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Diffusion Tensor Spectroscopic Imaging of the Human Brain in Children and Adults

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Abstract

Purpose—We developed diffusion tensor spectroscopic imaging (DTSI), based on proton-echo-planar-spectroscopic-imaging (PEPSI), and evaluated the feasibility of mapping brain metabolite diffusion in adults and children.

Methods—PRESS prelocalized DTSI at 3 Tesla was performed using navigator-based correction of movement-related phase errors and cardiac gating with compensation for TR-related variability in T₁-saturation. Mean diffusivity (MD) and fractional anisotropy (FA) of total N-acetyl-aspartate (tNAA), total creatine (tCr), and total choline (tCho) were measured in 8 adults (17–60 years) and 10 children (3–24 months) using $b_{\max}=1734 \text{ s/mm}^2$, 1 cc and 4.5 cc voxel sizes, with nominal scan times of 17 min and 8:24 min. Residual movement related phase encoding ghosting (PEG) was used as a regressor across scans to correct overestimation of MD.

Results—After correction for PEG, metabolite slice-averaged MD estimated at 20% PEG were lower ($p<0.042$) for adults ($0.17/0.20/0.18\times 10^{-3} \text{ mm}^2/\text{s}$) than for children ($0.26/0.27/0.24\times 10^{-3} \text{ mm}^2/\text{s}$). Extrapolated to 0% PEG, the MD estimates decreased further ($0.09/0.11/0.11\times 10^{-3} \text{ mm}^2/\text{s}$ versus $0.15/0.16/0.15\times 10^{-3} \text{ mm}^2/\text{s}$). Slice-averaged FA of tNAA ($p=0.049$), tCr ($p=0.067$) and tCho ($p=0.003$) were higher in children.

Conclusion—This high-speed DTSI approach with PEG regression allows estimation of metabolite MD and FA with improved tolerance to movement. Our preliminary data suggesting age-related changes support DTSI as a sensitive technique for investigating intra-cellular markers of biological processes.

Keywords

Magnetic resonance spectroscopy; magnetic resonance spectroscopic imaging; diffusion tensor; human brain; age dependence

Introduction

Measurement of brain metabolite diffusion has the potential to provide valuable information about intracellular changes associated with normal aging, as well as diverse pathological processes, including ischemia, multiple sclerosis, brain tumors, and developmental disorders, such as autism spectrum disorder¹. The apparent diffusion coefficient (ADC) of metabolites reflects intracellular biophysical properties, including viscosity, cell swelling, restriction in subcellular structures, and cytoplasmic streaming². Metabolite fractional anisotropy (FA) provides additional directional information for characterizing the intracellular environment³. The specificity of metabolite diffusion to the intracellular compartment provides unique information that cannot be obtained by diffusion tensor imaging (DTI), which measures water diffusion in both intra- and extracellular compartments^{1,4,5}.

Our initial demonstration of metabolite diffusion magnetic resonance spectroscopy (MRS) in the human brain⁶ has been extended by others to measure the mean diffusivity (MD) of metabolites (=trace/3 of the ADC tensor), and display metabolite ADC contrast between white matter and gray matter in adult healthy subjects^{3,7}. In these reports, N-acetyl-aspartate (NAA) ADC values were found to be significantly higher in white matter as compared to gray matter, which may reflect that NAA, mainly found in neurons, follows a more restricted path across axons than in gray matter regions^{2,3,7–10}. Significantly higher ADC for creatine (Cr) were also found in white matter as compared with gray matter; however, the biophysical mechanism underlying this finding has not been established¹¹. Ellegood et al.¹² showed a lower NAA FA in gray matter as compared to white matter at a very high b-value ($b=5018$ s/mm²), consistent with more isotropic diffusion in neuronal cell bodies.

To date, most human studies investigating metabolite diffusion have used single voxel localization methods due to the high motion sensitivity of conventional MR spectroscopic imaging (MRSI) techniques^{3,4,6–8}. More recently, diffusion sensitive MRSI methods have been developed. We introduced a navigator-based phase correction method for diffusion sensitive spectroscopic imaging to minimize phase-encoded artifacts¹³. However, residual sensitivity to rotational movement prevented the quantification of the metabolite ADC¹³. Other investigators introduced accelerated acquisition techniques to minimize motion artifacts. For example, Bito et al. demonstrated diffusion-weighted MRSI in rat brains using a combination of line-scan acquisition, spatial-spectral encoding with an oscillating sinusoidal echo-planar gradient, and phase correction based on the residual water signal¹⁴. A recent study by Ercan et al. used a dual echo encoded diffusion sensitive MRSI method with reacquisition of the phase encoding (PE) steps affected by rotational head movement to measure a gray/white matter gradient of metabolite diffusion in a central brain region in human subjects¹⁰. Reacquisition was performed based on the amplitude of the navigator

signal. This approach empirically established a prescan-based amplitude threshold (85%), below which a k-space point was reacquired, increasing the acquisition time up to 81%. Although a significant improvement in data quality was demonstrated, residual phase encoding ghosting (PEG) was not assessed.

