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A flow-diffusion model of oxygen transport for quantitative mapping of cerebral metabolic rate of oxygen ($CMRO_2$) with single gas calibrated fMRI

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Abstract

One promising approach for mapping $CMRO_2$ is dual-calibrated functional MRI (dc-fMRI). This method exploits the Fick Principle to combine estimates of CBF from ASL, and OEF derived from BOLD-ASL measurements during arterial O_2 and CO_2 modulations. Multiple gas modulations are required to decouple OEF and deoxyhemoglobin-sensitive blood volume. We propose an alternative single gas calibrated fMRI framework, integrating a model of oxygen transport, that links blood volume and CBF to OEF and creates a mapping between the maximum BOLD signal, CBF and OEF (and $CMRO_2$). Simulations demonstrated the method's viability within physiological ranges of mitochondrial oxygen pressure, P_mO_2, and mean capillary transit time. A dc-fMRI experiment, performed on 20 healthy subjects using O_2 and CO_2 challenges, was used to validate the approach. The validation conveyed expected estimates of model parameters (e.g., low P_mO_2), with spatially uniform OEF maps (grey matter, GM, OEF spatial standard deviation ≈ 0.13). GM OEF estimates obtained with hypercapnia calibrated fMRI correlated with dc-fMRI ($r = 0.65$, $p = 2{\cdot}10^{-3}$). For 12 subjects, OEF measured with dc-fMRI and the single gas calibration method were correlated with whole-brain OEF derived from phase measures in the superior sagittal sinus ($r = 0.58$, $p = 0.048$; $r = 0.64$, $p = 0.025$ respectively). Simplified calibrated fMRI using hypercapnia holds promise for clinical application.

Keywords

Calibrated functional magnetic resonance imaging (calibrated fMRI), cerebral metabolic rate of oxygen (CMRO2), hypercapnia, hyperoxia, oxygen transport modelling

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Introduction

Oxidative metabolism provides most of the brain's energy and is altered in a variety of pathologies such as neurodegenerative and neuroinflammatory diseases, stroke, epilepsy, and migraine.[1] Magnetic resonance imaging (MRI) approaches for mapping baseline ($_0$) cerebral metabolic rate of oxygen ($CMRO_{2,0}$)[2–8] exploit the Fick Principle, that expresses CMRO[2] as the product of oxygen delivery (the product of oxygen concentration in arterial blood, C_aO_2, and cerebral blood flow, CBF) and oxygen extraction fraction (OEF) measured in either the macrovascular or the microvascular compartment. Macrovascular CBF_0 can be estimated from volume flow rate in large feeding arteries or draining veins using flow encoding sequences,[2] whereas OEF_0 can be assessed in draining veins using sequences that measure magnetic susceptibility or blood T_2 predominantly affected by the presence of deoxyhemoglobin (dHb).[8,9] Since large vessels feed or drain significant portions of brain, such measures deliver global or regional information at best. Microvascular CBF_0 can be mapped using perfusion-weighted sequences such as Arterial Spin Labelling (ASL). One drawback of ASL is its low contrast in white matter (WM). Moreover, mapping microvascular OEF_0 is challenging because baseline magnetic susceptibility and MR relaxation parameters within a voxel with a small vascular compartment are not uniquely affected by dHb.

Among others,[10,11] dual-calibrated functional MRI (dc-fMRI)[4,12–15] is a promising approach for OEF_0 and $CMRO_{2,0}$ mapping. While measuring CBF_0 with ASL, dc-fMRI estimates OEF_0 from the blood oxygen level dependent (BOLD) signal sensitivity to dHb_0. dc-fMRI uses BOLD-ASL recordings, biophysical modelling of BOLD signal[16] and assumed isometabolic hypercapnic and hyperoxic modulations of CBF and C_aO_2 through respiratory stimuli. BOLD sensitivity to dHb_0 is encoded in the maximum BOLD increase, M, corresponding to complete dHb removal. The two respiratory stimuli decouple the contribution to M of OEF_0 and the dHb_0-sensitive cerebral blood volume, $CBV_{v,0}$, when Hb concentration in blood [Hb][4,14] is known. Although dc-fMRI has been applied in exemplar clinical studies,[17–21] its adoption is limited by the low signal to noise ratio (SNR)[22] and by the complex gas challenge paradigm required.

Here, we introduce a new calibrated fMRI framework that estimates OEF_0 with only one measurement of M based on one manipulation of brain physiology and a flow-diffusion model of oxygen transport.[13,23,24] The model describes the steady-state oxygen diffusion from the capillaries into the tissue (equal to $CMRO_2$) as proportional to the product of the mean capillary transit time (mean CTT, MCTT) and the pressure gradient between the capillary bed and the mitochondria (where the proportionality constant is the effective tissue permeability to oxygen, k). Since MCTT can be expressed as the ratio between capillary blood volume (CBV_{cap}) and CBF, the flow-diffusion model can be incorporated in the formulation of M by substituting $CBV_{v,0}$ for an appropriately scaled $CBV_{cap,0}$ (with ρ being the scaling factor). This substitution replaces one unknown variable, $CBV_{v,0}$, with two unknowns, one proportionality constant, being a function of ρ and k, and the oxygen pressure at the mitochondria ($P_mO_{2,0}$). The advantage of the model lies in the tight distributions of the new parameters and on the reduced effect of their variabilities in the estimate of OEF_0, creating a probabilistic mapping of M, $C_aO_{2,0}$ and CBF_0 with OEF_0 and $CMRO_{2,0}$ as the parameters to be inferred.

