Published in final edited form as:

Matrix Biol. 2011 April ; 30(3): 188–194. doi:10.1016/j.matbio.2011.03.004.

Material and mechanical properties of bones deficient for fibrillin-1 or fibrillin-2 microfibrils

Emilio Arteaga-Solis^{a,*}, Lee Sui-Arteaga^a, Minwook Kim^b, Mitchell B. Schaffler^{c,**}, Karl J. Jepsen^c, Nancy Pleshko^d, and Francesco Ramirez^{a,†}

^a Department of Pharmacology and Systems Therapeutics, Mount Sinai School of Medicine, One Gustave L Levy Place, Box 1603, New York, NY 10029, USA

^b McKay Orthopaedic Research Laboratory, University of Pennsylvania, 3451 Walnut Street, Philadelphia, PA, 19104, USA

^c Department of Orthopedics, Mount Sinai School of Medicine, One Gustave L Levy Place, Box 1603, New York, NY 10029, USA

^d Department of Mechanical Engineering, Temple University, 1801 N Broad Street, Philadelphia, PA 19122, USA

Abstract

The contribution of non-collagenous components of the extracellular matrix to bone strength is largely undefined. Here we report that deficiency of fibrillin-1 or fibrillin-2 microfibrils causes distinct changes in bone material and mechanical properties. Morphometric examination of mice with hypomorphic or null mutations in fibrillin-1 or fibrillin-2, respectively, revealed appreciable differences in the postnatal shaping and growth of long bones. Fourier transform infrared imaging spectroscopy indicated that fibrillin-1 plays a predominantly greater role than fibrillin-2 in determining the material properties of bones. Biomechanical tests demonstrated that fibrillin-2 exerts a greater positive influence on the mechanical properties of bone than fibrillin-1 assemblies. Published evidence indirectly supports the notion that the above findings are mostly, if not exclusively, related to the differential control of TGF β family signaling by fibrillin proteins. Our study therefore advance our understanding of the role that extracellular microfibrils play in bone physiology and implicitly, in the pathogenesis of bone loss in human diseases caused by mutations in fibrillin-1 or -2.

Keywords

bone material and mechanical properties; congenital contractural arachnodactyly; fibrillin; Marfan syndrome; TGF β

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[†]Corresponding author: francesco.ramirez@mssm.edu; phone (212) 241-7237; fax (212) 996-7214.

^{*}Pulmonary Medicine Division of the Department of Pediatrics at Columbia University College of Physician and Surgeons, New York, NY.

^{**}Department of Biomedical Engineering at the City College of New York, NY

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1. Introduction

Fibrillin-1 and -2 are ubiquitously expressed glycoproteins that give rise to filamentous assemblies (microfibrils) with an average diameter of 10 nm (Ramirez, 2009). Fibrillin microfibrils associate or interact with several other extracellular matrix (ECM) proteins, including microfibril-associated glycoproteins (MAPGs) and latent TGF β -binding proteins (LTBPs), as well as with cross-linked elastin molecules in the elastic fibers (Ramirez and Rifkin, 2009). Fibrillin assemblies thus constitute the non-collagenous architectural elements of soft and hard tissue matrices (Ramirez, 2009). Immunohistological, biochemical and genetic findings indicate that fibrillin assemblies can also influence cell behavior principally by binding LTBP-associated latent TGF β complexes and BMP pro-peptides (Dallas et al., 1995; Arteaga-Solis et al., 2001; Neptune et al., 2003; Isogai et al., 2003; Sengle et al., 2008). Genetic studies, in particular, have demonstrated the importance of fibrillin deposition for the proper storage, distribution, release and activation of locally produced TGF β and BMP molecules (Ramirez and Rifkin, 2009). Mutations in fibrillin-1 or -2 cause two clinically distinct disorders of the connective tissue, Marfan syndrome (MFS; OMIM-154700) and congenital contractural arachnodactyly (CCA; OMIM-121050) respectively (Ramirez and Dietz, 2007). Mouse models of MFS have associated promiscuous activation of latent TGF β complexes with the progression of cardiovascular, lung and skeletal muscle abnormalities (Neptune et al., 2003; Ng et al., 2004; Habashi et al., 2006; Cohn et al., 2007), whereas loss of fibrillin-2 synthesis has been correlated with impairment of BMP-driven bone patterning of mouse autopods (Arteaga-Solis et al., 2001). More recently, deficiency of either fibrillin-1 or -2 in mice was demonstrated to decrease bone mineral density (BMD), a trait shared by MFS and CCA (Ramirez and Arteaga-Solis, 2008), through distinct alterations of local TGF β and BMP bioavailability without affecting the number of osteoblasts and osteoclasts (Nistala et al., 2010a; Nistala et al., 2010b and 2010c). The investigations have also excluded a direct structural role of fibrillin microfibrils in supporting mineral deposition in the bone matrix (Nistala et al., 2010a). On the one hand, loss of fibrillin-2 enhances the activation of otherwise matrix-bound latent TGF β complexes with the consequence of inhibiting osteoblast maturation, while concurrently increasing osteoblast-supported osteoclast activity (Nistala et al., 2010a and 2010b). On the other hand, loss or underexpression of fibrillin-1 elevates both local TGF β and BMP signaling with the net result of accelerating osteoblast maturation, while still enhancing osteoblast-driven osteoclast activity (Nistala et al. 2010a and 2010c). Decreased BMD is also a clinical finding in mice deficient for MAGP-1, a ubiquitous component of fibrillin microfibrils (Craft et al., 2010). In this case, however, the phenotype is accounted for by defective bone resorption due to a greater number of osteoclasts probably secondary to an augmented response of marrow macrophage cells to pro-osteoclastogenic signals (Craft et al., 2010).

