HHS Public Access

Author manuscript

Magn Reson Imaging. Author manuscript; available in PMC 2019 October 10.

Published in final edited form as:

Magn Reson Imaging. 2017 December ; 44: 72–81. doi:10.1016/j.mri.2017.08.001.

Transverse relaxation of cerebrospinal fluid depends on glucose concentration

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Abstract

Purpose: To evaluate the biophysical processes that generate specific T_2 values and their relationship to specific cerebrospinal fluid (CSF) content.

Materials and methods: CSF T_{2s} were measured *ex vivo* (14.1 T) from isolated CSF collected from human, rat and non-human primate. CSF T_{2s} were also measured *in vivo* at different field strength in human (3 and 7 T) and rodent (1, 4.7, 9,4 and 11.7 T) using different pulse sequences. Then, relaxivities of CSF constituents were measured, *in vitro*, to determine the major molecule responsible for shortening CSF T_2 (2 s) compared to saline T_2 (3 s). The impact of this major molecule on CSF T_2 was then validated in rodent, *in vivo*, by the simultaneous measurement of the major molecule concentration and CSF T_2.

Results: *Ex vivo* CSF T_2 was about 2.0 s at 14.1 T for all species. *In vivo* human CSF T_2 approached *ex vivo* values at 3 T (2.0 s) but was significantly shorter at 7 T (0.9 s). *In vivo* rodent CSF T_2 decreased with increasing magnetic field and T_2 values similar to the *in vitro* ones were reached at 1 T (1.6 s). Glucose had the largest contribution of shortening CSF T_2 *in vitro*. This result was validated in rodent *in vivo*, showing that an acute change in CSF glucose by infusion of glucose into the blood, can be monitored *via* changes in CSF T_2 values.

Conclusion: This study opens the possibility of monitoring glucose regulation of CSF at the resolution of MRI by quantitating T_2.

Keywords

MRI; Relaxation time; CSF; Human; Monkey; Rodent

1. Introduction

Quantification of both longitudinal (T_1) and transverse relaxation times (T_2) has been widely used to help diagnosis and monitor the treatment of multiple diseases. Recently, there has been an increase in interest in quantitating relaxation times to provide more information. Examples include using relaxation time measurements to quantitate myelination in white

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matter to distinguish stages of multiple sclerosis [1], estimating iron levels during the progression of Alzheimer's disease [2], to segment brain throughout neurodevelopment [3,4], and to monitor hippocampal damage due to epilepsy [5,6]. Although quantitating relaxation times is a powerful tool, the underlying biophysical processes that ultimately generate T_1 and T_2 values and their relationships to tissue and CSF biochemistry, remain poorly understood.

The literature reports a range of values for brain relaxation times. In healthy brain tissue, there are relatively consistent results for T_1 measured at 1.5 T and above (0.9 s − 1.4 s) depending on tissue type and magnetic field. There are also relatively consistent results for T_2 of brain tissue ranging from 0.05–0.1 s also depending on field strength and specific sequences used [7–9]. Remarkably, CSF T_2 values in the literature are very wide, ranging from 0.2 to 2.2 s (11-fold variation). Like tissue T_1 and T_2, CSF T_1 values are relatively more consistent ranging from 2.8 to 4.6 s (2-fold variation). Table 1 gives a few examples of CSF T_2 reported in the literature. In all cases, T_2 is shorter than T_1 indicating that constituents of the CSF are having a significant effect on relaxation times. There is no report that indicates which constituents of CSF explain the shorter T_2 *vs* T_1. The literature describes CSF composition as a dilute mix of proteins (14–40 mg/dL [10]), lipids (1.03 mg/dL; [11]), glucose (50–79 mg/dL; [12]) and metals (3 mg/dL counting all 22 metals reported in the literature [13]). There are over 2630 proteins in healthy patient CSF [10] and the three most abundant ones are albumin (20 mg/dL), IgG (2 mg/dL; [14,15]) and transferrin (2 mg/dL; [16]). Other abundant CSF proteins such as IgA, IgM, haptoglobin and α2-macroglobulin have a concentration lower than 1 mg/dL [17]. Lipids are mostly represented by phospholipids (0.4–0.6 mg/dL), cholesterol (0.3–0.5 mg/dL), glycolipids (0.07–0.09 mg/dL) and lipoproteins (ApoE =0.5 mg/dL). The most concentrated metals in CSF are Mg (3 mg/dL), Fe (0.007 mg/dL) and Zn (0.005 mg/dL) [13].

The purpose of the present work was to determine the T_2 of CSF *ex vivo* and *in vivo* and explore the possible reasons for such a large discrepancy of T_2 values in the literature. Furthermore, it was determined which constituents of CSF are involved in generating these specific T_2 values.

2. Materials and methods

The Institutional Review Board approved all human study protocols, and informed consent was received from all participants. MRI and CSF studies were performed on human subjects recruited for natural history studies of multiple sclerosis and HTLV-1 Associated Myelopathy/tropical spastic paraparesis (HAM/TSP). All animal experiments were performed in accordance with NIH guidelines and were approved by the Animal Care and Use Committee of the National Institute of Neurological Disorder and Stroke, National Institutes of Health (Bethesda, MD USA).

2.1. Ex vivo and in vitro MRI experiments

2.1.1. Ex vivo CSF samples—CSF samples were collected from 3 different species: 3 healthy human subjects (all males, age of 36.7 ± 10 years), 3 rhesus macaques (2 males, 1 female, mean age 9 ± 4 years), and 4 CD rats with a permanent intracisternal cannulation

(all males, 3 months old; Charles River, USA). CSF was collected by lumbar puncture in humans in the context of ongoing clinical studies at the NINDS, NIH. CSF was obtained from non-human primates by transcutaneous cisterna magna puncture (one-time collection, 1–2 mL of CSF) and CSF from rats was obtained *via* a daily CSF collection (a maximum of 100 μL of CSF/collection was allowed) using the permanent intracisternal cannulation until reaching a final volume of 500 μL. Sampling periods was 24 h apart to allow for CSF production.

2.1.2. Standard in vitro solutions for T_2 comparisons—To correlate T_2 values with metal, protein and glucose concentration, the following solutions were prepared in 0.9% NaCl: bovine serum albumin (1.5–30 μM; BSA, Bio Rad, USA), $ZnCl_2$ (0–1 mM; Sigma-Aldrich, USA), $FeCl_2$ (0–1 mM; Sigma-Aldrich, USA), $MnCl_2$ (0–80 μM; Sigma-Aldrich, USA), $CuCl_2$ (0–1 mM, Sigma-Aldrich, USA) and glucose (0– 1 mM; Sigma-Aldrich, USA). BSA was used to mimic protein content of CSF. The pH range of these solutions was between 6.2 and 7.4.

To evaluate whether chemical exchange due to pH explains T_2 differences at different field strength, glucose and BSA solutions were prepared in 0.9% NaCl at a physiological concentration of 4 mM and 0.01 mM, respectively (see Fig. 4). The pH was adjusted to 2 by adding few drops of HCl 50 mM and to 10 by adding few drops of NaOH 50 mM.

2.1.3. Ex vivo and in vitro MRI acquisition—All *ex vivo* and *in vitro* experiments were performed in a vertical bore, 14.1 T MRI system (Bruker Biospin, Billerica, MA) using a volume transmit/receive coil. *Ex vivo* CSF and *in vitro* BSA and glucose solutions were also measured at 4.7 T MRI using a volume transmit and receive coil. In order to maintain constant temperature of the samples, the internal MRI temperature was set at 37 °C. Solutions (CSF or solute) were transferred in a 1 cm NMR glass tube and placed in a 37 °C water bath for at least 20 min prior to each experiment. Each tube was homogenized by vortex and directly placed in the NMR coil. A 3D image was performed to determine the sample position in the MRI. T_2 measurement of the entire sample was performed with a multiecho Car-Purcell-Meiboom-Gill (CPMG) spectroscopy sequence with TR = 20,000 ms, TE(2048) = 2/2048 ms (intervals of 1 ms) and time of acquisition (TA) = 2 min 40 s. This eliminated the presence of any residual imaging gradients. T_1 mapping was performed for *ex vivo* CSF samples only with a 2D RARE-VTR sequence with TR(6) = 500–20,000 ms; TE = 7.6 ms; TA = 9 min 25 and resolution of 0.625 × 0.625 mm.

