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Altered distributions of bone tissue mineral and collagen properties in women with fragility fractures

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ABSTRACT

Heterogeneity of bone tissue properties is emerging as a potential indicator of altered bone quality in pathologic tissue. The objective of this study was to compare the distributions of tissue properties in women with and without histories of fragility fractures using Fourier transform infrared (FTIR) imaging. We extended a prior study that examined the relationship of the mean FTIR properties to fracture risk by analyzing in detail the widths and the tails of the distributions of FTIR properties in biopsies from fracture and non-fracture cohorts. The mineral and matrix properties of cortical and trabecular iliac crest tissue were compared in biopsies from women with a history of fragility fracture (+Fx; n = 21, age: mean 54 ± SD 15 y) and with no history of fragility fracture (−Fx; n = 12, age: 57 ± 5 y). A subset of the patients included in the −Fx group were taking estrogen-plus-progestin hormone replacement therapy (HRT) (−Fx + HRT n = 8, age: 58 ± 5 y) and were analyzed separately from patients with no history of HRT (−Fx − HRT n = 4, age: 56 ± 7 y). When the FTIR parameter mean values were examined by treatment group, the trabecular tissue of −Fx−HRT patients had a lower mineral:matrix ratio (M:M) and collagen maturity (XLR) than that of −Fx + HRT patients (−22% M:M, −18% XLR) and +Fx patients (−17% M:M, −18% XLR). Across multiple FTIR parameters, tissue from the −Fx − HRT group had smaller low-tail (5th percentile) values than that from the −Fx + HRT or +Fx groups. In trabecular collagen maturity and crystallinity (XST), the −Fx − HRT group had smaller low-tail values than those in the –Fx + HRT group (−16% XLR, −5% XST) and the +Fx group (−17% XLR, −7% XST). The relatively low values of trabecular mineral:matrix ratio and collagen maturity and smaller low-tail values of collagen maturity and crystallinity observed in the −Fx − HRT group are characteristic of younger tissue. Taken together, our data suggest that the presence of newly formed tissue that includes small/imperfect crystals and immature crosslinks, as well as moderately mature tissue, is an important characteristic of healthy, fracture-resistant bone. Finally, the larger mean and low-tail values of mineral:matrix ratio and collagen maturity noted in our −Fx + HRT vs. −Fx−HRT biopsies are consistent with greater tissue age and greater BMD arising from decreased osteoclastic resorption in HRT-treated patients.

1. Introduction

Fragility fracture risk depends on bone quantity, which is quantified clinically by areal bone mineral density (BMD), and bone quality, which encompasses geometric, microarchitectural, and material factors that contribute to whole-bone fracture resistance. Large epidemiologic studies have demonstrated that variation in BMD accounts for approximately 60% of fragility fracture risk [1,2], suggesting that the significant remaining proportion of fracture risk not explained by BMD may be explained by variations in bone quality. Factors that contribute to bone quality span multiple levels of bone structural hierarchy and include cancellous microarchitecture, microdamage density and morphology, tissue mineralization, and collagen crosslinking [3].

Fourier transform infrared (FTIR) imaging is capable of characterizing both the organic and the inorganic components of bone tissue in a spatially resolved fashion, and its outcomes are indices of bone quality that have been shown to account for fragility fracture incidence not predicted by BMD [4]. The following FTIR parameters, reviewed in detail elsewhere [5], have been defined and validated to quantify bone mineral and collagen properties: the mineral:matrix ratio, which measures bone mineral content [6]; the carbonate:phosphate ratio, which reflects the extent of carbonate substitution in the hydroxyapatite crystal lattice [7]; the collagen maturity (XLR), which indicates the ratio of nonreducible to reducible enzymatic collagen crosslinking [8]; and the crystallinity (XST), which is related to the mineral crystal size and perfection [9]. A prior study comparing bone tissue composition

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in women with and without fragility fractures using FTIR imaging identified that the mean values of several FTIR parameters–including the mineral:matrix ratio and collagen maturity in cortical regions as well as the collagen maturity and crystallinity in trabecular regions–were statistically associated with fragility fracture risk independently of BMD [4]. However, the cortical and trabecular regions do not exhibit a single homogeneous composition; rather, they are characterized by a heterogeneous distribution of compositional properties.

Spatial variation in bone tissue properties, i.e., heterogeneity, is emerging as a potential indicator of bone quality in healthy and pathologic tissue [10–13]. A narrower distribution of tissue properties at the micrometer scale may contribute to increased fracture risk through a reduction in the tissue's intrinsic toughening mechanisms [14], as has been demonstrated at the ~10 nm length scale [12]. Although several studies have noted differences in the distributions of tissue material properties in patients with and without histories of fragility fractures, their results diverge concerning which group exhibits greater heterogeneity. In a quantitative backscattered electron imaging (qBEI) analysis of bone tissue from patients with and without fractures, the mineralization distributions of iliac crest trabeculae from patients with fractures had smaller coefficients of variation than those of patients without fractures [15]. Femoral neck tissue of patients with fragility fractures characterized with FTIR imaging had narrower distributions of mineral:matrix ratio and carbonate:phosphate ratio but wider distributions of crystallinity compared to that of fracture-free cadaveric controls [16]. Finally, a microradiograpic study of the femoral neck showed that the heterogeneity of mineralization was greater in patients with a history of fragility fracture vs. non-fracture controls [17]. In summary, consistent trends in tissue heterogeneity with fracture status have not yet emerged, likely owing to confounding variables such as patient/donor age, tissue age (i.e., time since tissue formation), anatomic site, and differing experimental techniques.

