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Benchmarking Transverse Spin Relaxation Based Oxygenation Measurements in the Brain during Hypercapnia and Hypoxia

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Abstract

Purpose—To simultaneously assess reproducibility of three MRI transverse relaxation parameters (R_2' , R_2^* and R_2) for brain tissue oxygenation mapping and to assess changes in these parameters with inhalation of gases that increase and decrease oxygenation, to identify the most sensitive parameter for imaging brain oxygenation.

Materials and Methods—48 healthy subjects (25M, ages 35 ± 8 yrs) were scanned at 3.0T, each with one of four gases (mildly and strongly hypercapnic and hypoxic) administered in a challenge paradigm, using a gas delivery setup designed for patient use. Cerebral blood flow mapping with arterial spin labeling, and simultaneous R_2' , R_2^* and R_2 mapping with Gradient-Echo Sampling of Free Induction Decay and Echo (GESFIDE) were performed. Reproducibility in air and gas-induced changes were evaluated using non-parametric analysis with correction for multiple comparisons.

Results—Our gas delivery setup achieved stable gas challenges as shown by physiological monitoring. Test-retest variability of R_2' , R_2^* , and R_2 were found to be 0.24 s^{-1} (8.6% of mean), 0.24 s^{-1} (1.3% of mean), and 0.15 s^{-1} (1.0% of mean), respectively. Strong hypoxia produced the most conclusive oxygenation-driven relaxation change, inducing increases in R_2' ($25 \pm 13\%$, $p=0.03$), R_2^* ($5 \pm 2\%$, $p=0.02$), and R_2 ($2 \pm 2\%$, NS).

Conclusion—We benchmarked the intra-scan test-retest variability in GESFIDE-based transverse relaxation rate mapping. Using a reliable framework for gas challenge paradigms, we recommend strong hypoxia for validating oxygenation mapping methods, and the use of tissue R_2' change, instead of R_2^* or R_2 , as a metric for studying brain tissue oxygenation using transverse relaxation methods.

Keywords

Oxygenation; transverse relaxation; R_2' ; hypercapnia; hypoxia

Introduction

Brain tissue oxygenation mapping provides invaluable information for studying normal brain physiology and function (1, 2), and evaluating pathological conditions including

cerebrovascular diseases and tumors (3, 4). Oxygenation can be assessed with several MRI-based methods, including quantification of transverse spin relaxation rates R_2' , R_2^* , and R_2 (reciprocals, respectively, of relaxation time constants T_2' , T_2^* , and T_2 ; related as $R_2^* = R_2 + R_2'$). These relaxation rates are sensitive to the susceptibility effect of paramagnetic deoxyhemoglobin within the imaging voxel, as described by the blood oxygenation level dependent (BOLD) principle (5). Relaxometry samples all tissue compartments, including blood, extra-cellular and intra-cellular water (6). Transverse relaxometry is insensitive to RF field inhomogeneity, and can offer vast improvements over measuring only signal magnitude.

Due to the relative simplicity of measuring R_2^* , it is the most common relaxation rate marker for susceptibility changes in tissues. Several studies have used R_2^* mapping to study brain tissue oxygenation changes in cerebrovascular pathologies (7–9), though comparison against positron emission tomography did not find a relationship between abnormal oxygen metabolism and increased R_2^* (10), and measurements are confounded by blood volume factors (11). Blood R_2 is also used to measure oxygenation in the brain (12), though measurement of localized tissue R_2 (13) is relatively limited, partly because it is affected by factors unrelated to oxygenation, including vasogenic edema (14), gliosis (15) and blood compartment changes (16). In theory, R_2' is independent of these factors and has been proposed as a better measure of tissue susceptibility changes due to oxygenation (17).

In this study, we aimed to concurrently assess the intra-scan air-breathing reproducibility of tissue R_2' , R_2^* , and R_2 in the human brain, and to quantify changes in these parameters when the subject is breathing different strengths of hypercapnic and hypoxic gases. Based on our results, we aimed to ascertain which relaxation parameter is most suitable for imaging brain oxygenation, both in terms of the magnitude and polarity of changes observed during gas challenges, and whether the changes can be definitively attributed to oxygenation and perfusion.

