Published in final edited form as:

Magn Reson Med. 2018 August ; 80(2): 521–528. doi:10.1002/mrm.27046.

Quantitative assessment of cerebral venous blood T_2 in mouse at 11.7T: Implementation, optimization, and age effect

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Abstract

Purpose—To develop a non-contrast-agent MRI technique to quantify cerebral venous T_2 in mice.

Methods—We implemented and optimized a T_2 -Relaxation-Under-Spin-Tagging (TRUST) sequence on an 11.7 Tesla animal imaging system. A flow-sensitive-alternating-inversion-recovery (FAIR) module was used to generate control and label images, pair-wise subtraction of which yielded blood signals. Then, a T_2 -preparation module was applied to produce T_2 -weighted images, from which blood T_2 was quantified. We conducted a series of technical studies to optimize the imaging slice position, inversion slab thickness, post-labeling delay, and repetition time. We also performed three physiological studies to examine the venous T_2 dependence on hyperoxia (N=4), anesthesia (N=3), and brain aging (N=5).

Results—Our technical studies suggested that, for efficient data acquisition with minimal bias in estimated T_2 , a preferred TRUST protocol was to place the imaging slice at the confluence of sagittal sinuses with an inversion-slab thickness of 2.5mm, a post-labeling delay of 1000ms and a TR of 3.5s. Venous T_2 values under normoxia and hyperoxia (inhaling pure oxygen) were 26.9 ± 1.7 and 32.3 ± 2.2 ms, respectively. Moreover, standard isoflurane anesthesia resulted in a higher venous T_2 compared to dexmedetomidine anesthesia (N=3, $p=0.01$) which is more commonly used in animal fMRI studies to preserve brain function. Venous T_2 exhibited a decrease with age (N=5, $p<0.001$).

Conclusion—We have developed and optimized a non-invasive method to quantify cerebral venous blood T_2 in mouse at 11.7T. This method may prove useful in studies of brain physiology and pathophysiology in animal models.

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Keywords

blood oxygenation; cerebral venous T₂; TRUST; hyperoxia; anesthesia effect; aging effect; mouse; MRI

INTRODUCTION

Brain oxygen extraction fraction (OEF) and cerebral metabolic rate of oxygen (CMRO₂) represent important biomarkers of neural activities and brain diseases. These physiological parameters are traditionally a niche market of positron emission tomography (PET) imaging, which in itself is a complex procedure and requires the injection/inhalation of ¹⁵O labeled radiotracers as well as continuous arterial blood sampling to measure the arterial input function (1,2). However, such procedures are not feasible for animal models such as mice because, among other obstacles, there is simply not enough blood to be sampled during the experiment given that total blood volume in mice is generally about 1~2 ml (3). Thus, little is known about oxygen homeostasis in mice. On the other hand, mouse experiments provide access to a wide variety of disease models (4–8) important for understanding disease mechanisms, studying disease evolution (9,10), and evaluating therapy feasibility and efficacy (11,12). Therefore, development of non-invasive and non-contrast-agent techniques to measure brain OEF and CMRO₂ is expected to have profound potentials in studies of animal disease models.

The present study aims to develop a T₂-based MRI technique for quantifying cerebral venous oxygenation (Y_v), which is a critical step toward measurements of OEF and CMRO₂ in live mice. Y_v is defined as the oxygenation fraction in the venous blood (13). With the knowledge that, at baseline hematocrit, blood T₂ depends primarily on oxygenation levels, T₂ measurement has been explored as a method to obtain Y_v along with a proper calibration plot (14). The T₂-Relaxation-Under-Spin-Tagging (TRUST) technique was originally developed as a non-contrast-agent MRI technique to estimate Y_v by measuring venous blood T₂ in human (15,16). It utilizes the spin-tagging principle (17,18) to separate blood signals from surrounding tissue signals, thereby minimizing partial volume effects and mitigating the influence of subjective voxel selections. Over the past few years, apart from the various technical studies on TRUST (19–23), it has been broadly studied technique has been applied extensively to neonatal studies (24,25), aging studies (26), and a wide range of neurological and psychiatric disorders including Alzheimer's disease (27), multiple sclerosis (28), Sickle cell disease (29), anorexia (30), cocaine addiction (31), cannabis users (32), hepatic encephalopathy (33), end-stage renal disease (34), acute mountain sickness (35,36), children primary nocturnal enuresis (37) etc.

Despite of these applications in humans, TRUST MRI has never been applied in mice. Here, we conducted a series of studies to implement and optimize the TRUST technique for the measurement of cerebral venous T₂ of mice in vivo. Several key imaging parameters in TRUST MRI including imaging slice location, inversion thickness, post-labeling delay, and TR were separately optimized. Changes in venous blood T₂ consequent to a hyperoxia

challenge and anesthesia type were assessed. The effect of aging on cerebral venous T_2 was characterized and the results were compared to those in humans.