2.2. In vivo MRI experiments

2.2.1. Human—*In vivo* CSF T_2 values were calculated from three healthy subjects (1 male and 2 females, mean age 39 ± 4 years).

A heavily T_2-weighted image was performed to visualize CSF without any contribution of brain tissue in the image. Conventional T_2 maps were obtained with a standard two point T_2 sequence and T_2 maps were obtained with a multiple TEs sequence that attempted to minimize applied field gradients. All these sequences were used on 2 healthy subjects (2 females, ages of 42 and 35 years) on a Siemens 3 T Skyra system (software version VD11) equipped with a 32-channel phased-array receive-only head coil. Conventional T_2 maps

were generated using a dual-echo fast-spin-echo sequence with TR = 3500 ms, TE = 11/101 ms, TA = 3.5 min, in-plane resolution 0.5 mm, and slice thickness 3 mm. The T_2 mapping was a 2D spin-echo sequence (Siemens product sequence) with TR = 10,000 ms and 32 TEs equally spaced between 30.5 and 945.5 ms, TA = 10.5 min, in-plane resolution 3 mm, and slice thickness 3 mm. Heavily T_2-weighted images were acquired using a three dimension, fast spin–echo sequence (FSE), prescan-normalize filter for receive coil homogeneity, TR = 4800 ms, TE = 752 ms, FA = 100°, scan acceleration factor GRAPPA = 2, echo-train-length = 421, fat-saturation mode = strong, 0.65 mm isotropic resolution, bandwidth = 543 Hz/Px, and TA = 4 min 43 s [18]. This last sequence was also used on a Siemens 7 T MRI on one healthy volunteer (40 y.o. male) with the same parameters except the resolution: in-plane resolution 1.5 mm, and slice thickness 1.5 mm.

2.2.2. Rodent—A total of fifteen rodents were used to perform heavily T_2-weighted images, conventional T_2 mapping (n = 3), and T_2-spectroscopy (n = 12) to remove the presence of any background imaging sequence gradientsFor all the scans, rodents were anesthetized with isoflurane (4% induction, 1.5% maintenance) and placed in a dedicated cradle equipped with byte and ear bars. Temperature and respiratory rate were monitored during the acquisition. A 3D image was performed to determine the animal position in the MRI.

2.2.2.1. Conventional T_2 mapping and heavily T_2 images.: Three rats (SD, 3 months old) were used for this experiment. Heavily T_2-weighted images and conventional T_2 mapping were performed on a 4.7 T MRI system (Bruker Avance III scanner and Bruker console) using a 72 mm ID birdcage coil and a surface coil to detect.

Three dimensional, heavily T_2-weighted images were acquired with a T_2-preparation (128 echoes, 3 ms spacing, M-LEV8 phase cycling) with a segmented FLASH readout [19], TR = 10 ms, TE = 384 ms, in-plane resolution 0.3 mm, and slice thickness 1 mm, TA = 24 min. Conventional T_2 maps were generated with a multi-slice-multi-echo (MSME) sequence, TR = 4000 ms, TE(20) = 10.95/219 ms (intervals of 10.95 ms), in-plane resolution 0.3 mm, slice thickness 1 mm and TA = 6 min 24 s. For the MSME sequence, we used crushers giving 2π phase dispersion across the slice (same gradient strength for all echoes), and a 1-ms windowed sinc pulse for the refocusing pulse.

2.2.2.2. T_2-spectroscopy.: Twelve mice (C57Bl6 male, 3 months old) were used to performed T_2-spectroscopy. T_2 quantitation was carried out using T_2 spectroscopy sequence on 4 different horizontal field strength MRIs: 1 T (Bruker, Icon), 4.7 T (Bruker MRI), 9.4 T (Bruker MRI) and 11.7 T (Bruker MRI). The experiments at 4.7, 9.4 and 11.7 T all used a large birdcage coil (72 mm I.d. at 4.7 and 89 mm at 9.4 and 11.7 T) for transmission and a 1 cm diameter surface coil for reception. This arrangement serves to localize the experiment, and in particular, reduces the signal from the aqueous part of the eye (to b 10% of the signal).

The spectroscopic evaluation of T_2 was obtained using a multiecho CPMG sequence with TR = 10,000 ms, TE(1024) = 2/2048 ms (intervals of 2 ms), Nav = 8, and TA = 1 min 20 s at 1 T; TR = 20,000 ms, TE(1024) = 2/2048 ms (intervals of 2 ms), Nav = 8 and TA = 2 min 40

s at 4.7 T, 9.4 T and 11.7 T. This spectroscopic evaluation of T_2 was performed *in vivo* (CSF flow) and *post mortem* (no CSF flow) to observe the eventual impact of CSF flow on CSF T_2 value. At the end of each *in vivo* scan, the mouse was euthanized by cerebral dislocation and immediately re-scanned to obtain the *post mortem* CSF T_2 value. Due to the short time between sacrificing the animal and MRI data collection it is not likely that the pH got acidic due to the long time scale of CSF acidification [20].

2.3. In vivo experiments to manipulate CSF glucose concentrations: glucose challenge

To determine if T_2 of CSF *in vivo* was sensitive to changes in glucose, experiments were done to manipulate CSF glucose in rodent. It is well established that CSF glucose changes with blood glucose concentrations [21], consequently, glucose was injected in the blood. Ten rats (SD, male, 3 months old) were used for this study. Five rats were fasted for 24 h before the experiment, and five other rats were fed *ad libitum*. The day of the experiment, rats were anesthetized with isoflurane (4% induction, 1.5% maintenance), and a non-survival surgery was performed to catheterize the femoral artery for blood collection and the jugular vein for glucose infusion. Two PE50 tubes of about 1 m each were used for the catheterization and blood clotting was avoided using 10% heparin (Hospira, USA) in the saline solution. Dead volume was about 0.4 mL. After surgery, the rat was placed in a dedicated cradle equipped with bite and ear bars. Temperature and respiratory rate were monitored during the acquisition.

Experiments were performed at 4.7 T (Bruker MRI), using a volume excitation and surface coil detection configuration. A 3D image was performed to determine the animal position in the MRI. T_2 measurements of the whole head was performed using a multi-echo CPMG sequence with TR = 20,000 ms, TE(1024) = 2/2048 ms (intervals of 2 ms). This measurement was repeated 3 times, every 30 min, for a total time of 1.5h. Before each T_2 measurement, glycemia was measured using a glucometer (OneTouch UtraMini, Lifescan, USA) and one-time usage test strips (OneTouch Utra, Lifescan, USA). For this measurement, blood was collecting from the femoral vein catheter going out of the MRI scanner. At the end of the first T_2 measurement, a bolus of glucose (0.4 mL, 20% glucose in water) was injected *via* the jugular vein catheter, followed by continuous glucose infusion (10% in water, 0.05 mL/min). The infusion was stopped at the end of the experiment, just after the end of the third T_2 measurement. The rat was then placed in a stereotaxic frame and the skin of the neck was shaved and opened. The cisterna magna was exposed by separating the neck muscles and then gently perforating with a 25G needle. CSF was then directly collected using a glass capillary and rapidly frozen at −20 °C for glucose analysis (see below).

2.4. MRI data analysis

2.4.1. Human—T_2 maps were calculated by performing a bi-exponential fit of the pixel intensities using the robust least absolute residuals method in Matlab (MathWorks, MA). CSF-ROIs were drawn by hand in the lateral ventricles. The median T_2 value within the CSF-ROI was reported as the CSF-T_2 value for each subject.