Materials and Methods

Subject Population

With approval from Stanford University's Institutional Review Board, 48 normal volunteers (25 male, ages 33 ± 6 yrs; 23 female, ages 36 ± 10 yrs) were scanned in this prospective study with informed consent and HIPAA compliance. All were screened for history of cerebrovascular disease, anemia, heart disease, or lung diseases that might affect blood and brain oxygenation. Volunteers were instructed to abstain from consuming caffeine for 12 hours before the scan, given the known effects of caffeine on cerebral blood flow (CBF) (18). Volunteers were not instructed to abstain from smoking prior to the scan, though all scans took place on a tobacco-free campus.

Imaging Protocol

All subjects were scanned at 3.0T (MR750, GE Healthcare, Waukesha WI). The imaging protocol consisted of three identical epochs (denoted as “Air 1”, “Air 2” and “Gas” in Figure 1). Air 1 and Air 2 epochs were performed consecutively during air breathing, while the Gas

epoch followed a transition period of 2-3 minutes after commencing gas breathing, to ensure stable gas environment and subject physiology. Each epoch included measurements of oxygenation-driven relaxation and CBF as described below.

Imaging Sequences

At the start of the experiment, axial T_1 -weighted 3D SPGR BRAVO images (TR/TE/TI 9.2/3.7/400ms, flip angle 13° , voxel size $0.9 \times 0.9 \times 1.2 \text{ mm}^3$) were acquired for anatomic imaging and segmentation. At the start of each epoch, quadratic shim was performed to optimize magnetic field homogeneity, so that detected variation in relaxation rate can be attributed to intrinsic changes in tissue susceptibility.

Each epoch consisted of two sequences. Relaxometry was performed using the massively multi-echo 2D Gradient Echo Sampling of Free Induction Decay Echoes (GESFIDE) sequence (Figure 2) (19), which allowed simultaneous measurement of R_2' , R_2^* , and R_2 . Parameters are: TE_{SE}/TR 100/2000ms, 40 echoes, TE 5-130ms, voxel size $1.9 \times 1.9 \times 1.5 \text{ mm}^3$, 14 slices with 1mm spacing and 34 mm-thick coverage superior to lateral ventricles, and scan duration 3min 30s. CBF was mapped using GE Healthcare product 3D pseudocontinuous arterial spin labeling (pcASL) sequence: TE/TR 10.6/4845ms, TL/PLD 1500/2025ms, stack-of-spirals fast spin-echo readout, resolution $3 \times 3 \times 4 \text{ mm}^3$, 36 slices with whole-brain coverage, and scan duration 4min 41s. Imaging parameters were chosen in accordance with current consensus guidelines (20).

Gas Challenge Setup

Subjects were assigned to one of four gas groups, identified as mild hypercapnia (mHC: targeting change in end-tidal partial pressure of oxygen, $P_{et}CO_2=7 \text{ mmHg}$), strong hypercapnia (sHC: targeting $P_{et}CO_2=10 \text{ mmHg}$), mild hypoxia (mHO: targeting change in peripheral oxygen saturation, $S_pO_2=-5\%$), and strong hypoxia (sHO: targeting $S_pO_2=-10\%$). Target physiological changes were chosen to be comparable to reported values in literature. Observing from past experimentation with this mask, that delivered gases would be diluted by entrained room air by a factor of 1-2, we used the following premixed medical-grade gases to achieve desired conditions:

1. Medical air (control): 21% oxygen and 79% nitrogen;
2. Mild hypercapnia: 21% oxygen, 5% carbon dioxide, and 74% nitrogen;
3. Strong hypercapnia: 21% oxygen, 10% carbon dioxide, and 69% nitrogen;
4. Mild hypoxia: 14% oxygen and 86% nitrogen; and
5. Strong hypoxia: 10% oxygen and 90% nitrogen.