METHODS

TRUST pulse sequence

As shown in Fig. 1a, the TRUST sequence for the mouse study starts with a presaturation module to suppress signals within the imaging slice. Then, a flow-sensitive-alternating-inversion-recovery (FAIR) module (17) is applied to invert the spins in the inversion slab (yellow box, Fig. 1b). For convenience, inflow spins outside the inversion slab are dubbed as fresh spins. A post-labeling delay (PLD) is applied after the FAIR module to allow fresh venous spins to flow into the image slice. At the end of the PLD period, a non-selective T_2 -preparation pulse train is used to modulate T_2 -weighting while minimizing the blood outflow effect (38). Immediately after the T_2 -preparation, signals within the image slice (red line, Fig. 1b) are excited and measured by a segmented echo-planar-imaging (EPI) acquisition scheme. Following signal acquisition, a post-saturation module is employed to remove spin magnetization history so that RF pulses and timings applied in the current TR period do not appreciably affect signal in the next TR (16). Following the post-saturation module, a period of time is waited to allow the longitudinal magnetization to partially recover before the next inversion pulse is applied, so that a repeatable initial signal level is achieved. The above scan is known as the “control” scan. A “label” scan is also performed in which the FAIR module is non-selective.

Signals within the image slice originate from two parts: stationary tissue and flowing blood. The stationary tissue signal is identical between the control and label scans. By contrast, fresh blood spins follow inverse recovery in label scan but are uninverted in the control scan. Thus, a pair-wise subtraction cancels out tissue signal while isolating pure blood signal. This difference signal, when measured at a range of T_2 -preparation durations, allows for mono-exponential fitting to estimate venous blood T_2 (T_{2v}).

MRI experiments

All MRI experiments were performed on an 11.7T Bruker Biospec system (Bruker, Ettlingen, Germany) with a horizontal bore as well as an actively shielded pulse field gradient (maximum intensity of 0.74 T/m). The institutional animal care and use committee approved the experimental protocol. A total of 18 mice were studied. Of these, five C57BL/6 younger mice with body weight of 18~20 g (age 16~18 weeks) and five older mice (age 86~93 weeks) with body weight of 30~35 g were used in the aging study. The remaining 8 BALB/c mice (age 22~28 weeks) with body weight of 20~26 g were used in the remaining studies.

Animal preparation—Unless otherwise specified, anesthesia was administered using 2.0% vaporized isoflurane for 15 min, then switched to 1.5% for another 15 min, and maintained at 1.0% until the end of experiments. Mice were positioned with a bite bar and ear pins at the 10th minute under 2.0% isoflurane inhalation, and were placed on a water-heated animal bed with temperature control. Respiration was free and monitored during the

experiment to ensure the mouse was alive. Images were acquired using a 72-mm quadrature volume resonator as transmitter, and a four-element (2×2) phased array coil as receiver. The B_0 field over the mouse brain was homogenized by a global shimming (up to 2nd order) based on a subject-specific pre-acquired field map. Seven sub-studies, including four technical, two physiological, and one aging study, were performed as detailed below.

Study I—Imaging slice position. In order to place the imaging slice, a time-of-flight (TOF) scan was first performed to visualize the mouse's arterial and venous blood vessels. The TOF scan used the following parameters: TR/TE = 17/3.3 ms, field of view (FOV) = 16 mm × 16 mm, matrix size = 256 × 256, 25 axial slices, slice thickness = 0.5 mm, and scan duration = 2 min. The confluences of sagittal sinuses were identified as the location where the straight, superior, and transverse sagittal sinuses join each other (according to the multi-slice TOF images). To determine the optimal imaging slice location, six consecutive slices centering around the confluence were chosen, as shown in Figure 2a, from rostral to caudal direction. The optimal slice position for TRUST MRI was determined based on the largest difference signal criterion (N=3). Other imaging parameters of the TRUST sequence were: field of view (FOV) 16 mm × 16 mm, matrix size 128 × 128, voxel size 125 μm × 125 μm, and imaging slice thickness 0.5 mm. A segmented gradient-echo EPI was utilized in TRUST acquisition with a TE of 6.5 ms, a phase-encoding bandwidth of 3.1 kHz, and an echo-train length of 5.2 ms. Partial Fourier or navigator echoes was not used in the acquisition. No parallel imaging was used and the images were reconstructed with a sum-of-squares method. Since we are interested in signal intensity rather than actual T_2 value in this sub-study, only the shortest effective TE (eTE) was acquired.

Study II—Inversion slab thickness. In the control scan of the TRUST sequence, a selective inversion pulse is used to invert spins in the imaging slice. The inversion pulse used a default adiabatic pulse provided by the Bruker system and was a hyperbolic secant pulse of 15 ms in duration. The nominal flip angle of the pulse was 180°. Inversion efficiency of this pulse was found to be 91.5±0.8% based on additional experiments (data not shown) in mouse brain (N=2). Due to imperfection in frequency profiles of radiofrequency (RF) pulses, the inversion slab is generally set to be slightly wider than the image slice to ensure sufficient inversion of the imaging slice but minimally affecting inflowing spins from outside the imaging plane. We therefore conducted a study to determine the optimal inversion slab thicknesses by investigating values of 1.5, 2.5, 3.5, 4.5, 6.5, and 8.5 mm. This corresponds to a fixed imaging slice thickness of 0.5 mm with varying additional inversion slab thickness of 0.5, 1.0, 1.5, 2.0, 3.0, and 4.0 mm on each side. The PLD was set to be 1000 ms. The optimal inversion thickness was determined based on the largest vessel signal while maintaining minimal tissue signal in the difference (i.e. control-label) image (N=3). The other imaging parameters were identical to those in Study I.

Study III—Post-labeling delay (PLD). PLD should be chosen such that fresh venous spins have reached the imaging slice yet the labeling effect has not decayed away. We therefore studied the dependence of the difference signal on PLD by testing 13 PLDs ranging from 200 to 2600 ms at a step of 200 ms (N=3). We note that our sequence used a post-saturation

module after signal acquisition and a fixed recovery time (4000 ms in this study) so that the longitudinal magnetization before the next TR is the same regardless the PLD values.

