JBMR SHORT REPORT

In vivo UTE-MRI reveals positive effects of raloxifene on skeletal bound water in skeletally mature beagle dogs.

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ABSTRACT

Raloxifene positively affects mechanical properties of the bone matrix in part through modification of skeletal bound water. The goal of this study was to determine if raloxifene-induced alterations in skeletal hydration could be measured *in vivo* using ultra-short echotime magnetic resonance imaging (UTE-MRI). Twelve skeletally mature female beagle dogs (n=6/group) were treated for 6 months with oral doses of saline vehicle (VEH, 1 ml/kg/day) or raloxifene (RAL, 0.5 mg/kg/day). Following six months of treatment, all animals underwent *in vivo* UTE-MRI of the proximal tibial cortical bone. UTE-MRI signal intensity versus echotime curves were analyzed by fitting a double exponential to determine the short and long relaxation times of water with the bone (dependent estimations of bound and free water, respectively). Raloxifene-treated animals had significantly higher bound water (+14%; $p = 0.05$) and lower free water (-20%) compared to vehicle-treated animals. These data provide the first evidence that drug-induced changes in skeletal hydration can be non-invasively assessed using UTE-MRI.

INTRODUCTION

Raloxifene significantly reduces fracture risk despite minimal effects on bone mineral density [1-3]. Preclinical studies in a dog model have documented a positive effect of raloxifene on material-level biomechanical properties (the properties of the tissue independent of bone mass) using both estimated material properties from whole bone tests (vertebra, femoral neck, rib) and direct assessment on beams from femoral bone [4,5]. Recent work has identified changes in skeletal hydration, specifically increases in matrix-bound water, as a key factor in this positive material-level adaption of bone. Treatment with raloxifene for one year in beagle dogs led to significantly more total skeletal water, assessed gravimetrically, and this was positively associated with the bone's mechanical properties [6]. Ultra-short echotime MRI (UTE-MRI) can differentiate hydration status of bone under various conditions [7-9]. More detailed assessment of raloxifene's effects on bone hydration using UTE-MRI revealed that *ex vivo* soaking of cortical bone (both dog and human) in raloxifene resulted in more matrix bound water compared to control of bone [6]. As UTE-MRI may have potential for clinical application [10], the goal of this study was to test the hypothesis that UTE-MRI can be used *in vivo* as a diagnostic indicator to detect changes in bone hydration caused by pharmacological interventions.

METHODS

Experimental design

Twelve skeletally mature female beagles (1-2 years old) were treated with one of two conditions for 6 months (n=6 per group): daily oral saline vehicle (1 mL/kg) or daily oral raloxifene (0.5 mg/kg). Raloxifene was dissolved in 10% hydroxypropyl-β-cyclodextrin and administered at a dose consistent with the clinical management of post-menopausal osteoporosis on a mg/kg basis. This dose has been shown previously to alter mechanical properties in this animal model

following 6 months [11] and one year of treatment [4,5,12]. After six months of treatment, all animals underwent *in vivo* UTE-MRI. All procedures were approved by the Indiana University School of Medicine Animal Care and Use Committee, and were conducted in accordance with NIH and USDA guidelines on animal care and use prior to the start of the study.

Ultra-short Echotime MRI (UTE-MRI)

Prior to imaging, anesthesia was induced with a combination of ketamine (8mg/kg) and diazepam (0.3mg/kg) via the cephalic vein. Anesthesia was maintained on 1-2% Isoflurane (balanced with medial grade oxygen) delivered at 2 L/min via mask. The hind limbs were immobilized in a custom configured splint that permitted precise placement of the two channel Miniflex® surface coils (Rapid MR International) laterally over the diaphysis inferior to the tibial plateau. The splint and surface coils were then secured to a custom configured leg stabilization platform permitting precise and repeatable iso-center alignment of the hindlimb and coils in the scanner. Each animal was scanned on an Siemens 3T Tim Trio MRI using an 3D UTE sequence with the following characteristics: TR (Time to repeat the sequence) 20 ms; TE1 (Echo time 1) variable (0.05, 0.06, 0.07, 0.08, 0.10, 0.12, 0.14, 0.20, 0.30, 0.40, 0.50, 0.60, 0.80, 1.0, 1.1 ms); TE2 (Echo time 2) 5 ms; Fat Saturation; Average 1, Excitation Flip Angle 50°; Normalization Filter; Acquisition Matrix 80x80x80; Field of View 50x50; Spatial Resolution 0.63x0.63x0.63 mm, and TA (Total acquisition time) 28 min. Fat saturation was applied to prevent signal oscillation with TE [6] and although this may slightly impact the measured bound water fraction [13], we felt it was a reasonable compromise to avoid potentially greater error due to signal oscillation.