The aforementioned genetic findings are in line with the broad distribution of morphologically distinct fibrillin assemblies in skeletal tissues (Keene et al, 1991 and 1997; Zhang et al. 1994 and 1995; Gigante et al. 1996; Kitahama et al., 2000; Dallas et al., 2000; Arteaga-Solis et al., 2001; Quondamatteo et al., 2002), with microfibril participation in the extracellular regulation of TGF β and BMP bioavailability (Isogai et al., 2003; Sengle et al., 2008), with the prominent storage of TGF β and BMP ligands in skeletal matrices (Mohan and Baylink, 1991) and with the discrete and overlapping contributions of TGF β and BMP molecules to bone metabolism (Alliston et al., 2008). Additional evidence from genetically engineered mice has also implicated TGF β signaling in regulating critical parameters of bone strength (Atti et al., 2002; Balooch et al., 2005). The scope of the present study was therefore two-fold. First, we sought to confirm and expand previous Raman microspectroscopy and nanoindentation investigations that have predicted a significant decrease in the biomechanical properties of bones without fibrillin-2 microfibrils (Kavukcuoglu et al., 2007a). Second, we compared and contrasted the material and

mechanical properties of bones that either lack fibrillin-2 or underexpress fibrillin-1. The results of our study demonstrate that fibrillin-1 and fibrillin-2 microfibrils differentially specify bone strength conceivably as a reflection of their distinct roles in modulating the local bioavailability of TGF β family signals during osteogenic differentiation.