Study IV—Repetition time (TR). The optimal choice of TR is dependent on a tradeoff between signal per image and the number of images within a given scan duration. Seven TRs of 2.0, 3.5, 5.0, 6.5, 8.0, 10.0, and 12.0 s were tested. The optimal TR was determined based on the maximum signal per unit time (N=2).

Study V—Sensitivity of the technique to oxygenation changes. The optimized TRUST protocol was performed under both normoxic (breathing room air) and hyperoxic (breathing 100% oxygen) conditions (N=4). According to previous studies in human (39), inhalation of 100% oxygen is expected to increase the venous blood oxygenation by 10%, but have minimal effects on heart rate, breathing rate, or arterial oxygen saturation (because arterial oxygen saturation is close to 100% even in room air breathing). The TRUST scan used the following sequence parameters: inversion slab thickness of 2.5 mm, PLD of 1000 ms, TR of 3.5 s, matrix size of 128×128, FOV of 16 mm×16 mm, slice thickness of 0.5 mm, EPI factor of 16, three eTE values of 0.25, 20, and 40 ms, scan duration =2.8 minutes. Venous T₂ values were compared across conditions.

Study VI—Anesthesia effects on venous T₂. TRUST protocol was performed under two anesthetic regimens. One was the standard isoflurane (ISO) protocol and the other a bolus of 0.2 mg/kg dexmedetomidine followed by continuous 0.5% isoflurane (40,41) (denoted as DEX+ISO). To allow wash-out of anesthesia history effects, the two experiments were performed on different days (N=3).

Study VII—Age effect on venous T₂. In a separate cohort of ten mice (5 younger mice at an age of 16~18 weeks and 5 older mice at an age of 86~93 weeks), we applied the optimized TRUST protocol to study the age influence on venous T₂. The TRUST measurements were repeated three times in each mouse to evaluate reproducibility.

Data analysis

All processing procedures were conducted with a custom-written graphic-user-interface (GUI) tool built on MATLAB (MathWorks, Natick, Massachusetts). For all studies described above, subtraction between the control and label images was performed. A region of interest (ROI) was manually drawn on the difference image to encompass the sinus confluence. Four voxels within the ROI with the largest difference signals were selected for spatial averaging. Studies I through IV focused on maximizing the difference signal intensity. For Studies V through VII, the TRUST sequence was performed at eTE of 0.25, 20, and 40 ms. Thus, a mono-exponential fitting of the difference signal yielded a T_{2v} estimation. The student t test was performed in some studies to compare T_{2v} under different conditions with a statistically significant level of p<0.05.

RESULTS

Imaging slice position

Figure 2b shows control, label, and difference (i.e. control-label) images at six different TRUST slice positions. Corresponding TOF angiogram slices are also shown. Difference signal intensities (averaged over 4 peak voxels) are shown in Figure 3. The highest signal intensity appeared in slice #3, the position of confluence of sagittal sinuses where major veins converge. Therefore, the TRUST slice was placed at the sinus confluence for the remainder of the study.

Inversion slab thickness

Figure 4a shows difference signal in blood and tissue regions-of-interest as a function of inversion slab thickness of the FAIR pulse. Based on the goal of maximizing blood and tissue signal difference, the optimal inversion slab thickness was determined to be 2.5 mm. It should also be pointed out that the signal curve is relatively flat up until an inversion slab thickness of 4.5 mm (Figure 4a), suggesting that the slab thickness is not a critical parameter and can take a range of values without significantly affecting the performance of the sequence. A sharp signal reduction is seen at a slab thickness of 6.5 mm and beyond, suggesting that the maximum distance the blood can travel within the PLD was between 4.5 and 6.5 mm.

Post-labeling delay (PLD)

Figure 4b shows control, label, and difference signals in the sagittal sinus as a function of PLD. It can be seen that a PLD of 1000 ms exhibits the highest difference signal.

Repetition time (TR)

Figure 4c displays the intensity of difference signal as a function of TR. Considering that scan duration is proportional to TR, we also displayed signal per unit time (i.e. difference signal divided by square root of TR), which provides an indicator of signal-to-noise ratio. Based on these results, a TR of 3.5 s is considered an optimal value.

The above studies serve to optimize the TRUST pulse sequence. The following studies aim to test its sensitivity in detecting changes.

Hyperoxia challenge

Figure 5 shows a complete dataset of TRUST MRI under normoxia. Monoexponential fitting of the difference signal in the sagittal sinus is also shown. T_{2v} values during normoxia and hyperoxia were found to be 26.9 ± 1.7 and 32.3 ± 2.2 ms, respectively. There was a significant (paired t test, $p < 0.001$) increase in T_{2v} due to hyperoxia.

Anesthesia effect

Figure 6 summarizes TRUST results under two different anesthetic regimens. T_{2v} values were significantly (paired t test, $p = 0.01$) lower when using the DEX+ISO scheme, compared to the ISO scheme.

Age effect

Figure 7 shows results of the aging study. T_{2v} values were significantly ($p<0.001$) lower in older (86~93 weeks) than in younger (16~18 weeks old) group. Coefficient of variation (CoV), calculated by standard deviation across repetitions divided by average, was found to be comparable ($p=0.26$) between the two groups.