Our gas delivery setup prioritized comfort and safety, and was implemented based on its feasibility for in-clinic use in future studies. Compressed medical air and one of the four gases were delivered at approximately 12 L/min via a bubble humidifier (Hudson RCI, Teleflex Inc., Research Triangle Park NC) and standard oxygen therapy tubing to each subject, using an MR-safe high oxygen delivery face mask (Ceretec Hi-Ox, Ceretec Inc., Garden Grove CA), as shown in Figure 3. A sampling nasal cannula (4000F, Salter Labs,

Arvin CA) was worn under the mask. A custom elastic strap (Figure 3) with quick-release buckles was worn during scan. As expected, leakage occurred and was dependent on subjects' facial geometry. In addition, for subject comfort and safety, we used a mask with an anti-suffocation valve, which entrained room air if the subject emptied the reservoir bag during deep inhalation or hyperventilation.

Physiological Monitoring

Extensive physiological recording was performed, including inspired and end-tidal oxygen fractional concentration and carbon dioxide partial pressure ($f_{in}O_2$, $f_{et}O_2$, $P_{in}CO_2$ and $P_{et}CO_2$) via nasal cannula, heart rate and peripheral oxygen saturation (S_pO_2) via fingertip photoplethysmography, and systolic, diastolic, and mean arterial pressure (MAP) from a brachial blood pressure cuff. All measurements were recorded by a medical monitor (Medrad Veris 8600, Bayer Healthcare, Whippany NJ) at 1s intervals.

Data Analysis

All data analysis was performed using custom Matlab (MathWorks Inc., Natick MA) code, including functions from SPM8 (Wellcome Trust Centre for Neuroimaging, United Kingdom) and FSL (Oxford Centre for Functional MRI of the Brain, United Kingdom) software packages. To avoid introducing errors through co-registration, all GESFIDE images were analyzed in their native space, with T_1 -weighted anatomical images co-registered (using SPM8) to each GESFIDE data set before undergoing segmentation (using FSL) into three regions of interest (ROIs): gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF). pcASL images and CBF maps automatically generated (20) by the scanner (MR750, GE Healthcare, Waukesha WI) were also co-registered to corresponding GESFIDE data sets. R_2' and R_2 maps were calculated from GESFIDE images using mono-exponential fitting of echoes from the "A" and "B" portions of the time curve illustrated in Figure 2, as per the standard GESFIDE method for R_2' mapping (19). R_2^* was calculated from the "A" portion.

Intra-scan reproducibility of oxygenation-driven relaxation and CBF measurements was evaluated from the two air epochs, and assumed to be applicable to gas breathing. Data sets were excluded from analysis if at least one GESFIDE data set from an air epoch displayed noticeable motion artifact, which manifested as Gibbs ringing or noticeable low-spatial frequency R_2' variations in a pattern (e.g., bands) that did not correspond to anatomy.

Statistical Analysis

Wilcoxon signed-rank test was used to test for significance in changes in all physiological and imaging measurements between epochs, such as between first and second air epochs, or between air and gas epochs. Wilcoxon rank-sum test was used in pairwise comparisons between gas groups. Multiple comparisons were corrected using the Holm-Bonferroni method, which controls family-wise error rate. The significance level of 0.05 was used for all tests. For reproducibility analysis, agreement between the medical air epoch measurements was measured using Lin's concordance correlation coefficient ρ_c (21) and Bland-Altman analysis.

Results

Subject Population

Several datasets with motion artifacts were discarded. The final data set consisted of 38 subjects (18 male, ages 34 ± 7 yrs; 20 female, ages 34 ± 9 yrs) for intra-scan reproducibility analysis, and 40 subjects (21 male, ages 33 ± 7 yrs; 19 female, ages 34 ± 9 yrs) for gas challenge analysis. Among reproducibility analysis data sets, 5 subjects were discarded as they were scanned on an outdated protocol with only one air epoch, and 5 others were discarded due to motion artifact during at least one air epoch. Among gas challenge data sets, 8 from the following groups had motion artifacts that visibly corrupted the images: mild hypoxia (2 subjects), strong hypercapnia (3 subjects), and strong hypoxia (3 subjects).