In the current study, we developed and applied a novel 2D method for mapping the full diffusion tensor of major singlet metabolite resonances. This diffusion tensor spectroscopic imaging (DTSI) technique was implemented using cardiac-gated proton-echo-planar-spectroscopic-imaging (PEPSI)^{15,16} with PRESS prelocalization¹⁷ of a central supraventricular brain region. Online correction of movement-related phase errors was performed with spatially localized echo-planar navigator signal acquisition and compensation for T₁-saturation variability from cardiac gating. We further employed a novel analysis approach that uses the level of residual PEG in the movement-corrected data as a regressor across subjects in order to estimate residual movement-related artifacts and obtain more robust measurements of metabolite MD and FA. As a proof of concept, we applied this approach to the investigation of age-dependent properties of the diffusion tensors of tNAA (N-acetyl-aspartate + N-acetyl-aspartyl glutamic acid), tCr (creatine + phosphocreatine), and tCho (glycerophosphocholine + phosphocholine) in healthy adults and children. Preliminary accounts of this work have been previously presented as abstracts^{18,19}.

Methods

Sites/ Equipment

The study was conducted at two sites: the University of New Mexico (UNM) in Albuquerque and the University of Washington (UW)/Seattle Children's Hospital in Seattle using Siemens 3 Tesla TIM Trio scanners (Siemens Medical Solutions, Inc.) equipped with 32 channel (UNM) and 12 channel (UW) head coils.

Phantom

A validation study was performed at UNM on a spherical spectroscopy phantom containing physiological concentrations of tNAA, tCr and tCho.

Subjects

Healthy adult controls (3 females/1 male), aged 24–54 years, were scanned at UNM; two were scanned twice to test for reproducibility of the acquisition. At the UW, five children at 3 months-of-age (2 males/3 females), three children at 6 months-of-age (1 male/2 females), two children at 24 months-of-age (1 male/1 female) and four adults (4 males) aged 17–60 years were scanned. Three of these children were additionally scanned longitudinally at 6 months-of-age. One of the longitudinal scans was repeated to investigate the reproducibility of the acquisition. The children were enrolled in an infant brain imaging study (IBIS) evaluating infants at increased familial risk for autism due to an older affected sibling (N=7) or at low familial risk for autism (N=3), with no family history of autism²⁰. The children had no medical problems, although there was the potential that some might develop autism when older. Institutionally approved informed consent was obtained from adult participants and parents of children.

Pulse sequence design

The PEPSI-based DTSI pulse sequence (Fig.1) employed point resolved spectroscopy (PRESS) volume pre-localization, which was augmented using eight outer volume suppression slices positioned around the PRESS box, WET water suppression²¹, an echo planar readout along the X-axis, and conventional PE along the Y-axis. Multiple slice-selective navigators consisting of echo-planar readout gradients in the Z-direction were acquired in order to monitor phase and frequency drifts, T_1 -saturation related changes in signal amplitude, and movement-related changes in signal phase and amplitude. For the water reference acquisition, the first navigator signal was measured during the first water suppression module immediately after the water excitation radiofrequency (RF) pulse and before the application of the PE gradient. The second navigator was measured during the second water suppression module. The third navigator was measured immediately after the slice excitation of the PRESS module. The fourth navigator was applied after the diffusion gradients and immediately before the PE gradients and the echo-planar spatial-spectral encoding module. The spatial resolution of the navigator readout was twice the in-plane resolution along the readout direction.

Motion correction

Translational movements, which introduce a global and spatially uniform phase change to the signal, were corrected using navigator-based phase correction of each k-space point¹³. Nonlinear movement, primarily due to cardiac-related brain pulsation, introduces spatially varying phase and amplitude changes. Effects of nonlinear movement were minimized through the use of cardiac or peripheral gating⁶. To minimize the sensitivity to changes in heart rate, a correction of fluctuations in longitudinal relaxation time (T_1) related signal saturation was developed. The TR dependence of the metabolite signal amplitude for the PRESS prelocalized double spin-echo acquisition was computed using equation 14.64 from Bernstein et al.²²:

$$S = M_0 \left(1 - 2e^{-\frac{TR - \left(\frac{TE_1 + TE_2}{2} \right)}{T_1}} \right) + 2e^{-\frac{TR - \frac{TE_1}{2}}{T_1}} - e^{-\frac{TR}{T_1}} e^{-\frac{TE_2}{T_2}} \quad [1]$$

where M_0 is the magnetization. TE_1 and TE_2 are the echo times after the first and second refocusing RF pulses, respectively. Signal rescaling was performed online from the third to the last PE step based on the measured TR:

$$F = \frac{S_n}{S_2} \quad [2]$$

where S_n represents the calculated signal at the n^{th} PE step and S_2 represents the calculated signal at the second PE step. The signal saturation difference between individual scans was corrected offline based on the TR that was measured after the second PE step in each of the scans:

$$G = \frac{S_{b>0}}{S_{b=0}} \quad [3]$$

where $S_{b>0}$ and $S_{b=0}$ are the signals calculated for the diffusion-weighted and the non-diffusion weighted scans, respectively. The combined correction, labeled as C, was:

$$C = F \times G \quad [4]$$

Overestimation of MD values with increasing amplitude of PEG (measured in the integrated PEPSI data) was modeled using nonlinear regression across all children and adults (see below).