DISCUSSION

To the best of our knowledge, the present study represents the first report of imaging cerebral venous T_2 in mice. We conducted a series of technical studies to optimize the TRUST MRI sequence for mouse imaging and applied the technique in studies of hyperoxia effects, anesthesia influence, and aging. The data revealed that TRUST MRI can estimate T_{2v} in less than 3 minutes with a CoV of approximately 5%. The technique showed an excellent sensitivity in detecting venous T_2 changes associated with physiological challenges and aging.

Measurement of venous oxygenation is a critical step toward non-invasive assessment of brain metabolism and energy consumption. To estimate cerebral metabolic rate of oxygen ($CMRO_2$), one can combine TRUST MRI with additional measures of arterial oxygenation and cerebral blood flow (CBF), which can be obtained with phase-contrast MRI or arterial-spin-labeling (ASL) (42). Previous studies in humans have demonstrated the clinical utility of these techniques in several neurologic diseases such as Alzheimer's disease and multiple sclerosis (27,43). Since mouse models of neurologic diseases represent an important research area in terms of mechanistic understanding, longitudinal trajectory characterization, and preclinical stage of drug efficacy testing, it is highly valuable to implement the TRUST and related techniques to mouse MR imaging and lays a foundation for seamless translation between preclinical and clinical imaging.

According to our optimization results, the imaging slice of TRUST MRI should be placed close to the confluence of sagittal sinuses. This finding is consistent with reports in human TRUST studies, where the imaging slice is usually positioned to intersect the superior sagittal sinus close to the confluence (15). This is most likely attributed to the rapid flow and large size of the vessel at this location. We also identified an optimal TR of 3500 ms and PLD of 1000 ms, which provide an excellent SNR for the given scan duration.

In human TRUST MRI which is typically performed at lower field strengths of 3T or 1.5T, the image acquisition is usually performed using a single-shot EPI. This is because T_2^* of venous blood at 3T is approximately 35 ms (44), which is relatively long compared to the echo-train-length of 10–20 ms. However, on 11.7T, the T_2^* of typical venous blood was found to be 8.9 ± 1.1 ms ($N=2$, unpublished observations), which limits the echo-train-length feasible at this field strength. As a result, the TRUST acquisition needs to be divided into several segments to minimize image distortion and signal voiding. The exact number of segments needed will depend on the expected T_2^* of the blood, which is in turn dependent on oxygenation level, flow velocity, hematocrit etc. Furthermore, the segment number or EPI factor is also related to bandwidth, partial Fourier factor, parallel imaging factor, and use of navigators, among other sequence parameters. Given the complex interplay of these factors,

the present study did not attempt to provide an optimal EPI factor. Instead, we use an empirical EPI factor of 16 (with an echo-train-length of 5.2 ms) throughout our investigation. Researchers are recommended to adjust the EPI factor of their study based on the expected T_2^* of the blood as well as their sequence echo-train-length.

The present study has primarily focused on gradient-echo EPI acquisitions. We have also tested to use a segmented spin-echo EPI (SE-EPI) acquisition scheme in TRUST MRI in five mice. When comparing signal stability between SE-EPI and GE-EPI TRUST data, it was found that the CoV between these two methods were comparable (Supporting Figure S1) and that the signal intensity of GE-EPI was slightly higher than that of SE-EPI. We also tested the feasibility of turbo-spin-echo (TSE) acquisition in TRUST MRI and observed that the long echo-train-length associated with TSE was prohibitive in measuring a reliable signal in our experiment. We have therefore focused on GE-EPI in the main components of this work.

Breathing of hyperoxic gas is expected to increase venous oxygenation primarily by augmenting the amount of dissolved O_2 in the arterial blood, thereby increasing the venous blood T_2 . Our result shows a venous T_2 increase of about 5.3 ms from normoxia to hyperoxia, suggesting that TRUST is sensitive to physiologically relevant T_{2v} changes.

We also demonstrated an age effect on T_{2v} . We found that mice of 86~93 weeks old, an age equivalent to approximately 70 years in humans (45), had a significantly lower blood T_{2v} compared to mice of 16~18 weeks old (equivalent to about 25 years old in humans (45)). An age-related reduction in venous T_2 is consistent with previous reports in humans, which was attributed to the combined effects of lower CBF and higher energy consumption (to offset the reduced neural efficiency) in the older population (46).

The present study has primarily reported the results in blood water T_{2v} , instead of oxygen saturation fraction (Y_v). This is because the conversion from T_2 to oxygenation requires a calibration plot that specifies the relationship between these two parameters. A calibration plot is usually obtained by in vitro experiments on blood samples (47–50). Such a calibration plot does not need to be performed for every subject or every study. That is, a calibration plot obtained from one study can be used for other studies as long as the imaging parameters are compatible. However, the calibration plot is field-strength specific. While it is in our plan to perform such in vitro calibration experiments now that the pulse sequence has been optimized, an ideal calibration plot for our data is not available at present. As a proof-of-principle demonstration, however, we adopted a calibration plot obtained on rats (rather than mice) at 11.7T using a multi-echo CPMG (rather than T_2 -preparation CPMG) sequence (47). According to this plot, venous oxygenations for normoxia and hyperoxia conditions were $75.8 \pm 3.9\%$ to $83.8 \pm 4.3\%$, respectively, showing an average 8.0% increase. This amount of increase is similar to that (about 10%) observed in humans (39). One discrepancy from the human data is that venous oxygenation under normoxia in healthy humans is between 50 and 75% (46), thus the measured value of the present study is on the higher end of that spectrum. Mismatches between the in vivo data and the calibration data may be part of the reason. Another explanation is that the mouse data were collected under anesthesia whereas the human experiments are performed under awake state. The anesthetic

