Absolute measurements of cerebral perfusion and oxygenation in rats with near-infrared spectroscopy

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Introduction

Brain microvascular pathology is a common finding in Alzheimer's disease and other dementias. However, the extent to which microvascular abnormalities cause or contribute to cognitive impairment is unclear. Dietary vascular risk factors, including poor folate status are potentially modifiable predictors of cognitive impairment among older adults. Folate deficiency in rat impairs cognition and causes cerebral microvascular damage, without concomitant neurodegeneration [1]. We hypothesized that folate deficiency might result in functional decrements in cerebral oxygen delivery and vascular reactivity. To test this hypothesis, we developed a non-invasive near-infrared spectroscopy (NIRS) system to obtain quantitative, dynamic measurements of absolute brain hemoglobin concentration and oxygen saturation and used it to demonstrate significant cerebrovascular impairments in a rat model of diet-induced vascular cognitive impairment. With respect to control animals, folate deficient (FD) rats featured significantly lower brain hemoglobin concentration (73±10 μM vs. 95±14 μM) and oxygen saturation (55%±7% vs. 66%±4%). By contrast, resting arterial oxygen saturation was the same for both groups (96%±2%), indicating that brain oxygenation decrements were independent of blood oxygen carrying capacity. Vasomotor reactivity in response to hypercapnia was also impaired in folate deficient rats. Our results implicate microvascular abnormality and diminished oxygen delivery as a mechanism of cognitive impairment .

Methods

- 12 Sprague Dawley rats were fed their assigned diets (control (n=6) or folate deficient (n=6) diets) for 20 weeks and NIRS measurements were conducted at weeks 10 and 20.
- Cerebral concentrations of oxy-hemoglobin ([HbO_2]), deoxy-hemoglobin ([Hb]) and oxygen saturation of hemoglobin (%StO_2) were measured by NIRS [2,3].
- Measurements were made in anesthetized rats, subjected to changes in inspired O_2 (Hypoxia) and CO_2 (Hypercapnia).
- Each condition was repeated 3-4 times in each rat, with continuous NIRS monitoring of baseline and response to hypoxic/hypercapnic challenge
- Differences in the capillary density and in the overall cross-section of blood vessels in the investigated tissue volume ($\Delta(V_b/V_t)/(V_b/V_t)$) was calculated using the following equation, which relates relative changes in total hemoglobin concentrations in tissue and in blood ($[HbT]_b$):

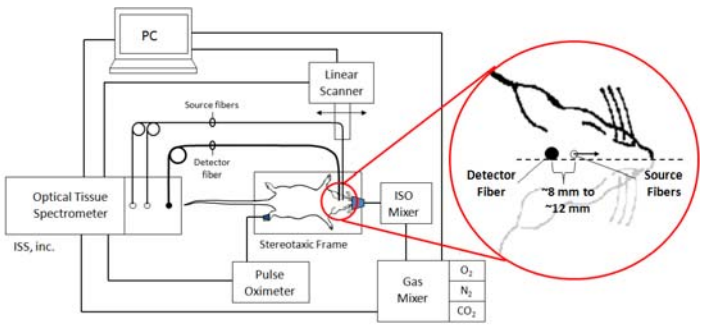
$$\frac{\Delta[\mathrm{HbT}]}{[\mathrm{HbT}]} = \frac{\Delta[\mathrm{HbT}]_b}{[\mathrm{HbT}]_b} + \frac{\Delta(V_b/V_t)}{(V_b/V_t)} \qquad (1)$$

- Vasomotor reactivity with respect to changes in blood volume (VMR_V) and flow (VMR_F) induced by hypercapnia challenge was calculated using the following equations:

$$\mathrm{VMR_V} \equiv \frac{\Delta[\mathrm{HbT}]}{[\mathrm{HbT}]_0} \qquad (2)\,, \qquad \mathrm{VMR_F} \equiv \frac{A}{\mathrm{SaO_2}\cdot[1-e^{-A}(A+1)]}\cdot\Delta\mathrm{StO_2} \qquad (3)$$

where A is a dimensionless constant that describes oxygen consumption in brain.

Figure 1 – Illustration of instrument design, experimental setup, optical probe placement on the rat's head, and the linear scanning scheme for absolute near-infrared spectroscopy measurements



Results

Figure 2 – Time traces of [Hb], [HbO_2], [HbT], StO_2 and SaO_2 during the hypoxia and hypercapnia protocols 10 and 20 weeks after the start of folate deficient diet (blue and red lines represent the mean values for each dietary group, whereas, dashed lines indicate the range corresponding to ± one standard error from the mean). A first striking result is the consistency of baseline values across animals within a group (control or folate deficient), and the reproducibility of baseline values measured at weeks 10 and 20. Absolute brain total hemoglobin concentration ([HbT]) and tissue oxygen saturation (StO_2) are significantly reduced by folate deficiency on week 10. These differences are maintained on week 20. Arterial oxygenation (SaO_2) unaffected. Relative changes in response to hypoxia and hypercapnia are similar.

