Introduction: Fluoride has been widely investigated for the treatment of osteoporosis because of its potential to increase bone mass. Its positive effects on bone mass, however, are often offset by its negative effects on bone material strength. Despite the large number of studies on fluoride, relatively little is known about the effects of fluoride on bone mineral, in part because in vivo studies do not allow the mineral effects of fluoride to be separated from the metabolic effects. An in vitro model of sodium fluoride exposure has been described as an alternative approach for studying fluoride, and results with this model have demonstrated that high levels of fluoride in bone markedly reduce its strength [1,2]. Our objective was to further characterize the in vitro model of sodium fluoride. We asked: Is there a “safe” level of fluoride in bone that does not result in decreased strength? Do the effects of in vitro fluoride that have been reported for wet bone also apply to dry bone? What changes in mineral structure are associated with in vitro fluoride exposure?

Methods: Eighty femora were dissected from 40 10-week old C57BL/6 female mice. All procedures were approved by our animal studies committee. Marrow was flushed from the medullary canal using phosphate buffered saline (PBS) and femora were soaked for 24 hours in a detergent solution [1]. Femora were then soaked for 12 hours in treatment solutions consisting of PBS with sodium fluoride (NaF) added to different concentrations. Femora were used for three experiments. For the first experiment, 40 femora were assigned to five groups: 0.0 M, 0.0015 M, 0.0045 M, 0.01 M and 0.04 M NaF. The concentrations of NaF were chosen to produce fluoride levels in bone similar to those reported in vivo. After treatment, the femurs were tested to failure in torsion at 1 deg/sec (Instron 8500R). Bones were kept wet during all steps. Ultimate torque, yield torque, ultimate rotation and torsional rigidity were determined from the torque-rotation curves. The bones were then ashed and fluoride content was determined using an ion probe. For the second experiment, 6 femora were assigned to three groups: 0.0 M, 0.0045 M and 0.04 M NaF. The bones were treated using the same conditions as the first experiment, except that they were dried in air for 48 hours prior to torsion testing. For the third experiment, 6 femora were assigned to one of two groups: 0.0 M and 2.0 M NaF. After treatment, bones were dried, sectioned and examined using Raman spectroscopy. In this technique, a microscope is used to focus a 1 μm diameter laser on the specimen surface. The inelastically scattered radiation produces spectra that characterize the molecular structure, analogous to spectra produced by x-ray diffraction.

Results: Analysis of variance indicated that bone fluoride content increased in a dose-dependent fashion: 1.8, 3.7, 5.2, 7.9 and 14.4 mg F/g ash for the 0.0, 0.0015, 0.0045, 0.01 and 0.04 M NaF groups, respectively (p<0.05). Mechanical properties of wet bone also changed in a dose-dependent fashion, with yield torque, ultimate torque and rigidity decreasing and ultimate rotation increasing with increasing NaF concentration (p<0.05; Fig. 1). The properties of dry bones were less affected by fluoride exposure than wet bones, but yield and ultimate force still decreased significantly (Fig. 2). Raman analysis revealed that fluoride exposure caused a small but reproducible shift in the position of the peak associated with the major phosphate vibration mode, indicating a structural change in the bone mineral (Fig. 3).

Discussion: Recent clinical studies have indicated that slow-release fluoride may increase bone mass without the negative effects associated with earlier formulations [3]. These findings have revived interest in the potential of fluoride for treating osteoporosis, and have suggested that low levels of fluoride in bone may not be detrimental. Based on our in vitro model, however, we conclude: 1) Mechanical properties are degraded in a dose-dependent fashion as fluoride content increases. We found no evidence for a “safe” level of fluoride in bone. 2) The effects of fluoride were observed on both wet and dry bones, indicating that the changes in wet mechanical properties reported previously with this model [1,2] were not due entirely to increased hydration. 3) Fluoride incorporation caused an upshift and band-narrowing in the Raman peak for phosphate, which suggests an increase in crystallinity as reported in other Raman studies of biominerals [4]. 4) The narrowing in crystallinity is consistent with x-ray diffraction data of bone following in vivo fluoride treatment [5], which suggests that the in vitro model produces similar changes to those that occur in vivo.


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