Gas Challenge Setup

Real-time recordings of S_pO_2 , $P_{in}CO_2$, $f_{in}O_2$, $P_{et}CO_2$ and $f_{et}O_2$ show that both normoxia and normocapnia were sustained during the air epochs, and that stable hypercapnia and hypoxia were indeed achieved during gas challenges (Figure 4 and Supplemental Figure S1). As expected, measured $f_{in}O_2$ and $P_{in}CO_2$ deviated from the administered gas mixtures due to room air entrainment. Therefore, the achieved changes in end-tidal gas content were lower than expected for the gas mixtures: $P_{et}CO_2$ of 10 ± 3 mmHg (sHC) and 7 ± 4 mmHg (mHC), and S_pO_2 of $-6 \pm 3\%$ (mHO) and $-11 \pm 3\%$ (sHO). Recordings of heart rate, respiratory rate, and blood pressure also confirm that subject physiology was stable during air breathing, and changed in a consistent and expected manner during gas challenges (Figure 4 and Supplemental Figure S1).

Pairwise comparisons of changes in physiological measurements were performed between the four gas conditions (Table 2). Notable findings include: 1) $f_{et}O_2$ was the only physiological change that significantly ($p < 0.05$) differentiated all pairs of groups, indicating its sensitivity to both hypercapnic and hypoxic challenges; 2) strong hypercapnia more significantly increased heart rate and diastolic blood pressure compared to other challenges. Full results are shown in Table 2.

Reproducibility

CBF was highly reproducible (Figure 5), with Lin's concordance correlation coefficient ρ_c of 0.95 in GM and 0.93 in WM. Among the relaxation rates, R_2^* and R_2 had very high reproducibility (Figure 5), with $\rho_c > 0.90$ for GM and WM. R_2' had lower correlation ($\rho_c = 0.69$ and 0.81 in GM and WM, respectively). The test-retest (Air 2 minus Air 1) differences between the two scans had overall standard deviation 3.6 mL/min/100 g or 6.7% (of mean value) for CBF, 0.24 s^{-1} or 8.6% for R_2' , 0.24 s^{-1} or 1.3% for R_2^* , and 0.15 s^{-1} or 1.0% for R_2 . Only CBF experienced a significant ($p = 0.021$) offset in the test-retest difference, but it was very small ($0.97 \text{ mL/min/100 g}$).

Comparison of measurements during air breathing also allows quantification of *inter-subject* variation. We found large baseline variation in the first air epoch measurements across subjects for CBF (19% [GM] and 18% [WM] standard deviation) and R_2' (18% [GM] and 9% [WM]), and less so for R_2^* (3.9% [GM] and 2.8% [WM]) and R_2 (4.4% [GM] and 2.1%

[WM]). Inter-subject variation in the second air epoch measurements was similar. Two physiological measurements, $f_{et}O_2$ and $P_{et}CO_2$, showed significant change ($p<0.01$ for both) between the air epochs, though the changes were extremely small (-0.3% for $f_{et}O_2$ and $+0.2\text{mmHg}$ for $P_{et}CO_2$), and is a result of the small variances due to the stable gas environment that allowed detection of these minimal changes. A full summary is presented in Supplemental Table 1.

Gas Challenges

Robust and significant ($p=0.03$) CBF increases were observed during hypercapnic challenges (Table 1). In GM, strong hypercapnia augmented CBF by $21\pm12\%$, and mild hypercapnia by $19\pm24\%$. We observed no significant change in CBF during hypoxia ($3\pm5\%$ with $p=0.52$ and $2\pm6\%$ with $p=1.00$ in GM during mild and strong hypoxia, respectively). Changes in WM CBF were similar but had smaller mean and standard deviation compared to GM. Figure 6 illustrates two representative data sets during strong hypercapnia and hypoxia.

Gas-induced R_2' changes are shown in Figure 7 and Table 1. In GM, the hypercapnic challenges did not show significant changes (sHC: $R_2'=-0.02\pm0.26\text{ s}^{-1}$, $p=1.00$; mHC:

$R_2'=-0.08\pm0.30\text{ s}^{-1}$, $p=1.00$), though the direction of the decrease would be consistent with increased tissue oxygenation. The two hypoxic challenges produced also produced dose-dependent R_2' increases (mHO: $R_2'=0.28\pm0.31\text{ s}^{-1}$, $p=0.16$; sHO: $R_2'=0.53\pm0.24\text{ s}^{-1}$, $p=0.03$). R_2^* displayed expected polarities for both hypercapnic and hypoxic challenges, and was significant ($p=0.02$) for strong hypoxia. R_2 had expected polarity and dose-dependent magnitude in all challenges, and was only significant ($p=0.03$ for both) in hypercapnia.