Data acquisition

Data were collected with a 90 ms TE, 2 s nominal TR, 32×32 voxels spatial matrix, and 20 mm slice thickness. At UNM, data were collected with a field-of-view (FOV) of 226 mm (1 cc nominal voxel size) and two signal averages, resulting in a nominal acquisition time of 2:24 min per gradient direction. The nominal total scan time was 17:08 min.

Data at UW were collected with single signal average to minimize overall acquisition time for the sleeping children, resulting in a nominal acquisition time of 1:12 min per gradient direction and a 8:24 min nominal total scan time. To match the signal-to-noise per unit time and unit volume at UNM, taking into consideration the difference in RF coil sensitivity described in Wiggins et al.²³, a larger FOV (480 mm) and nominal voxel size (4.5 cc) were used at UW.

The echo-planar spatial-spectral readout was performed using partial ramp sampling with 8 μ s ramp sampling delay, two-fold temporal oversampling, a 1087 Hz reconstructed spectral width (after separation of even and odd echoes), and a 1 Hz digital spectral resolution as described previously¹⁶. Diffusion gradients with 25 ms duration for the positive gradient lobe followed by a 1 ms negative gradient lobe were applied. Time delays following the diffusion gradients were used to reduce short-term eddy current effects. The maximum gradient amplitude was 23 mT/m along two orthogonal gradient axes, resulting in a 1734 s/mm² maximum b-value. Data were acquired with six different gradient directions in addition to a reference scan without diffusion gradients: (0,-Y,-Z), (-X,-Y,0), (-X,0,-Z), (X,-Y,0), (0,Y,-Z) and (X,0,-Z). To assess DTSI intra-session reproducibility, the scan with the (X,-Y,0) diffusion gradient direction was repeated at the end of each child's session to compare PEGs.

Cardiac-gating at UNM was performed using electrocardiogram (ECG) recordings with a 250 ms time delay between systole and the beginning of the pulse sequence. This mitigated pulsatile motion effects from cardiac-related brain and cerebrospinal fluid pulsations. Cardiac-gating using pulse oximetry and the same delay time was employed at UW. For children, the pulse sensor was attached to the big toe of the right foot.

Water-suppressed data were collected at both sites using a PRESS volume localization in AC/PC orientation that encompassed both gray matter and white matter in the medial centrum semiovale. In addition, water un-suppressed DTSI data from the same slice localization were collected at UNM, both in the phantom and in vivo using a single signal average.

Data reconstruction (Fig.2)

Reconstruction of the raw data was performed online as described previously¹⁶ using re-gridding and decimation of the ramp sampled data, spatial-spectral reconstruction with even-odd echo separation, multi-coil combination, zero-order phase correction based on the residual water signal, navigator-based correction of phase errors and amplitude fluctuations, and T₁-saturation correction. A spatial hamming filter (50% width) was applied to the k-space data, resulting in effective voxel sizes of 1.5 cc for the UNM data and 6.75 cc for the UW data.

Phase correction—Correction of movement-related phase instability in individual acquisitions was performed on a coil-by-coil basis using the 4th navigator, following our previously described approach⁶. Corrections were applied separately to the even and odd readout lines. Spatial reconstruction of the navigator acquisition employed automatic detection of the center of the PEPSI slice in the first PE step across the multi-coil data. This was done by enforcing consistency between the multi-coil data, i.e. using the most frequent peak position of coil elements with a signal-to-noise ratio (SNR) greater than 5:1.

Additionally, the reference location could be updated at every TR or every PE step in the case of multiple signal averages.

Amplitude correction—We investigated the performance of a coil-by-coil normalization for the amplitude fluctuations of the 4th navigator with respect to the first PE step. Data with an SNR below 2:1 were left uncorrected to avoid excessive noise enhancement. At the completion of data acquisition, the signal amplitudes were further normalized with respect to the strongest navigator signal amplitude across all PE steps. A ratio between the standard deviations (SD) of the amplitudes for the 3rd and 4th navigators was calculated across coils. K-space data with a ratio above 1.0 were discarded.

