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# Correlation between brain volume change and T2 relaxation time induced by dehydration and rehydration: Implications for monitoring atrophy in clinical studies

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ABSTRACT

Brain volume change measured from magnetic resonance imaging (MRI) provides a widely used and useful in vivo measure of irreversible tissue loss. These measurements, however, can be influenced by reversible factors such as shifts in brain water content. Given the strong effect of water on T2 relaxation, we investigated whether an estimate of T2 relaxation time would correlate with brain volume changes induced by physiologically manipulating hydration status. We used a clinically feasible estimate of T2 ("pseudo-T2") computed from a dual turbo spin-echo MRI sequence and correlated pseudo-T2 changes to percent brain volume changes in 12 healthy subjects after dehydration overnight (16-hour thirsting) and rehydration (drinking 1.5 L of water).

We found that the brain volume significantly increased between the dehydrated and rehydrated states (mean brain volume change = 0.36%, $p = 0.0001$) but did not change significantly during the dehydration interval (mean brain volume change = 0.04%, $p = 0.57$). The changes in brain volume and pseudo-T2 significantly correlated with each other, with marginal and conditional correlations ($R^2$) of 0.44 and 0.65, respectively.

Our results show that pseudo-T2 may be used in conjunction with the measures of brain volume to distinguish reversible water fluctuations and irreversible brain tissue loss (atrophy) and to investigate disease mechanisms related to neuro-inflammation, e.g., in multiple sclerosis, where edema-related water fluctuations may occur with disease activity and anti-inflammatory treatment.



## 1. Introduction

Chronic brain atrophy has long been appreciated in neurological diseases such as Alzheimer's disease (Alzheimer et al., 1995) and multiple sclerosis (MS) (Dawson, 1916) from post-mortem autopsy studies. Today such brain tissue loss can be measured noninvasively in vivo using magnetic resonance imaging (MRI) and image analysis tools. Compared to traditional manual or semi-automated image analysis procedures, advanced registration-based algorithms have greatly increased the sensitivity to even small brain volume changes.

An important physiologic contributor of reversible brain volume change is fluctuations in hydration status, which can be seen in various conditions, including water intake and thirsting (Duning et al., 2005; Streitbürger et al., 2012). In the study by Duning et al. (2005), hydration (drinking water) and dehydration (thirsting) led to significant brain volume change (+0.75% and −0.55%, respectively). This observed volume change is larger than the annualized rates of brain atrophy from normal aging of nonelderly adults, where the average rates range from approximately 0.1 to 0.3%/year (Fisher et al., 2008; Fotenos et al., 2005; Scahill et al., 2003). The results of these studies suggest that in short-term longitudinal studies, variability in subjects' hydration status could significantly affect the outcome of brain atrophy measurements (Sampat et al., 2010).

Another condition where the water-related fluctuation is believed to occur is in inflammatory brain edema (Zivadinov et al., 2008). Several MS clinical trials show that brain volume loss is accelerated after the initiation of anti-inflammatory therapy and that this acceleration disappears in the second year of therapy (Miller et al., 2007; Rudick et al., 1999). This phenomenon is often termed "pseudoatrophy" and is hypothesized to be the result of resolution of inflammatory edema, an idea supported by the association of pseudoatrophy patterns with gadolinium-enhancing lesions (Molyneux et al., 2000). A tissue-specific volumetric study has shown that the pattern of pseudoatrophy observed in the intramuscular interferon beta-1a phase III clinical trial (Rudick et al., 1999) appears to be mainly driven by white matter, suggesting that the suppression of inflammatory white matter lesions may be related to the volumetric change (Nakamura et al., 2010). While the effect of shifting hydration level is typically uncontrolled and somewhat random, thus adding noise, the pseudoatrophy effect adds bias because a particular group, e.g., treated vs. placebo, can be more affected.

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Overall, the pseudoatrophy effect complicates the interpretation of brain atrophy results as it counteracts or even overshadows the expected treatment effect in clinical trials and has led some to assess the treatment effect on atrophy only from the second year on (Arnold and De Stefano, 2013; Miller et al., 2007; Rudick et al., 1999).