Image Analysis

Image volumes for both variable (TE1) and fixed (TE2) echo times were imported, segmented, and quantified using Analyze 11.0 (AnalyzeDirect). Marrow and cortical bone for each image series per animal were segmented on the shortest TE1 image using a region growing technique, where the distal and proximal limits were prescribed at a fixed distance from the center of the FOV. Segmented regions were then extracted for all TE1 and TE2 images volumes, thereby permitting secondary analysis of the UTE signal. To correct for receiver gain offset differences between successive images, the following scaling was applied:

$$F(TE1,j) = \frac{Max[M(TE2,j)]}{M(TE2,j)} \quad (1)$$

$$C_c(TE1,j) = C(TE1,j) * F(TE1,j) \quad (2)$$

Where, F(TE1, j), M(TE2, j), C(TE1, j), and C_c(TE1, j) are the correction factors at the "*jth*" TE1, average marrow intensity at the "*jth*" TE2, average cortical bone intensity at the "*jth*" TE1, and corrected image intensity at the "*jth*" TE1. To improve model fits in low signal to noise data, images were corrected according to the methods of Miller and Joseph [14] and individually modeled using a double exponential decay [7], with the following modifications:

$$S(TE1,k) = ae^{(\frac{-TE1}{T2_B^*})} + be^{(\frac{-TE1}{T2_F^*})} \quad (3)$$

$$\%B(k) = \frac{a}{(a+b)}100 \quad (4)$$

$$\%F(k) = 100 - \%B(k) \quad (5)$$

Where, TE1, a, $T2_B^*$, b, $T2_F^*$, and $S(TE1,k)$ are the variable TE as described above, intercept for the bound fraction, $T2^*$ for the bound fraction, intercept for the free fraction, $T2^*$ for the free fraction, and the noise-free signal decay for the "*kth*" subject. In order to compute the percent

bound (%B(k)) and free (%F(k)) water in the system for the "*kth*" subject, Eqns 4 and 5 were employed.

Statistics

UTE-MRI data were evaluated using unpaired Students T-tests. Based on our previous work showed improvement in hydration of raloxifene-treated bone, a one-tailed t-test was used. For all statistical tests, *a priori* α-levels were set at 0.05.

RESULTS

Images acquired over the TE1 range from 0.05 to 1.1 ms resulted in high signal to noise ratios which ranged from 3.89±0.207 to 2.41±0.093, respectively (**Figure 1A-B**). Standardized segmentation of UTE images resulted in uniform cortical (170.0±10.5 mm^3) and marrow (62.2±3.79 mm^3) volume of interest, and when individually modeled yielded highly consistent normalized signal as a function of TE1 (**Figure 1C**). The free and bound T2* time constants for vehicle and raloxifene were 0.204±0.027, 5.02±1.39, 0.264±0.021, and 7.84±1.59 ms, respectively. UTE-MRI assessment of bound and free water was assessed in the cortical bone of the proximal tibia (**Figure 2A&B**). Raloxifene treatment for 6 months led to significantly more bound water (+14%) and significantly less free water (-20%) when compared to vehicle-treated animals (p=0.05, n=6/grp).

DISCUSSION

Given the emerging interest in UTE-MRI as a tool to assess bone hydration [7-10] and the recent evidence from our lab that raloxifene positively affects bone hydration [6] we undertook *in vivo* measures of free/bound water in animals treated with raloxifene (or vehicle). Our results

provide exciting and novel data showing that raloxifene leads to higher bound water compared to control animals and that this is detectable using *in vivo* UTE-MRI scanning.

Our analysis showed raloxifene treatment resulted in higher bound water – consistent with our previous work [6]. In addition, the rate constants from the UTE-MRI were consistent with previous data in ex vivo bone samples [7]. The mechanisms underlying raloxifene's positive effects on bound water remain to be determined. Our previous work points to the increased bound water at the collagen/mineral interface, effectively increasing the ability of the mineral and collagen to dissipate energy and toughen the matrix. This effect occurs independent of bone turnover and is cell-independent [6]; although there are clearly cell-dependent effects of raloxifene [15] and these could be contributing to changes in hydration.