2.4.2. Rodent—T_2 mapping: T_2 values were calculated by performing a mono-exponential fit of the pixel or echo intensities. ROIs were drawn on the lateral ventricles, and the T_2 maps were derived by fitting.

T_2 measurement by spectroscopy: The T_2 data was fitted using a linear least squares with nonnegativity constraints method (NNLS; Matlab lsqnonneg function, MathWorks; [22]). For the data presented here, no regularization was used. T_2 values were assigned to 1000 bins with an equal separation of 2 ms (covers 2–2000 ms) for the fitting. We obtained a spectrum with three groups of peaks. The first group was assigned to brain and muscle tissue T_2, the second was assigned to fat T_2 and the third was assigned to CSF T_2 (Fig. 2B). The average value of peak assigned to CSF was used to quantitate the T_2.

2.4.3. Solutions (CSF and solute)—T_2 was extracted from spectroscopic measurements using NNLS method [23]. Only a single component was found in the solution data and the average value of this component was used.

For each solute (proteins, metals and glucose), ΔR_2 ($1/T_2$; s^{-1}) values were plotted against solute concentration (mM). Relaxivity r_2 ($mM^{-1}{\cdot}s^{-1}$) was then computed ($\Delta R_2 = r_2 \times$ [solute]). Each solute's concentration in healthy human CSF was measured. Based on this concentration, ΔR_2 value in human healthy CSF for each solute was calculated using $\Delta R_2 = r_2 \times [BSA] + b$ assuming the *in vitro* values for relaxivities.

CSF protein concentration was quantified according to the method of Bradford (Quick start Bradford protein kit, Bio Rad, USA). A reference of 1 $mg{\cdot}mL^{-1}$ of BSA was used [24]. CSF paramagnetic metal (Cu, Fe, Mn, and Zn) concentration was quantified by inductively coupled plasma mass spectrometry technique (IC-PMS, Exova, California). CSF glucose concentration was performed using a colorimetric technique (Gluc kit, Sigma, USA). A reference of 1 $mg{\cdot}mL^{-1}$ of glucose was used. T_1 mapping: T_1 values were calculated by performing a mono-exponential fit of echo intensities. ROIs were manually drawn in the area cor-responding to liquid in the tube and T_1 map was derived by fitting.

3. Results

3.1. Ex vivo CSF T_2 measurement

Data from human, rodent and non-human primate (NHP) are shown in Table 2. Saline was used as a control and had a T_2 of about 3.0 s. The average human CSF T_2 was 2.1 s. Rodent and NHP T_2s were 1.8 s and 1.9 s, respectively. Representative signal T_2 decay is shown in Sup Fig. 1.

3.2. CSF T_2 values from MRI are sequence and magnetic field dependent

Heavily T_2-weighted images of human brain (Fig. 1A) and rodent brain (Fig. 1C) show high signal within the CSF surrounding the brain and inside the ventricles. The contrast to noise ratio between CSF and tissue was ~ 30:1 for human and ~ 16:1 for rodent. A conventional T_2 mapping measured a T_2 of 887 ± 50 ms for human CSF at 3 T and 174 ± 27 ms for rodent CSF at 4.7 T (Fig. 1B and D). Representative T_2 decay curve for human and signal T_2 decay for rodent are shown in Sup Figs 2 and 3, respectively.

Changes in the T_2 mapping sequence to minimize applied imaging gradients led to an increased CSF T_2 measured from human brain of 2.02 s at 3 T (Fig. 2A). This is consistent with the *ex vivo* T_2 measurement at low field (4.7 T) and indicates that judicious use of MRI sequence parameters can get an accurate T_2 at 3 T. Interestingly, the apparent human CSF T_2 dropped to 850 ms at 7 T even though the sequence was the same as that used at 3 T. Representative T_2 curves are shown in Sup Fig. 2.

To explore the reasons for the apparent shorter T_2 at high field, the rodent brain was studied. To eliminate the possibility of MRI sequence causing effects, a spectroscopic T_2 measurement was made from the entire rodent head (Fig. 2B). The long T_2 of CSF compared to fat or tissue T_2 enabled an unambiguous assignment of CSF in the T_2 relaxograms. An apparent T_2 of 0.4 s was measured at 11.7 T significantly shorter than the *ex vivo* measurement. CSF T_2 was measured at 9.4, 4.7, and 1 T to determine the effects of magnetic field. The T_2 of rodent CSF *in vivo* was 1.5 s at 1 T approaching the *ex vivo* measurement of 1.8 s. Representative signal T_2 decay is shown in Sup Fig. 4.

CSF flow in and out of the slice or through residual field gradients would be expected to shorten CSF T_2. To test this hypothesis, CSF T_2 was measured in live rodent (CSF flowing) and in *post mortem* rodent (no CSF flow). CSF T_2 values were about the same in live and *post mortem* rodent at 1 T where residual magnetic field gradients are small (1.6 s; Fig. 2C). There was an increasing difference as the field strength increased and at 11.7 T there was a 77% difference in T_2 from live (0.4 s) and *post-mortem* rodent (0.7 s; Fig. 2C).

Data for the magnetic field dependence of *ex vivo* and *in vivo* rodent and human CSF R_2 as well as saline R_2 is plotted in Fig. 3. There is almost no change of saline R_2 measured at 4.7 T and 14.1 T (ratio of 0.94) and a bigger change was observed for *ex vivo* CSF of human and rodent between 4.7 T and 14.1 T (ratio of 0.85 and 0.70, respectively). Both *in vivo* human and rodent CSF R_2s decrease with decreasing field strength. The *in vivo* human CSF R_2 approaches the *ex vivo* values by 3 T and the *in vivo* rodent CSF R_2 approaches the *ex vivo* values only at 1 T.

3.3. What causes the shortening of CSF T_2 as compared to saline T_2?

To test whether paramagnetic metals, ions, proteins, and/or glucose can explain the T_2 relaxivity of CSF as compared to saline, *in vitro* relaxivities were measured. BSA was used as a surrogate for CSF protein. Relaxivity data for Zn, Cu, Fe, Mn, glucose and BSA is shown in Fig. 4. The concentration of each of the relevant species was measured in CSF samples (Fig. 4B). The concentration of metals was too low to significantly modify CSF T_2 relaxivity (b 0.5%). Proteins can explain approximately 15% of the shortening of CSF T_2. Interestingly, the glucose concentration is high enough to cause most of the shortening to CSF T_2 with respect to saline T_2. Indeed, the human CSF R_2 of approximately 0.5 is explained primarily by the R_2 of saline (approximately 0.3) plus the R_2 of glucose (approximately 0.2). The T_2 relaxivity of glucose is quite low (0.067 mM^{-2} s^{-1}) but the very long T_2 of CSF enables the high concentrations of glucose (~ 3 mM) to dominate the T_2 relaxivity of CSF.

3.4. Chemical exchange does not explain the in vivo vs ex vivo T_2 discrepancy at high field

A major mechanism to explain relaxation by glucose is exchange of protons between glucose and water. To test whether chemical exchange can explain the difference of T_2 values at high field between *in vivo* and *ex vivo* (data shown in Fig. 3), *in vitro* relaxivities of glucose and BSA solutions at different pH were measured. These data were compared to *in vivo* and *ex vivo* rodent data, as well as saline and shown in Fig. 5. The range of glucose solutions R_{2s} at pH 2, 7 and 10 were between 0.27 ± 0.02 and 0.65 ± 0.03 at 14.1 T and between 0.36 ± 0.01 and 0.59 ± 0.02 at 4.7 T. For the BSA, the range of R_2 at pH 2, 7 and 10 were between 0.26 ± 0.02 and 0.39 ± 0.04 at 14.1 T and between 0.34 ± 0.03 and 0.41 ± 0.01 at 4.7 T. These data overlap with *ex vivo* CSF R_2 (0.56 ± 0.01 at 14.1 T and 0.39 ± 0.03 at 4.7 T). Glucose R_2, at different pH are very different from *in vivo* CSF R_2 at high field (2.6± 0.1 at 11.7 T) suggesting that chemical exchange is not a major contributor to the T_2 difference between *in vivo* and *ex vivo* data at high field.

