NMR assessment on bone simulated under microgravity

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Introduction

Microgravity-induced bone loss has been suggested to be similar to disuse-osteoporosis on Earth, which constitutes a challenging public health problem. No current non-destructive method can provide the microstructural changes in bone particularly on cortical bone. Recently, the authors have applied low field nuclear magnetic resonance (NMR) spin-spin relaxation technique and computational analysis method to determine the porosity, pore size distribution and microdamage of cortical bone [1-3]. The studies by the authors have shown that this technology can be used to characterize microstructural changes as well as bone water distribution (bound and mobile water) changes of weightless treated (simulating a microgravity condition) turkey and mouse cortical bone. We further determinate that the NMR spin-spin relaxation time (T_2) spectrum derived parameters can be used as descriptions of bone quality (e.g. matrix water distribution and porosity size distributions) and, alone or in combination with current techniques (bone mineral density measurements), more accurately predict bone mechanical properties.

Methods

Bone sample preparation Two kinds of animal samples were collected and prepared for designed experiments from SUNY. Cortical bones of the mid-diaphyses of the ulnae of 1-year-old male turkeys were dissected from freshly slaughtered animals. Eight samples were categorized from normal (or control) and four samples were 4-week disuse treated by functionally isolated osteotomies (disuse). A total of 12 mouse long bone samples including 6 disuse bones harvested from disuse and 6 contralateral controls were used. The bone samples were cut 2-cm long using a diamond saw and the soft tissue attachment was carefully cleaned up from endosteal and periosteal surfaces.

NMR system NMR test was on a Southwest Research Institute (SwRI) built 0.5 to 40 MHz broad-line NMR system with an electromagnet 19 inches in diameter with a 4-inch gap, and was set up for a proton frequency of 2.3 MHz and 27 MHz. Determination of porosity and pore size distribution The total volumes (V_B) of each bone sample were determined by Archimedes' principle. The NMR CPMG signal am-

plitudes were normalized to equivalent volumes of pore water by calibration with the NMR signal amplitude of a known volume of water (V_l). Therefore, the porosity of the bone was calculated as V_l/V_B. All the obtained T_2 relaxation data were computerized to inversion T_2 relaxation spectra and the spectra can be transformed to the pore size distribution with the longer T_2 relaxation times corresponding to larger pores [4].

Determination of water distribution The FID signal is due to the liquid, bound and solid phase protons inside the bone. Using the T_2 relaxation data obtained from FID measurement, the inversion T_2 relaxation distribution patterns shown three peaks in the spectrum, from left to right, result from protons in the solid component, in bound water, and in mobile water, respectively.

Validation of NMR porosity and pore size with SEM results Each bone was then examined by a backscatter scanning electron microscopy (Model 1810D, AMRAY, Bedford, M.A.) at 8 equal pies with 50x and 120x magnification. The images were then processed using custom-written software on PV-WAVE.

Results and Discussions

A strong correlation between NMR determined porosity and SEM measured data was observed. In this study, our results show that the average porosity is increases and the pore size is shifted to the larger size in disuse group than in normal group. The average of bound water to mobile water in disuse bone group is decreased than in normal group. The results also show the parameter of the average porosity times the average ratio of bound water to mobile water may be a constant for both normal and disuse bone groups. These parameters could be used as measures of bone quality since they combine measures of both bone porosity and water distribution, and the derived results can be used for further bone mechanical property and quality studies.

Acknowledgment

This work is supported by NIH R01-AR049286-01A1 and SwRI project R9397.

References

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