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Research Summary Overview

Investigation of the Impact of Body Temperature and Post-Mortem Interval on Magnetic Resonance Imaging (MRI) of Unfixed Tissue

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PURPOSE AND DESIGN OF PROJECT

Due to the excellent sensitivity of Magnetic Resonance (MR) imaging to subtle differences in soft tissues, MR enables non-invasive anatomical imaging with superior soft tissue contrast relative to x-ray computed tomography (CT). Soft tissue contrast in MR is not determined primarily by tissue density (as in x-ray imaging) but instead by T1 and T2, tissue-specific parameters that characterize the timing of the MR signal. Further, user-specified parameters TR and TE control the timing of the MR acquisition protocol. Together, T1, T2, TR, and TE determine the MR image contrast – i.e., whether two different tissues are distinguishable. In the medico-legal death investigation setting, normal temperature-dependent and post-mortem interval (PMI)-dependent changes in MR image contrast, not encountered in clinical MR of live subjects, have the potential to confound the identification of pathology or injury. Our over-arching hypothesis is that post-mortem Magnetic Resonance (PMMR) will eventually find broader application, to forensic imaging and other disciplines, if the dependence of image contrast on temperature and normal PM changes is better-understood.

In this study, our objective was to study temperature-dependent and PM changes in the MR imaging characteristics of normal mammalian tissue in a thorough and systematic way, with the long-term goal of optimizing PMMR protocols for future use in forensic investigation. To

achieve this objective, this basic research study was designed to be carried out in three phases:

Phase 1) Measure the tissue-specific MR parameters T1, T2, and the apparent diffusion coefficient (ADC) as a function of temperature for a variety of ex vivo mammalian tissues;

Phase 2) Utilize this knowledge: a) to identify tissue parameters for use as non-invasive

thermometers, essential for optimizing imaging with respect to sample temperature, and b) to develop procedures for improving PMMR imaging protocols for use in future studies; **Phase 3)** Measure the tissue-specific MR parameters T1, T2, and the apparent diffusion coefficient (ADC) as a function of PMI for 3 different decomposition temperatures using intact animal subjects.

METHODS AND SUMMARY OF RESULTS

Methods and Data Analysis: Ex vivo mammalian tissues were placed in 4 oz. snap-seal containers or into 6-well plates with covers. Whole animal heads were wrapped in plastic. Sample temperature was controlled using an incubator (Shel Lab Model LI27, Sheldon Manufacturing), freezer packs, and/or isothermal heating pads (Deltaphase Isothermal Pads, Braintree Scientific) and measured using a hand-held digital thermometer (HH147 with type T thermocouple, Omega Engineering) or an MR-compatible fiber optic thermometry system (FOB104 with FOBS-30 cables, Omega Engineering) to log the temperature of samples in the MR scanner. Small ex vivo samples were further enclosed in an insulating box, and large samples (whole heads) were wrapped in bubble wrap to stabilize the temperature during scanning. Samples were imaged in a 1.5 Tesla Siemens Sonata MR scanner. To determine T1 and T2 (single-echo), the samples were imaged using a standard spin echo acquisition sequence by varying TR (TR = 200, 400, 800, 1600, 3200 ms; TE=11 ms), or by varying TE (TR=3200 ms, TE=7, 11, 25, 40, 80, 120 ms), over the temperature range 1-39°C. A multi-echo T2 sequence was also performed (TR=3200 ms, with 13 evenly spaced TE values, 6.7 - 128 ms). From the image data, regions-of-interest (ROIs) were selected corresponding to each tissue. Using the Mean Curve application on the scanner, the mean signal intensity, standard deviation, and area of each ROI were determined and saved in ASCII format. ASCII data was then imported into Matlab to determine T1, T2 single-echo, and T2 multi-echo as follows: for each tissue, mean signal was plotted vs. TR (or TE) and fit with the appropriate function to extract the relaxation time constant T1 (or T2). **(Equations, statistical parameters, and a list**

of other experimental parameters archived for each measurement are provided in Appendix I.)