3.5. CSF T_2 depends on serum glucose concentration

The strong contribution of glucose to the CSF relaxivity opens the possibility of measuring changes in CSF glucose by measuring changes in CSF T_2. An *in vivo* experiment was performed to determine if CSF T_2 changes with changes in CSF glucose concentration. To increase the size of the glucose change caused by infusion, five animals were fasted to lower blood and CSF glucose concentrations and five other animals were fed *ad libitum*. One of the "fed *ad libitum*" rat could not be used, consequently the total of rats was nine. Experiments would have been best done at 1 T where the measured T_2 is longest (best dynamic range), however, due to availability of MRI time, these experiments were performed at 4.7 T. For each rat, CSF puncture was performed at the end of the experiment, before euthanasia; because of the technical difficulty of CSF puncture only seven CSF samples were obtained from the nine rats that were used in this experiment. The protocol used and the R_2 results are show in Fig. 5. There was good correlation between the R_2 measured in both the blood glucose (Fig. 5B) and CSF glucose (Fig. 6C). The correlation between CSF T_2 and blood glucose (glycemia) had a $R_2 = 0.71$ (p b 0.001; Fig. B) and the correlation between CSF T_2 and CSF glucose (glycorrhachia) had a $R_2 = 0.93$ (p b 0.005; Fig. 6C). Thus, CSF T_2 values change when CSF glucose is varied in this model of increased blood glucose.

The ratio of rodent CSF glucose and serum glucose was 0.41 (Fig. 6D) which is close to the value of 0.6 previously measured in rodent and human [21,25]. The apparent T_2 relaxivity for glucose derived from this experiment was 0.13 mM^{-1} s^{-1} (slope of line in Fig. 6C), higher than the *in vitro* value of 0.07 mM^{-1} s^{-1}. These discrepancies are likely due to the fact that the protocol was not designed to make sure glucose concentrations in the CSF had fully equilibrated and were sampled at the same time as T_2 was measured.

4. Discussion

In this study, the transverse relaxation time (T_2) of CSF was measured *ex vivo* and *in vivo* to determine the values for CSF T_2 and understand the factors that may lead to N 10-fold

variation in CSF T_2s in the literature (Table 1). *In vitro* CSF T_2 measurements were approximately 2 s for human, non-human primate and rodent. A similar value for T_2 of CSF could be obtained from human brain at 3 T and from rodent brain at 1 T. Higher field measurements led to apparent shorter T_2 most likely due to residual field gradients in the CSF caused by the brain tissue at the higher fields. While the *ex vivo* T_2 value of CSF was long, it was still shorter than the T_2 of saline. Glucose was identified as the predominant CSF component which can explain the shorter CSF T_2 *in vitro* as compared to saline. This opens the possibility of using CSF T_2 to monitor CSF glucose. This was confirmed *in vivo* in rodent by glucose infusion and simultaneous CSF T_2 measurement, opening the possibility of studying glucose regulation of CSF at MRI resolution.

4.1. In vivo CSF T_2 depends on field strength and sequences parameters

Ex vivo, CSF T_2 was much longer than a conventional *in vivo* CSF T_2 measurements using standard FSE or MSME sequences both in human and rodent brain. This result agrees with previous concerns raised in the literature. Indeed, it has been shown that conventional T_2 mapping sequences could be affected by residual field gradients and spurious echoes from these sequences [26,27]. Therefore, minimizing the residual imaging gradients using an adequate sequence and decreasing residual tissue gradients by using lower magnetic field strength as we did in our study, led to longer apparent T_2 measurements of CSF. Indeed, human *in vivo* CSF T_2 approached the *in vitro* data at 3 T while the rodent *in vivo* CSF T_2 approached *in vitro* data at 1 T. Longer echo times should lead to shorter apparent T_2s due to longer diffusion distances for water in the residual gradients. Flow of CSF in residual gradients could also shorten T_2 [28]. Therefore, in our study we showed that eliminating CSF flow in the *post-mortem* rodent brain lengthened the measured T_2 excepted at 1 T, where residual tissue gradients had a minimal effect on T_2.

A spectroscopy pulse sequence was used in rodents to eliminate imaging gradients completely which achieved the longest T_2's measured here. However, even at 1 T rodent CSF T_2 values *in vivo* did not reach the long values of T_2 measured *ex vivo* implying that tissue gradients still contribute to the shortening of T_2. Also, tissue gradient are expected to have larger impact on rodents CSF T_2 due to the small size of the CSF spaces compare to that in humans. In the human brain, a simple multiple TE spin echo acquisition with a long TR led to the longest T_2s as compared to clinically conventional used two point T_2 mapping pulse sequence based on a fast spin echo. At 3 T, human CSF measured was about 2 s which is on the low end of values obtained *ex vivo* (2–2.8 s).

Implementing a spectroscopic measurement of human CSF T_2, as was done for the rodents, would allow us to assess how many residual imaging gradients decreased the T_2 at 3 T. At 7 T, it is clear that tissue gradients begin to shorten the apparent CSF T_2 in humans since the CSF T_2 dropped from 2 to 0.85 s even when using the same MRI pulse sequence as used at 3 T.

Previous data from both, human and rodent, show a large range of values for T_2. Typically, these measurements did not attempt to minimize residual gradients that might affect T_2 measurements [28,29,30]. One report has longer CSF T_2 values [30,31,32,33] which were measured at low field and likely used imaging pulse sequences with lower applied field

gradients available at the time. *In vitro* human CSF T_2 values have been reported to be 2.64 s at 2.33 T [34] and 1.82 at 0.25 T [35] consistent with the results in the present study.

4.2. CSF T_2 depends on glucose concentration

Even though CSF has a very low content of biological molecules, the *ex vivo* T_2 of CSF in this study was still shorter than the T_2 of saline. In contrast, CSF T_1 was very similar to saline T_1. It is well known that relaxation agents tend to have a higher T_2 relaxivity than T_1 relaxivity. To understand the origin of the shorter T_2 of CSF as compared to saline, the relaxivity of major constituents of CSF were measured and compared to measured concentrations in CSF. The results indicate that metals concentrations were too low to significantly change the CSF T_2 while protein concentration was high enough to have a small effect on CSF T_2. In this study, we used albumin relaxivity as a representative of CSF protein. Thus, differences in protein relaxivities will probably affect this determination to some extent. Glucose is the component with the highest concentration in CSF and interestingly, the T_2 relaxivity of glucose is large enough to affect CSF T_2 proportionally to glucose CSF concentration. These results suggest that CSF T_2 can be used as a measure for CSF glucose concentration. Consistent with this, a change in CSF T_2 was measured as CSF glucose was varied in the rodent brain by varying the blood glucose level with infusion of glucose. This opens the possibility of studying regulation of glucose levels in human CSF at the MRI resolution.

There has been recent interest in using glucose as a contrast agent for MRI. Yadav et al., showed that T_2 of blood depends on glucose concentration [36]. They found a glucose T_2 relaxivity of 0.02, 0.06, and 0.08 $mM^{-1}\ s^{-1}$ at 3.0, 7.0, and 11.7 T, respectively. The relaxivity in blood at 11.7 T was 0.09 $mM^{-1}\ s^{-1}$. In agreement with their results, a relaxivity of glucose in water of 0.07 $mM^{-1}\ s^{-1}$ at 14.1 T and 0.04$mM^{-1}\ s^{-1}$ at 4.7 T was measured. There is a field dependence for all solutions most likely due to chemical exchange effects. For CSF, we show that exchange can be important for explaining the difference between *in vivo* and *in vitro* T_2 relaxivity but this is not large enough to explain differences at high field where the background gradient effects most likely explain the difference between *in vivo* and *in vitro* T_2. However, for accurate quantitation of glucose from T_2, care will have to be taken to account for factors that may affect exchange such as CSF pH. Yadav et al. performed an *in vivo* study after a glucose bolus injection while imaging the liver, and demonstrated a 10% drop in signal intensity after glucose infusion followed by recovery of the signal intensity after about 50–100 s. They concluded that glucose can be used as a T_2 contrast agent for MRI at concentrations that are already approved for human use.