To decipher the significance of brain volume loss after initiating anti-inflammatory therapy, we need to distinguish the irreversible component of brain tissue loss (true atrophy) from reversible fluctuation in brain volume (pseudoatrophy). In the current study, we used a two-point estimate of T2-relaxation time as a marker of brain water content. T2 relaxation time is associated with the tissue water content and can be quantitatively measured using multi-echo MR sequences (Whittall et al., 1997). Quantitative multi-echo T2-relaxation measurements are, however, not generally feasible in clinical studies because they typically require 16 or more echoes and long acquisition times with partial brain coverage. By contrast, a dual-echo sequence offers a semi-quantitative but clinically feasible estimate of bulk T2-relaxation times; in this study, we term a two-point estimate of T2-relaxation "pseudo-T2" or pT2, to differentiate it from fully quantitative techniques that model multi-exponential relaxation due to multiple water compartments. Previous studies have shown that pT2 is not numerically the same as the multi-echo T2 relaxation time (Okujava et al., 2002; Rajagopalan et al., 2013) but is strongly correlated ($r > 0.88$) with multi-echo T2 (Okujava et al., 2002) as well as being precise and highly reproducible (Townsend et al., 2004) (0.27% scan–rescan difference for whole brain (Derakhshan et al., 2010)).

In the current study, we investigated the ability of pT2 methodology to explain brain volume fluctuations induced by varying hydration levels. Our hypotheses were (1) that dehydration and rehydration would affect both brain water content, as measured by pT2, and brain volume, as measured by TBM, and (2) that pT2 change and brain volume change are correlated. To evaluate that, we set up a dehydration–hydration protocol similar to that of Duning et al. (2005).

## 2. Methods

### 2.1. Subjects

Fourteen healthy subjects (2 women) underwent MRI scanning. Their average age was 32.85 years (standard deviation: 7.41, range: 24–46 years). The inclusion criteria were: no previous history of neurologic, metabolic, or psychiatric disorders and no use of recreational or prescription drugs. All subjects provided informed consent to participate in the study, and the study was approved by the Research Ethics Board of the Montreal Neurological Institute and Hospital.

### 2.2. MRI

The subjects were imaged on a 1.5 T MRI scanner (Siemens Sonata) in three different epochs: a) baseline MRI, performed a few days or weeks prior to dehydration; b) dehydration MRI performed after 16 h of relative fasting overnight, during which subjects were instructed to refrain from drinking and to ingest only dry solid foods; and c) immediately after the dehydration scan, subjects drank 1.5 L of water over 90 min, followed by the rehydration scan. This dehydration and rehydration protocol is modified from the study by Duning et al. (2005) in that the duration of rehydration increased from 20–30 to 90 min.

For each subject, we acquired a structural 3D T1-weighted spoiled gradient-recalled echo image (Fast, Low-Angle SHot, FLASH) [echo time (TE): 9.2 ms, repetition time (TR): 22 ms, voxel size: $1.2 \times 1.2 \times 1.2$ mm$^3$, scan time 10:22 min] for the measurement of volume change and one set of dual-echo T2-weighted fast spin echo images [TEs: 12 and 83 ms, TR: 2070 ms, slice thickness: 3 mm, field-of-view: 250 mm, matrix $= 256 \times 256$; echo train length: 5, scan time: 5:33 min] to estimate pT2.

### 2.3. Image analysis

#### 2.3.1. Pre-processing

$T_1$-weighted MRI images were corrected for geometric distortion using a nonlinear deformation field obtained from Lego® phantoms (Fonov et al., 2010) and for field inhomogeneity using the N3 method (Sled et al., 1998).

#### 2.3.2. Pseudo-T2 calculation

The dual-echo T2-weighted sequence was used to produce a pT2 map using the equation $pT2 = (TE_2 - TE_1) / \ln(S_1 / S_2)$ where $S_1$ and $S_2$ are the measured image intensities at each echo time, $TE_1$ and $TE_2$ (Derakhshan et al., 2010; Duncan et al., 1996). All images were registered to a standard space defined by the MNI-152 atlas using a six parameter rigid registration (Collins et al., 1994). The brain was extracted using FSL BET (Smith, 2002), and the extracted brain tissue was segmented using SIENAX's FAST (Zhang et al., 2001). A brain parenchymal mask (excluding CSF) was created by combining gray matter and white matter probability maps and thresholding at 50% to exclude voxels with low probability of containing tissue (typically due to partial volume with CSF).

#### 2.3.3. Volume change measurement

We used a type of TBM called the pairwise Jacobian integration method to measure the volume change in the brain parenchyma (Nakamura et al., 2013; Nakamura et al., 2014). Briefly, the pairwise Jacobian integration performed the following procedures: (a) linear alignment of the pre-processed image pair with 12-parameter skull-based symmetric registration (Jenkinson et al., 2002); (b) image resampling in halfway-space; (c) nonlinear alignment of the resampled images using ANTS (Avants et al., 2008); (d) calculation of the Jacobian determinants for each voxel; and (e) averaging of the Jacobian determinants within the brain parenchymal mask, which was a combination of the gray matter and white matter masks obtained by FSL SIENAX (Zhang et al., 2001) and thresholded at 50%. The resulting metric from the Jacobian integration method is a percent of brain volume change (PBVC).