T₁-saturation correction—In order to mitigate signal fluctuations from cardiac-gating²⁴, an effective water T₁ was determined by applying saturation correction to the first navigator data and selecting the T₁-value that minimized the SD across all PE steps. Correction of metabolite data was performed using an average T₁-value for tNAA, tCr, and tCho based on the effective water T₁-value (using empirically determined ratios of metabolite to water T₁), that minimized the spatial SD of the metabolite ADC. These values were in the range reported by Ethofer et al.²⁵. Sensitivity of the T₁-correction to errors in T₁-estimates and the effects on calculated ADC and FA were investigated in one adult subject by varying T₁-values of water from 800 ms to 3000 ms.

Post-processing (Fig.2)

Evaluation of PEG—After subtracting the average background signal, the mean amplitude of PEG in the spectrally integrated data was measured in anterior and posterior regions within the PRESS volume. Relative PEG levels were determined by computing ratios of the mean amplitude of PEG over the mean signal inside the PRESS volume. These levels were computed individually for each of the six diffusion gradient directions and then averaged. Additionally, the coefficient of variation (C.V.) of the navigator amplitude was computed across the nine central PE steps, after averaging across all RF channels over three of the UNM scans. The effect of PEG on the C.V. was assessed along each of the six diffusion gradient scans.

Spectral quantification—There were 20 and 77 voxels inside the PRESS box at the UW and UNM sites, respectively. LCModel fitting²⁶ was restricted to a subset of voxels inside the PRESS volume prelocalization to avoid chemical shift related displacement artifacts at the edges of the volume. The central 6 voxels of the UW data sets and the central 24 voxels of the UNM data sets were selected, which covered comparable volumes of interest. The simulated basis-set contained 18 metabolites, as described¹⁶. Spectral fitting was performed using a Cramer-Rao Lower Bound (CRLB) threshold of 20%.

Diffusion tensor computation—The absolute concentration values from LCModel were saved as NIfTI images, using custom MATLAB and Perl scripts. MD and FA maps for each metabolite and water scan (for UNM data) were computed using the MedINRIA software package and saved as NIfTI images²⁷. In addition to the full tensor analysis, ADC using only one gradient direction was calculated. The selected gradient direction was (X,-Y,0), which consistently resulted in the smallest PEG across UW data. For participants with a repeated (X,-Y,0) scan, the scan with the minimum PEG was selected for MD and ADC estimations. Spatial averages and SD of the ADC and FA maps within the PRESS box were computed for children and adults using a custom MATLAB script.

Statistical analysis

Adult scans from both sites were pooled together for the statistical analysis as no significant MD and PEG site differences were found (see Results). The group-averaged PEG for all directions was computed for both the adult and child pools. A two-tailed t-test was used to assess group differences in PEG. Using a regression-based approach that accounts for auto-correlations from repeated scans, age dependence of MD and FA values was assessed using age as a binary variable (adults and children) and the level of the PEG as a covariate (IBM-SPSS and SAS v9.4 software). An exponential least squares model resulted in the best fit to the apparent MD as a function of the amplitude of PEG. MD was estimated using equation (5):

$$\ln(MD(i, j, k)) = \beta_0(i) + \beta_1(i) \times \alpha_1 + \beta_2(i) \times \xi + b(j) + (i, j, k) \quad [5]$$

where i is the metabolite parameter, j is the subject parameter, k is the time parameter, $\beta_0(i)$ is the $\ln(MD)$ intercept, $\beta_1(i)$ accounts for age differences, α_1 is the binary age variable,

$\beta_2(i)$ is the regression slope coefficient for PEG, ξ accounts for the level of PEG, $b(j)$ is the random person effect due to scan repetitions, and (i, j, k) accounts for residual variations not explained by the model. (i, j, k) was assumed to be independent and normally distributed with separate variances for each metabolite. FA was estimated using equation (6):

$$FA(i, j, k) = \beta_3(i) + \beta_1(i) \times \alpha_1 + \beta_5(i) \times \xi + b(j) + (i, j, k) \quad [6]$$

where $\beta_3(i)$ is the intercept, $\beta_4(i)$ accounts for age differences, and $\beta_5(i)$ is the regression slope coefficient for PEG. The remaining parameters are as described above.

The model was tested by assessing the age-dependence of MD and FA slopes as a function of the level of PEG. The mean and SD of slice-averaged MD and FA were reported as a function of PEG. Furthermore, the effect of gender was assessed while controlling for PEG. Finally, MD values and confidence intervals were estimated at 20% PEG and extrapolated to 0% PEG using an age-independent slope, as no significant age differences were found (see Results). The extrapolation to 0 % PEG provided an estimate of MD for the conditions of no movement artifacts. We further report the group-averaged MD for children and adults without correction for PEG (uncorrected MD). In addition, we examined the age-dependence of the ADC that was computed using a single gradient direction (X,-Y,0) with minimum PEG (see above) to further validate the methodology.

