A METHOD FOR MEASURING THE CONTRIBUTION OF LIPIDS TO EXCHANGE-INDUCED FREQUENCY CONTRAST WITHOUT REFERENCE CHEMICALS

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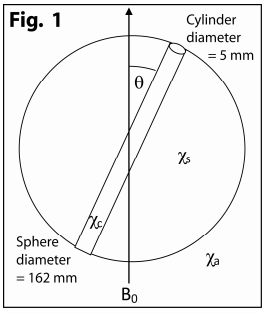
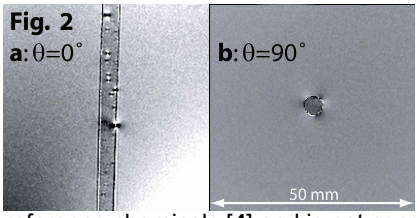
Introduction: Chemical exchange of protons between water and off-resonance molecular sites has been shown to contribute to white-gray matter (WM-GM) resonance frequency contrast in brain tissue [**1**]. It is still unknown which molecular species are primarily responsible for this exchange-induced frequency ($\mathbf{f_e}$) contrast. Studies in protein solutions [**2,3**] showed f_e shifts in direct proportion to the protein concentration, prompting some to attribute tissue f_e contrast to exchanging NH and OH protons on proteins [**2**]. In addition, there is evidence that lipids, particularly galactocerebrosides (GC), could contribute to the f_e contrast [**4**]. So far, f_e measurements have relied on internal frequency reference chemicals [**1-4**]. Unfortunately, these chemicals have been found to interact [**5**] with proteins (TSP [**3,6**]), with water itself (dioxane [**7**]), and potentially even with lipids [**8**], causing frequency shifts which may confound f_e estimates. Therefore, to accurately determine the f_e shifts induced by various molecules and allow investigation of their contributions to tissue f_e contrast, a method is needed which does not rely on internal reference chemicals. Here we aimed to develop a phantom-based method to measure the contribution of GC to f_e.



Methods: It is difficult to separate exchange-induced frequency shifts from those induced by magnetic susceptibility (χ) differences, particularly when the χ values of the chemicals are unknown. Instead of using an internal frequency reference chemical, we used the analytical expression for the frequency shift inside a phantom of simple geometry to obtain both $\Delta\chi$ and f_e. A schematic diagram of the phantom is shown in Fig. 1. The cylinder, an interchangeable thin-walled glass tube, contains GC vesicles (or saline) of χ_c, the sphere contains saline of χ_s and the air outside the sphere has χ_a. The frequency shifts inside the cylinder, including Lorentz sphere corrections, are given by Equation 1 [**3,9**]. **Eq. 1**

$$\frac{\Delta f}{f_0} = \frac{4\pi}{6}\left((\chi_c - \chi_s)\left(3\cos^2\theta - 1\right)\right) + \frac{4\pi\chi_a}{3} + \frac{f_e}{f_0}$$