Another approach to detect glucose as a possible MRI contrast agent is to measure chemical exchange of protons from glucose to water using the chemical exchange saturation transfer (CEST) MRI. Glucose, used as a CEST agent (glucoCEST), was first described by two groups: Walker-Samuel et al. and Chan et al. and shown to be detectable in rodent brain [37,38]. Recent work by Xu et al., used natural D-glucose as a contrast agent for CEST [39]. They explored the feasibility of using D-glucose for dynamic perfusion imaging to detect malignant mouse brain tumors based on blood-brain-barrier breakdown after an intravenous bolus of D-glucose. The time-resolved glucose signal changes were detected using

glucoCEST MRI. They showed that dynamic glucoCEST MRI is a feasible technique for studying brain tumor enhancement, reflecting differences in tumor blood volume and permeability with respect to normal brain. The long T_1 of CSF allows glucoCEST to be performed in CSF. In general, CEST experiments are much less sensitive than T_2 weighted MRI, however, they do not suffer from the issues of residual gradients that affect CSF T_2 measurements. CEST studies to determine glucose may complement CSF T_2 changes especially in cases where increased protein due to tissue degradation or increased cellularity may also affect CSF T_2.

In conclusion, this work demonstrates that quantitating CSF T_2 is very difficult due to the long T_2 of CSF. Any residual gradients either from the imaging sequence or residual tissue gradients especially at higher magnetic fields leads to an apparent shortening of the measured T_2. Interestingly, both the very long T_2 of CSF and the fact that glucose is the dominant contributor to the relaxation properties of CSF lead to the intriguing possibility that regulation of CSF glucose can be studied by simply using T_2 weighted MRI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This study was supported by the Intramural Research Program of the National Institute of Neurologic Disorders and Stroke (NINDS) (BN42639), National Institutes of Health.

Abbreviations:

CNR	contrast to noise ratio
CPMG	multiecho Car-Purcell-Meiboom-Gill
CSF	cerebrospinal fluid
T_1	longitudinal relaxation time
T_2	transversal relaxation time

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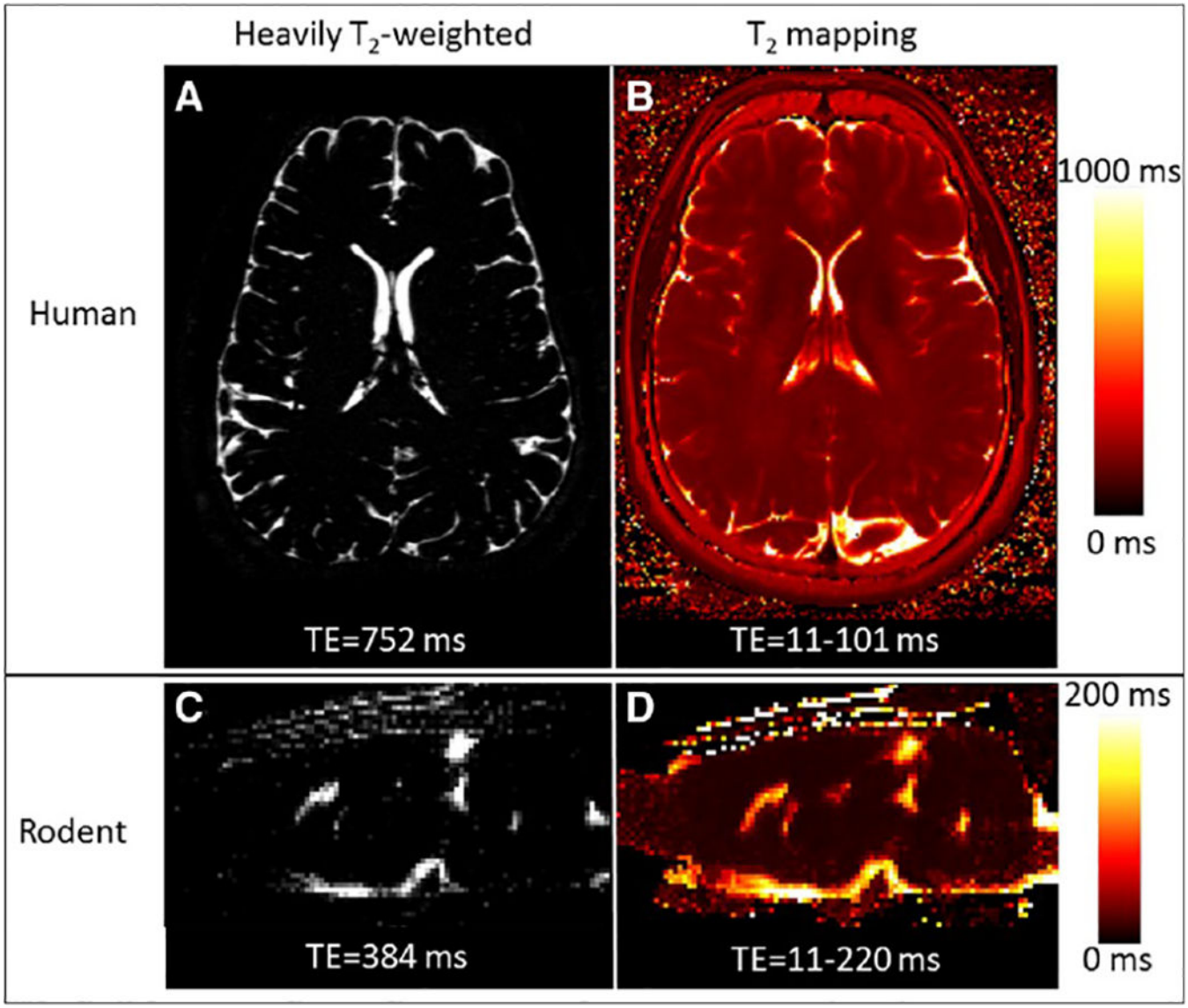
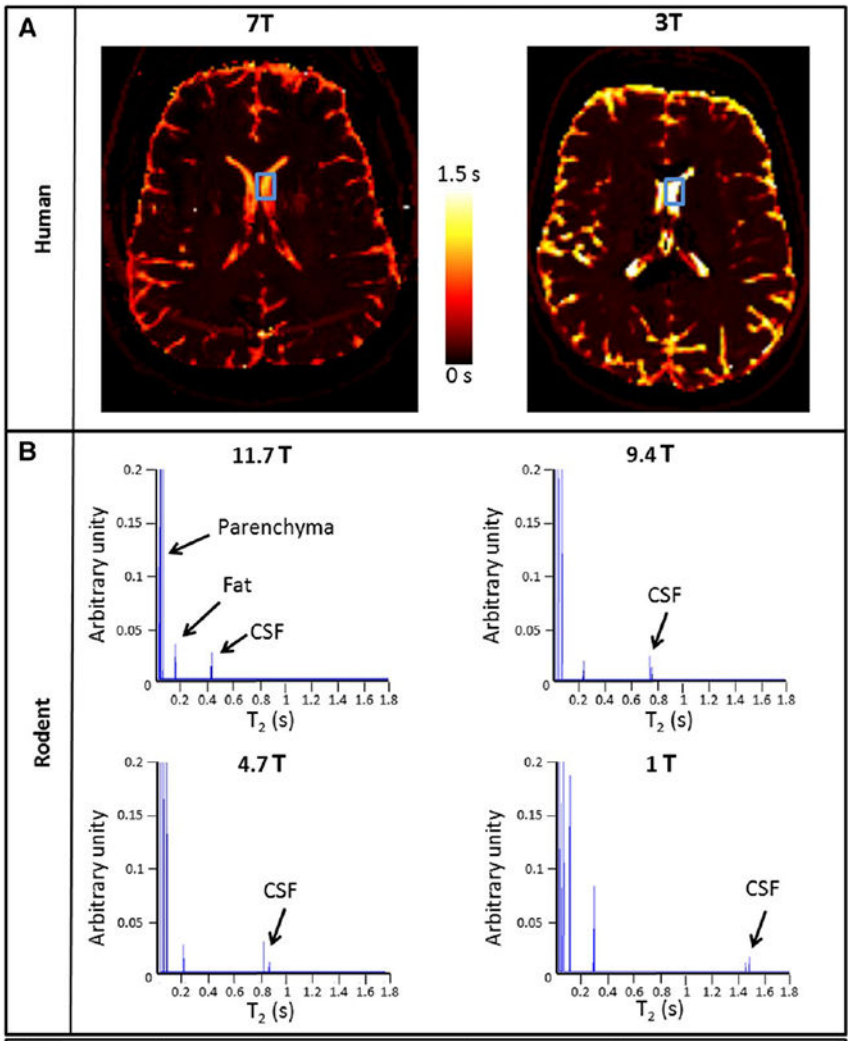