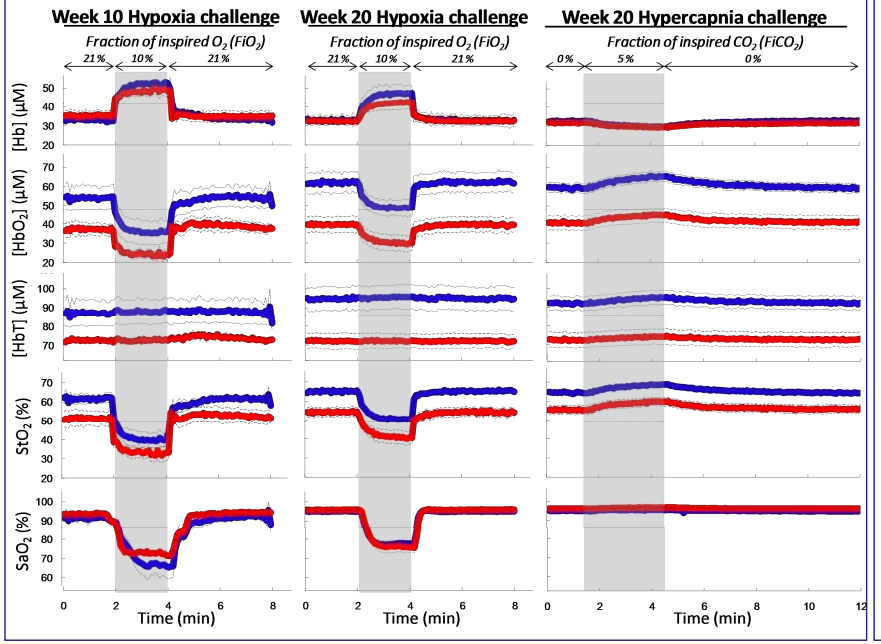


Figure 3 – Illustration of the differences in animal groups and changes within each group between weeks 10 and 20 in cerebral tissue saturation (StO_2) and concentration of hemoglobin ([HbT]), blood concentration of hemoglobin ($[HbT]_b$), and partial blood volume (V_b/V_t) Eq. (1). FD rats have significantly lower cerebral partial blood volume compared to control rats. However, partial blood volume in FD rats appears to recover to control levels by week 20, suggesting microvascular remodeling between weeks 10 and 20.

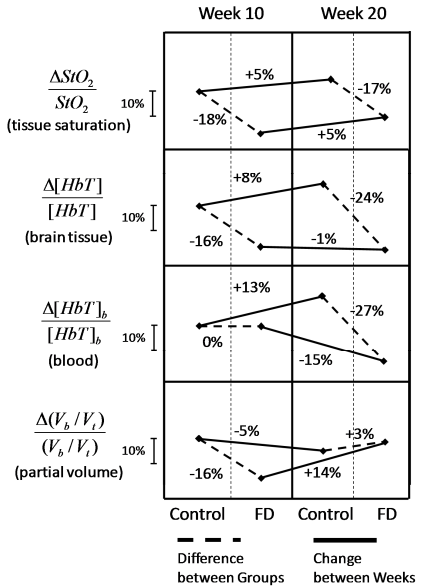
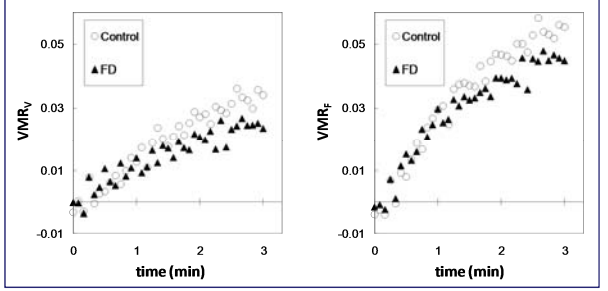


Figure 4 – Vasomotor reactivity in response to hypercapnia associated with vascular volume changes (VMR_V) or blood flow velocity changes (VMR_F). Folate deficient (FD) rats feature that vasomotor response is significantly dampened in the FD rats by the end of the challenge period with respect to both blood volume and blood flow velocity. First one minute of the hypercapnia challenge did not show statistical difference between FD and control rats (p>0.05); however response to these changes at the final thirty seconds of the hypercapnia challenge were significantly lower in FD than control rats (p<0.001)



Discussion

- The ability to perform non-invasive, accurate, reproducible, and robust absolute measurements of cerebral tissue hemoglobin concentration and saturation can be of paramount importance for the characterization of hemodynamic cerebral responses as a mechanism of neurological dysfunction.
- We have demonstrated the critical importance of obtaining absolute measurements of these parameters, as opposed to technically easier relative measurements.
- We were able to show that folate deficiency can induce a significant deficit in central nervous system (CNS) oxygen delivery and blood perfusion, as indicated by lower [HbT] and [HbO2], and (StO2) despite normal (SaO2), which could explain the cognitive deficits observed in this model.
- The anatomical validation of NIRS based CBV estimates and structural microvascular correlates of the functional NIRS measures remains to be determined.

Acknowledgments

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