ADC measurements were performed using a standard Siemens ADC sequence, with the number of b-values increased from 3 to 6 ($b = 0, 500, 1000, 1500, 2000, 2500 \text{ s/mm}^2$), in an attempt to improve sensitivity to low diffusion rates encountered in PM samples. The ADC map (an image of the resulting ADC values) was generated automatically on the scanner. From the ADC maps, the ADC of brain tissue was determined by manually selecting large ROIs containing both gray and white matter (but not ventricles, air, or other tissue types) using the Mean Curve utility on the scanner, or using ROI tools in a PACS viewer (iSite Enterprise, Philips; or K-PACS v1.6.0, IMAGE Information Systems). For each ADC determination, 3 or 4 ROIs were selected (e.g., dorsal, ventral, right, and left ROIs from an axial slice through the mid-brain), and the average ADC within each ROI was obtained. The reported ADC value (and uncertainty) at a given temperature or time-point was computed as the mean (and standard deviation) of the 3 or 4 average ADC values.

Additional experimental details, relevant to specific studies, are included below with the summary of the results of each study.

Phase 1 focused on the measurement of the MR relaxation parameters T1 and T2, and the apparent diffusion coefficient (ADC), as a function of temperature for a variety of fresh (<2 day PMI) mammalian tissues. The study was expanded to include measurements of formalin-fixed and frozen-thawed ex vivo mammalian tissues, and measurements of brain relaxation parameters in living human subjects, for comparison to PM animal brain tissue.

1.A Fresh Tissue: Data from fresh tissue (<2 day PMI, never frozen) was obtained representing 26 different mammalian tissue types from 21 animal subjects and 5 species (cow, dog, pig, and sheep). Study results, approximately 1525 measurements of each relaxation time (T1, T2 single-echo, T2 multi-echo), as well sample characteristics and statistical parameters, are archived in a Microsoft Access database. ***Brief summary of results: The data***

demonstrate how the relaxation parameters vary with temperature (over the 0-40 °C range) for each tissue type. Overall, T1 tends to increase linearly with increasing temperature for most tissue types, with the exception of tissues with high lipid contents (fat, liver, and bone marrow), which show nearly constant T1 over the range of temperatures studied. Overall, T2 values (single-echo and multi-echo) tend to be fairly constant as a function of temperature for nearly all tissue types, with the exception of fat and bone marrow which show a significant decrease in T2 as the temperature is reduced. (Data provided in Appendix A)

1.B Thermal-history of Fat and Muscle: A more detailed study of the dependence of fat and muscle relaxation times (T1 and T2) for porcine tissue (N=3) was performed as a function of temperature comparing the temperature-dependence of T1 and T2 under 4 different thermal cycles: (1) slow cooling (on day 1, starting 2 hours PM); (2) gradual warming (on day 2, following gradual cooling on day 1 and overnight refrigeration); (3) gradual warming (on day 2, after immediate refrigeration for 24 hours); and (4) gradual warming (on day 2) after 12 hours at room temperature followed by 12 hours of refrigeration. On day 1, fresh, still warm PM tissue was obtained at a local slaughterhouse in the morning. The tissue was immediately divided and packaged in insulated containers for transport to the scanner (within 15 minutes PM). The tissue used for thermal cycles 1, 2, and 3 was maintained at “body” temperature during transport using warming pads; the tissue used for thermal cycle 4 was immediately placed on ice packs. **Brief summary of results:** *All muscle and fat relaxation parameters showed some degree of hysteresis (i.e., the value of T1 or T2 at a given temperature depended to some extent on the thermal history of the sample.) Most significantly, fat that was cooled below 15 °C showed a reduced value of T2 upon warming, relative to fat that was never cooled below 15 °C. (Data provided in Appendix B)*