Fig. 1.

In vivo human and rodent CSF T_2 measurement. Human imaging was performed on a 3 T Siemens Skyra and rodent imaging on a 4.7 T Bruker. Heavily T_2-w images are shown in panel A (TE = 752 ms) and C (TE = 384 ms). B. Human T_2 mapping was performed using a FSE sequence with TR = 3500 ms, TE(2) = 11/101 ms, in-plane resolution 0.5 mm, and slice thickness 3 mm. D. Rodent T_2 mapping was performed using a MSME sequence, TR = 4000 ms, TE(20) = 10.95/219 ms (intervals of 10.95 ms), in-plane resolution 0.3 mm, and slice thickness 1 mm. ROIs were manually drawn in the lateral ventricles and the resulting T_2 values were 887 ± 50 ms for human CSF and 174 ± 27 for rodent CSF.



C

	Field (T)	TE (ms)	*In vivo* T_2 (s)	Post mortem T_2 (s)
Human	7	X32 [30.5 – 945.5]	0.85 ± 0.09	-
	3	X32 [30.5 – 945.5]	2.02 ± 0.13	-
Rodent	11.7	X1024 [2 - 2048]	0.39 ± 0.01	0.69 ± 0.09
	9.4	X1024 [2 - 2048]	0.67 ± 0.03	0.77 ± 0.07
	4.7	X1024 [2 - 2048]	0.95 ± 0.10	1.03 ± 0.21
	1	X1024 [2 - 2048]	1.61 ± 0.09	1.61 ± 0.33

Fig. 2.

In vivo human and rodent CSF T_2 measurement at different field strength. A. CSF T_2 was measured at different field strengths. In humans, T_2 mapping was performed at 7 T and 3 T. Images show an apparently longer CSF T_2 at 3 T than at 7 T (according to the color scale). The blue boxes represent the ROIs drown in the lateral ventricles. B. In rodent, spectroscopic sequences were used to measure CSF T_2 of the whole head at 11.7 T, 9.4 T, 4.7 T, and 1 T. There are 3 peaks in the spectra, the first corresponding to parenchyma with very short T_2 (0–0.1 s), the second to fat with short T_2 (0.1–0.2 s), and the last to CSF with

the longest T_2 (0.3–2 s). C. Quantification of CSF T_2 *in vivo* shows longer T_2 values at low field in both Human and rodent. For rodent, CSF T_2 *post mortem* got longer at higher field likely due to CSF flow in the brain/head gradients.

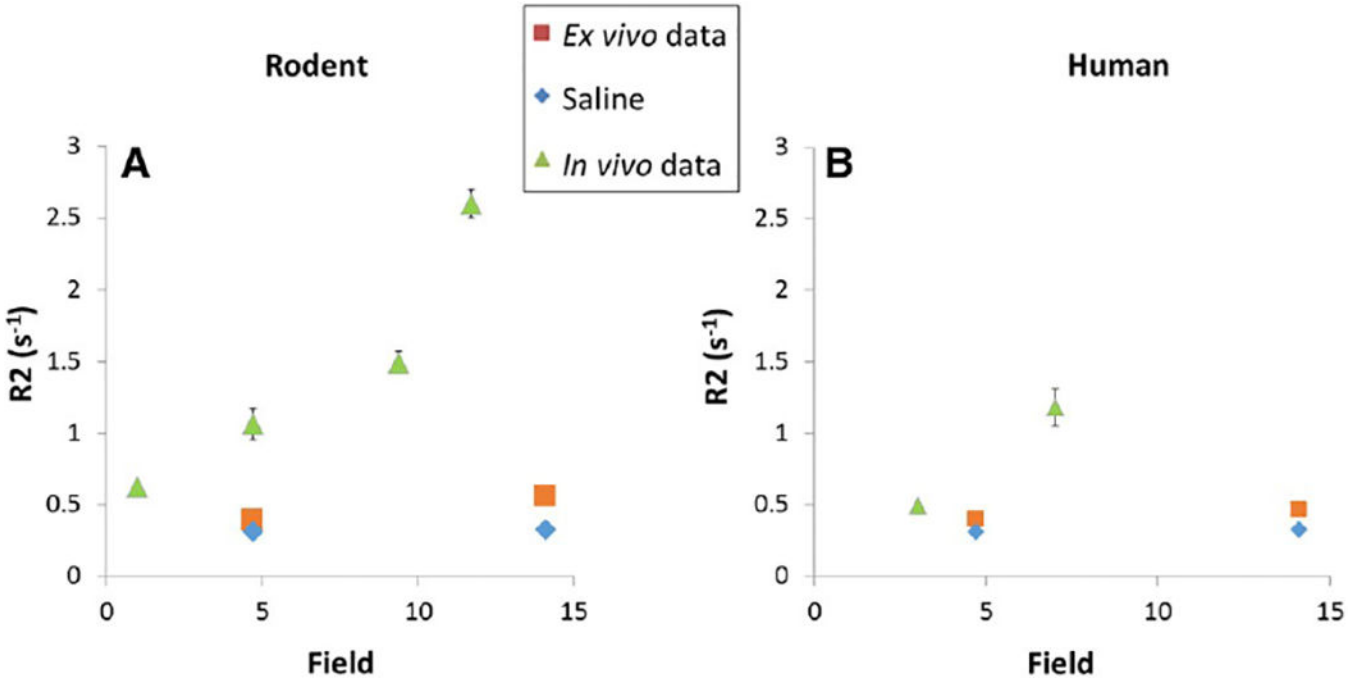
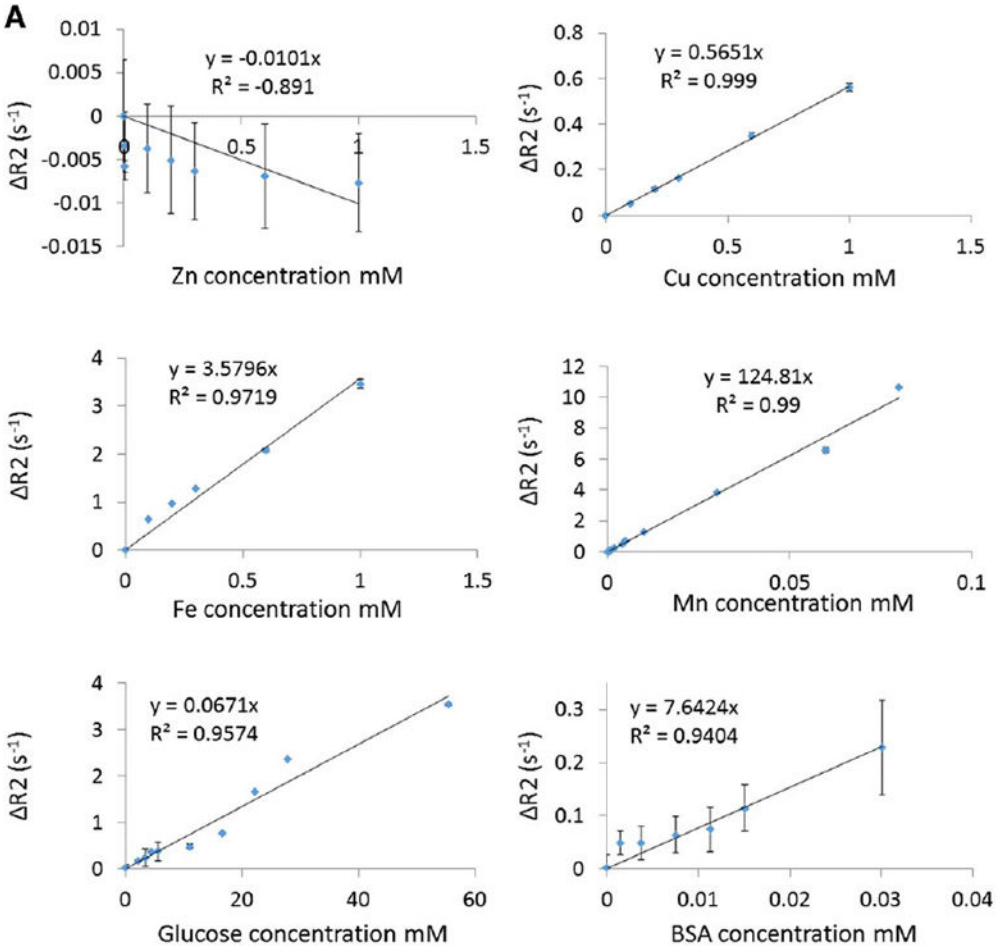