The current study using UTE-MRI was only able to assess relative amounts of water (bound/free). Thus, it's possible that changes in bound water were influenced by changes in free water. Although we were not able to directly measure porosity, a major determinant of free water, in these animals we have previously documented the intracortical turnover rate of tibia in this age dog is 1-2% per year [16,17]. Suppression of intracortical remodeling, as would be expected with raloxifene, would therefore produce minimal changes in porosity, especially over 6 months (the remodeling cycle in a dog is ~ 3 months long [18]). Based on this, it is unlikely free water changes would account for the entire difference between groups quantified in the current work. Future work should employ standards that allow absolute volumes of free/bound water using UTE-MRI and/or should confirm these *in vivo* findings with *ex vivo* analyses by NMR.

These novel data show for the first time that drug-induced modulation of bone water can be detected *in vivo* using UTE-MRI. Alterations in water, both increases and decreases, have been shown to be associated with biomechanical properties [6,19]. Having the ability to quality

changes in hydration non-invasively will allow more detailed assessment to track not only how raloxifene is altering bone properties, by how other interventions affect bone hydration, both in preclinical and clinical studies.

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Figure Legends

Figure 1. Images of tibia cross-section (arrow), surrounded by muscle, from the transverse UTE-MRI at a TE1 of 0.05 (A) and 1.1 ms (B) illustrating the image quality over the range of TE1 studied. Panel C provides a chart of average normalized signal for with model fits (solid lines) for VEH (n=6, solid) RAL (n=6, open). Data presented as means ± standard error of the mean.

Figure 2. Raloxifene leads to higher bound water (A) and lower free water (B) in the cortical bone following 6 months of treatment. *In vivo* assessment of hydration was done using UTE-MRI. n= 6 animals per treatment group. Data presented as means ± standard error of the mean. * p = 0.05 between groups using a one-tailed t-test as described in the statistics section.

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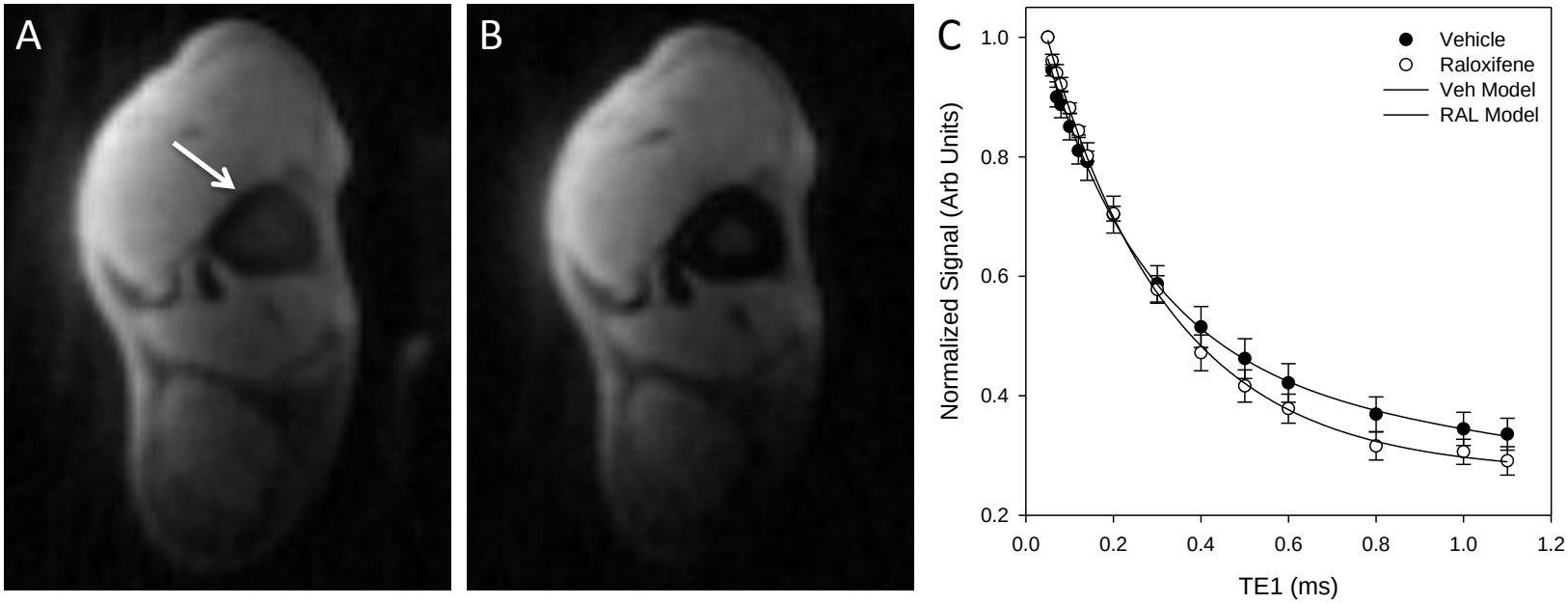
A
B
C
Vehicle
Raloxifene
Veh Model
RAL Model
Normalized Signal (Arb Units)
TE1 (ms)
1.0
0.8
0.6
0.4
0.2
0.0
0.2
0.4
0.6
0.8
1.0
1.2