Relative percentage changes in measurements (denoted as % CBF, % R_2' , Table 1) show similar trends, but also shows that R_2' experienced greater relative change than R_2^* and R_2 in hypoxia (mHO: % R_2' $13\pm13\%$, % R_2^* $2\pm2\%$, and % R_2 $1\pm1\%$; sHO: % R_2' $25\pm13\%$, % R_2^* $5\pm2\%$, and % R_2 $2\pm2\%$). Measurements in WM show similar trends, though with smaller values and reduced statistical significance.

Pairwise comparison was performed for each measurement between all gas challenges. In GM, notable findings include: 1) there was no statistically significant difference between strong and mild hypercapnia, or between strong and mild hypoxia, although R_2' magnitude suggested dose-dependence; 2) strong hypoxia induced a significant ($p=0.01$) increase in tissue R_2' relative to the hypercapnic challenges; 3) tissue R_2^* and R_2 were more sensitive to differences between gas challenges. Also, for hypercapnia, changes in R_2^* were largely accounted for by changes in R_2 . Full results are shown in Table 3.

Discussion

This study successfully measured the intra-scan air-breathing reproducibility of CBF and all three transverse relaxation rates simultaneously, as well as the impact of different extents of hypercapnia and hypoxia on these measurements, using clinically friendly sequences and equipment. In doing so, we have produced valuable benchmarking information for future

studies, supported some prior publications, and underlined many practical considerations in clinical applications.

Rigorous physiological monitoring in this study validated our gas challenge setup, which produced stable normoxic normocapnia and desired extents of hypercapnia and hypoxia. Intra-scan reproducibility studies indicate the minimum changes that can be confidently attributed to an external perturbation in an individual subject during a challenge experiment. During the air epochs, measured differences in CBF and relaxation parameters are attributed to fluctuations in unobserved physiological variables and noise from the imaging sequences. Our measured CBF variability was comparable to a previous study (22), while there are no published simultaneous assessment of the reproducibility of these three transverse relaxation rates. Our GESFIDE relaxometry method fits R_2^* and R_2^*B from the measured signal curve, and then calculates R_2' and R_2 . As a result, fitting error is additively propagated to both R_2' and R_2 in identical ways. R_2' was found to have the lowest reproducibility, though we should note that ASL measurements of CBF had comparable variability. In addition, absolute test-retest differences in R_2' and R_2^* were comparable, so the difference in percentage reproducibility originated from different magnitudes of baseline measurement, with baseline R_2' being about 20-25% of R_2^* and R_2 . Moreover, despite our attempt to obtain a homogenous population by limiting enrolment to relatively young, normal volunteers, we also observed large inter-subject variation, which is also similar or lower than that of CBF measurements. Therefore, researchers investigating R_2' as a biomarker need to quantify and report reproducibility, and use intra-subject control data (e.g., contralateral measurements) wherever possible.

According to Yablonskiy and Haacke's quantitative BOLD (qBOLD) biophysical model (23), R_2' is modeled as proportional to the product of deoxygenated blood volume (DBV) and SO_2 . Therefore, if we were to calculate SO_2 from independent measurements of R_2' and DBV, with intra- or inter-scan variability denoted by standard deviations $\delta R_2'\%$ and $\delta DBV\%$ respectively, the corresponding variability in SO_2 will range between $\min\{\delta R_2', \delta DBV\}\%$ and $(\delta R_2' + \delta DBV)\%$. In other words, the intra-scan test-retest difference of SO_2 calculated using GESFIDE-measured R_2' will be at least 8.6% of the mean SO_2 measurement. δDBV may be approximated with δCBV , the variability of cerebral blood volume (CBV). In literature, intra-scan test-retest δCBV has been measured to be around 22% in the whole brain for Dynamic Susceptibility Contrast (DSC) MRI at different field strengths (24), and to be 4-5% (25) but regionally highly variable (26) for the non-contrast vascular-space-occupancy (VASO) method.