Results

Method validation in phantom

PEG and eddy current artifacts were negligible in the phantom. Mean MD values of tNAA ($0.73 \pm 0.7 \times 10^{-3} \text{ mm}^2/\text{s}$) in the phantom at 20 °C were consistent with previously reported values⁷ while water results were slightly lower ($1.84 \pm 0.07 \times 10^{-3} \text{ mm}^2/\text{s}$). FAs measured in the phantom for tNAA (0.11 ± 0.05) and water (0.04 ± 0.02) represent the experimental threshold for detecting diffusion anisotropy.

Method validation *in vivo*

One adult and one child scan from UW were discarded due to very low SNR (<2) and high PEG (>43%). The PRESS volume was placed well within brain tissue to avoid lipid contamination (Fig.3a). Navigator correction was highly effective in reducing PEG (Fig.3b) and restoring the spatial localization to within the PRESS box (Fig. 3c), with spectral quality (Fig. 3d,e) comparable to that of non-diffusion weighted data. High b-value metabolite images demonstrate that comparable image quality was obtained along all 6 diffusion gradient directions (Fig.4). PEG showed a dependence on the diffusion gradient direction (Supporting FigureS1, $p=0.04$) which may reflect orientation-dependent differences in sensitivity to rotational and non-rigid body movement. The three gradient directions with the smallest amount of PEG in UNM data were (X,0,-Z), (-X,-Y,0) and (X,-Y,0) (Supporting FigureS1), with less than 0.01% difference between them. They also resulted in the smallest navigator amplitude fluctuation (Supporting FigureS2). Navigator amplitude fluctuations across the remaining three gradient directions were 23 % higher than the first three gradient

directions ($p=0.002$). PEG was positively correlated with the navigator amplitude fluctuations ($R^2 = 0.93$) (Supporting FigureS2).

Combining phase and amplitude corrections provided comparable localization performance to that of phase correction only; however, noise amplification resulted in a slight overestimation of MD. As a result, the combined amplitude and phase correction method was not utilized.

TR variability due to ECG-gating resulted in scan time variability of up to 17%, and up to a 7% difference in metabolite signal saturation prior to T_1 correction. The sensitivity analysis showed that the MD of tNAA and tCr increased by 0.074% for each 1% increase in water T_1 , while FA values of tNAA and tCr decreased by 0.039%. The results for tCho were less consistent due to SNR limitations and proximity to the water resonance.

The spectral quality in adults and children was comparable and spatially uniform for most gradient directions except for (-X,0,-Z), which exhibited artifacts at the edges of the PRESS box. CRLBs of the LCModel fits were on average less than 15% for all subjects. The difference in SNR values in the children between high b-value data (5.2 ± 2.2) and low b-value data (10.0 ± 2.4) was larger than for SNR differences comparing adult high b-value data (4.6 ± 1.3) to low b-value data (6.6 ± 1.9). The spectral linewidth in non-diffusion-weighted data in children (4.5 ± 2.4 Hz) and adults (4.2 ± 0.9 Hz) were not significantly different ($p=0.66$). For diffusion-weighted data, spectral linewidth differences between children (4.9 ± 1.7 Hz) and (4.7 ± 1.0 Hz) adults were also non-significant ($p=0.85$).

Adult data acquired at the two sites did not show significant differences in metabolite MD (tNAA, $p=0.73$; tCr, $p=0.64$; tCho, $p=0.64$) or PEG ($p>0.69$). Inter-session reproducibility (acquired on different days in two adults at UNM) showed variability of 27% in slice-averaged MD, which reflects variability in PEG levels (39%). Intra-scan reproducibility in UW data in children, measured using the repetition of the (X,-Y,0) scan, showed no significant difference in PEGs between the two scans ($p=0.724$).

MD and FA maps of tissue water in adults showed clear distinctions between gray and white matter (Fig.5a); this distinction was less pronounced in the metabolite maps, in part due to limitations of SNR and residual PEG after phase correction (Fig.5a). MD and FA maps in children were more uniform, consistent with smaller cerebrospinal fluid spaces and lower gray/white matter MRI contrast as compared to adults (Fig.5b).

Age dependence of metabolite diffusion

PEG levels in adults (8.77%–42.1%) had a slightly lower range than in children (14.9%–49.2%). The mean PEG averaged across all children ($32.3\pm9.1\%$) was significantly higher than in adults ($24.2\pm10.6\%$; $p=0.041$). When plotting metabolite MD as a function of PEG, exponential functions were found to provide the best fit for both adults and children. Exponential fitting showed average values for R^2 and mean square error of 0.70 and 0.25, respectively (Fig.6). The slopes of the $\ln(\text{MD})$ as a function of PEG for all metabolites were not significantly different for adults and children ($p>0.5$).