agent isoflurane has been reported to suppress the brain's oxygen metabolism by 38% and increase CBF by 25% in humans at the 1.5 minimum alveolar concentration (equivalent to 1.5% for mice) (51). Collectively, venous oxygenation under isoflurane anesthesia is expected to be considerably higher than awake state. A rough estimation based on a CBF increases by 15% and CMRO₂ decrease by 25% (for 1.0% isoflurane used in this study) suggests that typical venous oxygenation in anesthetized mouse should be 67~83%, which is in good agreement with our experimental data.

To further support our notion of an effect of anesthesia on venous oxygenation, we conducted Study VI, where TRUST MRI was performed with different anesthetic regimens. Dexmedetomidine is a common sedative for functional MRI research in rodents. In particular, the combination of dexmedetomidine with low-dosage isoflurane has been proven to preserve functional responses to stimulus (40). In contrast to isoflurane, dexmedetomidine has been reported to maintain the oxygen extraction fraction (52). Therefore, it is expected that DEX+ISO administration will be associated with a lower Y_v in comparison with ISO. Consistent with this expectation, venous oxygenation saturation under DEX+ISO was found to be about 50%. These results also underscore the importance of consistent anesthetic strategies in making comparison across experiments or studies.

The present study has a few limitations. Although the TRUST technique has been shown to be useful in a variety of brain diseases (27,28,33), a limitation of this method is that it provides an estimation of global venous oxygenation only, with no spatial information. Further technical improvements are needed to extend the global measurements into spatially resolved maps. Possible approaches include the addition of velocity-selective or phase-contrast modules (53–55). Another limitation is that we optimized TRUST imaging parameters sequentially with one study focusing on one parameter only. However, it is possible that the optimal value of one parameter depends on other parameters. One could vary multiple parameters in a single study, e.g. varying slice location while varying slab thickness, to determine the optimal parameter set. However, this will lead to prohibitively long scan time. We also note that, in the analysis of the data, we empirically used four peak voxels for the estimation of blood T_2 in accordance with our previous human studies. We have also tested to use more voxels (up to 8) and the results were similar ($p>0.05$ for all comparisons). Finally, we would like to point out that the optimized protocol proposed above was based on young mice under isoflurane anesthesia. It is possible that, for experiments under other conditions, adjustments of the imaging parameters are needed for best results. Based on our data using alternative anesthetic method (i.e. DEX+ISO) and aged animals, our general impression is that the proposed protocol can also yield satisfactory results under those conditions. For example, the CoV of T_2 estimation was $6.0\pm2.1\%$ under DEX+ISO anesthesia and, in aged animals, the CoV was $4.5\pm2.1\%$. These values are in a similar range as those from young mice under the standard isoflurane anesthesia.

CONCLUSIONS

We implemented and optimized a non-invasive MRI technique, T_2 -Relaxation-Under-Spin-Tagging, to measure cerebral venous blood T_2 in mouse at 11.7T. This technique can be performed within 3 minutes with a test-retest variability of about 5%. Physiological studies

showed that this technique is sensitive to oxygenation changes associated with hyperoxia challenge, anesthetic agent, and cerebral aging. This method holds promises in studies of brain physiology and pathophysiology in animal models.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

NIH R01 MH084021, NIH R01 NS067015, NIH R01 AG042753, NIH R01 AG047972, NIH R21 NS095342, NIH R21 NS085634, and NIH P41 EB015909.