2. Results

2.1. Morphology of fibrillin-deficient long bones

Previous analyses have shown that expression of fibrillin genes in the forming skeleton begins before mesenchyme differentiation and continues throughout bone formation and growth with the accumulation of distinct macromolecular assemblies, such as uninterrupted elastic fibers running along the entire length of the perichondral/periosteal matrix of long bones, circumferential bundles of microfibrils wrapped around the Ranvier's groove, and compact fiber-like microfibrils deposited immediately around chondrocytes, osteocytes and osteons, at the endochondral surface and within the trabecular matrix (Keene et al, 1991 and 1997; Zhang et al. 1994 and 1995; Gigante et al., 1996; Kitahama et al., 2000; Dallas et al., 2000; Arteaga-Solis et al., 2001; Quondamatteo et al., 2002). The focus of the present study was to examine key parameters of bone strength in mice deficient for fibrillin-1 or -2 microfibrils (n=6–10 per genotype and assay) after they reached peak bone mass (Price et al., 2005). X-ray radiographs of 4 month-old mice under-expressing fibrillin-1 (*Fbn1*^{mgR/mgR} mice) or lacking fibrillin-2 (*Fbn2*^{-/-} mice) yielded a first approximate account of the skeletal differences between the mutant strains and their respective wild-type littermates (Fig. 1A, upper panels). Microcomputed tomography (μ CT) similarly provided a gross visual indication of morphological differences between wild-type and mutant femurs (Fig. 1A lower panels), which subsequent morphometric analyses detailed more rigorously. Specifically whole bone measurements showed that the femurs of *Fbn1*^{mgR/mgR} and *Fbn2*^{-/-} mice are respectively 8% longer and 4.5% shorter than normal (Table 1). Increased bone length of *Fbn1*^{mgR/mgR} femurs phenocopies the major skeletal trait of MFS (Ramirez and Arteaga-Solis, 2008). Measurement of the medial-lateral and anterior-posterior axes in mid-diaphyseal cross-sections revealed additional morphological differences. On the one hand, *Fbn1*^{mgR/mgR} femurs are fairly normal in width and shape, but they have a thinner cortex and a larger endosteal cavity (Fig. 1B and Table 1). By contrast, the femurs of *Fbn2*^{-/-} mice are smaller in width, unusually round in shape, and with a relatively thicker cortical area and smaller endosteal cavity (Fig. 1B and Table 1). Unlike the thinner cortical bone of *Fbn1*^{mgR/mgR} mice, however, the combination of greater cortical thickness and smaller periosteal diameter of *Fbn2*^{-/-} femurs results in a total cortical area nearly identical to that of the wild-type counterparts (Fig. 1B and Table 1). In contrast to previous analyses of *Fbn1*^{mgR/mgR} and *Fbn2*^{-/-} vertebrae (Nistala et al. 2010a and 2010c), there was no statistically significant differences in trabecular space, number and thickness or in trabecular bone volume/total volume between the mutant and wild-type femurs (Table 1). A trend toward lower BMD was instead noted in the trabecular bone of both *Fbn1*^{mgR/mgR} and *Fbn2*^{-/-} femurs (Table 1), which however did not reach the statistical significance of reduced BMD in fibrillin-1 or -2 deficient vertebrae (Nistala et al. 2010a and 2010c). Lastly, comparative measurements of the height of the columnar and hypertrophic zones in the growth plates of newborn mutant and wild-type mice failed to identify changes that could relate the observed variations in adult bone morphology with defective endochondral bone development (Fig. 1C and D).

2.2. Material properties of fibrillin-deficient long bones

Fourier transform infrared imaging spectroscopy (FT-IRIS) was next employed on mid-diaphyseal cortical bone and trabecular from adult *Fbn1*^{mgR/mgR} and *Fbn2*^{-/-} mice (n=6–8 per genotype and assay) in order to assess potential changes of bone mineral and matrix

ultrastructure *in vivo*. For cortical bone, the analysis revealed no significant differences in mineral carbonate content, collagen maturity (PHR 1660/1690) or crystallinity, as well as a tendency of the mineral:matrix ratio to be less in *Fbn1^{mgR/mgR}* than wild-type femurs (6.53 ± 0.35 vs 7.1 ± 0.74 ; $p=0.06$) (Fig. 2 and Table 2). Similarly, mineral orientation (anisotropy ratio) had an appreciable tendency to be less in *Fbn1^{mgR/mgR}* than wild-type cortical bones (1.2 ± 0.37 vs 1.68 ± 0.50 ; $p=0.09$) (Table 2). Infrared raw and 2nd derivative spectra from non-mineralized matrix regions of wild-type and *Fbn1^{mgR/mgR}* bone samples were also identical, implying a similar protein ultrastructure in the two genotypes. No differences were found in trabecular bone properties between the two genotypes. FT-IRIS analyses of *Fbn2^{-/-}* bones yielded no appreciable differences in any of the above parameters when compared to control samples for both cortical and trabecular bone (Fig. 2 and Table 2). Failure of detecting changes of mineral:matrix ratio in *Fbn2^{-/-}* cortical bone contrasts previous Raman spectroscopy findings, which have occasionally identified focal increments of mineral:matrix ratio and crystallinity in mid-cortical regions (Kavukcuoglu et al., 2007a). We believe that the discrepancy probably reflects the sensitivity of the two methodologies. With this consideration in mind, we interpreted the FT-IRIS data to suggest that fibrillin-1 has a greater role than fibrillin-2 in determining bone material properties.

