Water and Fat Suppressed Proton Projection MRI (WASPI) Study on Bone Specimens after Proton-Deuteron Exchange

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Introduction

With increasing use of solid state MRI and UTE sequences to image bone, questions have arisen as to the nature of the molecular species giving rise to the short-T_2 proton signal. When such images are used for quantitative tissue measurements, such as bone matrix density, it is critical to identify those tissue molecular constituents (for example, bulk water, bound water, and covalently bonded collagen protons) which contribute to the signal. Bone matrix, the organic fraction of bone consisting largely of protein (mostly collagen) and containing significant water, is a complex material in which water and protein exist in a wide range of motional states, with water undergoing molecular exchange between compartments and some protein protons (hydroxyls, amides, amines, etc.) undergoing chemical exchange with water. A recent study [1] showed that the Water- And fat-Suppressed Projection Image (WASPI) proton signal of bone matrix is uncorrelated with the bone water content (defined as that water removable by heating under specified conditions in a vacuum oven) but is highly correlated with two independent and widely used chemical measurements of the protein content, which is exactly what one desires if the intent is to measure bone matrix density. Deuterium exchange has been widely used to classify the chemical and motional states of hydrogen in tendon [2] and bone [3, 4]. In this study we use deuterium exchange to identify the source of proton signal in WASPI.

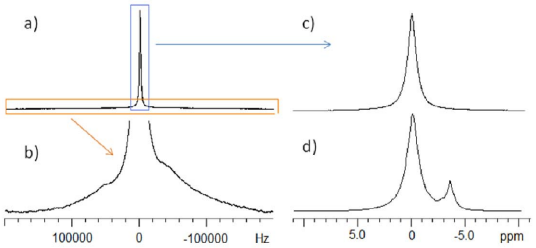

Figure 1: Representative proton NMR spectra (a) of cortical bovine bone specimens contain resonances from (b) broad solid components and (c) narrow mobile components including bone water. A sample with a strong –3.50 ppm fat resonance is shown in (d).

Materials and Methods

Cortical bone specimens (length 10 mm, width 5-7 mm, thickness 3 mm) were cut from the midshaft cortices of bovine femora, while trabecular bone specimens with similar dimensions were cut from the epiphysis. A group of cortical bone specimens (exchanged group) were immersed in 99% deuterium oxide up to 7 days, with periodic replacement of the D_2O. Another group of cortical bone specimens (heated group) was heated at 110 °C for 48 hours. Single pulse NMR spectroscopy with short or long receiver delay, total MRI (all fluid and solid signals) and WASPI (only solid signals) were carried out with a Bruker 4.7T system on the exchanged group at various D_2O immersion times and on the heated group before and after heating. A 20% PEO/PMMA blend calibration phantom [1] was imaged alongside the bone specimens as an intensity standard.

Results and Discussion

Single pulse NMR spectra with a long receiver delay (150 μs) detected only liquid signals from the sample, showing two peaks (water at 0 and fat at –3.5 ppm) in trabecular samples, while only one peak (water at 0 ppm) was observable in cortical bone samples (Fig. 1c, d). This indicated that there was very little fat in the cortical bone samples. To reduce interference from fat, we used cortical bone in most experiments. Single pulse NMR spectra with a short receiver delay (10 μs) of cortical bone specimens showed a broad peak (Fig. 1a), which was fitted to sum of two lorentzian peaks at short D_2O immersion time, a broad resonance arising from collagen and other macromolecules MMs (Fig. 1b) and a narrow resonance arising from mobile proton components (Fig. 1c). At long D_2O immersion time, a fat resonance became apparent and was included in the fit. Peak area fractions at different exchange times are shown in Table 1. It was found that peak area fractions stabilized after 7 days of D_2O exchange; hence the proton signal is only due to nonexchangeable protons which are 60.6% of the total original protons. The WASPI signal can be observed after 7 days of exchange and the average image intensity was 29.4% of that before exchange (Fig. 2f). Therefore it can be deduced that the WASPI signal after 7 days of D_2O exchange came from the nonexchangeable protons on collagen and other macromolecules (and any nonexchangeable protons in the mineral) since bone water is maximally exchanged with D_2O. According to reference [2], the mobile side chains of collagen and MMs have proton T_2 on the order of a few hundred microseconds which increases with hydration. WASPI can observe the signals of these protons on macromolecules.

D_2O Exchange Time	Mobile Proton (%)	Fat (%)	Collagen and MM (%)	Total (%)
0	40.6	-	59.4	100
6hr	20.7	-	57.2	77.9
7days	7.0	2.9	50.7	60.6

Table 1: Corresponding peak area percentages at different D_2O exchange time by fitting ^1H NMR spectra of a cortical bone specimen.

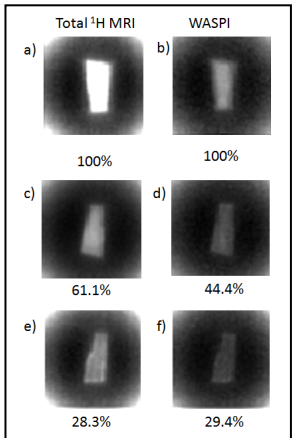

Figure 2: Total proton MRI and WASPI of the bone specimen before D_2O exchange (a-b) and after D_2O exchange 6 hours (c-d) and 7 days (e-f). The numbers are average image intensities as percentages of those before D_2O exchange.

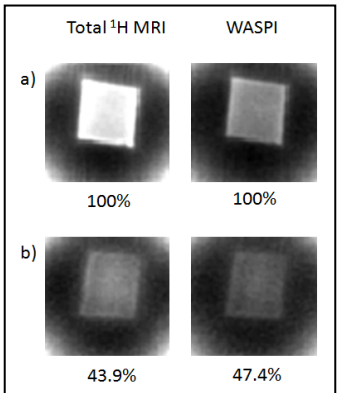

Figure 3: Total proton MRI and WASPI of the bone specimen before (a) and after heating at 110°C for 48 hours (b).

The WASPI image intensity of unexchanged bone (Fig. 2b) is similar to that of the total MRI intensity after 6 hr of exchange (Fig. 2c), indicating that most of the rapidly exchangeable water is suppressed by WASPI. Mobile proton components continued to exchange after 6 hr (Table 1) and the WASPI signal intensity observed after 6 hr (Fig. 2d) was higher than that of 7 days of exchange (Fig. 2f), which indicated that the signals in Fig. 2d arise not only from nonexchangeable protons on collagen and other macromolecules, but also from the mobile components which can be exchanged with D_2O slowly such as most tightly bound (least exchangeable) water. Similarly, the WASPI signal can be observed in heated bone. This signal arises from the proton pool not evaporated at 110°C (Fig. 3).

Quantitative evaluation of the WASPI signal distribution among collagen proton (resonance linewidth > 2 kHz) and tightly bound water proton (resonance linewidth ~ 1 kHz) can be obtained by comparing the WASPI intensity following exchange at various times and before and after heating. However, a complicating factor in this analysis is that the proton T_1 and T_2 of matrix constituents are strong functions of both hydration and proton content. Quantitative analysis must take these relaxation effects into account, which is in progress.

Conclusions

WASPI imaging can detect bone matrix signals from a proton pool which does not exchange with D_2O and which is not lost by heating; both of these procedures would result in substantial loss of bone water. Thus the WASPI image signal must result at least in part from the solid components of bone matrix such as collagen and other macromolecules.

References

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