Measurement of Phosphorus Content in Normal and Osteo-malacic Rabbit Bone by Solid-State 3D Radial Imaging

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Introduction

Osteomalacia is a disease caused by vitamin *D* deficiency and, similar to osteoporosis, can lead to fractures. A hallmark of osteomalacia is hypomineralization, which reduces bone stiffness and strength. True mineral density (degree of bone mineralization – *DMB*) is difficult to measure with any modality since it requires knowledge of both bone mass and bone volume. It has recently been shown that ^{31}P solid-state MRI has the potential to measure *DMB* [1]. In this work, we advance the hypothesis that *DMB* can be measured with sufficient precision to distinguish osteomalacic from normally mineralized bone. Toward this goal, we obtained 3*D* ^{31}P images at 162 *MHz* in the tibia of hypophosphatemic rabbits and normophosphatemic controls. Results were also compared with DMB obtained by quantitative micro-CT.

Materials and Methods

The extremely short T_2 (~100 μs) and the long T_1 (~ 50*s* at 9.4*T*) of ^{31}P in bone preclude conventional spin warp techniques for imaging. Here, a 3D radial projection reconstruction (PR) sequence was designed for operation at 162 *MHz*. In order to capture the center of *k*-space before a significant portion of the signal has decayed, a fraction of the signal was sampled during gradient ramping and regridding was used prior to 3D Fourier reconstruction. 2626 gradient directions were sampled on a series of equally spaced parallel rings in 65 azimuthal angle increments so as to be uniformly distributed on a sphere [2]. Imaging parameters were: RF pulse flip angle = 5.6 deg, TR=0.25*s*; FOV=3x3x3 cm^3; receive bandwidth = 200*kHz*; NEX=8; scan time ~ 88 *min*. Images were reconstructed by regridding onto a matrix of 108x108x108 voxels, yielding 278x278x278 μm^3 voxel size. The pulse sequence was implemented on a 9.4*T* vertical-bore superconducting system equipped with standard 100G/cm gradients (DMX-400, Bruker Instruments, USA), allowing for a ramping time of 100 μs in conjunction with a custom-made NMR probe (2.7 *cm* long x 1.2 *cm* diameter solenoidal *rf* coil). A reference capillary containing 2.5*M* K_2HPO_4 was co-imaged. Phosphorus (*P*) in bone was quantified (in wet weight %) from the mean signal intensity obtained from the center 5 slices in all images relative to K_2HPO_4. Micro-CT measurements were done on an MS-8 instrument (GE Medical Systems, formerly EVS Corp., London, Ontario, Canada) jointly with K_2HPO_4 solutions of varying concentration for calibration. Seven hundred twenty-one 2D views were collected at 0.5^0 increments, corresponding to one full rotation of the specimen and the data reconstructed with the manufacturer's cone-beam recon program. Scan time was 2 hrs for 3 averages per view yielding a final voxel size of 32x32x64 μm^3.

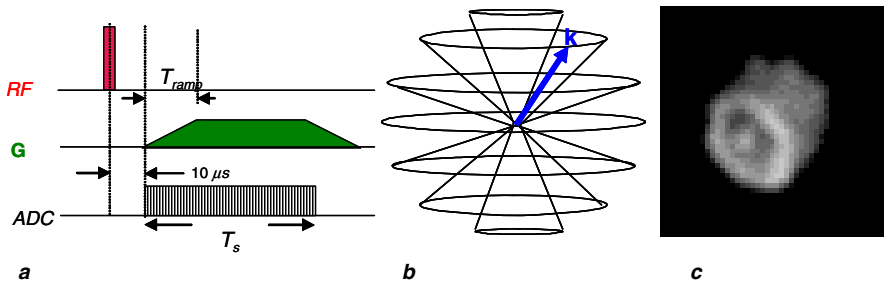

Fig.1 a) 3D radial projection imaging sequence using ramp sampling. b) Sampling on concentric cones for uniform mapping of k-space. c) Volume rendered image of one of the specimens obtained by 3D radial imaging.

20 female 5-week old New Zealand White rabbits were divided into four groups of which 5 were fed normal diet for 8 weeks (control (CO1) group); 5 a low *P* diet (0.09%) for the same period to induce osteomalacia (TR1 group). Further, 5 animals received a low-*P* diet for 8 weeks at which time they switched to normal diet for 6 weeks (TR2 group) for the purpose of examining the effect of recovery from hypophosphatemia. Finally, 5 animals received normal diet for the entire 14 weeks (CO2 group). After 8 weeks the CO1 and TR1 group of animals were euthanized. The CO2 and TR2 group were euthanized after 14 weeks. Cortical bone specimens of 1.5 *cm* length were harvested from the right and left tibiae (for ^{31}P NMR and μ-CT, respectively) and all soft tissue was removed.

Results and Conclusion

Fig. 1c shows a 3D volume-rendered image of one of the specimens. ^{31}P NMR-derived scatter plots of *P* content comparing CO1/TR1 groups are displayed in Fig. 2a, clearly indicating a significant difference between the normal and osteomalacic bone (14.41 ± 0.84 *vs.* 13.13 ± 0.74 (% wet weight); *p*=0.02). Fig.2b shows the effect of recovery by comparing CO2 to TR2 (11.94 ± 1.04 *vs.* 11.32 ± 0.59 (% wet weight); *p*=0.14). *P* content estimated from the present method and *DMB* obtained from μ-CT was highly correlated in all the groups (r^2=0.76; p=0.0001, Fig. 2c). The data indicate that subtle differences in phosphorus content and thus mineral density can be measured *ex vivo* in small animal bone. The method might be applicable to studies *in vivo* in laboratory animals and possibly in humans.

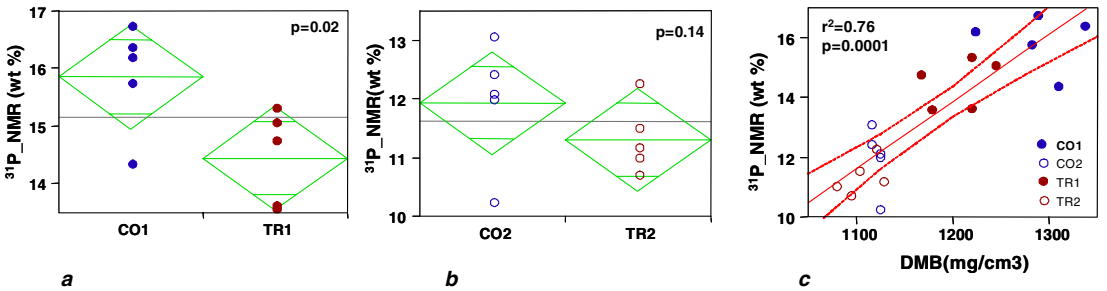

Fig.2 a) Comparison of *P* content between normal) and osteomalacic groups (CO1 vs. TR1) showing lower values in TR1; b) same for groups CO2 and TR2 showing effect of recovery (no significant group difference); c) correlation between μ-CT-derived *DMB* and *P* content measured by ^{31}P NMR.

References

1) Wu Y et al, Calcif Tissue Int 1998; **62**; 512-518. **2)** Glover G H et al, JMRI 1992; **2**; 47-52.

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