2.3. Biomechanics of fibrillin-deficient long bones

The last set of experiments monitored the biomechanical properties of adult *Fbn1^{mgR/mgR}* and *Fbn2^{-/-}* femurs by loading to failure mutant and wild-type bones in a 4-point bending apparatus ($n \geq 9$ per genotype). In accordance with previous data from nanomechanics analyses (Kavukcuoglu et al., 2007a), significant differences in some of the biomechanical endpoints were observed in the *Fbn2^{-/-}* compared to wild-type bones (Table 3). Specifically, 4 month-old *Fbn2^{-/-}* femurs reported a 29% decrease in maximum load ($p < 0.0003$), a 25% decrease in total work ($p < 0.04$) and a 30% decrease in stiffness ($p < 0.0004$). Although appreciably evident, the decrease in the post-yield deformation alone was not statistically significant ($p > 0.05$). Consistent with these observations, morphometric analyses revealed a 40% reduction in the polar moments of inertia (J), an indication of resistance to bending loads, in the *Fbn2* mutant compared to wild-type femurs (Table 1). Furthermore, calculation of the bone strength index, which is based on measuring bone torsional and bending strength relative to bone length, revealed that *Fbn2^{-/-}* femurs are 31% weaker than control samples ($p < 0.003$) (Table 1). In sharp contrast to *Fbn2^{-/-}* bones, those harvested from adult *Fbn1^{mgR/mgR}* mice showed no differences in stiffness, total work and post-yield deformation compared to those of wild-type littermates (Table 3). Sole exception was a 23% increase in maximum load between the two genotypes ($p < 0.008$) (Table 3). Taken together, these observations supported the notion that fibrillin-2 microfibrils have a greater influence on the mechanical properties of bone than fibrillin-1 assemblies.

3. Discussion

In contrast to the wealth of information about collagen I fibers (Bonadio et al., 1993; Jepsen et al., 1997; Camacho et al., 1999; Phillips et al., 2000; Miller et al., 2007), the contribution of non-collagenous molecules to bone strength is limited to non-architectural elements of the bone matrix (Boskey et al., 1998, 2002, 2003 and 2005; Kavukcuoglu et al., 2007b; Mochida et al., 2009; Thurner et al., 2010). Here we documented that deficiency of fibrillin-1 or -2 microfibrils, the non-collagenous component of the bone architectural matrix (Ramirez, 2009), differentially influence the morphology, mechanical properties and to a lesser extent, the material quality of long bones. This finding extends previous studies showing that fibrillin-1 and -2 microfibrils influence bone patterning and osteoblast maturation differently because of their discrete roles in modulating local TGF β and BMP signaling (Arteaga-Solis et al., 2001; Nistala et al., 2010a, 2010b and 2010c). Our data,

together with those from mice with graded deficiencies of TGF β signaling (Atti et al., 2002; Balooch et al., 2005), support the notion that extracellular microfibrils contribute to bone strength mostly, if not exclusively, by regulating the local bioavailability of TGF β family members. In this respect, it would be of interest to clarify whether similar perturbations of TGF β family signaling are involved in the bone loss phenotype of mice deficient for the microfibril-associated protein MAGP-1 (Craft et al., 2010).

Earlier Raman spectroscopy studies have shown an appreciable trend for a greater than normal mineral:matrix ratio and crystallinity in the mid-cortical region of *Fbn2*^{-/-} bones, as well as less type-B carbonate substitution in the endosteal region that are focally distributed throughout the whole bone cross-section (Kavukcuoglu et al., 2007a). Moreover, nanoindentation analyses of *Fbn2*^{-/-} bones have identified statistically significant reductions in elastic modulus and hardness compared to wild-type samples (Kavukcuoglu et al., 2007a). The border line significance of the Raman spectroscopy findings, together with the strong inferential value of the nanoindentation data, was originally interpreted as indirect evidence that fibrillin-2 microfibrils predominantly specify the mechanical rather than the material properties of bone (Kavukcuoglu et al., 2007a). Non-uniform occurrence of material changes in *Fbn2*^{-/-} bones probably accounts for the apparent discrepancy between the FT-IRIS findings described here and the Raman spectroscopy data of previous investigations (Kavukcuoglu et al., 2007a). On the other hand, lower resistance of *Fbn2*^{-/-} bones to fracture supports earlier nanoscale evidence suggesting a role of fibrillin-2 microfibrils in bone integrity (Kavukcuoglu et al., 2007a). Greater fragility and promiscuous TGF β signaling of *Fbn2*^{-/-} bones parallels the lower fracture toughness of transgenic mice that overexpress TGF β 2 (Balooch et al., 2005; Nistala et al., 2010a).

