



Impact Of Intermittent Hypercapnia On Glymphatic Clearance In Healthy Adults And Patients With Alzheimer's Disease

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Background

A failure of the glymphatic clearance of proteins and peptides implicated in neurodegeneration may contribute to the pathophysiology of Alzheimer's disease (AD).^{1,2} Efficient waste clearance relies on vasomotion (intraslow constriction and dilation of blood vessels) to drive cerebrospinal fluid (CSF) through the interstitial space and clear waste from the brain.³⁻⁵ As such, augmenting vasomotion may increase clearance of proteins implicated in neurodegeneration and serve as a disease-modifying intervention. Our recent work suggests that intermittent hypercapnia (IHC) enhances intraslow vasomotion and promotes CSF inflow and clearance in healthy controls (HC) and patients with Parkinson's disease (proposed mechanisms illustrated in Figure 1).⁶ The current study evaluated whether intermittent hypercapnia (exposure to low levels of CO₂ in ON-OFF cycles) can be used to induce intraslow oscillations in cerebral blood flow, CSF inflow, and clearance of proteins and peptides implicated in AD from the brain.

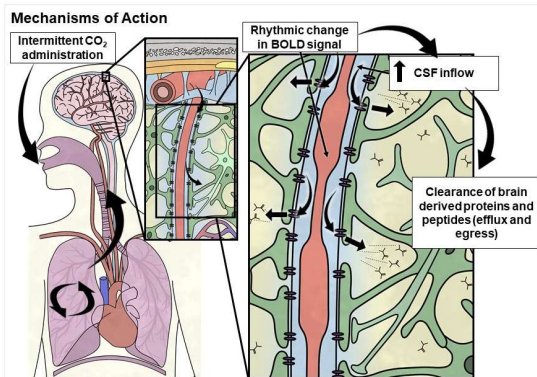


Figure 1. Hypothesized Mechanisms of Action. Intermittent hypercapnia (IHC) stimulates vasomotion that drives CSF into the perivascular and interstitial space (influx), clearing proteins and peptides implicated in PD and metabolic waste such as lactate clearance (efflux). *Original figure.*

Methods

Participants

- Eight HC and 4 patients positive for plasma pTau217⁷ ages 50-89 underwent a baseline period of normal breathing followed by three IHC sessions (each session 8 cycles of 35 s ON and 35 s OFF; Figure 4) while undergoing blood-oxygen-level-dependent (BOLD) imaging.
- Five blood draws via an intravenous catheter (IV) were obtained at two baselines, 45 min (earliest point after the participants completed IHC and were out of the MRI), 90 min, and 150 minutes after the start of the intervention to evaluate whether increased concentrations of plasma biomarkers could be detected in plasma following IHC.
- The HD-X analyzer (Quanterix) ultrasensitive single molecule array (SIMOA) technology was used to obtain plasma concentrations of:
 - Brain derived Tau (BD-Tau)
 - Amyloid-β 40 and 42 (Aβ40; Aβ42)
 - Glial Fibrillary Acidic Protein (GFAP)
 - Neurofilament Light Chain (NFL)
- Diagnostic:
 - Phosphorylated Tau 217 (pTau217)

Study Overview



Figure 2. Study Overview. Participants underwent three intermittent hypercapnia (IHC) sessions (each session 8 cycles of 35 s ON and 35 s OFF 30 min total) in the MRI with 5 blood draws via an intravenous catheter (IV) obtained at two baselines, 45, 90, and 150 minutes after the start of the intervention. Blood-oxygen-level-dependent (BOLD) imaging was collected for a baseline period of 120 s followed by 8 cycles of IHC. Symptoms were assessed at pre-specified intervals throughout the procedures.