1.C Tissue Preservation: We expanded the tissue relaxation study to include both formalin-fixed and frozen-thawed tissue for 9 tissue types (aorta, fat, kidney cortex, kidney medulla, liver, lung, cardiac muscle, skeletal muscle, and spleen) from 2 species (cow, pig), for

comparison to fresh (never frozen) tissues. Samples (2-5 g each) were either fixed by immersion in 10% neutral buffered formalin or frozen in a -20°C freezer for at least two weeks; then transferred to a refrigerator the night before measurements. The database contains >600 measurements of T1 and T2 single-echo, and > 400 measurements of T2 multi-echo, obtained from fixed and frozen-thawed tissue samples. **Brief summary of results:** *For most tissues (non-lipid-containing), tissue preservation (freeze-thaw or formalin-fixation) resulted in a decrease in T1. T1 values tended to be slightly shorter for frozen-thawed tissue compared to T1 values for fresh tissue at the same temperature. Formalin-fixation resulted in an even greater reduction in T1 and a weaker temperature dependence. The exceptions to this pattern were the lipid-rich tissues (liver and fat); on average, significant changes in T1 due to preservation were not observed for liver and fat. Freezing-thawing and formalin-fixation had minimal effects on the T2 values for all tissue types studied. (Data provided in Appendix C)*

1.D In Vivo Brain: A small in vivo human brain MR study (5 subjects) was performed to obtain values of T1, T2 single-echo and T2 multi-echo of cortical gray matter, white matter, and deep gray matter structures under normal in vivo conditions, for comparison to PM data. The purpose of this small study was to obtain measurements from live subjects using the same measurement protocols employed for PM specimens, to ensure that any observed differences between PM and in vivo tissue parameters were not due to differences in measurement protocol. **Brief summary of results:** *The in vivo human brain measurements of T1, T2 single-echo, T2 multi-echo, and relative proton density (white/gray), were in good agreement with the PM animal brain results obtained at comparable temperatures (35-37 °C) using the same protocol. Differences between our PM results and published in vivo data (obtained by others), which we previously noted, appear to be due to differences in the measurement technique. Therefore, we conclude that temperature-dependent effects (convergence of relaxation times and decrease of the relative spin density of white matter, as temperature decreases) are the*

primary cause of poor contrast in fresh PM brain MRI - other PM factors, such as hypoxia, do not seem to have a significant effect. (Data provided in Appendix D)

1.E Brain ADC: We limited the ADC study to the temperature-dependence and PMI-dependence of the ADC of brain tissue. Successful ADC measurements were performed for brain (measured in situ, using whole heads). We experimented with obtaining ADC values from other tissues using small, intact animals, but generally, the ADC data obtained from PM tissues, except for brain, are too low to obtain reliable results. Diffusion is a thermally-activated process, i.e., diffusion occurs more rapidly at higher temperatures. Therefore the apparent diffusion coefficient (ADC) is expected to depend on temperature according to the Arrhenius equation: $ADC \propto \exp(-E_a/kT)$, where E_a is the activation energy, k is Boltzmann's constant, and T is the absolute temperature (Kelvins). According to this equation, if the ADC value (vertical logarithmic axis) is plotted versus $1/T$ (horizontal linear axis), the result should be a straight line with a negative slope. ADC values were also plotted versus PMI (linear-linear) to assess the PMI dependence. ***Brief summary of results:*** *ADC measured as function of temperature from 4 pig brains and 3 sheep brains all fall on a single straight line on a semi-log plot independent of PMI or thermal cycling; whereas no consistent pattern was observed by plotting ADC vs. PMI. These data demonstrate that our previous observation of ADC increasing with increasing PMI was actually the result sample warming, in samples refrigerated prior to incubation and ADC measurement at room temperature). After controlling for temperature, no PMI-dependence of the ADC of brain tissue was observed. (Data provided in Appendix E)*

Phase 2 focused on using the knowledge gained through measurements of T1, T2, and ADC to: A) identify tissue parameters for use as non-invasive thermometers, essential for optimizing imaging with respect to sample temperature, and B) develop procedures for improving PMMR imaging protocols.