Regression analysis showed that the age-dependence of the MD was significant for all metabolites (tNAA, tCr, tCho: $p=0.009$; $p=0.015$; $p=0.042$). No significant gender effect was found ($p>0.20$). MD values corrected for PEG were considerably lower than uncorrected MD values (Table 1). For adults, the mean MD estimated in a supraventricular slice at 20% PEG were in ranges reported in previous studies⁹: tNAA (0.17×10^{-3} mm²/s), tCr (0.20×10^{-3} mm²/s), and tCho (0.18×10^{-3} mm²/s). In children, estimated MDs at 20% PEG were higher than for adults: tNAA (0.26×10^{-3} mm²/s), tCr (0.27×10^{-3} mm²/s), and tCho (0.24×10^{-3} mm²/s). Extrapolated MDs in adults at 0% PEG for tNAA (0.09×10^{-3} mm²/s), tCr (0.11×10^{-3} mm²/s), and tCho (0.11×10^{-3} mm²/s) were on average smaller than reported in previous studies²⁸. Extrapolated MDs in children at 0% PEG for tNAA (0.15×10^{-3} mm²/s), tCr (0.16×10^{-3} mm²/s), and tCho (0.15×10^{-3} mm²/s) remained higher than in adults. Slice-averaged MD (0.75×10^{-3} mm²/s) and FA (0.22) of tissue water were consistent with previous diffusion tensor MRI studies in adults²⁹.

Regression analysis showed no significant dependence of FA on the PEG for any of the metabolites ($p>0.172$). FA values of tCr in adults showed a trend level difference as compared to those in children ($p=0.067$), whereas FAs were lower in adults as compared to children for tNAA ($p=0.049$) and tCho ($p=0.003$) (Table 2).

Computation of the ADC using the single gradient direction (X,-Y,0) with minimum PEG, served as a secondary assessment of residual motion effect when computing MD. The resulting age-dependent-ADC found for all metabolites (tNAA, $p=0.006$; tCr, $p=0.002$; tCho, $p=0.001$) mirrors the results obtained with the MD and thus suggests that movement effects in MD, after regression of PEG, were small.

Discussion

In this study, we demonstrate the feasibility of mapping the diffusion tensor of metabolites in young children and adults using high-speed DTSI. In order to systematically analyze the overestimation of metabolite MD values caused by residual PEG, we introduce a new regression-based approach. Previous work has shown age-dependent decreases in the ADC of tissue water^{29–31}; our DTSI approach specifically probed the intracellular MD of metabolites with findings of age-related differences that were lower in adults compared to children.

The extrapolated metabolite MD values at 0% PEG in adults are smaller than ADC values reported in most of the previous studies²⁸, which suggests that, due to the effects of residual movement, experimental MD and ADC values measured *in vivo* may overestimate true physiological values. Ellegood et al.³ reported adult metabolite MD values in the range of $0.15\text{--}0.17\times10^{-3}$ mm²/s for gray matter and $0.19\text{--}0.30\times10^{-3}$ mm²/s for white matter. In our study, the experimental metabolite MDs in adults estimated at 20% PEG are in the range of $0.17\text{--}0.20\times10^{-3}$ mm²/s, reflecting the mixture of gray and white matter in the PRESS volume. Ercan et al.¹⁰ reported adult metabolite ADC ranging from $0.11\text{--}0.13\times10^{-3}$ mm²/s (gray matter) and $0.13\text{--}0.17\times10^{-3}$ mm²/s (white matter), using only three gradient directions, and after discarding voxels with CRLB values higher than 10%. Interestingly, in

the current study, adult MD values extrapolated to 0% PEG were found to be slightly lower ($0.9\text{--}0.11\times 10^{-3}\text{ mm}^2/\text{s}$).

Average PEG was significantly higher in children than in adults, even though the children were asleep during data acquisition. Residual brain motion and a faster cardiac rate in children may have limited the effectiveness of the navigator-based motion correction in these subjects. A recent study using multi-shot diffusion-weighted MRI has shown that, since the brain is always in motion, cardiac-gating does not fully compensate phase errors due to non-rigid body movement³². Sensitivity to movement, albeit at a smaller level, continues to be a concern even in single-shot DTI of water and may impact measurement of the age-dependence of water diffusion³³, especially when using advanced methods, such as HARDI and diffusion spectrum imaging with large b-values.

Our FA values in adults are consistent with previous MRS studies, which have shown that both the magnitude of FA in gray/white matter and their ratio are different for metabolites and tissue water¹². However, the lack of age-dependence of FA for tCr and the slight decrease with age of FA for tNAA and tCho may reflect sensitivity constraints of MRSI related to the large voxel size³⁴ and the limited volume of acquisition (due to PRESS box prelocalization). These FA metabolite findings are in the opposite direction of numerous DTI studies of regional water diffusion changes across the lifespan, which in general have reported increasing FA (and decreasing MD) with age^{31,30}. Interestingly, a longitudinal study of subjects aged 5–32 years showed that an increase in white matter volume over time was accompanied by a conservation of total brain volume³⁵. Thus, the small age-related FA decrease of tNAA and tCho observed in our study may suggest that these metabolites were mostly located in astrocytes and neuronal cell bodies.

