-1}$ peak intensity ratio (collagen cross-linking), and $1030 \text{ cm}^{-1}/1020 \text{ cm}^{-1}$ peak intensity ratio (mineral crystallinity). The change in pixel population averages for each of the parameters was compared with one-way analysis of variance (ANOVA) (Instat, GraphPad Corp., Carlsbad, CA).

RESULTS

All three groups in the MORE study showed increases in the mineral/matrix ratio in trabecular and cortical bone (Fig 3, Table 3), but no changes were found among groups. Crystallinity and the collagen cross-link parameter did not differ throughout the 2-year period and did not differ between treatment groups (Table 3).

DISCUSSION

These experimental data reveal how mineral analyses can be used to probe the effects of therapies on osteoporotic bone mineral properties. Although the 2-year treatment with raloxifene did not cause a significant change in the infrared parameters when the three treatment groups were compared, the treatments (including supplementation with calcium and vitamin D in the placebo group) resulted in improvement of mineral properties with time. These data are in agreement with the mean degree of mineralization of bone data, which similarly showed no changes between the raloxifene groups and the placebo groups.¹² The effects seen in the placebo group confirm previous observations that this supplementation can reduce some of the bone loss in menopausal women.^{12,37,86} The distribution of mineral was not different when the groups were compared, suggesting that the decrease in fracture risk compared with the placebo group in the larger population study was not caused by changes in the mineral parameters. It is probable that the decreased bone turnover in the raloxifene-treated groups reduced perforation of bone plates and therefore the bone strength on the tissue mechanical level. However, longer-term studies and a larger sample size probably will be needed to validate that possibility.

From the data presented in this review, it seems that a broad distribution of mineral crystal sizes, increased mineral content, and improved microarchitecture are associated with improved mechanical properties. Dual energy x-ray absorptiometry measurements can only provide information on BMD, and this is the most extensively used surrogate in clinical trials.⁴⁰ However, when biopsies are available, measurement of crystal and architectural properties can provide information that may reveal the efficacy of therapeutics. The observation that the biopsies in the MORE study (placebo and treatment) showed improvement in mineral parameters after 2 years makes longer-term analyses of similar parameters essential and suggests a need for evaluation of mineral properties in additional therapeutic trials.

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Fig 1.
A typical spectrum of trabecular bone, taken from a biopsy in a placebo-treated patient in the MORE study is shown. The spectral features of interest are indicated. The absorbance is a dimensionless ratio.

Fig 2A-G.

Images here were taken from the same placebo-control sample as shown in Figure 1 using infrared imaging spectroscopy. (A) The distribution of mineral intensities as revealed by the integrated area under the mineral phosphate band (900-1200 cm^{-1}) in the raw data. (B) The ratio of the integrated areas of the mineral phosphate band to the amide I peak shows the anatomic distribution of the mineral/matrix ratio. The axes are in pixels, where each pixel is 6.25 μm . (C) The ratio of the intensity of subbands at 1030 cm^{-1} and 1020 cm^{-1} shows the distribution of the crystallinity parameter. (D) The ratio of intensity of subbands at 1660 and 1690 cm^{-1} show a parameter related to the ratio of nonreducible and reducible collagen cross-links. (E) Pixel distribution for the image of mineral/matrix ratio in Figure 2B is shown. Note the distribution is skewed to the left. (F) Pixel distribution for the image of crystallinity in Figure C is shown. The distribution is skewed to the left, showing the presence of relatively more small crystal-lites than larger ones. (G) Pixel distribution for the image of collagen cross link ratio shown in Figure D is shown. Note the peak is sharp, showing limited variation in collagen maturity in the controls. The bin size for these histograms was set at 50.

Fig 3.

Changes in mineral/matrix ratio in individual patients after two years of treatment with raloxifene is shown in this graph. The y-axis shows the mineral/matrix ratio (ratio of the integrated areas of the phosphate band to that of the amide I band) with each unique symbol in a given figure representing the same patient at baseline (1995) and 2 years after treatment (1997) for cortical and trabecular bone. The solid lines connect the baseline trabecular or cortical data to the 2-year time point for each patient.

