The impact of normoxic and hyperoxic baseline periods in block paradigms of hypercarbic cerebrovascular reactivity studies

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Target audience: Researchers interested in mechanisms of hypercapnic cerebrovascular reactivity contrast and preparation of related methods for clinical trials.

Purpose: Identification of cerebrovascular compromise in the clinic is frequently performed using catheter angiography, which provides high levels of sensitivity to intra-luminal change but is insensitive to tissue-level hemodynamics. Additionally, this method is sub-optimal for longitudinal monitoring due to repeat radiation and contrast exposure. Alternatively, hypercarbic-normoxic (HC-NO; e.g., 5%CO_2; 21%O_2, 74%N_2) BOLD fMRI can be used to assess cerebrovascular reactivity (CVR) and parenchymal reserve capacity[1,2,3]. As a significant proportion of patients for whom assessment of CVR is desirable are hypoxic, and may be operating near reserve, capacity, the safety of HC-NO gas administration is of concern. A hypercarbic-hyperoxic (HC-HO; e.g., 5%CO_2/95% O_2) challenge is alternatively possible, and in a recent trial of 92 patients with symptomatic cerebrovascular disease, HC-HO was found to elicit no adverse events and provided a surrogate marker of cerebrovascular reserve consistent with intracranial vasculopathy[4]. However, HC-HO gas introduces several experimental confounds, including elevated PaO_2 and PvO_2, reduced blood water T_1, and non-specific rebinding of O_2 dissolved in plasma to dHb in veins[4,5]. Here, the aim was to determine the feasibility of controlling for such confounds by administering baseline hyperoxia (HO; 95% O_2 / 5% N_2) rather medical grade room air (RA; 21% O_2 / 79% N_2) interleaved with HC-HO. The hypothesis to be investigated was that HC-HO stimuli interleaved with a HO baseline would provide comparable contrast to HC-NO stimuli.

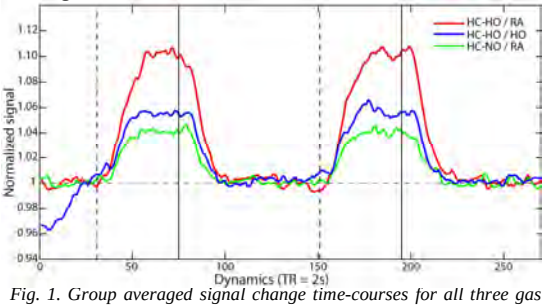

Fig. 1. Group averaged signal change time-courses for all three gas pairings. Vertical dashed lines indicate vasoactive stimulus onset, while solid vertical lines indicate vasoactive stimulus cessation. Note that signal increase is evidenced during initial onset of baseline HO (blue) as HO effects stabilize.

Methods: *Experiment.* MRA-confirmed healthy volunteers (n=12; 3F/9M; age=28.8±3.7 yrs) provided informed, written consent in accordance with local IRB guidelines and were scanned at 3T (Philips). Participants were fitted with a nasal cannula for $EtCO_2$ monitoring and a nonrebreathing oxygen mask for gas administration. The stimulus paradigm consisted of a 29 min scan during which subjects were presented with two blocks each of the following gas pairings, RA (21% O_2 / 79% N_2) interleaved with HC-NO (5% CO_2; 21% O_2, 74% N_2), RA interleaved with HC-HO (5% CO_2 / 95% O_2), and HO (95% O_2 / 5% N_2) interleaved with HC-HO, while BOLD data (TR/TE=2000/35 ms; spatial resolution 3x3x4 mm^3) were acquired. Subjects were randomly assigned to one of three stimulus orderings. *Analysis.* BOLD data were slice-time, motion, and baseline drift corrected. Signal changes and z-statistics were calculated. Z-statistics were calculated for each of the gas pairings, from which the resulting maps were thresholded at Z > 2, binarized, and multiplied to produce a mask indicating common activated voxels across all conditions. This mask was then used to extract signal changes and Z-statistic values. Extracted values were analyzed using Pearson correlations and Student's t-tests.

Results: The group averaged BOLD signal change time-course is shown in **Fig. 1**. Stimulus reproducibility was assessed across stimulus block pair signal changes (**Fig. 2a-c**) through a Pearson's correlation and demonstrates that stimuli were significantly correlated (R^2 = 0.77, P<.0001; **Fig. 2d**). T-tests demonstrate that mean percent signal changes (HC-NO/RA=4.10±0.92; HC-HO/HO=5.20±1.85; HC-HO/RA=9.91±2.21) are significantly different across all conditions (HC-NO/RA vs. HC-HO/HO: P=.017; HC-HO/HO vs. HC-HO/RA: P<.0001; HC-NO/RA vs. HC-HO/RA P<.0001; **Fig. 3a**), but that by administering baseline HO, the amount of signal change is significantly reduced for HC-HO. Furthermore, analysis of Z-statistic images indicates that Z-statistics do not differ between HC-NO/RA and HC-HO/HO, but HC-HO/RA is significantly greater (P=.0006 and P=.004, respectively; **Fig. 3b**).