Our measurements of hypercapnia-induced CBF increase were mostly consistent with values reported in literature (22, 27, 28), though CBF per mmHg of $P_{et}CO_2$ was unexpectedly lower in strong hypercapnia than in mild hypercapnia. On the other hand, prior studies reported a wide range of CBF changes in acute hypoxia, from small reductions to ~20% increases (29–31). In our work, we observed no significant CBF changes ($p=0.05$ and 1.00 for mild and strong hypoxia respectively), possibly due to the short duration of hypoxia.

During hypoxia, we observed positive and increasing R_2' with increasing strength of challenge. This is consistent with theory. According to the BOLD principle, Yablonskiy and

Haacke's biophysical model (23), and the Grubb relation between CBF and CBV (32), we expect R_2' to *increase* with CBF *increase* if SO_2 remains constant, and to *increase* with SO_2 *decrease* if CBF remains constant. Our observation was that the hypoxic challenge may have achieved the latter condition, where tissue SO_2 decreases due to reduction in oxygen supply without concomitant CBF change.

However, our hypercapnic data found no significant R_2' changes (all $p > 0.05$), possibly due to competing blood volume and oxygenation effects. During hypercapnia, CBF increases without any metabolic increases, leading to oversupply of oxygenated blood, increase in tissue SO_2 , and reduction in R_2' . Concurrently, increasing CBF is expected to increase CBV via the Grubb relationship, leading to an increase in the blood volume term in the Yablonskiy and Haacke model, thereby reducing R_2' . Since hypercapnia produced opposing effects of increased SO_2 and increased blood volume, we believe that it is a suboptimal oxygenation challenge. An additional confounding factor is the Bohr effect during hypercapnia, where the lower pH would reduce SO_2 . Without blood sampling, we cannot quantify this competing effect. An alternative gas challenge for increasing tissue oxygenation is hyperoxia, which has been reported to cause insignificant reduction in CBF with appropriate T_1 correction for ASL-based measurements (33), removing one of the opposing effects and resulting in a net R_2' reduction. We plan to test this challenge with our setup in the future.

Blood R_2 also experiences these competing effects (16), so we expect brain tissue R_2 to face similar problems. Our tissue R_2 measurements in hypercapnia suggest that R_2 is more weighted by SO_2 change than R_2' , but R_2 in hypoxia was smaller than R_2' despite the absence of blood volume change. This suggests that the oxygenation-based component of tissue R_2 has a more complicated model than R_2' . Due to the lack of theoretical foundation for tissue R_2 modeling, it is limited in utility.

Additionally, due to the sensitivity of tissue R_2 to non-oxygenation factors, baseline R_2 is limited as a biomarker in the presence of pathology. Though this study is performed in normal volunteers, the findings contribute towards the knowledge base needed for translating relaxometry-based oxygenation mapping to patients.

By magnitude alone, R_2^* is most sensitive, producing strongly polar responses to all four gas challenges. However, due to the dependence of $R_2^* (= R_2' + R_2)$ on R_2 , it is similarly poorly modeled and has limited utility in patients with brain pathologies.

Lastly, we note that percentage R_2' was by far the largest during hypoxia. This suggests that relative R_2' change is potentially a good parameter for studying oxygenation changes in challenge paradigms where blood volume changes are either negligible or quantified to isolate the effect of oxygenation change. This is particularly true in focal changes, such as lesions in ischemic stroke, where pathology is likely to impact R_2 and therefore R_2^* .

While we had a relatively large sample size, we did not perform multiple gas challenges in the same individual, so we cannot attribute inter-group differences to gas challenges alone. It is possible, though unlikely, that physiology of subjects could have been unbalanced at the group level, potentially confounding our results. We did not measure CBV change in this

study, so we could not estimate its effect on spin relaxation. A non-contrast and therefore repeatable measurement of relative CBV is theoretically possible using the VASO technique (34). In pathologies, tissue composition may also change, altering the baseline measurements of relaxation rates and potentially affecting the relaxation rate changes due to challenges. Therefore, separate CBV quantitation is crucial in pathologies.