### 2.4. Statistical analysis

To confirm the effects of dehydration and rehydration on brain volume, PBVC was modeled using a general linear mixed model (GLMM) as the result of the interval (baseline–dehydration or dehydration to rehydration) and a subject-specific random effect.

The mean pT2 in brain tissue was calculated for each subject at each imaging session, and then differences between these means were computed corresponding to the baseline–dehydration and dehydration–rehydration intervals for each subject. PBVC was calculated between baseline and dehydration as well as dehydration and rehydration for each subject. PBVC was modeled using a GLMM with the pT2 change as a fixed effect and a subject-specific random effect.

The statistical analysis was performed using custom software written in Python (Python Software Foundation, http://python.org), using the MINC tools (MINC tools, McConnell Brain Imaging Centre, Montreal), the Scientific Python package (Scipy, http://www.scipy.org), the RPy2 module (RPy2, http://rpy.sourceforge.net) and the R statistical software (R-Team, 2012). GLMMs were calculated with the lme4 R package (Bates and Maechler, 2009). $p$-Values for the random effects and overall model fit were calculated using $\chi^2$-tests. The significance of fixed effects was computed using $f$ tests with denominator degrees of freedom estimated with a Satterthwaite approximation, using the R package MixMod (Kuznetsova and Brockhoff, 2012). $R^2$ values for the mixed models were calculated according to the procedure suggested by Nakagawa and Schielzeth (2013), where a marginal $R^2$ measures the variance explained

by the fixed effects alone and the conditional $R^2$ includes the contribution of the random effects. The marginal $R^2$ is primarily relevant to cross-sectional studies while the conditional $R^2$ applies to longitudinal designs.

One subject had poor quality imaging due to residual geometric distortion and another exhibited outlying changes in both PBVC and pT2. These were eliminated from the analysis.

## 3. Results

Fig. 1(a) shows examples of PD-, T2-, and T1-weighted images as well as computed pT2 maps from one subject for the 3 epochs. Fig. 1(b) shows the Jacobian determinant maps calculated by nonlinear registration of baseline structural scans from all subjects using ANTS (Avants et al.,