Fig. 3.

R_2 differences between saline, *ex vivo* and *in vivo* rodent and Human CSF at different field. A. Rodent CSF and saline T_2 measurement were performed using a multi-echo CPMG sequence with TR = 10,000 ms at 1 T and TR = 20,000 ms at 4.7 T, 9.4 T and 14.1 T; TE(1024) = 2/2048 ms (intervals of 2 ms) at 1, 4.7 and 9.4 T and TE(2048) = 2/2048 ms (intervals of 1 ms) at 14.1 T. Scans were performed at 1, 4.7. 9.4 and 11.7 T for *in vivo* data and at 14.1 and 4.7 T for *ex vivo* data. B. Human CSF T_2 measurements were performed using two different sequences. *In vivo* CSF T_2 measurements were performed by a multi-contrast spin echo sequence with TR = 10,000 ms and 32 TEs equally spaced between 30.5 and 945.5 ms. *Ex vivo* CSF and saline T_2 measurements were performed using the same multi-echo CPMG sequence detailed above for rodent.

A



B

Elements	Relaxivity r2 ($mM^{-1}.s^{-1}$)	Concentration in human CSF (mM)	Calculated R2 (s^{-1}) in human CSF
Zn	$-1.0.10^{-2}$	$2.6.10^{-4} \pm 4.4.10^{-5}$	$-2.6.10^{-6} \pm 4.5.10^{-7}$
Cu	0.6	$1.7.10^{-4} \pm 2.4.10^{-5}$	$1.0.10^{-4} \pm 1.4.10^{-5}$
Fe	3.6	$< 5.4.10^{-4}$	$< 1.9.10^{-3}$
Mn	124.8	$< 1.8.10^{-5}$	$< 2.3.10^{-3}$
Proteins (BSA)	7.6	$8.9.10^{-3} \pm 1.2.10^{-3}$	$6.8.10^{-2} \pm 1.1.10^{-2}$
Glucose	$6.7.10^{-2}$	3.4 ± 0.4	0.23 ± 0.03
Human CSF	-	-	0.47 ± 0.02

Fig. 4.

CSF compounds that can substantially change human CSF T_2 relaxivity. All T_2 measurements were performed on a 14.1 T Bruker MRI system at 37 °C using a CPMG sequence with TE(2048) = 2/2048 ms (intervals of 1 ms). A. Example of ΔR_2 plotted against glucose (n = 3) and BSA concentration (n = 3). The regression equation is written for each graph, showing the relaxivity r_2 for each species represented in this figure. B. Table showing the relaxivity r_2, the concentration and the calculated R_2 in human CSF for each element. Relaxivity r_2 was computed for each species (proteins, metals and glucose) from the plot

between ΔR_2 and solute concentration. The concentration of metals in human CSF was determined by IC-PMS and the one of BSA and glucose was quantified using colorimetric methods. Using r_2 and concentration values, we calculated the R_2 for each element in human CSF (example for the BSA: $R_2 = r_2 \times$ [BSA concentration]; $R_2 = 7.6 \times 8.9.10^{-3}$; $R_2 = 6.8.10^{-2}\ s^{-1}$).

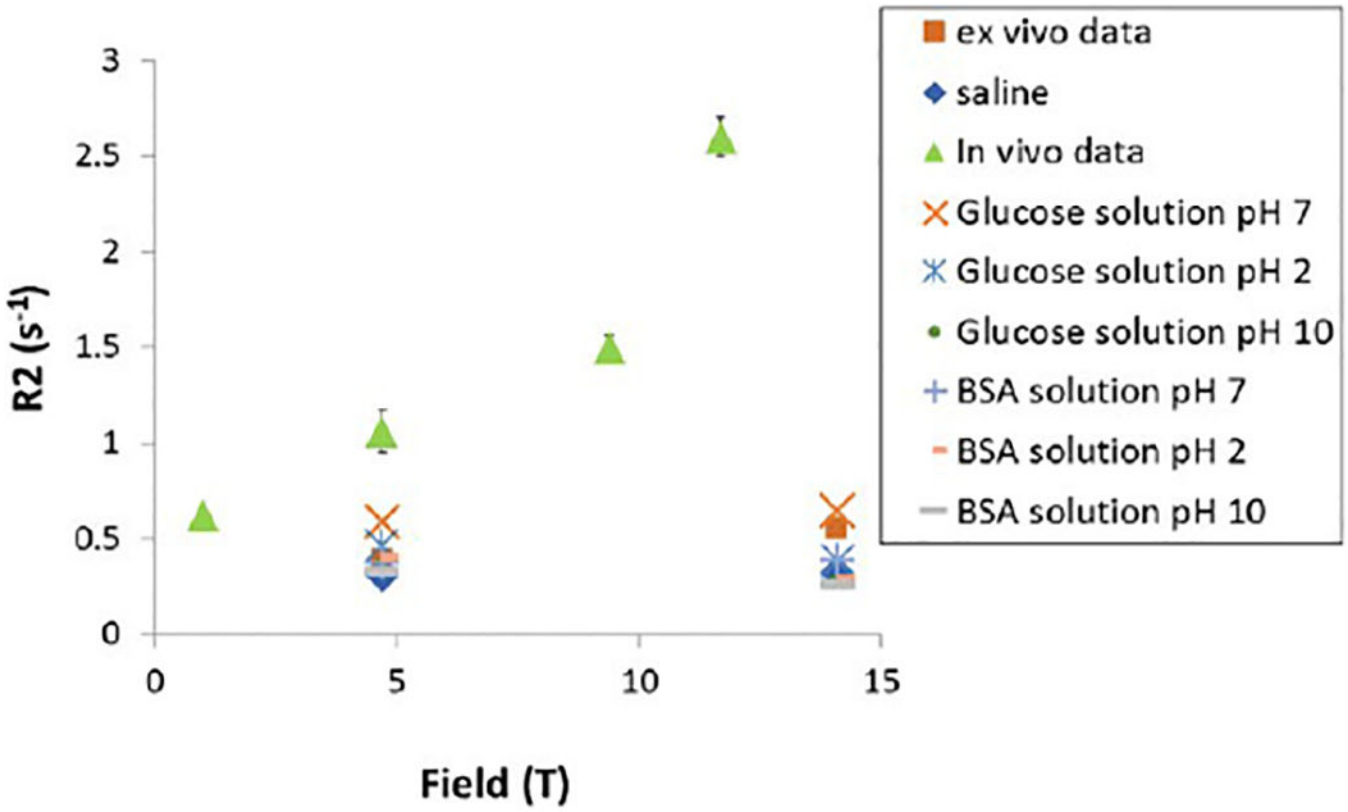


Fig. 5.