2.A Non-invasive MR Thermometry: Based on the review of data obtained during Phase 1 (Appendices A and E), the parameters with the strongest and most reproducible

temperature-dependence were found to be the ADC of brain tissue and the T1 values for cardiac muscle, skeletal muscle, and lung parenchyma. Based on these results, we predict that these parameters will be the most reliable parameters to measure for purposes of non-invasive thermometry. Not only was a strong dependence on temperature observed, but nearly the same dependence was observed independent of the sample temperature history (e.g., whether the sample was being cooled or re-warmed). Therefore, if a measurement of one of these parameters is performed, the temperature can be reliably estimated (with an uncertainty of approximately +/- 5 deg C). Measuring the value of two or more of these parameters would result in a temperature estimate with higher reliability. This project was focused on the MR characteristics of solid tissues and organs; however, we occasionally measured relaxation parameters from bodily fluids observed strong temperature dependences for the T1 of blood, bile, and vitreous fluid (in the orbits), based on a small number of measurements. We recommend future study (using a larger sample size) of these fluids, to establish their suitability as non-invasive thermometers.

2.B MR Protocol Optimization: We developed computer codes (in Matlab) to enable the optimization of MR acquisition parameters and the computation of expected tissue contrast, based on the T1 and T2 data obtained in Phase 1 and the equations presented in Appendix I. Three Matlab programs were developed: Program 1) Computes the acquisition parameter value (TE or TR) that maximizes image contrast between two tissues, based on the T1 and T2 values for each tissue at some temperature of interest; Program 2) Computes the contrast between two tissues for the current values of the acquisition parameters TE and TR, based on the T1 and T2 values for each tissue at the current temperature; then computes a new TR and new TE value that result in equivalent contrast at some new temperature (based on T1 and T2 for each tissue at the new temperature); Program 3) Computes tissue signal levels (and contrast between pairs) for more than 2 tissue types for a particular choice of TE and TR, given the T1 and T2 values for each tissue at the temperature of interest. **(Matlab codes provided in Appendix F)**

Phase 3 focused on studying the impact of PMI on MR tissue parameters, and evaluating whether PMMR measurements might enable non-invasive PMI determinations in the future.

PMI Study: To investigate the impact of PMI on MR relaxation parameters, we incubated whole ~500 g male Sprague-Dawley rats at either 4 °C (refrigerator temperature, N=3), 18.5 °C (ambient temperature, N=3), or 35 °C (normal body temperature, N=3), performing measurements over 200-800 hours (until the specimen was too decomposed to identify the organs). More than 1160 measurements of each relaxation parameter (T1, T2 single-echo, and T2 double-echo) were performed to characterize 10 tissue types (brain, heart, kidney cortex, kidney medulla, liver, lung, skeletal muscle, spleen, subcutaneous fat, and visceral fat) at the same temperature as the incubation temperature for a given specimen; thus, temperature was constant, except for a brief period of initial cooling to 4 °C or 18.5 °C. **Brief summary of results:** *As noted in section 1.E. above, our analysis of the temperature-dependence and PMI-dependence of the ADC of brain tissue demonstrated that ADC varies with temperature, but not PMI. (See Appendix E) In the constant temperature experiments conducted on intact rats, we found that relaxation times for most tissues did not vary significantly over the first 24 hours PM. The exceptions were the T1 values for heart, kidney medulla, liver, and lung, and the T2 single-echo value for liver incubated at 35 °C. Considering longer PMI values (200-800 hours), several tissues (brain, kidney cortex, kidney medulla, and lung) showed a significant variation in T2 (single-echo and multi-echo) with PMI; the strongest PMI-dependence is observed for the highest incubation temperature (35 °C) due to faster decomposition, while the PMI-dependence is very weak for the refrigerated specimens. The T1 values of heart, kidney cortex, kidney medulla, and lung showed a marked decrease over 200 hours but leveled off to a constant value at longer PMI, for all temperatures considered. The T1 of brain showed a similar decrease over 200 hours at 35 °C, but was stable at 18.5 °C and 4 °C.*