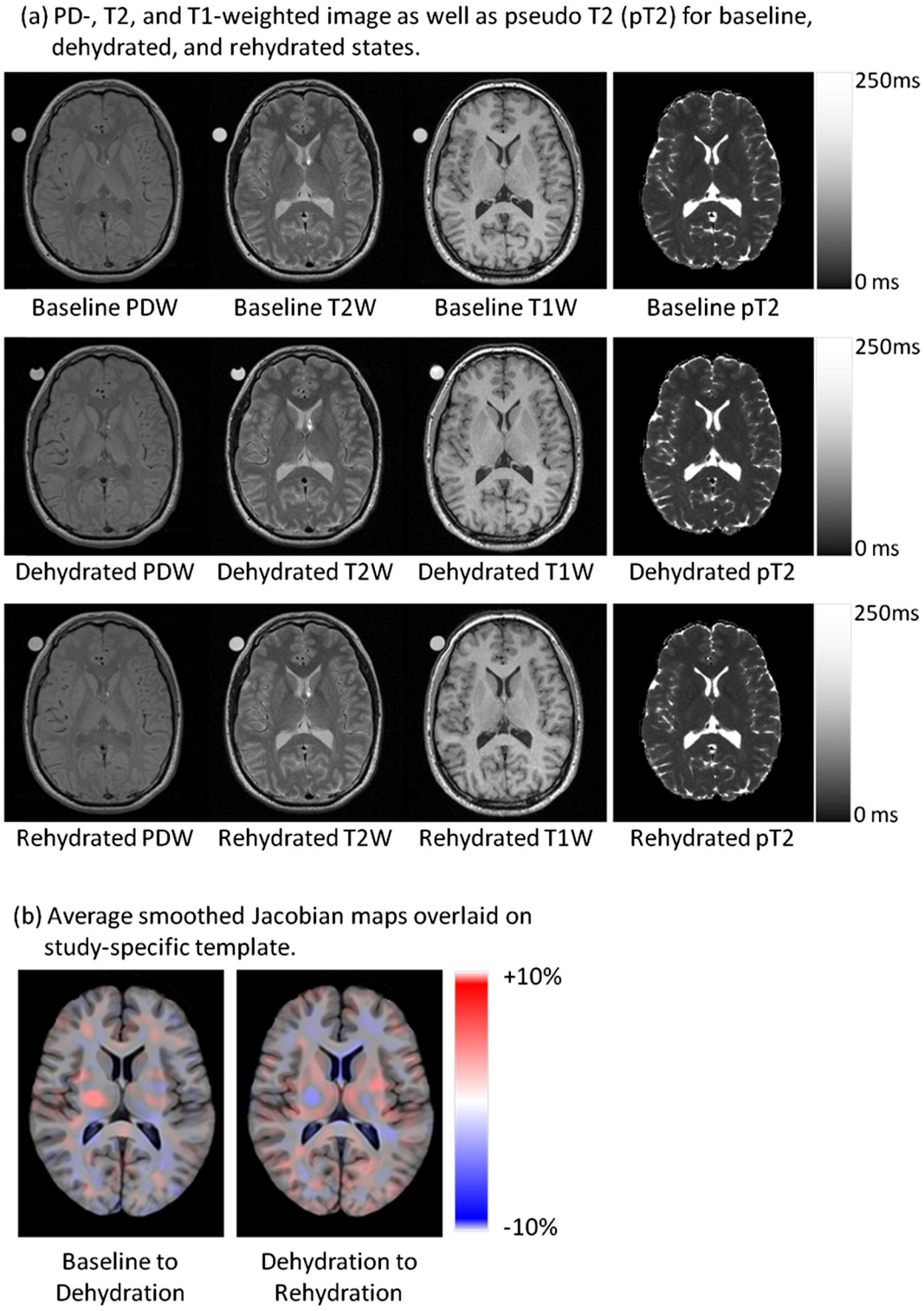


**Fig. 1.** (a) Example raw and pT2 images. From left to right: PD-weighted scan, T2-weighted scan, T1-weighted scan, and pT2 (pseudo-T2) map within the brain mask from one subject. Each row represents epoch (baseline, dehydrated, and rehydrated) where the images are not registered but chosen for similar slices. (b) The Jacobian maps averaged from all subjects, smoothed with an 8 mm-FWHM, and overlaid on the study-specific template.

2008), smoothing with an 8 mm-FWHM Gaussian kernel, averaging, and mapping on the study-specific template.

The average (SD) delay between baseline and dehydration MRI was 12.4 (7.7) days (range = 3–28 days) while the average (SD) delay between dehydration and rehydration was 2.2 (0.2) h (1.8–2.5).

We found excellent correlation between brain volume changes and pT2. As shown in Fig. 2, increases and decreases in brain volume were strongly correlated to increases and decreases in T2 relaxation times, respectively.

The model describing PBVC in terms of interval fit significantly ($p = 0.0053$) although the random effect was not significant ($p = 1.0$). Due to the negligible random effect, the marginal and conditional $R^2$ values were the same, 0.26. The baseline–dehydration interval did not have a significant effect on brain volume (mean = 0.04%, $p = 0.57$) but the dehydration–rehydration interval was associated with a significant increase in brain volume (mean = 0.36%, $p = 0.0001$).

The model describing the relationship between PBVC and pT2 fit significantly ($p = 0.0001$) with a marginal $R^2 = 0.44$ and conditional $R^2 = 0.65$. The random effect was not significant ($p = 0.21$) although the amount of variance explained suggests that longitudinal designs may still be worthwhile in larger studies. PBVC was significantly associated with pT2 change ($p = 0.0002$) according to the formula $\Delta BV = 0.116\ \Delta pT2$ ($\Delta BV$ in %, $\Delta pT2$ in ms), with a unit change in pT2 (1 ms) predicting a 0.116% of change in brain volume.

## 4. Discussion

We used 16 h of dehydration, followed by 90 min of intensive rehydration, with MRI acquisition in both epochs, to evaluate the relationship between brain volume change and pseudo-T2 relaxation time change, estimated from a dual-echo turbo spin-echo sequence. The results show that brain volume changes with hydration status and that the change in brain volume significantly correlates with brain water content as measured by pT2. These results suggest that pT2 is sensitive to reversible brain water fluctuations, and the full multi-echo sequence may not be necessary. Since a dual-echo sequence requires relatively short acquisition time and is available on clinical scanners, the measurement of pT2 provides a clinically feasible marker of brain water content.

