INTRODUCTION
Diseases of articular cartilage (AC), such as osteoarthritis (OA), impact up to a quarter of the Australian population. The avascular nature of cartilage and its limited ability to self-repair makes early diagnosis and treatment of OA an important factor in reducing the healthcare burden, particularly for an aging population. Our work aims to develop a robust MRI methodology for evaluation of cartilage ECM within an individual joint for early diagnosis of OA. As an extension to our work on water diffusion and relaxation times in isolated cartilage to interrogate the molecular hydrodynamics of water in AC [1], we aimed to investigate the metabolite distribution within the cartilage matrix and the influence of water on metabolite signals, using solid state NMR.

BACKGROUND
Articular cartilage, an avascular connective tissue lining articulating surfaces of the long bones, is comprised predominantly of extracellular biopolymers. Healthy adult human cartilage is 2 – 4 mm thick and contains a sparse population of chondrocytes within an extracellular matrix of collagen (15 - 20%), proteoglycans (3 – 10%) lipids (1 – 5%) and water (65 – 80%). Understanding of the characteristics responsible for the load bearing efficiency of AC and the factors leading to its degradation are incomplete.

Whilst DTI shows the structural alignment of collagen in AC and T2 relaxation measurements [1] suggest that the dependence of alignment of collagen in AC [2], the need for AC structural integrity makes solid state NMR an ideal tool to study the metabolic profile and chemical interactions involved in functional AC. We examined the contribution of water in different functional 'compartments' using 1H-MAS, 13C-MAS and 13C-CPMAS NMR of bovine patellar and femoral cartilage incubated in D2O to remove free water. Previous reports have described the metabolic profile in AC using MAS NMR [3-6] but the influence of freezing and thawing on the metabolite distribution has not been reported. Spectra recorded from fresh cartilage were compared with those from frozen tissue.

Evidence for water compartmentation 1H MAS NMR spectra of cartilage were dominated by a large water signal, with asymmetric spinning side bands. The asymmetry of the spinning sidebands is consistent with the presence of anisotropic water environments within cartilage. The inset peaks show the second spinning side band on each side of the water peak.

Water replacement in cartilage Cartilage samples were incubated for up to 3 hours in D2O prior to measurement of 1H (A) and 13C MAS NMR spectra. 'Dehydration' by exchange of D2O for water resulted in a reduction of signal intensity in 1H (A) and 13C-CP-MAS (B) measurements but not single pulse 13C measurements (C), indicating that water protons are participating in the polarization transfer.

Storage effects Many studies of cartilage report that tissue was frozen and 'stored until required' after collection of samples from an abattoir. When cartilage was soaked in D2O and the 13C NMR spectrum of the supernatant recorded, there was a difference in metabolites released into the incubation solution, depending on whether or not the tissue had been frozen. The figure below shows the differences in the profile of metabolites released from fresh (A), compared to cartilage frozen for short-term (1 week - B) and long-term (6 months - C) storage at -20°C. The duration of freezing did not alter the metabolites released into D2O, but the act of freezing did. In structural and functional studies, the differences in metabolite release following freezing may alter cartilage performance or account for differences between studies.

Conclusions MAS-NMR measurements of 1H and D2O exchange provide evidence for water compartmentation within cartilage. Replacement of mobile tissue water with D2O results in residual signal from hydrogen in bound environments which could include non-exchanging water. MAS-13C NMR measurements of patella and femur cartilage show different metabolic profiles. This may reflect the functional performance of the cartilage but also provides a window into the changes that may be produced by load bearing. Freezing of cartilage for even short periods of time at -20°C results in changes to the diffusional egress of metabolites from the matrix into surrounding solution. This may not be important for structural studies of cartilage but the potential loss of metabolites due to frozen storage should be considered when cartilage function is examined.

References