Cerebral oxygen sensing and the integrated regulation of hypoxic vasodilatation

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Modern humans are the most encephalised of all species, with a brain that has more than tripled in size over the last 3 million years, much thanks to a dramatically enlarged neocortex. Yet being so big doesn't come cheap and fuelling the brain is energetically expensive. Indeed, despite weighing less than 2 % body mass, we allocate a disproportionate 20 % of the body's resting basal oxygen (O_2) budget to maintaining its "dark energy", equating to a cerebral metabolic rate of oxygen ($CMRO_2$) of ~3 mL/min/100g, more than 10 times that expected from its mass alone. Any interruption in the continuous supply of precious O_2 can spell disaster for the brain given its almost exclusive reliance on aerobic metabolism and baffling lack of energy reserve (Bailey, 2019).

Since its O_2 supply is indeed so delicate, it would seem intuitive for evolution to favour feedback mechanisms capable of "sensing" subtle changes in blood O_2 concentration and transmitting signals to the cerebrovasculature coupling local cerebral O_2 delivery (cDO_2) to tissue metabolic demand. Yet precisely how the brain "titrates" cDO_2, the product of cerebral blood flow (CBF) and arterial O_2 content (caO_2), fundamental components of the Fick Principle underlying convective cerebral O_2 transport, remains enigmatic. What is clear is that too little or too much of this paradoxically toxic, mutagenic gas can ultimately kill you; the brain balances precariously on a bioenergetic tightrope (Bailey, 2019) (Figure 1).

In this issue of *Experimental Physiology*, Howe *et al*. (2019) went to considerable lengths to explore precisely this, elegantly highlighting the brain's "balancing act" in response to differential manipulations in red blood cell (RBC) mass during acclimatisation to terrestrial high-altitude (HA) hypoxia and following acute hypervolemic haemodilution. Specifically, they sought to isolate to what extent haemoconcentration contributes to changes in CBF independent of ventilatory adaptation throughout the course of acclimatisation, concluding that (the) "CBF response…is generated (primarily) by diuresis and erythropoiesis-mediated increases in haemoglobin (Hb) and haematocrit (Hct), while the remaining contribution…is likely attributable to ventilatory acclimatization". The authors consistently observed inverse relationships between changes in caO_2 (and not PaO_2) and CBF such that cDO_2 remained invariant throughout, that is, well preserved, consistent with the conservation of mass principle (Ainslie *et al.*, 2016; Bailey *et al.*, 2017).

That CBF is primarily governed by compensatory changes in caO_2 and not PaO_2 (i.e. "bound" in terms of saturation and carrying capacity and not "free" O_2) to maintain convective O_2 delivery extends earlier works incorporating haemodilution/carbon monoxide (CO) interventions and in acute/chronic anaemia (reviewed in Hoiland *et al.*, 2016). Furthermore, isovolumic haemodilution is associated with blunted cerebral hypoxic vasodilatation and CDO_2 attributable to attenuated desaturation (reviewed in Ainslie *et al.*, 2016; Hoiland *et al.*, 2016). Of note in the current study, albeit in the face of hypervolemia, the authors observed an equivalent suppression in cerebral perfusion (upon mathematical correction for full restoration of Hct) independent of (unaltered) arterial O_2 saturation (Howe *et al.*, 2019).

Collectively, these findings single out the erythrocyte as an important regulator of cerebral hypoxic vasodilation with Hb [more specifically, the allosteric shift from relaxed (oxygenated) to tense (deoxygenated) state] implicated as the hypoxic sensor capable of releasing vasoactive metabolites

from neurons, astrocytes, pericytes and smooth muscle cells. Considerable evidence supports an increasingly important role for nitric oxide (NO) with the stable metabolites nitrite (NO_2^-) and *S*-nitrosohaemoglobin (SNO-Hb) prime candidates given their ability to conserve and transfer bioactivity "long-distance" within the cerebral microcirculation (Bailey *et al.*, 2017) (Figure 1).

However, there are some incongruencies, indeed la "bete noire" lies with the fact that pharmacological blockade of NO fails to fully attenuate the CO- or systemic hypoxia-induced vasodilatation during exercise (Gonzalez-Alonso *et al.*, 2001). Equally, mathematical approximation of the deoxyHb "stimulus" [i.e. deoxyHb mass to blood volume (BV) ratio] estimated as [1-(SaO_2/100) × total Hb mass (Hb concentration × BV)/BV] based on the current data reported by Howe *et al.*, (2019), indicates that haemodilution should have invoked less (NO-mediated) vasodilatation (24 *vs*. pre-haemodilution control value of 27 g/L), excluding potential NO-mediated contributions caused by blood viscosity-induced alterations in shear-stress (see below). Thus, NO (deoxyHb) paints a powerful picture, but it's clearly not the only artist at play; spare a thought for blood viscosity!

