Introduction
Injection of hypertonic sodium chloride (NaCl) solutions into the masseter muscle is employed as an experimental model of acute muscle pain (1). One mechanism which is thought to contribute to the pain caused by these intramuscular injections is osmotically induced changes to the muscle tissue itself. Measurements of transverse magnetization relaxation decays offer a method to study muscle tissue (2,3) and could offer a means to study the biophysical effect of hypertonic solutions on muscle tissue in vivo. We have previously found that injection of NaCl solutions into muscle resulted in biexponential transverse (T2) relaxation decays, with a fast and a slow component which were attributed to intracellular and extracellular water protons, respectively. We have exploited the biexponential behavior of the T2 relaxation decays to determine whether a relationship exists between the osmolarity of intramuscularly injected solutions and the calculated extracellular water component of in vivo muscle.

Methods
Adult male Sprague Dawley rats (n=10) were anesthetized with isoflurane. Sodium chloride solutions (0.1 ml at 0.45, 0.9, 3 and 6%, n=5 for each osmotic strength) were injected bilaterally into the masseter muscle. Magnetic resonance imaging was performed on a Bruker Biospec (Bruker Instruments, Inc., Billerica, MA) operating at 2 T. A volume coil was used as a transmitter and a homemade surface coil (3x4cm) acted as a receiver. Coronal multislice T2 weighted images (repetition time TR=2s, echo time TE=40ms) were acquired after injection of NaCl solution to allow identification of the injection site. A slice selective CPMG imaging sequence (TR=2s, 32 echoes, 10 ms echo spacing) was acquired on two slices within the region of edema. The non negative least square technique was used to confirm the biexponential behavior of the relaxation decay curves. Fits of the signal decay with echo time to a biexponential function were performed using a Levenberg Marquardt non linear least squares algorithm. The values of the normalized amplitudes of the fast and slowly relaxing components for different concentrations of NaCl were compared by Analysis of Variance and post-hoc Dunnett’s method, as appropriate.

Results
Monoexponential decays with T2 times approximately equal to 35 ms were observed within in vivo muscle before injection of NaCl solutions. After injection, biexponential decays were observed. The T2 relaxation times and normalized amplitudes of the two components for all ROIs (4 x 4 pixels) within the injection site were calculated with the Levenberg Marquardt algorithm. The fast component (T2: 20-40 ms, percentile fraction: approximately 50%) and a slow component (T2: 150-400 ms) were assigned to intracellular and extracellular water protons, respectively. To determine whether a relationship existed between the osmolarity of intramuscularly injected solutions and the calculated extracellular water component of in vivo muscle, we chose the ROI within the injection site with the maximal normalized amplitude for the slowly relaxing component, i.e. with the maximal extracellular water content. The bar graph in Figure 1 shows the results of this analysis. The maximal extracellular water content with 0.45% NaCl solutions was slightly smaller than with 0.9% NaCl solutions. However, there was a statistically significant increase of the maximal extracellular water content, relative to 0.9% NaCl solutions, when 3 or 6% NaCl solutions were injected (p<0.05, ANOVA and Dunnett’s method). One week after injection of these solutions there was no longer any visible evidence of edema on T2 weighted images.

Discussion
In this study, in vivo measurements of T2 relaxation by magnetic resonance imaging were performed to investigate the distribution of water in rat masseter muscle tissue after intramuscular injection of NaCl solutions of varying toxicity. Analysis of relaxation decay curves within injection sites revealed that there were two components for the image derived T2 decay curves. We examined the effects of NaCl solutions of varying osmolarity on the relaxation behaviour of muscle tissue, specifically on the amplitudes of the two components. The ability to distinguish the intra- and extracellular volume components with good spatial resolution in vivo allowed us to study the effects of solutions of varying osmolarities on these two volumes. The calculated extracellular water content, after injection of isotonic solutions was 50%. The extracellular water content increased to 60% and 70%, respectively, after injection of hypertonic solutions of 3% and 6%, and decreased to approximately 45% after injection of hypotonic solutions. The results obtained appear to be consistent with in vitro studies which have shown that myocyte volume is decreased in strongly hypertonic solutions to a comparable extent (4). The apparent decrease in extracellular volume after injection of 0.45% NaCl solutions into the masseter muscle was considerably less than expected from in vitro results. The difference probably reflect the greater mechanical constraints on the expansion of myocyte volume in in vivo muscle than in in vitro muscle fiber preparations. Nevertheless, it seems reasonable to conclude that injection of NaCl solutions of varying osmotic strength modify masseter myocyte volume in vivo to an extent great enough to be resolvable with CPMG imaging sequences. Further, given that the extracellular volume increases proportionally to the osmotic strength, it may be feasible to use this technique as an indirect in vivo measure of changes in the concentration of intramuscularly injected solutions over time.

References