Additionally, in our study, we selected GESFIDE imaging parameters to prioritize high spatial resolution and short echo time spacing. The small voxel size resulted in low spatial SNR in the R_2' measurements, so that analysis was performed for large ROIs at group level, instead of voxel-wise in individual subjects, where imaging parameters would need to be optimized. Finally, we did not perform T_1 mapping or correction in calculating CBF from pcASL data during different gas epochs and across subjects. Various modeling and experimental studies (35–38) have shown that blood T_1 is strongly dependent on hematocrit, weakly but non-negligibly dependent on deoxyhemoglobin concentration, and strongly dependent on the amount of dissolved molecular oxygen (O_2). The lack of hematocrit correction in this study likely added noise to our results. Although the amount of dissolved O_2 is already low during air-breathing and would not be increased by either hypercapnia or hypoxia, the corresponding deoxyhemoglobin concentration changes may have biased the calculated CBF values. Specifically, during hypoxia, actual CBF may have been slightly higher than reported. This effect competes against the impact of decreasing oxygenation on transverse relaxation rates.

In summary, this study utilized a reliable setup for gas challenge paradigms, assessed the intra-scan reproducibility of transverse relaxation rate measurements for the first time, and demonstrated changes in these parameters with hypercapnia and hypoxia in a large cohort. From our measurements, we recommend the use of strong hypoxia for studying oxygenation mapping methods in healthy volunteers over hypercapnia, which creates competing effects and potentially inconclusive measurements. We also recommend the use of R_2' change, over R_2^* or R_2 , for studying brain tissue oxygenation. Future studies should concentrate on improving R_2' mapping sequences to evaluate focal oxygenation changes in individuals. T_1 quantification should also be performed for quantitative oxygenation studies involving ASL, relaxometry, and any strong gas challenge.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Imaging protocol. Initial anatomical imaging was performed using a T₁-weighted brain volume (BRAVO) sequence. During initial air breathing, reproducibility of oxygenation (measured using 2D GESFIDE) and CBF (using ASL) was performed in succession to test reproducibility. Following this, one of four gases was administered to perturb oxygenation, and following a 2-3 minute period to allow brain oxygenation levels to stabilize, a final set of oxygenation and CBF imaging was performed. At the start of each epoch, high-order quadratic shimming was performed to minimize extrinsic susceptibility effects.

Figure 2.

a) GESFIDE sequence's idealized signal evolution. Each circle represents a collected image, such that 40 echoes in total are acquired during one 3 min 30 s acquisition. b) Sequence diagram corresponding to the multiple echoes. c) Representative images corresponding to the echo times of the solid symbols on signal curve of a).

Figure 3.

a) Gas challenge hardware setup, with the Ceretec Hi-Ox mask separated into its components, and arrows indicating direct of gas flow. The subject breathes through the facemask. The anti-suffocation valve permitted inflow of room air only when there is a negative pressure differential in the mask relative to the room environment. b) Photo of the customized elastic strap that improves facemask seal.

Figure 4.
Changes in physiological measurements due to each gas challenge: strong hypercapnia (sHC), mild hypercapnia (mHC), mild hypoxia (mHO), and strong hypoxia (sHO).

Figure 5.

Correlation and Bland-Altman plots for CBF, R_2' , R_2^* and R_2 in air. R_2' had relatively lower reproducibility compared to R_2^* and R_2 , with the standard deviation of intra-scan difference of approximately 8.6% of mean. CBF had high reproducibility, though our measured baseline GM/WM CBF ratio was $157 \pm 6\%$ —lower than previously reported pcASL measurements with different imaging parameters (39, 40).

Figure 6.

Representative data sets from a) strong hypercapnic and b) strong hypoxic gas challenges. Despite the low SNR of the GESFIDE data sets, we observe no reduction in R_2' in spite of CBF augmentation during hypercapnia, and elevation in R_2' with no change in CBF during hypoxia.

Figure 7. Bar charts illustrating CBF, R_2' , R_2^* , and R_2 (a: absolute change; b: percentage change) with error bars indicating standard deviation.

