Determination of the in vitro limit of detection for pulmonary surfactant using proton magnetic resonance spectroscopy at 1.5T

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Purpose
To establish the lowest concentration of a solution of dipalmitoyl phosphatidylcholine which can be detected in vitro by proton magnetic resonance spectroscopy at 1.5T.

Background
Pulmonary surfactant is a phospholipid and protein mixture which is normally excreted by the fetal lungs from around 23 weeks gestation and plays a vital role after birth in reducing surface tension at the alveolar tissue-air interface. Lecithin (consisting of dipalmitoyl and other disaturated phosphatidylcholines) is the major active component of pulmonary surfactant. In cases where there is poor development of fetal lungs (pulmonary hypoplasia), or where there is a good chance a baby will be delivered prematurely, the ability to assess how well the lungs are likely to function after birth is of value for counselling parents and also in the planning of perinatal care. Fetal magnetic resonance (MR) imaging can be used to investigate lung volumes and thoracic anatomy in many causes of pulmonary hypoplasia, however there is at present no established method for quantitative MR assessment of fetal lung fluid composition. This study examines the technical feasibility of measuring the concentration of lecithin by proton magnetic resonance spectroscopy (H-MRS) using a 1.5T clinical imaging system.

Methods and materials
A 12.8mM solution of lecithin (Product P5911: D-L-α-dipalmitoyl phosphatidylcholine, Sigma-Aldrich Company Ltd, Gillingham UK) was prepared using chloroform as a solvent (Product 100776B: Chloroform AnalaR, VWR International Ltd, Lutterworth UK). Initial attempts to dissolve the compound in both water and saline were unsuccessful, providing only a transient suspension which was unsuitable for magnetic resonance spectroscopy. Further serial dilutions of lecithin solution were prepared at 9.7mM, 5.5mM, 5mM and 2.9mM concentrations, along with a blank reference sample of chloroform without lecithin. Point resolved spectroscopy (PRESS) sequences were used with an 8-channel head coil on a 1.5T clinical imaging system (GE HDx Signa: GE Healthcare, Slough UK). A range of sequence parameters were used to acquire spectra for each of the samples as follows: TE 144ms; TR 1500ms; single cubic voxels of linear dimension 1cm, 1.25cm and 1.5cm; and either 128, 256 or 512 spectral acquisitions. Signal-to-noise ratio (SNR) was measured for the characteristic peak attributed to the choline group of the lecithin, and these measurements were used to estimate a lower limit of detection for lecithin in chloroform solution.

Results
The largest spectral peak seen in all samples was attributed to chloroform and was assigned a chemical shift of 7.25ppm (see figure 1). Several other much smaller peaks were also seen in the blank chloroform sample however, and these were attributed to the presence of 1% ethanol used to stabilise laboratory-grade chloroform solvent. Samples containing lecithin displayed a characteristic spectral peak corresponding to the choline moiety at 3.2ppm and also a larger peak at 1.3ppm. The latter peak was attributed to $(CH_2)_n$ groups within the saturated fatty acid side chains of lecithin. This peak was not used in subsequent analysis as this region of *in vivo* spectra is often difficult to interpret due to the presence of other broad non-specific lipid macromolecule peaks. The results of forty individual measurements of SNR for the choline peak of lecithin are shown in figure 2.

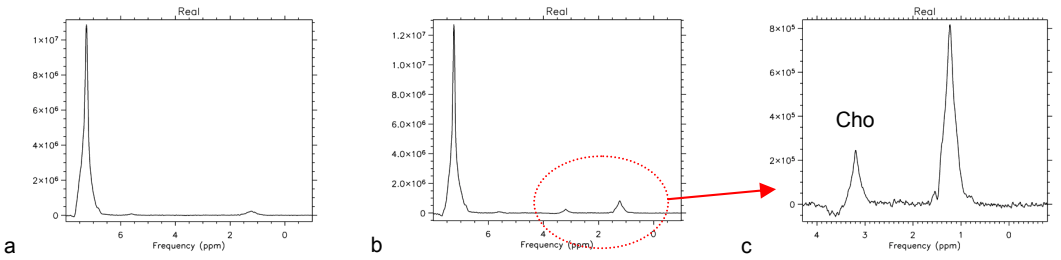

Figure 1: a) In vitro spectrum of chloroform solvent; b) In vitro spectrum of 12.8mM lecithin in chloroform; c) Same spectrum as b in more detail - the characteristic peak at 3.2ppm corresponds to choline (Cho). PRESS Sequence parameters used in all three spectra: TE144, TR1500, voxel size $(1.5cm)^3$, 256 spectral acquisitions.

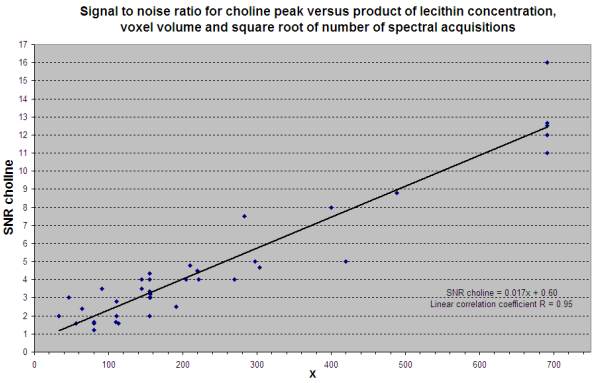

Figure 2: SNR for the choline peak of lecithin in chloroform solution versus the product (x) of lecithin concentration/mM (c), voxel volume/cm^3 (v) and the square root of the number of spectral acquisitions (n). A line of best fit is drawn, with a linear correlation coefficient (R) of 0.95 (SNR choline = 0.017x + 0.60).

Discussion
The effective lower limit of detection for the choline spectral peak is determined by the SNR achieved, i.e. when the SNR approaches 1 then the choline can no longer be detected. In practice, SNR values considerably higher than this would be required to form the basis of a robust clinical test. Nevertheless, extrapolating the experimental data back to a SNR of 1 suggests that when using typical clinical scanning parameters of a $3.375cm^3$ voxel and 256 spectral acquisitions (scan time 7:00 minutes) the minimum detectable concentration would be approximately 0.5mM. The mean physiological concentration of lecithin in amniotic fluid which is thought to correspond to mature fetal lung tissue is around 0.05mM, a factor of 10 below the limit of detection determined in this study. In order to achieve the necessary 10-fold increase in sensitivity the voxel dimensions would either have to be increased at least to those of a 3.0cm cube (for 256 spectral acquisitions), or the number of spectral acquisitions increased to more than 16384 for a 1.5cm voxel (scan time around eight hours). Such parameters are not considered to be clinically practicable in fetal imaging. There may be some increase in signal to noise ratio achievable by using alternative coil geometry or positioning, particularly situating the coil very close to the region of interest, however this would not necessarily always be an option in vivo, depending on fetal lie and the thickness of the maternal abdominal wall. Scanning at higher field strength is not currently advised in pregnancy.

Conclusion
The lower limit of detection of lecithin in vitro is estimated to be around 0.5mM using typical clinical sequence parameters at 1.5T. This is well above the physiological concentrations found in amniotic fluid samples. Non-invasive measurement of amniotic fluid lecithin concentration by 1.5T proton magnetic resonance spectroscopy using current clinical imaging parameters is therefore not considered to be practicable.