References

1. Kudomi N, Hayashi T, Teramoto N, Watabe H, Kawachi N, Ohta Y, Kim KM, Iida H. Rapid quantitative measurement of CMRO(2) and CBF by dual administration of (15)O-labeled oxygen and water during a single PET scan—a validation study and error analysis in anesthetized monkeys. *J Cereb Blood Flow Metab.* 2005; 25(9):1209–1224. [PubMed: 15874976]
2. Muzik O, Mangner TJ, Leonard WR, Kumar A, Janisse J, Granneman JG. 15O PET measurement of blood flow and oxygen consumption in cold-activated human brown fat. *J Nucl Med.* 2013; 54(4): 523–531. [PubMed: 23362317]
3. Riches AC, Sharp JG, Thomas DB, Smith SV. Blood volume determination in the mouse. *J Physiol.* 1973; 228(2):279–284. [PubMed: 4687099]
4. Arap W, Pasqualini R, Ruoslahti E. Cancer treatment by targeted drug delivery to tumor vasculature in a mouse model. *Science.* 1998; 279(5349):377–380. [PubMed: 9430587]
5. Huber KM, Gallagher SM, Warren ST, Bear MF. Altered synaptic plasticity in a mouse model of fragile X mental retardation. *Proc Natl Acad Sci U S A.* 2002; 99(11):7746–7750. [PubMed: 12032354]
6. Tsankova NM, Berton O, Renthal W, Kumar A, Neve RL, Nestler EJ. Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. *Nat Neurosci.* 2006; 9(4):519–525. [PubMed: 16501568]
7. Budde MD, Kim JH, Liang HF, Russell JH, Cross AH, Song SK. Axonal injury detected by in vivo diffusion tensor imaging correlates with neurological disability in a mouse model of multiple sclerosis. *NMR Biomed.* 2008; 21(6):589–597. [PubMed: 18041806]
8. Hamm JP, Peterka DS, Gogos JA, Yuste R. Altered Cortical Ensembles in Mouse Models of Schizophrenia. *Neuron.* 2017; 94(1):153–167. [PubMed: 28384469]
9. Maeda J, Ji B, Irie T, Tomiyama T, Maruyama M, Okauchi T, Staufenbiel M, Iwata N, Ono M, Saido TC, Suzuki K, Mori H, Higuchi M, Suhara T. Longitudinal, quantitative assessment of amyloid, neuroinflammation, and anti-amyloid treatment in a living mouse model of Alzheimer's disease enabled by positron emission tomography. *J Neurosci.* 2007; 27(41):10957–10968. [PubMed: 17928437]
10. Zhang J, Peng Q, Li Q, Jahanshad N, Hou Z, Jiang M, Masuda N, Langbehn DR, Miller MI, Mori S, Ross CA, Duan W. Longitudinal characterization of brain atrophy of a Huntington's disease mouse model by automated morphological analyses of magnetic resonance images. *Neuroimage.* 2010; 49(3):2340–2351. [PubMed: 19850133]
11. Chesi M, Matthews GM, Garbitt VM, Palmer SE, Shortt J, Lefebvre M, Stewart AK, Johnstone RW, Bergsagel PL. Drug response in a genetically engineered mouse model of multiple myeloma is predictive of clinical efficacy. *Blood.* 2012; 120(2):376–385. [PubMed: 22451422]
12. Scheer N, Ross J, Rode A, Zevnik B, Niehaves S, Faust N, Wolf CR. A novel panel of mouse models to evaluate the role of human pregnane X receptor and constitutive androstane receptor in drug response. *J Clin Invest.* 2008; 118(9):3228–3239. [PubMed: 18677425]

13. Golay X, Silvennoinen MJ, Zhou J, Clingman CS, Kauppinen RA, Pekar JJ, van Zijl PC. Measurement of tissue oxygen extraction ratios from venous blood T(2): increased precision and validation of principle. *Magn Reson Med*. 2001; 46(2):282–291. [PubMed: 11477631]
14. Wright GA, Hu BS, Macovski A. 1991 I.I. Rabi Award. Estimating oxygen saturation of blood in vivo with MR imaging at 1.5 T. *J Magn Reson Imaging*. 1991; 1(3):275–283. [PubMed: 1802140]
15. Lu H, Ge Y. Quantitative evaluation of oxygenation in venous vessels using T2-Relaxation-Under-Spin-Tagging MRI. *Magn Reson Med*. 2008; 60(2):357–363. [PubMed: 18666116]
16. Xu F, Uh J, Liu P, Lu H. On improving the speed and reliability of T2-relaxation-under-spin-tagging (TRUST) MRI. *Magn Reson Med*. 2012; 68(1):198–204. [PubMed: 22127845]
17. Kim SG. Quantification of relative cerebral blood flow change by flow-sensitive alternating inversion recovery (FAIR) technique: application to functional mapping. *Magn Reson Med*. 1995; 34(3):293–301. [PubMed: 7500865]
18. Detre JA, Alsop DC. Perfusion magnetic resonance imaging with continuous arterial spin labeling: methods and clinical applications in the central nervous system. *Eur J Radiol*. 1999; 30(2):115–124. [PubMed: 10401592]
19. Rodgers ZB, Englund EK, Langham MC, Magland JF, Wehrli FW. Rapid T2- and susceptibility-based CMRO2 quantification with interleaved TRUST (iTRUST). *Neuroimage*. 2015; 106:441–450. [PubMed: 25449740]
20. Barhoum S, Rodgers ZB, Langham M, Magland JF, Li C, Wehrli FW. Comparison of MRI methods for measuring whole-brain venous oxygen saturation. *Magn Reson Med*. 2015; 73(6):2122–2128. [PubMed: 24975122]
21. Stout JN, Tisdall MD, McDaniel P, Gagoski B, Bolar DS, Grant PE, Adalsteinsson E. Assessing the effects of subject motion on T2 relaxation under spin tagging (TRUST) cerebral oxygenation measurements using volume navigators. *Magn Reson Med*. 2017; doi: 10.1002/mrm.26616
22. O'Brien C, Okell T, Jezzard P. Spatially localised measurements of oxygen extraction fraction using modified T2-relaxation-under-spin-tagging (SL-TRUST). *Proc Intl Soc Mag Reson Med*. 2017; 25:1897.
23. Bush A, Borzage M, Detterich J, Kato RM, Meiselman HJ, Coates T, Wood JC. Empirical model of human blood transverse relaxation at 3 T improves MRI T2 oximetry. *Magn Reson Med*. 2017; 77(6):2364–2371. [PubMed: 27385283]
24. Liu P, Huang H, Rollins N, Chalak LF, Jeon T, Halovanic C, Lu H. Quantitative assessment of global cerebral metabolic rate of oxygen (CMRO2) in neonates using MRI. *NMR Biomed*. 2014; 27(3):332–340. [PubMed: 24399806]
25. Liu P, Chalak LF, Lu H. Non-invasive assessment of neonatal brain oxygen metabolism: A review of newly available techniques. *Early Hum Dev*. 2014; 90(10):695–701. [PubMed: 25028136]
26. Peng SL, Dumas JA, Park DC, Liu P, Filbey FM, McAdams CJ, Pinkham AE, Adinoff B, Zhang R, Lu H. Age-related increase of resting metabolic rate in the human brain. *Neuroimage*. 2014; 98:176–183. [PubMed: 24814209]
27. Thomas BP, Sheng M, Tseng BY, Tarumi T, Martin-Cook K, Womack KB, Cullum MC, Levine BD, Zhang R, Lu H. Reduced global brain metabolism but maintained vascular function in amnesic mild cognitive impairment. *J Cereb Blood Flow Metab*. 2017; 37(4):1508–1516. [PubMed: 27389176]
28. Ge Y, Zhang Z, Lu H, Tang L, Jaggi H, Herbert J, Babb JS, Rusinek H, Grossman RI. Characterizing brain oxygen metabolism in patients with multiple sclerosis with T2-relaxation-under-spin-tagging MRI. *J Cereb Blood Flow Metab*. 2012; 32(3):403–412. [PubMed: 22252237]
29. Jordan LC, Gindville MC, Scott AO, Juttukonda MR, Strother MK, Kassim AA, Chen SC, Lu H, Pruthi S, Shyr Y, Donahue MJ. Non-invasive imaging of oxygen extraction fraction in adults with sickle cell anaemia. *Brain*. 2016; 139:738–750. [PubMed: 26823369]
30. Sheng M, Lu H, Liu P, Thomas BP, McAdams CJ. Cerebral perfusion differences in women currently with and recovered from anorexia nervosa. *Psychiatry Res*. 2015; 232(2):175–183. [PubMed: 25795596]
31. Liu P, Lu H, Filbey FM, Tamminga CA, Cao Y, Adinoff B. MRI assessment of cerebral oxygen metabolism in cocaine-addicted individuals: hypoactivity and dose dependence. *NMR Biomed*. 2014; 27(6):726–732. [PubMed: 24757009]

