Comparison of ASL measures of cerebrovascular reactivity to CO2 using different respiratory manipulations
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Introduction: ASL measurements of the cerebrovascular reactivity to CO2 (CVR) may provide a clinical index of the cerebral vasculature’s health status1 and are also an essential component of calibrated MRI to estimate the rate of oxidative metabolism in the brain2. CVR is usually expressed as the increase in cerebral blood flow (CBF) per mmHg increase in end-tidal PCO2 (PETCO2) that are measured during a respiratory manipulation. A common method for inducing hypercapnia, i.e. CO2 increases, consists of administering air mixtures with a fixed concentration of CO2 (FI). In FI-based manipulations the PETCO2 changes depend on different physiological parameters, rendering reproducibility of the stimulus challenging. Moreover, accelerated breathing during hypercapnia can lead to incidental changes in CO2 and O2 levels. Based on a specially designed sequential rebreathing circuit and incorporating a physiological modeling of CO2 fluxes in the body, the RespirAct system, an implementation of the PC method, can target PETCO2 and PETO2 levels individually. Another approach that has been used to induce hypercapnia is to have subjects to execute a breath hold (BH). Although very simple, requiring minimal equipment and setup time, BH methods are limited to subjects who are able to understand and comply with the breath-hold instructions. Disadvantages include the associated hypoxia and difficulty estimating the changes in PETCO2 for the normalization of CBF responses. We sought to determine whether these different types of respiratory manipulation lead to consistent measurements of CVR. 

Methods: Ten young healthy subjects were scanned in a Siemens 3T using pseudo-continuous ASL (pCASL) to measure CBF, during 4 different respiratory manipulations of ~10 min duration each (Fig. 1). pCASL parameters were: labeling time = 1.5s, delay = 0.9s, TR/TE = 3000/10ms, 11 slices with 7x4x4mm resolution. In the PC manipulation a commercial system (RespirAct™, Thornhill Research Inc.) was used to control PETCO2 and PETO2. The system was programmed to increase the subjects’ PETCO2 by 5mmHg in two different instances of 2m:20s while keeping PETO2 unchanged. In the FI, the paradigm followed the same block design as in PC, with subjects breathing air during the baseline condition and a 5% CO2 air mixture to stimulate hypercapnia. In the BH, subjects hold their breath for 20s in 12 different instances, separated by 30s of paced breathing (PB). In the latter 3 manipulations subjects were instructed to pace their breath at 16 breaths per min (bpm). In a fourth manipulation that mimicked the block design of PC and FI, the baseline condition consisted of hyperventilation (HV) – with subjects breathing at 24bpm and stimuli of 2 compounded blocks of breath-hold. Respiratory levels of CO2 (and O2) were continuously monitored using a nasal cannula and a gas sampler/analyser (Biopac MP150). The baseline levels and amplitude of the hypercapnic responses were obtained through linear modeling of the end-tidal points in the capnographs (Fig. 2, left panel: capnographs in grey, models in solid black). As to the CBF measures, the ASL image series were first motion corrected, flow series were obtained by subtracting tag-control images and the difference series were then fit with a GLM consisting of the monitored values of PETCO2 plus a 3rd order polynomial representing the drift terms and a constant offset (Fig 2, right panel: ASL signal in grey, models in black). The missing values of PETCO2 during breath-holds were completed with the estimates obtained with the capnograph modeling. The ASL signal was converted to physiological units of CBF as in ref 5. The GLM fit yielded estimates of the baseline CBF and related increases. CBF was averaged within a grey matter probability ROI obtained with the segmentation of anatomical acquisitions (MPRAGE of 1mm³ resolution). The resting "normal" PETCO2 and CBF levels were obtained from additional scans in which subjects breathed air spontaneously. End-tidal and CBF/CVR values were compared during conditions and against the resting levels using paired t-tests.

Results: Results are summarized in Fig. 3. Differences between ΔPETCO2 were generally statistically significant, with the exception of the pair FI and HV (B). Baseline levels of PETCO2 were significantly different than resting values (A). Whereas FI, BH and HV had baseline PETCO2 levels that were lower than the spontaneously arising values, the PC manipulation had levels that were higher. Baseline PETCO2 levels were, as expected, lowest in HV, where ventilatory rate was 50% higher than in the rest of the manipulations. The paced breathing of 16bpm caused subjects to go hypoxic in the FI and BH scans, and baseline PETCO2 did not differ significantly between the respective manipulations. PETCO2 levels much below 40 mmHg were difficult to achieve with the RespirAct protocol. Changes in O2 were all significantly different from zero (D). In PC and FI, changes were minimal and virtually indistinguishable. Baseline levels of PETO2 were above resting levels for HV, but they were all significantly different from zero (F). Since PETO2 increases mostly due to the HV response. Baseline CBF levels were generally correlated with baseline PETCO2, with PC having considerably higher values than the other manipulations, amongst whom the differences were not statistically significant. When CVR was expressed in terms of the absolute change in CBF per mmHg change in PETCO2 there were significant differences in the values given by the different methods (H). Absolute CVR values were correlated with the resting PETCO2 associated with each manipulation. When expressed as the percent change in CBF per unit of change in PETCO2, PC, FI, and BH yielded comparable CVR estimates that did not differ by a statistically significant amount (G). The HV manipulation, again, gave a significantly lower CVR, indicating that CBF responses are significantly attenuated for lower doses of CO2.

Discussion: Reproducibility of the hypercapnic stimuli in all 4 manipulations were comparable. Incidental changes in O2 were well controlled in the FI manipulation using the paced breathing. In BH, the hypoxia may have stretched the respective CVR value, but the effect seems to be minor. Values of CVR expressed in terms of the absolute change in CBF per unit of change in PETCO2 depended on the range of PETCO2 levels considered, indicating that the non-linearity of the CBF-CO2 dose-response curve exerts significant influence on the CVR reactivity observed using ASL. Variability between methods was reduced when CVR was expressed in terms of the percent change in CBF, but with HV producing a significantly lower, but less robust estimate. We conclude that manipulations involving significant hypocapnic baseline should be avoided.