Because external bone size in adult mice is largely determined by 2–3 weeks of age (Price et al., 2005), the slender phenotype (narrow relative to length) of 4 month-old *Fbn2*^{-/-} femurs suggests that loss of fibrillin-2 impairs sub-periosteal expansion and thus, osteoblast activity during postnatal growth. Such a postulate is entirely consistent with prior genetic evidence showing that fibrillin-2 deficiency inhibits osteoblast maturation by enhancing latent TGF β activation (Nistala et al., 2010a). Increased cortical thickness of *Fbn2*^{-/-} mice is expected for a slender diaphyseal bone (Jepsen et al. 2007), suggesting that loss of fibrillin-2 did not perturb the morphological component of normal buffering mechanisms. However, the FT-IRIS data refuted the expectation that slender bones should also be associated with increased tissue mineralization to increased material stiffness. Association between adult mineralization and slenderness is established during early postnatal growth (Jepsen et al., 2009). For example, *Gdf7* deficiency in mice has been reported to impair sub-periosteal expansion of femurs, but neither longitudinal growth nor compensatory mechanisms leading to a slender phenotype with normal strength (Maloul et al., 2006). Mutations that disrupt growth after this postnatal window and lead to slender adult phenotypes do not show the compensatory benefit of increased tissue-quality (Yakar et al., 2009). Taken together, the lack of association between slender adult femurs and reduced tissue-quality of *Fbn2*^{-/-} bones strongly suggest that fibrillin-2 may exert its primary effect after 2–4 weeks of age. The available data do not however exclude that differences in the femur length could in principle relate back to differences in the weight, and implicitly the nutrition, of the mutant mice. Notwithstanding this last point, it is at least conceivable to conclude that restriction of TGF β signaling by fibrillin-2 microfibrils may contribute to acquisition and maintenance of bone mass as well as resistance to fracture based on the following considerations: first, TGF β signaling plays a central role in both bone formation and bone strength (Atti et al., 2002; Balooch et al., 2005; Alliston et al., 2008) and second, fibrillin-2 microfibrils negatively regulate TGF β activity during osteogenic differentiation without directly impacting bone matrix mineralization (Nistala et al., 2010a).

Absence of periosteal expansion and increased marrow space in *Fbn1^{mgR/mgR}* femurs are respectively in line with normal osteogenic differentiation and osteoblast-supported osteoclast activity (Nistala et al., 2010c). Endocortical changes were small and by engineering convention they would be expected to have minimal effects on bone stiffness. Bone mass, morphology, architecture and material quality are all critical determinants of bone compliance (Heaney, 2003). Reduced bone mass and altered bone architecture are common traits of *Fbn1^{mgR/mgR}* and *Fbn2^{-/-}* mice, which otherwise differ in bone morphology and to a lesser extent, bone material properties. The diverse molecular structure of *Fbn1^{mgR/mgR}* and *Fbn2^{-/-}* bone matrices is probably the main determinant of the distinct macromechanical properties of the mutant long bones. The strikingly different morphology of femurs deficient for fibrillin-1 or -2 may also account for distinct responses to mechanical bending. These morphological differences are likely to reflect changes in TGF β and/or BMP signaling, which are probably confined to postnatal growth as preliminary histological evidence excluded a developmental defect. Conditional inactivation of *Fbn1* gene expression selectively in cartilage or bone and before or after embryonic development will eventually test the validity of these and related hypotheses.

4. Experimental procedures

4.1. Animals

Fbn1^{mgR/mgR} and *Fbn2^{-/-}* mice were bred onto C57Blk/6 and 129/SvEv genetic backgrounds, respectively, and genotyped as described (Arteaga-Solis et al., 2001; Pereira et al., 1999). The choice of two different genetic backgrounds was dictated by the greater survival of *Fbn1^{mgR/mgR}*/C57Blk/6 mice and the larger litter size of *Fbn2^{-/-}*/129/SvEv mice. Mutant bones were compared to those from the respective age- and sex-matched wild-type littermates so to minimize potential differences in bone properties between the two genetic backgrounds. Female mice were employed in all the analyses to eliminate gender-related differences and also correlate the findings to those of previous studies (Wallace et al., 2006; Nistala et al. 2010a and 2010c). Mice were handled and euthanized in accordance with national and institutional guidelines.