32. Filbey FM, Aslan S, Lu H, Peng SL. Residual Effects of THC via Novel Measures of Brain Perfusion and Metabolism in a Large Group of Chronic Cannabis Users. *Neuropsychopharmacology*. 2017; doi: 10.1038/npp.2017.1044
33. Zheng G, Lu H, Yu W, Luo S, Liu Y, Liu W, Liu H, Wu L, Zheng L, Kong X, Zhang LJ, Lu GM. Severity-specific alterations in CBF, OEF and CMRO2 in cirrhotic patients with hepatic encephalopathy. *Eur Radiol*. 2017; doi: 10.1007/s00330-00017-04809-00339
34. Zheng G, Wen J, Lu H, Lou Y, Pan Z, Liu W, Liu H, Li X, Zhang Z, Chen H, Kong X, Luo S, Jiang X, Liu Y, Zhang Z, Zhang LJ, Lu GM. Elevated global cerebral blood flow, oxygen extraction fraction and unchanged metabolic rate of oxygen in young adults with end-stage renal disease: an MRI study. *Eur Radiol*. 2016; 26(6):1732–1741. [PubMed: 26334507]
35. Smith ZM, Krizay E, Guo J, Shin DD, Scadeng M, Dubowitz DJ. Sustained high-altitude hypoxia increases cerebral oxygen metabolism. *J Appl Physiol*. 2013; 114(1):11–18. [PubMed: 23019310]
36. Wang K, Smith ZM, Buxton RB, Swenson ER, Dubowitz DJ. Acetazolamide during acute hypoxia improves tissue oxygenation in the human brain. *J Appl Physiol*. 2015; 119(12):1494–1500. [PubMed: 26472861]
37. Yu B, Huang M, Zhang X, Ma H, Peng M, Guo Q. Noninvasive imaging of brain oxygen metabolism in children with primary nocturnal enuresis during natural sleep. *Hum Brain Mapp*. 2017; 38(5):2532–2539. [PubMed: 28195439]
38. Botnar RM, Stuber M, Danias PG, Kissinger KV, Manning WJ. Improved coronary artery definition with T2-weighted, free-breathing, three-dimensional coronary MRA. *Circulation*. 1999; 99(24):3139–3148. [PubMed: 10377077]
39. Xu F, Liu P, Pascual JM, Xiao G, Lu H. Effect of hypoxia and hyperoxia on cerebral blood flow, blood oxygenation, and oxidative metabolism. *J Cereb Blood Flow Metab*. 2012; 32(10):1909–1918. [PubMed: 22739621]
40. Fukuda M, Vazquez AL, Zong X, Kim SG. Effects of the alpha(2)-adrenergic receptor agonist dexmedetomidine on neural, vascular and BOLD fMRI responses in the somatosensory cortex. *Eur J Neurosci*. 2013; 37(1):80–95. [PubMed: 23106361]
41. Adamczak JM, Farr TD, Seehafer JU, Kalthoff D, Hoehn M. High field BOLD response to forepaw stimulation in the mouse. *Neuroimage*. 2010; 51(2):704–712. [PubMed: 20211267]
42. Debacker CS, Daoust A, Kohler S, Voiron J, Warnking JM, Barbier EL. Impact of tissue T1 on perfusion measurement with arterial spin labeling. *Magn Reson Med*. 2017; 77(4):1656–1664. [PubMed: 27136322]
43. Le Heron CJ, Wright SL, Melzer TR, Myall DJ, MacAskill MR, Livingston L, Keenan RJ, Watts R, Dalrymple-Alford JC, Anderson TJ. Comparing cerebral perfusion in Alzheimer's disease and Parkinson's disease dementia: an ASL-MRI study. *J Cereb Blood Flow Metab*. 2014; 34(6):964–970. [PubMed: 24619276]
44. Mao D, Li Y, Liu P, Peng SL, Pillai JJ, Lu H. Three-dimensional mapping of brain venous oxygenation using R2* oximetry. *Magn Reson Med*. 2017; doi: 10.1002/mrm.26763
45. Dutta S, Sengupta P. Men and mice: Relating their ages. *Life Sci*. 2016; 152:244–248. [PubMed: 26596563]
46. Lu H, Xu F, Rodrigue KM, Kennedy KM, Cheng Y, Flicker B, Hebrank AC, Uh J, Park DC. Alterations in cerebral metabolic rate and blood supply across the adult lifespan. *Cereb Cortex*. 2011; 21(6):1426–1434. [PubMed: 21051551]
47. Lin AL, Qin Q, Zhao X, Duong TQ. Blood longitudinal (T1) and transverse (T2) relaxation time constants at 11. 7 Tesla. *MAGMA*. 2012; 25(3):245–249. [PubMed: 22071580]
48. Silvennoinen MJ, Clingman CS, Golay X, Kauppinen RA, van Zijl PC. Comparison of the dependence of blood R2 and R2* on oxygen saturation at 1.5 and 4. 7 Tesla. *Magn Reson Med*. 2003; 49(1):47–60. [PubMed: 12509819]
49. Lu H, Xu F, Grgac K, Liu P, Qin Q, van Zijl P. Calibration and validation of TRUST MRI for the estimation of cerebral blood oxygenation. *Magn Reson Med*. 2012; 67(1):42–49. [PubMed: 21590721]
50. Grgac K, Li W, Huang A, Qin Q, van Zijl PC. Transverse water relaxation in whole blood and erythrocytes at 3T, 7T, 9.4T, 11.7T and 16. 4T; determination of intracellular hemoglobin and extracellular albumin relaxivities. *Magn Reson Imaging*. 2017; 38:234–249. [PubMed: 27993533]