The liver T1 undergoes large oscillations over time (decreases, increases, and decreases again) with the oscillations occurring more rapidly for higher incubation temperatures. Notably, almost no variation of T1 or T2 on PMI was observed for skeletal muscle. Overall, the T2 (single-echo and multi-echo) of brain tissue showed the strongest and most regular dependence on PMI and appears to be the best candidate for use in estimating PMI non-invasively, provided the decomposition temperature is known. (Complete data provided in Appendix G)

PRODUCTS

- Invited review article: Adolphi NL. An Equation-free Introduction to Post-mortem MR Image Contrast and Pulse Sequence Optimization. *J. Forensic Radiol. Imaging* 2016;4:27–34. (DOI: <http://dx.doi.org/10.1016/j.jofri.2015.12.007>) (**Appendix H**)
- A searchable data base containing more than 10,000 individual measurements (T1, T2 single-echo, T2 multi-echo, and ADC values and statistical parameters) for a variety of tissue types (white matter, kidney cortex, liver, etc) and tissue conditions (fresh, fixed, frozen-thawed), obtained over a range of temperatures and PMI values (**data available upon request**)
- Three Matlab programs enabling computation of MR image contrast and optimization of MR protocols based on the values in the data base (**Appendix F**)
- Five manuscripts in progress: 1) survey of T1 & T2 vs. temperature for non-brain tissues; 2) explanation of anomalies in PM fat/muscle contrast; 3) comparison of fresh, frozen-thawed, and fixed tissue with regard to MR relaxation times, tissue weight, and CT density; 4) dependence of brain tissue T1, T2, and ADC on temperature and PMI (including comparison to live, human imaging results); 5) survey of T1 and T2 vs. PMI – at 3 fixed decomposition temperatures – based on data from intact PM animal subjects

IMPLICATIONS FOR FORENSIC RESEARCH AND PRACTICE

The successful completion of this basic research project will support the continuing development of advanced imaging methods for medicolegal death investigation. PMMR can

provide soft tissue information, non-invasively, that is not available by x-ray or CT. The full realization of PMMR in forensic investigation may lead to more reliable diagnoses of soft tissue injuries or pathology. The current findings (temperature- and PMI-dependence of fundamental parameters) are the basis for new studies aimed at: 1) validating our image optimization procedures in human decedents, 2) applying PMMR to forensic cases, and 3) comparing PMMR findings with those of PMCT and standard autopsy. We anticipate that our systematic study of how common tissue preservation methods affect the physical properties and imaging characteristics of post-mortem tissue will be valuable to both researchers and practitioners. Refrigeration, freezing, and formalin-fixation are methods commonly used in research and clinical medicine, and these methods and sample conditions are also commonly encountered in the autopsy setting. Further, our study of the effect of post-mortem changes on MR image parameters may someday lead to new methods for estimating body temperature and/or PMI. A long term goal of these studies is to develop a method for estimating PMI, over the range of days to weeks, by a *non-invasive, quantitative* PMMR assessment of specific post-mortem soft tissue changes, such as changes in the T2 of brain tissue.

At the New Mexico Office of the Medical Investigator, we are now including an optimized T1-weighted brain imaging protocol, developed under this award, in examinations of human decedents receiving PMMR. In addition to using improved acquisition protocols in our practice, we expect that the results of these studies, as well as our participation in the Data Acquisition Working Group of the International Society for Forensic Radiology and Imaging (ISFRI), will impact the transfer of MR imaging technology to the forensic community in the future. At this time, the results of these studies have been disseminated in presentations at international meetings, published abstracts, and an invited review article, which provides practical guidance for optimizing MR protocols. However, it is still too soon to gauge the impact that this study - and further research that builds on these results - will ultimately have on the practice of medico-legal death investigation.