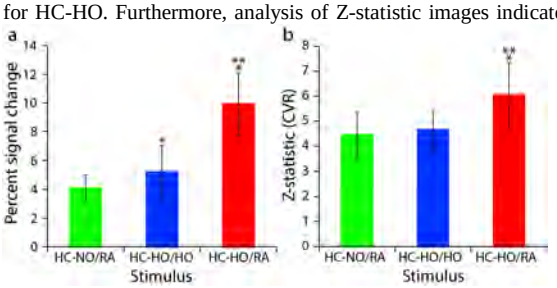

*Fig. 3. a) Percent signal change bar graphs for the 3 gas pairings. b) Z-statistic (CVR) bar graphs for the 3 gas pairings. * = significantly greater than HC-HO/RA; ** = significantly greater than HC-HO/HO. Signal changes are significantly different across all conditions, while Z-statistics reduce effects of HO and only demonstrate significant differences when not accounting for HO effects in vasocactive stimuli by administration of baseline HO.*

Discussion: The primary findings from this study are that (i) administration of baseline HO prior to a HC-HO stimulus significantly reduces the BOLD signal change relative to HC-HO interleaved with RA, (ii) analysis of Z-statistic images significantly reduces any residual effect of HO during HC-HO administration not accounted for by baseline HO, and (iii) administration of common vasoactive gas stimuli yield reproducible signal changes using the decribed setup. These results provide evidence that some of the concerns associated with CVR assessment by HC-HO administration during BOLD imaging (i.e., artificially elevated BOLD-signal due to rebinding of excess venous O_2 to hemoglobin) can be reduced by administering a baseline HO gas with equivalent O_2 content to that of the HC-HO stimulus. It should be noted that administration of baseline HO does not fully account for non-CVR related HC-HO signal changes. In a parallel analysis we have modeled[6] this effect and found it is primarily dependent on the increase of the baseline signal, with additional contributions from extravascular decreases in R_2* caused by HO. As extravascular water constitutes approximately 95% of the MR voxel and 70% of the BOLD effect at the 3T[7], the increased venous and arterial HbO_2 during HO causes larger R_2* changes in the surrounding tissue during HC-HO than during HO alone. Previous evidence has indicated that use of a general linear model to yield Z-statistic maps aids in reducing or eliminating the effect of HO not related to CVR[4], resulting in significant spatial correlations with perfusion[8]. Here we further demonstrate this method reduces non-CVR related signal during administration of HC-HO with baseline HO.

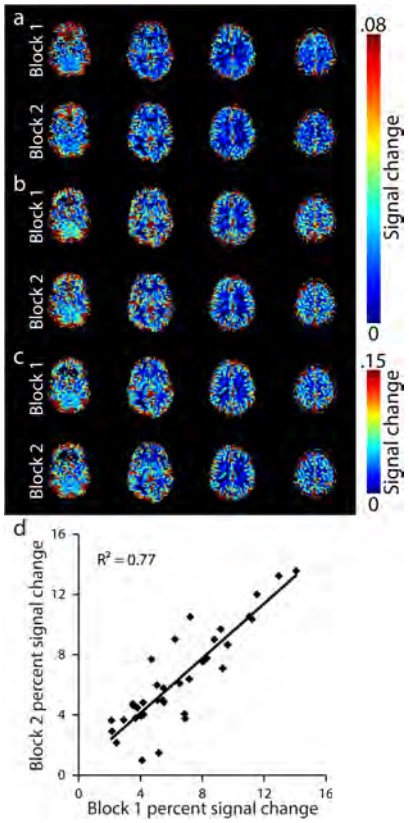

Fig. 2. a-c) Representative signal change maps from one subject for the 3 gas pairings: (a) HC-NO/RA, (b)HC-HO/HO, and (c) HC-HO/RA. d) Scatter plot of signal changes for first and second blocks of vasoactive gas stimuli, indicating significant reproducibility.

Conclusion: Reduction of non-CVR related changes associated with HC-HO vasoactive stimuli can be achieved by administration of a baseline HO gas, and sequential measurements with different levels of hyperoxia can provide information about extravascular R_2* changes unique to vascular stimuli.

References/Funding: 1. Spano, V.R.,et al. Radiology. 2013; 266(2): 592-598. **2.** Yezhuvath, U.S., et al., NMR Biomed. 2009; 22(7): 779-786. **3.** Wise, R.G., et al., JCBFM. 2007; 27(8): 1521-1532. **4.** Donahue, M.J., et al., Stroke. 2014; 45(8): 2335-2341. **5.** Hare, H.V., et al. JCBFM. 2013; 33(11): 1799-1805. **6.** Lu, H., et al. JCBFM. 2004; 24(7): 7664-770. **7.** Donahue, M.J., et al., NMR Biomed. 2010; 24(1): 25-34. **8.** Faraco, C.C., et al., MRM. 2014. NIH/NINDS 5R01NS078828