To measure any f_e due to GC, the tube was filled with GC and Δf from a repeat experiment with a saline-filled tube ($\Delta\chi = \chi_c - \chi_s = 0$, $f_e = 0$) was subtracted to remove the χ_a term in Eq.1. GC was at 25 mM, which is intermediate between GM and WM GC concentrations [**10**]. 50 mM Palmitoyl oleoyl phosphatidyl choline (POPC) was included because pure GC does not form stable vesicles and the WM POPC:GC molar ratio is close to 2:1 [**10**]. Lipid stock solutions were combined for the chosen concentrations and the solvents were removed by evaporation under a vacuum. To form multi-lamellar vesicles, an authentic model for WM cell membranes, the lipid films were rehydrated in the same phosphate-buffered saline as in the sphere with 5 freeze-thaw cycles as in [**11**]. Multislice coronal (x,z) GRE images were acquired at 7 Tesla (f_0 = 300 MHz), at room temperature, with the cylinder at θ = 0° and 90° to B_0, TE = 12, 30 ms, 0.25 x 0.25 mm^2 voxels, 25 averages, 10 slices, thickness = 0.8/2 mm for θ = 0°/90° respectively, gap = 25%, and shim currents set to zero. The GC-filled tube was replaced with an identical saline-filled tube, ensuring that the phantom was placed in the same position and imaged with identical parameters. Images were acquired at two angles to separate f_e from $\Delta\chi$ which has an orientation-dependent contribution (Eq. 1). Fixing the shim and the phantom position for each angle obviated the need for spatial-high-pass filtering (to remove large-scale background fields) which can bias frequency measurements. Each magnitude GC image (TE = 12 ms) was linearly registered to the corresponding saline image. The registration parameters were applied to the phase images (scaled to frequency) at each TE. In a 200-x-200-voxel central square far from the sphere edges, regions of interest (ROI) were drawn inside the cylinder, excluding air bubbles, and in a homogeneous background region to allow correction for any global frequency offset between the GC and saline acquisitions. The corrected ROI mean saline frequency was subtracted from the ROI mean GC frequency in each slice at each TE to give Δf_{GC} at θ = 0° and 90°. f_e and $\Delta\chi$ were calculated using Eq. 1.



Results: Central regions of coregistered GC – saline frequency difference images (scaled ± 10 ppb, TE = 12 ms) at θ = 0° and 90° are shown in Fig. 2. The net GC frequency shifts inside the cylinder were –3.013 ± 0.051 (ROI Mean ± SE over TEs and slices) and –3.984 ± 0.109 ppb at θ = 0° and 90°, respectively. These values gave $\Delta\chi$ = 0.155 ± 0.121 ppb and f_e = –3.66 ± 0.163 ppb for GC. This small, positive lipid $\Delta\chi$ is unexpected given that lipid-rich myelin is thought to have a negative χ relative to other tissues [**12**] although the χ measured here is relative to an unknown saline χ_s. The negative f_e value is opposite to that obtained in experiments using reference chemicals [**4**] and is not consistent with the positive WM-GM f_e measured in brain tissue experiments [**1**] although it is possible that the GC and POPC f_e values may partly cancel each other.

Discussion and Conclusions: A method has been developed to allow precise reference-chemical-free measurements of f_e. The method separates out $\Delta\chi$ by exploiting the orientation dependence of χ-induced frequency shifts. An alternative method to obtain $\Delta\chi$ would involve fitting the residual GC–saline dipolar field pattern outside the cylinder at θ = 90°. That method was not used here because the χ of the tube glass was much larger than that of saline so slight misregistrations of the GC and saline acquisitions lead to large artifactual residual field patterns, making it difficult to extract $\Delta\chi_c$ with sufficient precision. We are investigating the precision of this alternative technique and it is likely that a tube material with a χ closer to that of saline could improve it. In future, this phantom could be filled with different chemicals and concentrations to facilitate investigation of their contributions to tissue f_e and χ-induced contrast.

References: **1.** Shmueli et al. *MRM* **2011** 65:35-43 **2.** Zhong et al. *Neuroimage* **2008** 40:4:1561-6 **3.** Luo et al. *J. Magn Reson* **2009** 202:102-8 **4.** Shmueli et al. *Proc ISMRM* **2011** 704 **5.** Leutritz et al. *Proc ISMRM* **2011** 4526 **6.** Shimizu et al. *J Biomol NMR* **1994** 4:859–862 **7.** Mizuno et al. *J Phys Chem B* **2003** 107:3972-8 **8.** Forti et al. *J Biomater Appl* **2006** 20:267-285 **9.** Barbara *J Mag Res A* **1994** 109:265-269 **10.** O'Brien & Sampson *J Lipid Res* **1965**, 6:537-544 **11.** Kucharczyk et al. *Radiology* **1994**, 192:521-9 **12.** Duyn et al. *PNAS* **2007** 104:11796-11801