Although our data acquisition method uses strong diffusion gradients, the effect of eddy current in our data is relatively small as evidenced in the phantom data and the similarity of metabolite maps obtained with six different diffusion gradient directions (see Fig.4) and primarily manifest as line broadening. It was further mitigated by restricting the volume of interest (VOI) to a PRESS prelocalization, well within brain tissue, and selection of a subset of voxels within the PRESS box for analysis.

Diffusion-weighted MRSI is highly sensitive to translational, rotational, and nonlinear movements, which each lead to distinct artifacts⁶. Translational movements introduce a globally and spatially uniform phase change of the signal that can be corrected using navigator-based phase correction at each k-space point¹³. Rotational movements also lead to phase changes that vary linearly in space perpendicular to the direction of the diffusion gradient(s) and the axis of rotation^{32,36,37}. Application of a diffusion gradient along the PE direction is a special case, which allows the phase change along the readout direction to be resolved, thus minimizing motion sensitivity. Although our data acquisition methodology can account for translational movement using phase correction and nonlinear movements using cardiac gating, it is limited in that it cannot completely correct for rotational movement.

In the current study, navigators were applied in a single direction (perpendicular to the imaging slice) to monitor movement perpendicular to the slice and to maximize the navigator signal amplitude and phase resolution. Reacquisition of PE steps affected by rotational movement based on the rotation-related amplitude loss of the navigator was recently introduced¹⁰. This approach empirically establishes the navigator reference signal amplitude from a maximum of five acquisitions during prescan and employs an amplitude threshold (85%) below which a k-space point is reacquired. Although a significant improvement of the data quality was demonstrated, a rigorous criterion for selecting the threshold was not established and the level of residual PEG was not reported. This approach, not used in the present study, also substantially increases the acquisition time (up to 81% in¹⁰). Here, we report the level of residual PEG and further demonstrate an associated exponential decay of MD. Our method also enables relatively short nominal scan times (8:24 min with a single average).

A number of limitations remain that need to be addressed. The use of navigators along multiple orthogonal spatial directions may help to resolve rotation-related phase gradients in multiple directions and result in a more complete correction of rigid body translations and rotations³⁸. To further reduce motion sensitivity, we are developing single-shot 2D spatial-1D spectral encoding using interleaved PE and parallel imaging³⁹, as well as combined compressed sensing and parallel imaging⁴⁰, to assess the feasibility of single-shot DTSI of an entire slice⁴¹. This approach can be combined with discarding, or reacquisition of, signal averages that are affected by rotational movement¹⁰ to achieve superior tolerance to movement effects. We are further exploring the feasibility of mapping MD in different slice locations and using a 3D acquisition¹⁸. The small sample size in this study limits the interpretation of age-related findings and did not allow us to investigate age dependence of MD and FA within subject groups. The regression approach was thus limited to between group differences. Despite efforts to match the PRESS volume selection between adults and children we could not exclude possible group differences in gray/white matter tissue composition in the VOI. We are thus planning in future studies to assess changes in metabolite diffusion between white matter and gray matter in adults and older children. This approach may provide greater specificity for monitoring age-related changes in MD values estimated at 0% PEG and allow for a more direct comparison with the results reported in¹⁰. The effect of restricted diffusion was not investigated in the current study due to limitations in scan time and maximum gradient moment, which also limited the minimum TE. A recent study in humans suggested that metabolites move rather freely at the diffusion time used in our study⁴². Further investigation of multiplet resonances at shorter diffusion times would require higher field strength and much stronger gradient systems, which are increasingly becoming available.

In summary, this study demonstrates the feasibility of acquiring slice localized 2D DTSI data from the human brain *in vivo*. Using regression analysis, we found slice-averaged MD and FA values in adults, estimated at 20% PEG, to be within the range of previously published values and estimated MDs at 0% PEG to be lower than in previous reports. There were significant differences in MD comparing adults and young children that parallel the age-dependence of water diffusion. However, due to the small sample size, these age-related differences require further validation in future studies. The measured age differences suggest

that DTSI may hold promise as a noninvasive research tool for assessing intracellular brain developmental processes and maturation *in vivo*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Schematic representation of the PEPSI pulse sequence with water-suppression (WS1, WS2, WS3), outer-volume-suppression (OVS), point resolved spectroscopy (PRESS), phase encoding (PE) on G_{ph} in the y-direction, echo-planar readout on G_r in the x-direction, and navigator (NAV1, NAV2, NAV3, and NAV4) modules.

Fig. 2.

