

## Reply to: “Does ammonia really disrupt brain oxygen homeostasis?”



To the Editor:

We are grateful for Drs. Sørensen and Vilstrup's interest<sup>1</sup> and thoughtful consideration of our recent study<sup>2</sup> and are encouraged that they agree with our substantive conclusions. We welcome this opportunity to reply to their concerns regarding our specific interpretation that decreased tissue  $pO_2$  could contribute to the metabolic disruption seen in hepatic encephalopathy (HE).

We understand they argue that observations of metabolic disruption, such as decreased cerebral blood flow (CBF), are secondary to a decrease in metabolic demand caused by ammonia reducing central nervous system activity. We agree that a reduction in metabolic demand is a significant component contributing to the neuronal dysfunction seen in patients with HE and believe that our hypotheses could be fully compatible. We propose that a negative feedback loop is constructed between the supply and demand of all metabolic substrates in the brain during the development of HE, as a result of the maladaptation of a number of homeostatic processes that would normally closely match energy demand and supply. Whether it is demand or supply that is first to drop, it still feeds into the same negative feedback loop that sees the brain less able to respond to the metabolic demands of neurons, leading to neurological dysfunction.

The first pillar of their concern relates to the baseline tissue  $pO_2$  observed in our model. Specifically, that it is not sufficient to induce significant metabolic disruption as some of the cohort display a  $pO_2$  above a proposed 'critical' range of 8.8-6.7 mmHg in healthy rats.<sup>3</sup> We posit that the absolute concentration of oxygen is immaterial to our hypothesis and our observation simply provides evidence that a major metabolic component (oxygen) is significantly lower in our model of HE when compared to sham subjects. Any reduction in  $pO_2$  would impair the brain's ability to maintain metabolic sufficiency.

We argue that the absolute concentration of tissue oxygen is not a reliable indicator for potential neuronal dysfunction. Firstly,  $pO_2$  measurements are not generalizable given the lack of homogeneity of tissue oxygenation within the brain and differences in experimental conditions (such as anesthetics and sampling locations) that would affect baseline tissue  $pO_2$  values. Additionally, the level of oxygen tension used by several studies to mimic hypoxia is in the range of 20–25 mmHg,<sup>4,5</sup> which is within the normal range of brain parenchymal  $pO_2$ .<sup>6</sup> However, there is substantial evidence that the brain responds to reductions in oxygen tension before reaching 8.8mmHg. Astrocytes are activated at  $\sim 17$ mmHg causing elevations in

intracellular calcium and release of ATP by exocytosis.<sup>7</sup> Further, in astrocytes, mild hypoxia activates a range of compensatory mechanisms that rebalance metabolism; including inhibition of mitochondrial respiration,<sup>8</sup> production of potent vasodilators,<sup>8,9</sup> and increases in the rate of lipid peroxidation, among other effects.<sup>7</sup> This may be important for local control of cerebral microcirculation when  $pO_2$  decreases in a particular microdomain of the brain. Chronic activation of this pathway is expected to have detrimental effects on metabolic supply matching, and consequently neuronal function. Our conclusions merely speculate on the possible effects that a constraint of the oxygen supply seen in our model may have on neuronal function given that the brain is an extremely energy intensive organ with several systems having evolved to ensure constant energy supply. Impairment of any one of these mechanisms would, at the very least, put pressure on the reserve of the others to effectively manage brain metabolic demand.

As for our interpretation of the observed low lactate concentration, we argue that the brain would reduce the production of lactate in response to a gradual downward pressure on both metabolic demand and supply in the brain. Increases in lactate would be indicative of extreme metabolic distress as there is sufficient tissue oxygenation even at 6.7 mmHg for some level of aerobic metabolism to be maintained. It is worth noting here that our model is one of *mild* HE. We agree that CBF data is essential to our understanding of this complex system, and we are in the process of addressing this unmet need. Further, we believe that care should be taken when interpreting decreased CMRglc in bile duct ligated (BDL) animals<sup>10</sup> as direct evidence of decreased CMRO<sub>2</sub>. Total glucose uptake measured by FDG-PET does not inform on the mitochondrial oxidative reactions, which can be maintained by alternative energy substrates.

Finally, although potentially contradictory to Drs. Sørensen and Vilstrup's data, recent *in vitro* (coculture of astrocytes and neurons) and *ex vivo* studies (brain slices from BDL rats), have shown that concentrations of ammonia as low as 5  $\mu$ M induce mitochondrial hyperpolarisation, lipid peroxidation, increased reactive oxygen species production, as well as profound neuronal death in the hippocampus.<sup>11</sup>

To summarize, we consider metabolic demand and supply closely coupled in the pathophysiology of HE. Simultaneous interventions on both would be the best approach to breaking the reinforcement of our proposed negative feedback loop and maximizing the possibility of complete reversal of neurological impairment after resolution of hyperammonemia.

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**Conflict of interest**

RJ has research collaborations with Takeda and Yaqrit and consults for Yaqrit. RJ is the founder of Yaqrit Limited, which is developing UCL inventions for treatment of patients with cirrhosis. RJ is an inventor of ornithine phenylacetate, which was licensed by UCL to Mallinckrodt. He is also the inventor of Yaq-001, DIALIVE, and Yaq-005, the patents for which have been licensed by his University into a UCL spinout company, Yaqrit Ltd. All other authors report no conflict of interest.

Please refer to the accompanying ICMJE disclosure forms for further details.

**Authors' contributions**

The manuscript was written by Patrick Hosford and Anna Hadjihambi, who together with Rajiv Jalan reviewed the final version critically.

**Supplementary data**

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jhepr.2022.100666>.

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