Indeed, notwithstanding contributions from other mediators including *β*-adrenergic receptor activation, prostaglandins, epoxyeicosatrienoic acids, ATP-sensitive potassium channels and adenosine, future researchers should give some thought to free radical-mediated activated coagulation and altered plasma viscosity (Figure 1). This makes intuitive sense given the inextricable relationship between Hb (primary determinant of caO_2) and Hct (major component of blood viscosity). However, despite an albeit limited body of literature, the weight of evidence favours caO_2 over viscosity, implying that cerebral O_2 transport is actively regulated and not simply a passive consequence of altered Hct and viscosity (Figure 1).

But let's get back to that (spared) thought. Emerging evidence suggests that hypoxia and exercise-induced activated coagulation is indeed subject to redox-regulation subsequent to a free radical-mediated increase in the bioactivity of coagulation factor VIII and von Willebrand factor notwithstanding the oxidative-inactivation of bioactive NO that can also trigger platelet aggregation and thrombin formation that collectively increase blood viscosity (Fall *et al.*, 2018). Indeed, it is becoming increasingly clear that hypoxia shifts the endothelial phenotype towards one in which anticoagulant properties decrease and pro-oxidative-inflammatory-nitrosative stress dominates the endovascular milieu (Bailey *et al.*, 2019). Likewise, a reduction in blood viscosity following haemodilution could potentially suppress vascular shear-induced endothelial NO-mediated cerebral vasodilatation, as rightfully highlighted by the current authors (Howe *et al.*, 2019).

The new information presented by Howe *et al*. (2019) makes an important contribution to a complex and clinically relevant topic while encouraging the need for further research. Human translational physiological approaches that take advantage of low and high viscosity replacement fluids, pharmacological NO blockade (neuronal and endothelial), regional (trans-cerebral) sampling of bioactive metabolites including blood gas analysis to quantify cerebral O_2 extraction (the "forgotten sister" and final link in convective cerebral O_2 transport) will ultimately help resolve the relative contributions of each of the pathways underpinning the integrated regulation of cerebral perfusion.

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Legend

Figure 1. The "push, pull and nudge" of cerebral oxygen delivery in hypoxia; a bioenergetic tightrope

Summary of the vascular, rheological, pulmonary and renal pathways underpinning the integrated regulation of cerebral blood flow (CBF) during differential manipulations in red blood cell (RBC) mass incurred by hypoxia (↑) or haemodilution (↓). Given that haemoglobin (Hb), the primary determinant of arterial oxygen content (caO_2) and haematocrit (Hct), the primary determinant of blood viscosity are intimately related, isolating their independent roles in the regulation of CBF has traditionally proven challenging. Both caO_2 (vascular "push") and viscosity (rheological "pull") are inversely related to CBF in the healthy brain consistent with the observations reported by Howe *et al.*, (2019) with the former considered the dominant stimulus notwithstanding comparatively minor, albeit important contributions from changes in acid-base balance (pulmonary/renal "nudge"). Viscosity is considered the primary contributor in the diseased (ischaemic) brain given that cerebral vasodilatation is (at or close to) maximal. A reduction in caO_2 such as that encountered during hypoxia and corresponding allosteric modification of Hb from the oxygenated to deoxygenated state allows for NO bioactivity to escape the RBC resulting in compensatory hypoxic vasodilatation (left-hand side). Equally, making blood "thinner" through haemodilution decreases Hct and plasma viscosity increasing cerebral perfusion consistent with the Hagen-Poiseuille equation, albeit constrained within the context of a compliant system (right-hand side). All three mechanisms are subject to redox-regulation and collectively strive to maintain cerebral O_2 delivery (CDO_2) and optimise O_2 transport/cellular partial pressure of O_2 (PO_2) given the brain's inherent vulnerability to energetic failure. However, it is important to note that haemodilution typically fails to adequately maintain CDO_2 in the clinical setting with patients at increased risk of acute neurological injury and

long-term impairment (reviewed in Hoiland *et al.*, 2016). Hatched, colour-coded (traffic light) boxes tentatively "rank" those mechanisms considered most important in the current publication (green, amber, red). $CMRO_2$, cerebral metabolic rate of oxygen; CBF, cerebral blood flow; caO_2/cvO_2, arterial/venous O_2 content; DO_2, O_2 delivery; oxy/deoxyHb, oxygenated/deoxygenated haemoglobin; NO_2^-, nitrite; ATP, adenosine triphosphate; SNO-Hb, *S*-nitrosohaemoglobin NO, nitric oxide; OXINOS, oxidative-inflammatory-nitrosative stress; Q, flow; P_1-P_2, pressure gradient; R, resistance; η, viscosity; L, length; r, radius.