51. Oshima T, Karasawa F, Okazaki Y, Wada H, Satoh T. Effects of sevoflurane on cerebral blood flow and cerebral metabolic rate of oxygen in human beings: a comparison with isoflurane. *Eur J Anaesthesiol.* 2003; 20(7):543–547. [PubMed: 12884987]
52. Wang X, Ji J, Fen L, Wang A. Effects of dexmedetomidine on cerebral blood flow in critically ill patients with or without traumatic brain injury: a prospective controlled trial. *Brain Inj.* 2013; 27(13–14):1617–1622. [PubMed: 24102571]
53. Bolar DS, Rosen BR, Sorensen AG, Adalsteinsson E. QUantitative Imaging of eXtraction of oxygen and Tissue consumption (QUIXOTIC) using venular-targeted velocity-selective spin labeling. *Magn Reson Med.* 2011; 66(6):1550–1562. [PubMed: 21674615]
54. Guo J, Wong EC. Venous oxygenation mapping using velocity-selective excitation and arterial nulling. *Magn Reson Med.* 2012; 68(5):1458–1471. [PubMed: 22294414]
55. Krishnamurthy LC, Liu P, Ge Y, Lu H. Vessel-specific quantification of blood oxygenation with T2-relaxation-under-phase-contrast MRI. *Magn Reson Med.* 2014; 71(3):978–989. [PubMed: 23568830]

Figure 1.

Illustration of TRUST MRI pulse sequence. (a) Sequence diagram. Sinc-shaped symbols represent frequency-selective RF pulses with flip angles indicated at the top (0.5 ms Gaussian pulse for 90° and 15 ms hyperbolic secant pulse for 180°). Open rectangles are non-selective pulses with higher ones denoting 180° refocusing pulses and smaller ones 90° excitation pulses. Within the T_2 -preparation module, the first and last pulses are applied in opposite phase. Effective TE (eTE) denotes the extent of T_2 weighting. Post-labeling delay (PLD) denotes the time between FAIR labeling and image excitation. (b) Representative positions of TRUST imaging slice (red box) and slice-selective inversion slab (yellow box) displayed on a sagittal localizer image. Note that the slice-selective inversion slab is usually large than the imaging slice to account for imperfection in RF profile.

Figure 2.

Optimization of imaging slice location. (a) Six candidate slice positions overlaid on a sagittal anatomical image. (b) Control, label, and difference images from each of the TRUST scans ($eTE=0.25$ ms), as well as a time-of-flight (TOF) angiogram for reference.

Figure 3.
Difference signal intensity as a function of slice location.

Figure 4.
Dependence of TRUST signal intensity on (a) inversion slab thickness, (b) post-labeling delay (PLD), and (c) TR.

Figure 5.

A complete data set of TRUST for the estimation of venous T_2 . Control, label, and difference images for three eTEs (0.25, 20, and 40 ms) along with a monoexponential fitting of the blood signal are shown.

Figure 6.
Venous T₂ measured under different anesthesia strategies (N=3).

Figure 7. Age-related differences in venous blood T_2 . (a) Venous T_2 in younger (N=5) and older groups (N=5). (b) Coefficient of variations (CoV) across measurements in younger and older groups.