While we found robust changes in brain volume from the dehydrated to rehydrated state, in agreement with the study by Duning et al. (2005)), we did not observe a statistically significant change from the baseline to dehydrated state, unlike Duning et al. This may have resulted from the fact that the baseline scan was performed several days or more before the thirsting period commenced, so that hydration state was not controlled (subjects may have exhibited various states of dehydration already, limiting the brain volume change that could be measured from overnight thirsting), while the dehydration to rehydration epoch was well-controlled by design. In our study, we applied phantom-based correction for geometric distortion, which is a known instrumental source of volumetric variations (Caramanos et al., 2010). Subjects were scanned at similar times; 13 of 14 baseline scans were acquired in the morning, and all dehydration scans were acquired in the morning.

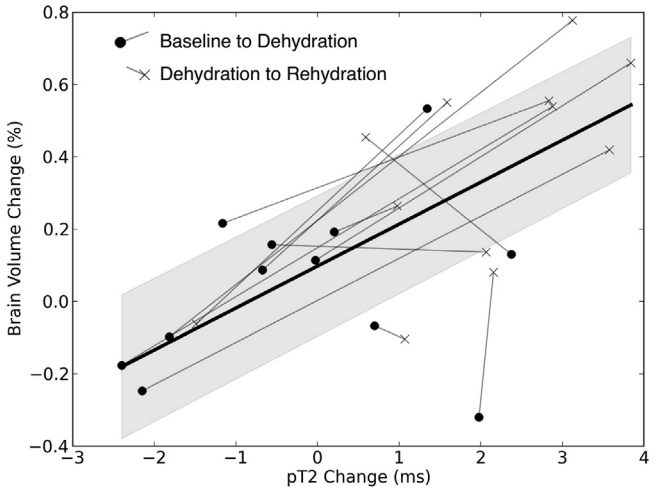


**Fig. 2.** Relationship between T2 relaxation time (pT2) change and percent brain volume change measured by the Jacobian integration method (PBVC). The change in these metrics between baseline and dehydrated scans is indicated by filled circles, and the change between dehydration and rehydration by crosses. Connected points indicate data from the same subject. The heavy line shows the model estimate, and the shaded area is the 95% confidence region.

Our previous experiments have shown that the scan–rescan absolute error of this TBM method was less than 0.2% while that of pT2 measurement was about 0.3% (Derakhshan et al., 2010). The volume change of 0.36% during dehydration–rehydration interval is well above the scan–rescan reproducibility error. These numbers are also in line with our finding of a conditional $R^2 = 0.65$, which indicates that more than half of the variance can be explained by pT2 given the dehydration or rehydration status. Assuming that there is no brain atrophy in healthy young subjects within a short time, the rest of the variance is likely the measurement error.

Our study has important implications for studies of brain atrophy, especially in MS clinical trials where so-called pseudoatrophy (accelerated decrease in brain volume on initiation of anti-inflammatory therapy) may be larger than the estimated treatment effect (Miller et al., 2007). The pT2 methodology can be used to distinguish whether a brain volume change may be due to reversible water-related fluctuations. Our finding shows that altered hydration status will affect both brain volume and pT2, and we hypothesize that tissue loss or atrophy will affect brain volume but not pT2. If reversible brain-water fluctuation accounts for the pseudoatrophy effect in MS clinical trials, brain atrophy outcomes should be measured after a delay, as in the study by Sormani et al. (2014). Frequent MRI scans can help to characterize the temporal pattern of brain volume change (Fisher et al., 2007). One could also include pT2 in the statistical model of brain volume change as a covariate to reduce the effect of water-related fluctuations. On the other hand, accelerated atrophy could be associated with tissue loss. For example, we have previously shown that accelerated brain atrophy after immune ablation and autologous hematopoietic stem cell transplantation for aggressive MS (Chen et al., 2006) is associated with accelerated brain atrophy soon after immunoablation (median of −3.2% in 2.4 months) without significant change in pT2, presumably reflecting either neurotoxicity or a change in the volume of inflammatory cells within the brain. While our pT2 technique cannot distinguish the different water compartments such as extracellular fluid, intracellular fluid and vascular fluid, it does allow us to quantify changes in bulk water, aiding the interpretation of changes in brain volume observed in clinical studies.

In conclusion, the measurement of pT2 can serve as a marker of changes in bulk brain water content and thus can help further investigate to what extent pseudoatrophy in multiple sclerosis may be related to shifts in brain fluid content.

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