Table 1

Mean and standard deviation for absolute and percentage CBF, R_2' , R_2^* , and R_2 in GM, along with p values assessing the significance of the values.

		sHC		mHC		mHO		sHO	
		Mean±SD	p	Mean±SD	p	Mean±SD	p	Mean±SD	p
CBF	mL/100 g/min	14±9	0.03 [#]	10±7	0.03 [#]	2±3	0.52	1±4	1.00
	%	21±12	0.03 [#]	19±24	0.03 [#]	3±5	0.42	2±6	1.00
R_2'	s ⁻¹	-0.02±0.26	1.00	-0.08±0.30	1.00	0.28±0.31	0.16	0.53±0.24	0.03 [#]
	%	-1±12	1.00	-3±15	1.00	13±13	0.16	25±13	0.03 [#]
R_2^*	s ⁻¹	-0.28±0.26	0.12	-0.28±0.39	0.24	0.37±0.33	0.12	0.80±0.40	0.02 [#]
	%	-2±2	0.12	-2±2	0.19	2±2	0.12	5±2	0.02 [#]
R_2	s ⁻¹	-0.26±0.14	0.03 [#]	-0.20±0.17	0.03 [#]	0.09±0.13	0.14	0.27±0.25	0.10
	%	-2±1	0.03 [#]	-1±1	0.03 [#]	1±1	0.14	2±2	0.10

All tests were corrected for multiple comparison using the Holm-Bonferroni method.

[#] indicates p<0.05.

Table 2

p-values for pairwise comparisons, between gas conditions (four gas challenges), of changes in physiological measurements.

	S _p O ₂	P _{in} CO ₂	f _{in} O ₂	P _{et} CO ₂	f _e O ₂	Heart Rate	Resp Rate	Sys BP	Dias BP	MAP
mHC-sHC	0.49	0.16	0.001 [#]	0.03 [#]	0.001 [#]	0.01 [#]	0.94	1.00	0.02 [#]	0.73
mHO-sHC	0.001 [#]	0.001 [#]	0.001 [#]	0.001 [#]	0.001 [#]	0.01 [#]	1.00	1.00	0.003 [#]	0.45
mHO-mHC	<0.001 [#]	<0.001 [#]	<0.001 [#]	<0.001 [#]	<0.001 [#]	0.99	1.00	1.00	0.83	1.00
sHO-sHC	0.001 [#]	0.001 [#]	0.001 [#]	0.001 [#]	0.001 [#]	0.99	1.00	1.00	0.04 [#]	0.45
sHO-mHC	<0.001 [#]	<0.001 [#]	<0.001 [#]	<0.001 [#]	<0.001 [#]	0.05	0.94	1.00	1.00	1.00
sHO-mHO	0.02 [#]	0.52	0.001 [#]	0.06	0.001 [#]	0.09	1.00	1.00	1.00	1.00

All tests were corrected for multiple comparisons using the Holm-Bonferroni method.

[#] indicates p<0.05.

Table 3

All p-values for pairwise comparison between gas groups, for absolute and relative percentage CBF, R_2' , R_2^* and R_2 measurements in GM.

	CBF		R_2'		R_2^*		R_2	
	Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative
mHC vs. sHC	0.77	0.42	0.57	0.62	0.85	0.85	0.43	0.43
mHO vs. sHC	0.01 [#]	0.01 [#]	0.07	0.10	0.01 [#]	0.01 [#]	0.00 [#]	0.00 [#]
mHO vs. mHC	0.01 [#]	0.01 [#]	0.07	0.10	0.01 [#]	0.01 [#]	0.00 [#]	0.00 [#]
sHO vs. sHC	0.01 [#]	0.01 [#]	0.01 [#]	0.01 [#]	0.00 [#]	0.00 [#]	0.00 [#]	0.00 [#]
sHO vs. mHC	0.01 [#]	0.01 [#]	0.00 [#]	0.01 [#]	0.00 [#]	0.00 [#]	0.00 [#]	0.00 [#]
sHO vs. mHO	0.77	0.73	0.13	0.18	0.05	0.09	0.21	0.21

All tests were corrected for multiple comparison using the Holm-Bonferroni method.

[#] indicates $p < 0.05$.