Effect of chemical exchange on rodent CSF R_2 values. T_2 measurement of rodent CSF *in vivo* and *ex vivo*, saline, glucose and BSA solutions were performed using a multi-echo CPMG sequence with TR = 10,000 ms at 1 T and TR = 20,000 ms at 4.7 T, 9.4 T and 14.1 T; TE(1024) = 2/2048 ms (intervals of 2 ms) at 1, 4.7 and 9.4 T and TE(2048) = 2/2048 ms (intervals of 1 ms) at 14.1 T. Scans were performed at 1, 4.7. 9.4 and 11.7 T for *in vivo* data and at 14.1 and 4.7 T for *ex vivo* data as well as for saline, glucose and BSA solutions. Glucose and BSA solutions were prepared in saline at a concentration of 4 mM and 0.01 mM, respectively, to mimic CSF physiological values. The pH of both, glucose and BSA solutions is at 7 and it was changed to be acidic (pH 2) and basic (pH 10).

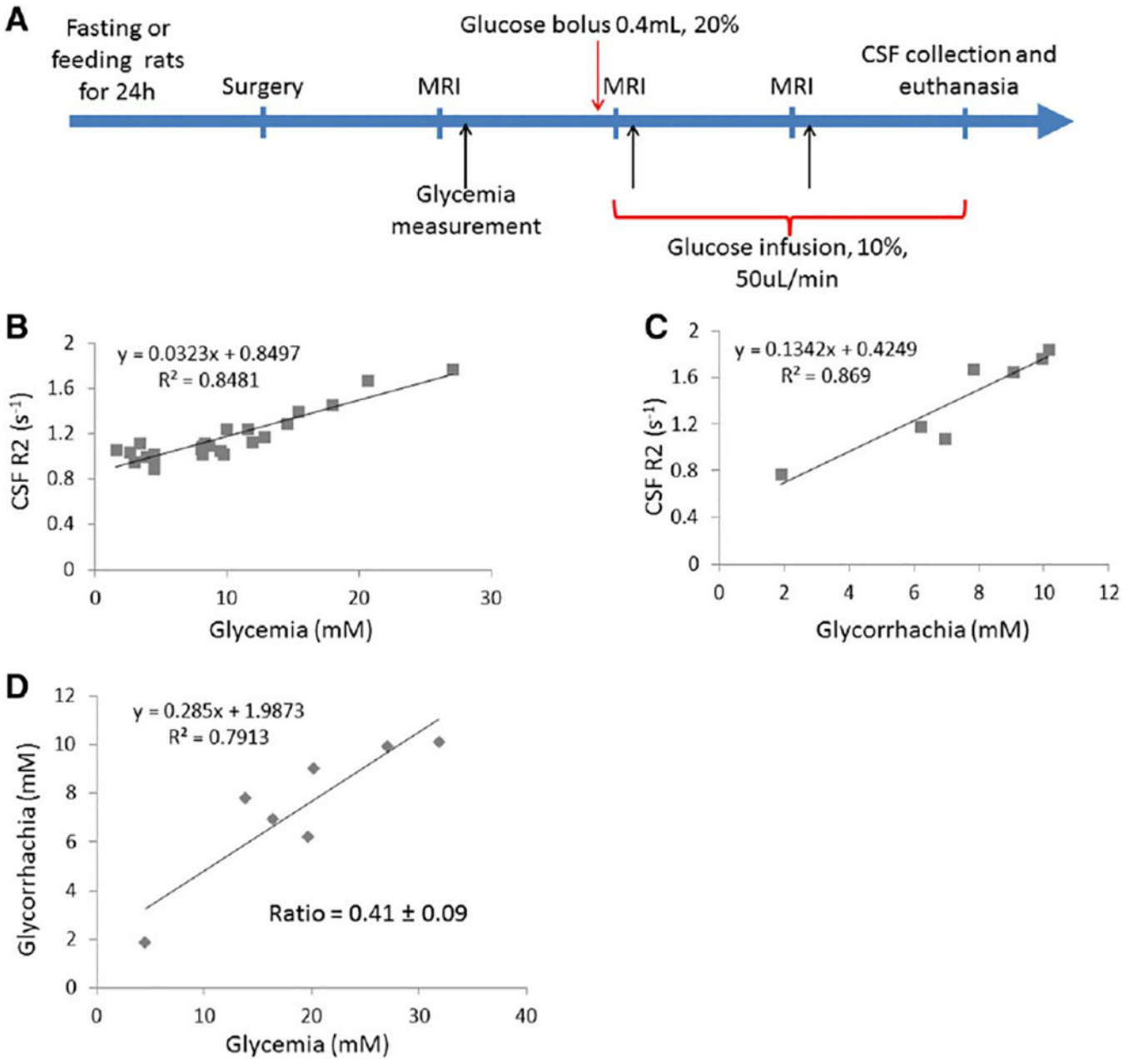


Fig. 6.

CSF T_2 depends on glycemia A. Experimental scheme. Surgery was performed under anesthesia to catheterize both jugular vein and femoral artery. Black arrow corresponds to serum glucose concentration measurement *via* the femoral artery. The rat is kept within the MRI scanner (4.7 T Bruker MRI system). B. CSF R_2 values measured at 3 time point as shown in A using the spectroscopy sequence, plotted against serum glucose concentration values (n = 9 rats). C. CSF R_2 values measured at the end of the experiment, just before CSF collection, plotted against CSF glucose concentration values (n = 7). CSF was collected just

after the last MRI acquisition and before euthanasia. D. Glycorrhachia as a function of glycemia (n = 7 rats). The ratio of glycorrhachia to glycemia is 0.42.

Table 1

CSF relaxation times T_1 and T_2 from different reports in the literature. Relaxation times were measured *in vivo* in healthy human at different field strengths.

Magnetic field (T)	**Human CSF *in vivo***		**Literature**
	T1 (s)	**T 2 (s)**	
0.15	4.36	1.76	Hopkins et al., 1986 [32]
0.35	2.72	0.17	Kjos et al., 1985 [40]
0.6	4.22	2.19	Hopkins et al., 1986 [32]
1.4	4.31	-	Hopkins et al., 1986 [32]
1.5	-	0.15	Larsson et al., 1989 [33]
3	-	0.50	Piechnik et al., 2009 [29]
4	4.55	0.70	Jezzard et al., 1996 [30]

Table 2

T_1 and T_2 of *ex vivo* CSF samples. CSF samples were collected from healthy humans, rats and monkeys. The relaxation times were measured at 37 °C on a 14.1 T Bruker scanner using a multi-echo CPMG sequence with TR = 20,000 ms, TE(2048) = 2/2048 ms (intervals of 1 ms). Values are represented as mean ± standard deviation.

Sample name	n	T_1 (s) at 14.1 T	T_2 (s) at
Saline	4	4.20 ± 0.29	3.07 ± 0.24
Human	3	4.22 ± 0.30	2.14 ± 0.11
Rodent	4	3.98 ± 0.03	1.79 ± 0.04
NHP	3	4.04 ± 0.21	1.87 ± 0.13