INTRODUCTION: To improve the clinical assessment of fracture risk, magnetic resonance methods are being developed to quantify concentrations of matrix-bound and pore water within bone [1,2] because such measurements can be more predictive of the material properties of bone than X-ray-derived metrics [3], which are only sensitive to the mineral phase. One material property particularly sensitive to hydration and presumably bound water is toughness [4]. While there is evidence that the amount of bound water in bone and the toughness of bone decreases with aging [5,6], it is unknown how the energy level of tissue hydration (e.g., loosely vs. tightly bound) at which aging affects water-matrix interactions. We hypothesized that air-drying for <15 min. affects the toughness of young human bone to a greater extent than elderly bone because in effect elderly bone has less low-energy matrix-bound water that significantly contributes to ductility.

METHODS: Pairs of human cortical bone strips were extracted from the femur mid-shaft (medial quadrant) acquired from male and female cadavers (10 young donors: 31 to 60 yr. of age, 47.3±11.2 yr. and 10 old donors: 77 to 99 yrs. of age, 86.1±7.8 yr.) and machined into ‘dog-bone’ tensile specimens (gauge region: 10 mm x 2 mm x 2 mm). The non-fixed tissue was stored in phosphate buffered saline at ~5°C until analysis. The middle segment of the gauge region (1.23 mm in length) was imaged at an isotropic voxel size of 6 μm using the Scanco μCT40. Upon contouring the image stack of bone cross-sections (700 μH/cm³), the intracortical porosity (1-BV/TV) was determined in order to assign the paired specimens to wet or partially dried group such that porosity was not greater or less in one group (paired test p=0.460). In addition, the cross-sectional area was TV divided by 1.23 mm. The tensile tests were performed at ~24°C (~30% humidity) using the Instron Dynamight system. All specimens were pulled-to-failure at 5 mm/min recording force from a 1000 N load cell and strain from an extensometer at 50 Hz. The toughness was the area under the engineering stress vs. strain curve. Water was dripped on those specimens assigned to the wet group. For the other group, the paired specimens were air-dried until they lost 1.5% of their original mass and the tensile tested without the drip (an additional ~3 min. of air drying). The wet mass, submerged mass, and post-testing mass were measured with an electronic balance. The broken pieces of the partially dried specimens were further dehydrated at ~70°C for 48-h and then at 103°C for 48-h measuring the mass after each step.

To confirm that the air-drying protocol removed bound water and not just pore water, a grip region of each broken tensile specimen (wet group only) was cut (5 mm x 5 mm x 2 mm) and analyzed by 1H Nuclear Magnetic Resonance (NMR) relaxometry that discriminates 3 unique proton pools based on the differences in the T2 relaxation rate of solid protons (matrix with T2 = ~57±4 μs), bound water protons (T2 = 4~35 μs), and a mixture of pore water and lipid protons (broad T2 > 1 ms) [1.3]. With a microsphere of water with a known volume as a reference, the volume fraction of total and bound water within each cut sample was quantified for 3 conditions: fully hydrated, partially hydrated (air-dried for ~15 min.), and nearly dehydrated (oven-dried at ~70°C for 48-h). A repeated measures two-way ANOVA was used determine whether there differences in the tensile properties of bone between young and old as well as between the wet group and partially dried group. A t-test determined aging differences in porosity, gravimetric measurements, and NMR properties. Results are provided as mean ± standard deviation.

RESULTS: The moderate drying protocol removed a similar volume of water from the young and old tissue (Young: 6.4±2.3 % by vol vs. Old: 6.5±1.3 % by vol). This loss of water decreased the toughness of bone for both young and old, and the age-related difference in toughness existed for both wet and partially dried groups (Fig 1). The absolute difference in toughness between paired wet and partially dried specimens was greater in the young group (Δ=0.993±0.671 MJ/m³) than in the old group (Δ=0.549±0.499 MJ/m³), but with a sample size of 10 and high variance, this did not reach statistical significance (p=0.111). In addition to removing the pore water, the drying protocol decreased the volume fraction of bound water (Fig 2). Low energy drying tended to remove more bound water in the young samples of bone (Δ = 0.0105±0.0062 vol fraction), but again this did not reach statistical significance (p=0.132). Oven drying at 70°C practically removed all the bound water that is detected by NMR (Fig 3). Interestingly, when measured gravimetrically on broken tensile specimens, the total water removed by oven drying at 70°C was greater in young bone (19.6±1.8 % by vol) than in old bone (16.3±1.2 % by vol, p=0.0002). In this particular sample of intracortical bone, there was no difference in porosity (p=0.794) or pore water (p=0.320) between the two age groups.

Lastly, oven drying at 103°C further removed water from young (23.8±1.8 % by vol) and old bone (23.2±1.4 % by vol) with no difference in the amount removed (p=0.455). This suggests that with aging there is a decrease in loosely-to-intermediately bound water but an increase in tightly bound water.

Fig. 1. Low energy drying decreased Young and old bone. decreased bound water in bone.

DISCUSSION: The present findings do not unequivocally support the hypothesis that elderly bone has less loosely bound water, and as such, is resistant to the effects of partial dehydration. Interestingly though, partially dried young bone had a similar toughness as wet old bone (Fig 1). Assuming pore water does not appreciably contribute to bone toughness, a minimal loss of 5%-10% of the bound water pool as measured by NMR caused a rather significant decrease in toughness. Thus, low-energy matrix-water interactions are likely important to bone toughness. Conceivably though, restricted movement of the incompressible water within bone pores could contribute to bone’s resistance to tensile loading, but the partial drying protocol did not affect peak strength (p=0.524). Although we only observed a trend in young bone having less loosely bound water than old bone, an age-related change in the energy of the water-matrix interactions does appear to exist since a greater amount of water was removed from the young bone than old bone when the tensile specimens were oven-dried at 70°C with no difference in water removal at 103°C. NMR assessment of bone is primarily sensitive to bound water that exists at temperatures below 70°C (Fig 3), and these water-matrix interactions (H-H bonding) are important to the toughness of bone.

Fig. 3. Drying at 70°C removed bound water as measured by NMR.

SIGNIFICANCE: Magnetic resonance analysis of the relaxation behavior of protons in bone is sensitive to water interacting with matrix at low-to-intermediate energy levels, and the age-related decrease in these water interactions likely contributes to a decline in bone toughness.

ACKNOWLEDGEMENTS: The Musculoskeletal Tissue Foundation and the Vanderbilt Donor Program donated cadaveric tissue. The study was funded in part by a VU Discovery Grant and NIH (EB001744).


Poster No. 0375  •  ORS 2012 Annual Meeting