This manuscript reports the validation of the novel single gas approach. We term the new approach using a hypercapnic stimulus, hc-fMRI+, and that using a hyperoxic stimulus, ho-fMRI+. The report is divided into four sections. The first section, by exploiting simulations, describes the advantages, the validity, and the robustness to noise of the framework. The second section investigates the new model *in-vivo* using a dc-fMRI experiment, employing alternating hypercapnic and hyperoxic gas challenges in healthy subjects. We use a global estimate of OEF_0 in the grey matter (GM), obtained with dc-fMRI analysis,[22] and we invert the single gas model using only the hypercapnic or the hyperoxic component of the experiment to investigate the distribution of the proportionality constant and $P_mO_{2,0}$ across subjects. The third section validates hc-fMRI+ and ho-fMRI+ against dc-fMRI.[22] To do so, the two parameters of the model are fixed to the average values obtained from the previous analysis, and the model is inverted to infer OEF_0. Finally, in the fourth section, GM OEF_0 estimates from the different fMRI approaches are compared to whole-brain OEF_0 inferred from a validated MRI sequence performing phase measures in the superior sagittal sinus (SSS) and conventionally termed 'OxFlow'.[2,25]

Methods

Analytical modeling

Here we summarize the analytical model derivation. Please refer to Supplementary Information for a more detailed description.

BOLD model and the Dual-Calibrated fMRI experiment. The rate of signal decay due to dHb, $R_2^*|_{dHb}$, within a voxel is represented by:[26,27]

$$R_2^*|_{dHb} = \mathrm{A} \cdot CBV_v \cdot \left((1 - S_vO_2) \cdot [Hb]\right)^\beta \quad (1)$$

where S_vO_2 is venous saturation, [Hb] is the concentration of hemoglobin in blood and CBV_v is the BOLD sensitive blood volume. β ($\beta = 1.3$ at 3 T) and A are field strength and vessel geometry dependent constants. For small perturbations of $R_2^*|_{dHb}$ and using the Grubb relation linking fractional changes in CBV_v and CBF, the steady-state BOLD signal can be expressed, within the Davis Model framework, as:[14,28]

$$\frac{\Delta BOLD}{BOLD_0} = TE \cdot A \cdot CBV_{v,0} \cdot \left((1 - S_vO_{2,0}) \cdot [Hb]\right)^\beta \cdot \left\{1 - \left(\frac{CBF}{CBF_0}\right)^\alpha \cdot \left(\frac{1 - S_vO_2}{1 - S_vO_{2,0}}\right)^\beta\right\} \quad (2)$$

with the maximum BOLD signal M being equal to:

$$M = TE \cdot A \cdot CBV_{v,0} \cdot \left((1 - S_vO_{2,0}) \cdot [Hb]\right)^\beta \quad (3)$$

The subscript $_0$ depicts baseline values, $\Delta BOLD/BOLD_0$ is the relative BOLD signal change, TE is the sequence echo-time and α is the Grubb exponent ($\alpha = 0.38$). During an isometabolic manipulation of brain physiology, equation (2) can be expressed as a function of OEF_0 as:

$$\frac{\Delta BOLD}{BOLD_0} = TE \cdot A \cdot CBV_{v,0} \cdot \left(\left(1 - \frac{C_aO_{2,0}}{\varphi \cdot [Hb]} \cdot (1 - OEF_0)\right) \cdot [Hb]\right)^\beta \cdot \left\{1 - \left(\frac{CBF}{CBF_0}\right)^\alpha \cdot \left(\frac{1 - \frac{C_aO_2}{\varphi \cdot [Hb]} \cdot \left(1 - \frac{OEF_0 \cdot CBF_0 \cdot C_aO_{2,0}}{CBF \cdot C_aO_2}\right)}{1 - \frac{C_{a0}O_2}{\varphi \cdot [Hb]} \cdot (1 - OEF_0)}\right)^\beta\right\} \quad (4)$$

with C_aO_2 being the oxygen concentration in arterial blood and φ being the oxygen binding capacity of hemoglobin ($\varphi = 1.34$ mL/g). Even when combining together A and $CBV_{v,0}$ in equation (4), the equation still has two unknowns making it not possible to solve for OEF_0 through one manipulation of brain physiology. dc-fMRI solves this by performing two independent manipulations: hypercapnia and hyperoxia. However, the approach suffers from low SNR, a problem that has been addressed by regularizing the inversion procedure for OEF_0[29] and by using, simulation-trained, machine learning approaches applied to raw recordings.[22]