The purpose of this study is to compare the distributions of bone tissue properties in women with and without fragility fractures using FTIR imaging. We extend a prior study that examined the relationship of the mean FTIR properties to fragility fracture incidence [4] by (1) analyzing in detail the distributions of mineral and matrix properties in the bone tissue of biopsies from the fragility fracture and non-fracture cohorts and (2) analyzing biopsies from patients with and without prior histories of hormone replacement therapy (HRT) separately to distinguish the effects of HRT treatment history and fracture status. In addition to characterizing the heterogeneity of the tissue properties using techniques previously applied to FTIRI data [13,18], we have also adapted methodology initially developed for analysis of the center, width, and tails of BMDD data assessed by qBEI [19], and applied it to FTIR imaging data. In this study we test the hypothesis that the bone tissue of patients with a history of fragility fracture is characterized by narrower distributions of mineral and matrix properties with attenuated tails shifted toward the center of the distribution, compared to that of patients with no history of fragility fracture.

2. Methods

The biopsies analyzed in this study are a subset of those analyzed previously [4]. The original study included 54 iliac crest biopsies from women with low-trauma fractures (+Fx, n = 32) and without fractures (−Fx, n = 22). Patients with or without a history of treatment with estrogen-plus-progestin hormone replacement therapy (HRT) were included; patients with a history of treatment with all other bone-active agents (e.g., teriparatide, alendronate) were excluded. The following data was provided for each patient: code number, age at biopsy, HRT status (yes/no = 1/0), spine and hip BMD, T-score, and presence (1) or absence (0) of fractures at the time of biopsy. For the patients with fractures, biopsies were acquired 6 months–5 years post-fracture. For each biopsy, three to nine cortical images and three to nine trabecular images from three 2–3-μm-thick sections were collected with an infrared imaging system (Spotlight 300, Perkin-Elmer Instruments, Waltham, MA, USA) [4].

Of the data from the original 54 biopsies, data from 33 were available for the current study (+Fx n = 21, age: mean 54 ± SD 15 y; −Fx n = 12, age: 57.3 ± 5.1 y). Eight of the patients included in the −Fx group in the original study were taking HRT; all were included in the current study and analyzed separately from patients with no history of HRT (−Fx + HRT n = 8, age: 58 ± 5 y; −Fx − HRT n = 4, age: 56 ± 7 y). Raw FTIR image data from the available 33 datasets were analyzed in a blinded fashion. Spectra were baseline corrected, and the PMMA spectral contribution was subtracted (ISys Chemical Imaging Analysis Software, Malvern Instruments Inc., Malvern, UK). The following FTIR parameters were calculated from the infrared spectrum collected at each pixel: the mineral:matrix ratio (area ratio of the phosphate ν_1 and amide I peaks), the carbonate:phosphate ratio (area ratio of the carbonate ν_2 and phosphate ν_1 peaks), the collagen maturity (XLR, intensity ratio of the 1660 cm^{-1} and 1690 cm^{-1} bands), and the crystallinity (XST, intensity ratio of the 1030 cm^{-1} and 1020 cm^{-1} bands).

The analyses yielded spectroscopic images showing the spatial variation of each FTIR parameter within the sample (Fig. 1). For each image, four histograms were generated, each representing the set of bone pixel values associated with one of the four FTIR parameters. Each histogram was fit with a Gaussian curve. Four outcomes were used to characterize each FTIR parameter distribution: (1) the mean of the distribution to assess average composition, (2) the full width at half maximum (FHWM) of the Gaussian curve fit to the distribution to assess compositional heterogeneity at the center of the distribution, and (3) the 5th percentile (low tail) and (4) the 95th percentile (high tail) values of the distribution to assess the left and right tails of the distribution, respectively [19].

Statistical analyses were performed separately for cortical and trabecular bone. Linear mixed models were used to assess the relationship between groups (−Fx − HRT, −Fx + HRT, and +Fx) and FTIR outcome measures while accounting for the repeated measurements made within each section (multiple FTIR images per section) and the multiple sections assessed per biopsy. Pairwise differences between groups were assessed with Tukey post-hoc tests. Differences in patient demographic and densitometric parameters (age, total hip BMD, lumbar spine BMD, hip t-score, spine t-score) among groups were assessed with Kruskal–Wallis tests. When significant, pairwise differences were assessed with Bonferroni-corrected pairwise Mann–Whitney U tests. Statistical analyses were performed in SAS version 9.3 (Cary, NC, USA), with a level of significance of 0.05.

3. Results

3.1. Demographic and densitometric data

The mean patient ages were similar across groups (Table 1). The lumbar spine BMD values were not different across groups, but the spine T scores differed across groups: the −Fx + HRT group had a substantially greater mean T score compared to that of the +Fx group (+206%, p = 0.006) (Table 1). The trends observed in total hip BMD and total hip T score paralleled those noted for the spine T score, with the greatest values observed in the −Fx + HRT group. Specifically, hip BMD and hip T score in the −Fx + HRT group were respectively 37% and 318% greater than the corresponding values in the +Fx group (hip BMD p = 0.016, hip T score p = 0.025) and trended toward a + 38% and +274% difference vs. the −Fx−HRT group (hip BMD p = 0.057, hip T score p = 0.056).

3.2. Distribution means

FTIR image data were first analyzed to determine the mean values of all four FTIR parameters. Across most of the FTIR parameters, the −Fx − HRT group tended to have the smallest values, while the −Fx + HRT and +Fx groups had larger values that were similar