4.2. Histology, μ CT, and histomorphometry

Femurs were processed, sectioned and stained to visualize growth plate morphology of the distal femur using standard histological protocols (Baron, 1983), as well as scanned using an eXplore Locus SP Pre-Clinical Specimen Micro-computed Tomography system (GE Healthcare, London, Ontario, Canada). Contralateral femurs were used to measure biomechanical properties as described below. Three-dimensional images of the entire femur were obtained at an 8.7 micron voxel size (acquisition parameters: 80kVp, 80uA, 3 second exposure time [$\sim$ 69kJ], 0.010" aluminum beam filter), assessed in a 2.5mm region of the mid-diaphysis located immediately distal to the third trochanter, and individually thresholded using a standard thresholding algorithm to segment bone and non-bone voxels (Otsu, 1979). Cross-sections were analyzed for morphological traits describing the amount of tissue (cortical area, marrow area, total area, and cortical thickness,) and the spatial distribution of tissue (diameters and polar moment of inertia). The polar moment of inertia (J) was determined from the histomorphometric data by adding the anteroposterior and mediolateral moments of inertia, such that $J = I_{ML} + I_{AP}$ (Jepsen et al., 2001). The Selker and Carter's whole bone strength index (Selker and Carter, 1989) was calculated as a measure of bone torsional and bending strength relative to bone strength.

4.3. FT-IRIS analyses

Femoral bones were embedded in polymethylmetacrylate (n=6–8 per genotype), sectioned at 2 μ thickness and mounted onto barium fluoride windows. Data in the mid-IR spectral range

were acquired from cortical bone at mid-diaphysis and from individual trabeculae using a Spotlight 300 FTIR imaging system (Perkin-Elmer, Shelton, CT) at 6.25 μm spatial and 4 cm^{-1} spectral resolution all regions. The integrated areas of absorbance bands characteristic of mineral and matrix collagen were calculated with regard to mineral-phosphate (916–1180 cm^{-1}) and collagen (1588–1712 cm^{-1}) using the ratio of mineral to matrix absorbance (Min:Mat) as an indicator of tissue density. Carbonate substitution in bone mineral was calculated as the integrated area of the absorbance (Carb:Pho) between 850–890 cm^{-1} over the mineral phosphate absorbance. The peak height ratio (PHR) of the phosphate contour at 1030/1020 cm^{-1} was utilized as an indicator of mineral crystallinity, and the PHR at 1660/1690 cm^{-1} an indicator of collagen crosslink maturity (Boskey and Pleshko-Camacho, 2007). Second derivative spectra of non-mineralized matrix regions were assessed for differences in secondary protein structure. Mineral orientation in *Fbn1^{mgR/mgR}* and WT control samples were assessed using polarized FT-IRIS. A wire grid infrared polarizer was placed between the BaF₂ window containing the tissue section and the impinging IR radiation. Data were acquired with the sample polarizer in an initial position 0°, and at 90° from that orientation, as previously described (Camacho et al., 1999). Evaluation of changes in the intensity of specific peak heights enabled calculation of a dichroic or anisotropy ratio, a quantity related to the degree of orientation of the particular bond. The anisotropy ratio for the apatitic mineral was assessed by the PHR of mineral sub-bands at 1034 and 1102 cm^{-1} measured at 0°, and at 90°. This measurement was performed to assess whether the mineralization abnormalities found in the *Fbn1* mice also included differences in mineral orientation, a result found in studies of another mutant mouse model (Tesch et al., 2003). All data were analyzed using ISys 3.1 software (Malvern Instruments Ltd., Columbia, MD).

4.4. Biomechanics tests

Isolated femurs were tested for whole bone failure properties by loading them in the posterior to anterior direction, such that the anterior quadrant was subjected to tensile loads. Prior to testing, the length of the femur (from the most proximal point on the femoral head to the most distal point on the medial condyle) was measured to the nearest 0.01 mm using a sliding caliper. Each femur was loaded to failure in 4-point bending apparatus; outer and inner load points were 6.35 mm and 2.25 mm apart, respectively (Jepsen et al., 2001). The load-deformation curves generated were analyzed for stiffness, maximum load, work to fracture and post-yield deformation; the last parameter calculates the deformation at failure minus the deformation at yield. Yield was defined as a 10% reduction in the secant stiffness (load range normalized for deflection range) relative to the initial tangent stiffness.