DTSI post-processing pipeline. Online navigator correction, intra-scan gating correction, and spatial-spectral reconstruction were performed on both water-suppressed (1) and water un-suppressed data (2). (1) LCModel fitting was performed on water suppressed data. Fitted spectra with CRLB above 20% or SNR below 3 were systematically rejected. The phase encoding (PEG) level was then calculated. (2) Inter-scan gating correction, spectral integration, and masking of the spectra results were performed on water un-suppressed data. Using MedINRIA software, a diffusion tensor analysis on both water suppressed and water un-suppressed data was performed in order to obtain MD and FA. An additional data reduction was performed on the data, using only the diffusion-weighted gradient direction that was the least affected by PEG, to compute a scalar metric of the brain metabolite ADC using MedINRIA software.

Fig. 3.

Performance of the navigator correction *in vivo*. a) PRESS volume selection with outer volume suppression slices. Diffusion-weighted spectroscopic images of tNAA were reconstructed (b) without and (c) with navigator correction, which strongly reduced phase encoding ghosting (PEG) along the phase encoding direction. (d) Localized spectrum at $b = 1734 \text{ s/mm}^2$ with LCModel fitting is overlaid in red. (e) The spectral array at $b = 1734 \text{ s/mm}^2$ showed consistent spectral quality across the entire PRESS volume preselection.

Fig. 4.

High b -value (1734 s/mm^2) total N-acetyl-aspartate (tNAA), total creatine (tCr), and total choline (tCho) maps from an adult scan (24 years-of-age) at the University of New Mexico (UNM) for the six different diffusion gradient directions: (a) $(-X, -Y, 0)$, (b) $(-X, 0, -Z)$, (c) $(0, -Y, -Z)$, (d) $(X, -Y, 0)$, (e) $(X, 0, -Z)$ and (f) $(0, Y, -Z)$. The metabolite concentration (S) is given in arbitrary units. The maps were 2-fold interpolated.

Fig 5.

MD and FA maps for total N-acetyl-aspartate (tNAA), total creatine (tCr), and total choline (tCho), and tissue water in an (a) adult brain from the University of New Mexico (UNM) (24 years-of-age; 1 cc nominal voxel size; 1.5 cc effective voxel size, maps were 2-fold interpolated) and (b) a child brain from the University of Washington (UW) (6 months-of-age; 4.5 cc nominal voxel size; 6.75 cc effective voxel size, maps were 4-fold interpolated). The PRESS volume selection included 77 voxels and 20 voxels, respectively, while the region of interest (red box) for spectral quantification corresponded to 24 voxels and 6 voxels in a) and b) respectively.

Fig. 6.

Exponential fit of MD values as a function of phase encoding ghosting (PEG) for total N-acetyl-aspartate (tNAA), total creatine (tCr), and total choline (tCho), using age as a binary variable (using Equation [5]). The shaded area represents the 95% confidence interval.

Estimated MD values in children and adults for total N-acetyl-aspartate (tNAA), total creatine (tCr), and total choline (tCho) without correction for phase encoding ghosting (PEG) and at 20% PEG and 0% PEG. The corresponding p-values of the t-test for group differences between children and adults, and the 95% confidence interval (CI) are listed below. The age range was 3–24 months for children scans (n=13) and 17–60 years for adult scans (n=9).

Table 1

	Children			Adults		
	tNAA	tCr	tCho	tNAA	tCr	tCho
Uncorrected MD ($\times 10^{-3}$ mm ² /s)	0.40	0.40	0.30	0.20	0.30	0.20
95% CI ($\times 10^{-3}$ mm ² /s)	0.30–0.50	0.30–0.50	0.20–0.40	0.10–0.30	0.20–0.40	0.10–0.30
Corrected MD at 20% PEG ($\times 10^{-3}$ mm ² /s)	0.26	0.27	0.24	0.17	0.20	0.18
95% CI ($\times 10^{-3}$ mm ² /s)	0.20–0.33	0.22–0.34	0.19–0.31	0.14–0.21	0.17–0.24	0.15–0.22
at 0% PEG ($\times 10^{-3}$ mm ² /s)	0.15	0.16	0.15	0.09	0.11	0.11
95% CI ($\times 10^{-3}$ mm ² /s)	0.09–0.26	0.11–0.25	0.09–0.25	0.06–0.14	0.08–0.16	0.07–0.17
p-value				0.009	0.015	0.042

Estimated FA values in children and adults, p-values of the t-test for group differences between children and adults, and 95 % confidence intervals (CIs). The age range was 3–24 months for children scans (n=13) and 17–60 years for adult scans (n=9).

Table 2

		Children			Adults		
		tNAA	tCr	tCho	tNAA	tCr	tCho
FA		0.61	0.66	0.68	0.57	0.63	0.57
95% CI ($\times 10^{-3}$ mm ² /s)		0.52–0.70	0.59–0.73	0.59–0.77	0.49–0.65	0.59–0.67	0.47–0.67
p-value					0.049	0.067	0.003