Table 1.
Parameters Analyzed in Vibrational Spectroscopic Analysis of Bone

Parameter	Infrared	Raman	Comments
Mineral:matrix ratio	Integrated area of ν_1, ν_3 (900-1200 cm^{-1}) phosphate/amide I (1575-1720 cm^{-1}) ¹⁰¹	Integrated area of ν_1 (920-980 cm^{-1}) phosphate band/amide I ($\sim 1665 \text{ cm}^{-1}$) or hydroxyproline (876 cm^{-1}) band ¹¹⁷	Linearly related to ash weight ^{44,101}
Carbonate:amide I	Integrated area of 855-890 cm^{-1} carbonate band/amide I band ^{78,104}	Integrated area of peaks centered at 1070 cm^{-1} /1665 cm^{-1} ^{11,98,117}	Can also be expressed as carbonate to phosphate area.
Crystallinity	Curvefit 1030:1020 cm^{-1} subband area or 1030:1020 intensity area ¹⁷	Line broadening of ν_1 phosphate band ³⁸	The 1030 band is seen in stoichiometric hydroxyapatite and the 1020 band in nonstoichiometric hydroxyapatite. The ratio was correlated to mineral crystal size based on xray diffraction line broadening analysis. ⁴⁸
Acid phosphate	Subband at 1117 cm^{-1} ^{115,105} normalized to 960 cm^{-1} subband or phosphate band	1007 or 873 band normalized to ν_1 or amide I bands ⁹⁸	
Collagen maturity	Curvefit 1660 cm^{-1} /1690 cm^{-1} subband area ratio or intensity ratio ^{17,97}	Factor analysis of amide I band ¹¹⁷	

Table 2.
Infrared Analyses of Changes in Bone Mineral Properties

Species	Property	Change in Property
Canine osteoporosis ^{81,101}	Mineral:matrix (cortical)	Decreased
Postmenopausal women ^{15,16,91,92,94,95}	Mineral:matrix (cortical and trabecular)	Decreased
	Crystallinity (cortical)	Increased
	Crystallinity (trabecular)	Increased
	Carbonate:phosphate (trabecular)	Increased
	Collagen maturity	Increased
Ovariectomized monkey ^{48,93}	Mineral:matrix (trabecular)	Decreased
	Crystallinity (trabecular)	Increased
Biglycan-deficient mouse ¹²⁹	Mineral:matrix	Decreased
Interleukin-deficient mouse ³⁹	Crystallinity	Increased
Ovariectomized rat ^{4,9,87,97,102,111}	Mineral:matrix	Decreased
Disuse osteoporosis in rat ³¹	Crystallinity	No change

Table 3:
Mineral and Matrix Properties before and after 2 Years of Treatment

Crystallinity	Cortical Collagen Cross- Links	Mineral:Matrix	Crystallinity	Trabecular Collagen Cross Links	Mineral:Matrix
Placebo					
1.433	3.967	4.633	B 1.300	3.333	3.100
1.200	3.733	4.533	A 1.200	3.367	4.167
1.500	3.167	3.900	B 1.200	2.900	3.133
1.200	3.500	4.233	A 1.333	2.667	3.900
1.200	3.433	4.200	B 1.100	2.800	3.500
1.200	3.533	4.533	A 1.267	3.367	3.867
1.300	3.600	3.967	B 1.100	2.833	3.533
1.500	3.200	4.467	A 1.233	3.233	3.933
1.433	3.667	4.600	B 1.267	3.333	3.867
1.400	4.033	3.933	A 1.333	3.333	3.633
60 mg raloxifene					
1.267	3.733	4.033	B 1.200	3.033	2.733
1.233	3.433	4.300	A 1.267	3.450	3.733
1.300	2.300	3.350	B 1.200	2.250	3.025
1.467	2.700	4.167	A 1.200	2.433	3.933
1.400	2.433	3.800	B 1.367	2.267	2.967
1.433	3.533	4.733	A 1.333	3.100	3.633
1.533	3.600	4.467	B 1.267	3.067	3.333
1.367	3.500	4.433	A 1.300	2.967	3.667
1.367	3.500	4.500	B 1.233	2.933	3.500
1.367	3.633	4.667	A 1.367	3.300	3.733
120 mg raloxifene					
1.433	3.433	4.533	B 1.333	2.900	3.267
1.400	3.300	4.200	A 1.233	3.367	3.800
1.400	2.750	3.650	B 1.300	2.100	3.133
1.450	2.600	3.875	A 1.300	2.633	3.500
1.500	2.967	4.767	B 1.433	2.533	3.933
1.433	2.700	5.000	A 1.233	2.700	4.233
1.267	2.967	4.933	B 1.333	2.400	3.233
1.400	3.133	4.533	A 1.200	2.567	3.233
1.533	3.233	2.233	B 1.100	3.200	3.400
1.533	2.933	4.467	A 1.300	2.633	3.367

B = Before; A = After