4.5. Statistical evaluations

Two-way analyses of variance was used to test statistical significance for FT-IRIS data and two tailed t-tests were used for biomechanics, μCT and polarized FT-IRIS analysis. All data were analyzed using SigmaStat 3.5 software (SPSS Inc., Chicago, IL) and differences were considered significant at $p < 0.05$.

Acknowledgments

The authors thank Ms. Karen Johnson for organizing the manuscript. This work was supported by NIH grants AR42044 and AR44927, the National Marfan Foundation, and the John M. Driscoll, Jr., M.D. Children's Fund and Irving Institute/Clinical Trials Office Pilot Award.

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Figure 1. Fibrillins in bone and skeletal differences of fibrillin-deficient mice

(A) Illustrative X-ray radiographs of 4-months-old *Fbn1^{mgR/mgR}* and *Fbn2^{-/-}* mice and wild-type (WT) littermates of the respective genetic backgrounds (upper panel), and illustrative μ CT images of the corresponding femurs (lower panels; scale bar = 1 mm). (B) Illustrative μ CT images through the mid-diaphysis of femurs from 4-month *Fbn1^{mgR/mgR}* and *Fbn2^{-/-}* mice, and wild-type (WT) littermates of the corresponding genetic backgrounds showing differences in shape and cortical thickness. The drawing on the left shows the medial-lateral (x) and anterior-posterior (y) axes of a representative mid-diaphyseal cross-section; the bar graphs on the right summarize the total cortical area of mid-diaphyseal cross-sections of the indicated genotypes with the asterisk indicating a statistically significant difference. (C) H&E stained distal femoral growth plates of 4 day-old *Fbn1^{mgR/mgR}* and *Fbn2^{-/-}* mice, and wild-type (WT) littermates of the corresponding genetic backgrounds. (D) Bar graphs summarize height measurements of columnar and hypertrophic zones (c and h in panel C, respectively) in femoral growth plates of the indicated genotypes.

Figure 2. Fibrillin-deficient cortical bones display distinct mineral:matrix distributions
 Illustrative infrared image of the distribution of Min:Mat, an indicator of tissue density, in cortical bone of (A) wild-type (WT) and (B) *Fbn1*^{mgR/mgR} mice, and of (C) WT mice and (D) *Fbn2*^{-/-} mice (Scale bar = 80 μ m).

Table 1

Physical measurements of 4 months femurs

Variable	WT (C57BL/6) (n=10)	Fbn1 ^{mgR/mgR} (n=10)	p	WT (129/SvEv) (n=7)	Fbn2 ^{-/-} (n=6)	p
Body weight (gm)	27.24 ± 2.50	28.24 ± 1.90	>0.5	20.06 ± 1.11	18.23 ± 1.11	<0.02
Length of femur (mm)	15.70 ± 0.21	16.96 ± 1.24	<0.003	15.40 ± 0.50	14.70 ± 0.40	<0.0003
Cortical Area (mm ²)	0.984 ± 0.030	0.854 ± 0.040	<0.007	0.707 ± 0.057	0.629 ± 0.079	>0.07
Periosteal Diameter (X axis) (mm)	1.988 ± 0.105	1.925 ± 0.017	>0.14	1.355 ± 0.080	1.099 ± 0.081	<0.0002
Periosteal Diameter (Y axis) (mm)	1.335 ± 0.064	1.343 ± 0.015	>0.4	1.226 ± 0.055	1.099 ± 0.056	<0.002
Average periosteal diameter (mm)	1.661 ± 0.086	1.634 ± 0.014	>0.27	1.290 ± 0.560	1.100 ± 0.070	<0.0003
Endosteal Diameter (X axis) (mm)	1.483 ± 0.074	1.513 ± 0.033	>0.2	0.944 ± 0.046	0.652 ± 0.041	<0.000001
Endosteal Diameter (Y axis) (mm)	0.935 ± 0.048	1.035 ± 0.013	<0.0035	0.791 ± 0.049	0.625 ± 0.066	<0.0007
Average endosteal diameter (mm)	1.209 ± 0.059	1.274 ± 0.010	<0.036	0.867 ± 0.040	0.639 ± 0.049	<0.000005
CT/OD	0.288 ± 0.003	0.222 ± 0.003	<0.003	0.328 ± 0.005	0.419 ± 0.013	<0.003
Polar Moment of Inertia (I) (mm ⁴)	11.803 ± 2.540	9.882 ± 0.395	>0.05	0.213 ± 0.0309	0.128 ± 0.032	<0.0006
Strength Index	651.069 ± 45.35	625.289 ± 39.85	>0.06	248.810 ± 28.790	171.460 ± 36.980	<0.003
Trabecular Space (mm)*	0.057 ± 0.030	0.089 ± 0.025	>0.05	0.066 ± 0.019	0.072 ± 0.001	>0.05
Trabecular Number (1/mm)*	12.79 ± 1.680	10.52 ± 2.48	>0.05	13.707 ± 3.531	11.97 ± 1.395	>0.05
Trabecular Thickness (mm)*	0.010 ± 0.002	0.011 ± 0.003	>0.05	0.0108 ± 0.002	0.012 ± 0.001	>0.05
Trabecular Bone Volume/Total Volume (%)*	12.51 ± 0.77	11.38 ± 0.972	>0.05	14.36 ± 2.487	14.38 ± 2.07	>0.05
Trabecular Bone Mineral Density (mg/cc)*	136.37 ± 28.04	123.47 ± 25.08	>0.05	170.486 ± 42.55	150.13 ± 40.77	>0.05