Overview of intermittent hypercapnia (IHC) paradigm and blood oxygen level dependent MRI data processing

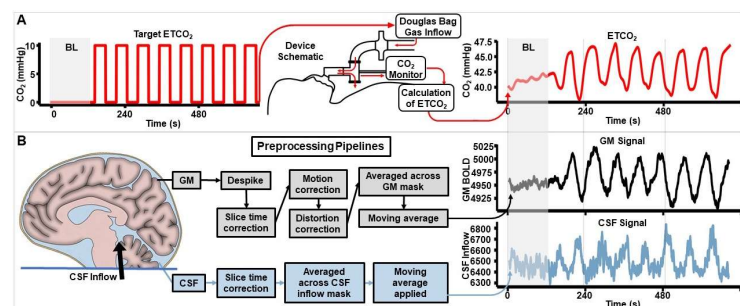


Figure 3. Overview of intermittent hypercapnia (IHC) paradigm and blood oxygen level dependent MRI data processing. **A.** The IHC paradigm exposes individuals to a medical grade gas mixture (5% CO₂, 21% O₂, BAL N₂), resulting in an increase in CO₂ for ON and OFF cycles. The exhaled or end-tidal CO₂ (ETCO₂) is used to evaluate the magnitude of the cerebrovascular response (reflecting vasomotion) and cerebrospinal fluid (CSF) flow to CO₂. **B.** The overall gray matter (GM) blood oxygen level dependent (BOLD) signal is used to quantify vasomotion. CSF inflow is detected at the lowest scan slice at the 4th ventricle.

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Acknowledgements

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Intermittent hypercapnia increases plasma concentrations of brain-derived proteins and peptides

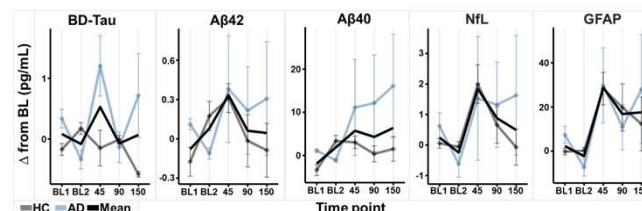


Figure 4. Plasma biomarker concentrations in response to intermittent hypercapnia (IHC). Two baseline (BL) and three post-IHC measurements were collected at ~45, 90 and 150 minutes following the start of IHC. Peak responses were observed at 45-minutes with significant increases of plasma concentrations observed across healthy controls (HC) and patients with Alzheimer's disease (AD). Linear mixed-effects model analysis of plasma biomarkers of neurodegeneration identified the greatest peak response at 45 minutes. Plasma concentrations increased from 4.5-18.5% over the average of the two baselines [HC and AD (n=12, except pTau217 and BD-Tau n=6); GFAP ($p=0.001$), NFL ($p=0.010$), Aβ42 ($p=0.016$), Aβ40 ($p=0.066$), BD-Tau ($p=0.107$), and pTau217 ($p=0.097$)].

Intermittent hypercapnia induces vasomotion and cerebrospinal fluid (CSF) inflow similar to patterns observed during non-rapid eye (NREM) sleep.

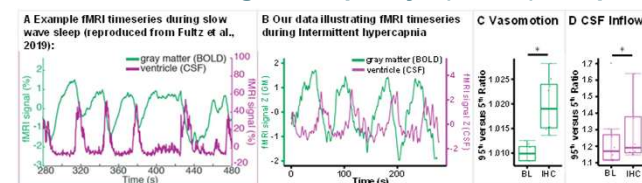


Figure 5. A. Fultz et al., (2019)⁸ demonstrated that low frequency oscillations in gray matter (GM) blood oxygen level dependent (BOLD) signal elicits increases in cerebrospinal fluid (CSF) inflow during slow wave sleep (Fultz et al., (2019) *Science*) **B.** Our data depicting the pattern of GM BOLD and CSF inflow in response to IHC is similar to that observed during NREM sleep, when glymphatic clearance is greatest. **C.** Our data showing the magnitude of GM BOLD and CSF inflow is significantly greater following IHC relative to baseline (BL).

Conclusions

- Intermittent hypercapnia increased intraslow oscillations in cerebral blood flow and CSF inflow in HC and patients with AD.
- Intermittent hypercapnia increased the appearance of biomarkers of neurodegeneration in the plasma of both HC and AD participants. Increased plasma concentrations suggest that these primarily brain derived proteins and peptides are cleared from the brain into systemic circulation following IHC.
- This suggests that intermittent hypercapnia may be used to clear toxic brain byproducts from the brain.
- Future work is necessary to evaluate these effects in a larger cohort as well as the safety, tolerability, and clinical efficacy of repeated administration.