Figure 1

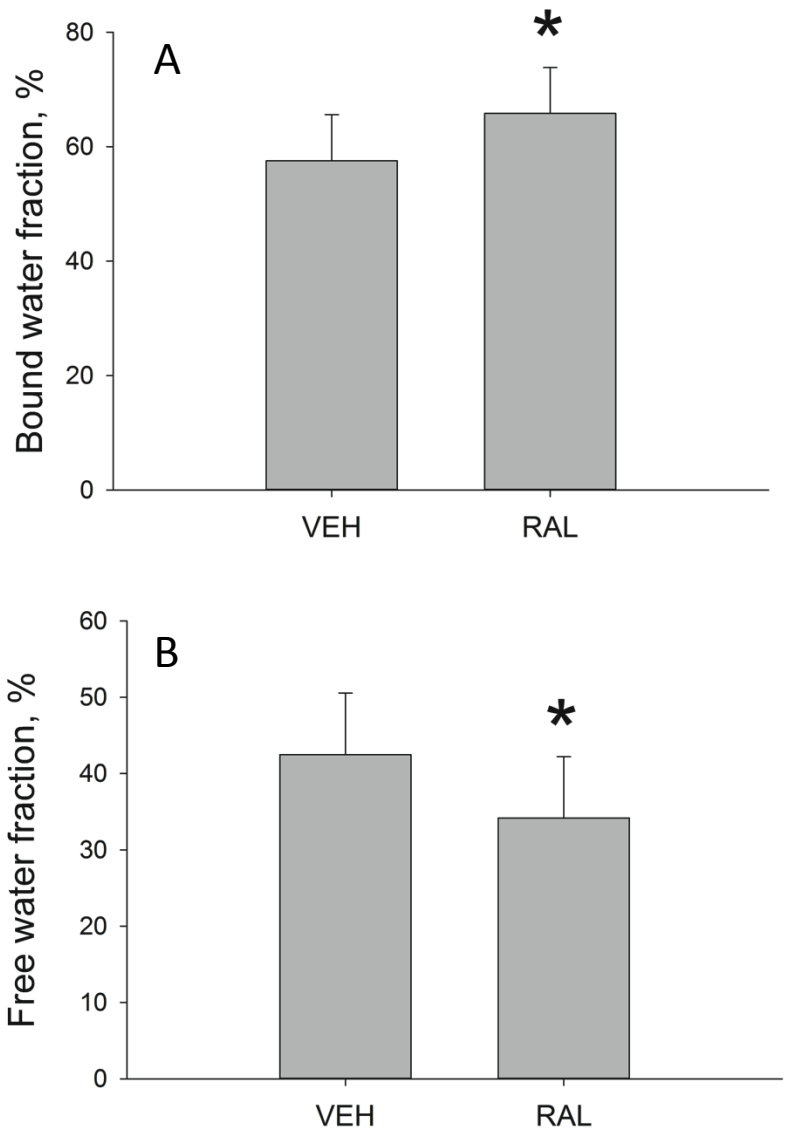
A
Bound water fraction, %
80
60
40
20
0
*
VEH
RAL
B
Free water fraction, %
60
50
40
30
20
10
0
*
VEH
RAL

Figure 2