CT/OD=Cortical thickness/Average periosteal diameter

* n=4 for each group

Table 2

FTIR imaging parameters of 4 months femurs

Variable	WT (C57Blk/6) (n=8)	Fbn1 ^{mgR/mgR} (n=8)	p	WT (129/SvEv) (n=6)	Fbn2 ^{-/-} (n=8)	p
Cortical Bone						
Min:Mat	7.10 ± 0.74	6.53 ± 0.35	0.06	7.22 ± 1.03	7.17 ± 0.59	>0.1
Car:Pho	0.0078 ± 0.0006	0.0082 ± 0.0004	>0.1	0.0082 ± 0.0007	0.0091 ± 0.0012	>0.1
PHR 1660/1690	3.41 ± 0.38	3.46 ± 0.32	>0.1	3.90 ± 0.37	3.80 ± 0.20	>0.1
Crystallinity	1.12 ± 0.03	1.15 ± 0.03	>0.1	1.15 ± 0.05	1.15 ± 0.04	>0.1
Anisotropy ratio	1.68 ± 0.50	1.20 ± 0.37	0.09	ND	ND	
Trabecular Bone						
Min:Mat	5.20 ± 0.14	5.0 ± 0.23	>0.1	5.05 ± 0.526	5.09 ± 0.4830	>0.1
Car:Pho	0.0078 ± 0.0002	0.0075 ± 0.0006	>0.1	0.0080 ± 0.0013	0.0083 ± 0.0009	>0.1
PHR 1660/1690	3.66 ± 0.15	3.69 ± 0.13	>0.1	3.89 ± 0.328	4.00 ± 0.3830	>0.1
Crystallinity	1.09 ± 0.029	1.07 ± 0.021	>0.1	1.06 ± 0.0358	1.05 ± 0.0240	>0.1
Anisotropy ratio	ND	ND		ND	ND	

Table 3

Biomechanical end points of 4 months femurs

Variable	WT (C57Blk/6) (n=10)	Fbn1 ^{mgR/mgR} (n=9)	p	WT (129/SvEv) (n=17)	Fbn2 ^{-/-} (n=13)	p
Maximum Load (N)	16.729 ± 2.467	20.611 ± 3.747	<0.008	28.170 ± 5.830	20.040 ± 3.490	<0.0003
Total Work (Nmm)	16.264 ± 7.182	14.243 ± 4.741	>0.05	13.280 ± 4.770	9.990 ± 3.560	<0.04
Post Yield Deformation (mm)	0.497 ± 0.281	0.326 ± 0.121	>0.05	0.142 ± 0.095	0.110 ± 0.097	>0.05
Stiffness (N/mm)	126.423 ± 14.521	132.566 ± 8.246	>0.05	175.450 ± 41.550	123.200 ± 26.180	<0.0004