Flow-Diffusion model of oxygen transport. A simple model can be used to describe the steady-state radial oxygen diffusion into the tissue along a straight cylindrical capillary of unit length:[24]

$$\frac{dC_{cap}O_2(x)}{dx} = -k \cdot T_{cap} \cdot \left(P_{cap}O_2(x) - P_mO_2\right) \quad (5)$$

where $C_{cap}O_2$ and $P_{cap}O_2$ are the concentration and the partial pressure of oxygen at a relative position x along the capillary and T_{cap} is the CTT. k, the effective permeability, combines the effects of the capillary wall and the surrounding brain tissue into a single interface between the plasma and a well-stirred oxygen pool at the mitochondria at end of the diffusion path, at which the pressure of oxygen is equal to P_mO_2.[13,30] CTT in the single straight capillary is then approximated by the MCTT in the capillary bed within the voxel. MCTT is expressed as the ratio between the capillary blood volume (CBV_{cap}) and CBF. Since $P_{cap}O_2$ and $C_{cap}O_2$ quickly equilibrate (less than a few milliseconds), depending upon the nonlinear nature of Hb binding to oxygen described mathematically by the Hill Equation:

$$SO_2 = \frac{1}{1 + \left(\frac{P_{50}}{PO_2}\right)^h} \quad (6)$$

the following can be obtained:

$$CBF \cdot \frac{dC_{cap}O_2(x)}{dx} = -k \cdot CBV_{cap} \cdot \left(P_{50} \cdot \sqrt[h]{\frac{C_{cap}O_2(x)}{\varphi \cdot [Hb] - C_{cap}O_2(x)}} - P_mO_2\right) \quad (7)$$

where P_{50} is the oxygen partial pressure when half of Hb is saturated (generally $P_{50} \approx 26$ mmHg; P_{50} can be inferred from a measure of end-tidal partial pressure of carbon dioxide, $P_{ET}CO_2$), and h is the Hill constant (h = 2.8). An approximated closed solution to the differential equation (7) can be made assuming a linear decrease of $C_{cap}O_2(x)$ and an average $C_{cap}O_2(x)$ equal to $\langle C_{cap}O_2(x)\rangle \approx \varphi \cdot [Hb] \cdot (S_aO_2 + S_vO_2)/2 = \varphi \cdot [Hb] \cdot (1\text{-}OEF/2)$, where S_aO_2 is the arterial oxygen saturation. Integrating equation (7) and equalizing the oxygen loss from the capillary to $CMRO_2$, the following is obtained:

$$CMRO_2 = CBF \cdot OEF \cdot CaO_2 = k \cdot CBV_{cap} \cdot \left(P_{50} \cdot \sqrt[h]{\frac{2}{OEF} - 1} - P_m O_2 \right) \quad (8)$$

Integration of the Flow-Diffusion model of oxygen transport into the BOLD model for calibrated-fMRI quantification of $CMRO_2$. CBV_{cap} is here assumed to be a fraction of CBV_v, i.e., $CBV_v = \rho \cdot CBV_{cap}$. Substituting CBV_{cap}, from equation (8) into equation (4), we obtain:

$$\frac{\Delta BOLD}{BOLD_0} = TE \cdot \frac{A \cdot \rho}{K} \cdot \frac{CBF_0 \cdot OEF_0 \cdot CaO_{2,0} \cdot \left(\left(1 - \frac{C_aO_{2,0}}{\varphi[Hb]} \cdot (1 - OEF_0) \right) \cdot [Hb] \right)^{\beta}}{\left(P_{50} \cdot \sqrt[h]{\frac{2}{OEF_0} - 1} - P_m O_{2,0} \right)} \cdot \left\{ 1 - \left(\frac{CBF}{CBF_0} \right)^{\alpha} \cdot \left(\frac{1 - \frac{C_aO_2}{\varphi[Hb]} \cdot \left(1 - \frac{OEF_0 \cdot CBF_0 \cdot C_aO_{2,0}}{CBF \cdot C_aO_2} \right)}{1 - \frac{C_aO_{2,0}}{\varphi[Hb]} \cdot (1 - OEF_0)} \right)^{\beta} \right\} \quad (9)$$

with the maximum BOLD signal M equal to:

$$M = TE \cdot \frac{A \cdot \rho}{K} \cdot \frac{CBF_0 \cdot OEF_0 \cdot CaO_{2,0} \cdot \left(\left(1 - \frac{C_aO_{2,0}}{\varphi[Hb]} \cdot (1 - OEF_0) \right) \cdot [Hb] \right)^{\beta}}{\left(P_{50} \cdot \sqrt[h]{\frac{2}{OEF_0} - 1} - P_m O_{2,0} \right)} \quad (10)$$

Equations (9) and (10) encode a non-linear mapping of measurable quantities M, $C_aO_{2,0}$ and CBF_0 with OEF_0, enabling OEF_0 (and hence $CMRO_{2,0}$) to be inferred using a single manipulation of brain physiology. Apart from the constants that can be indirectly inferred (e.g., P_{50}, [Hb]), assumed (e.g., φ, β) or controlled (e.g., TE), the mapping depends on the non-measurable quantities: A, ρ, k and $P_mO_{2,0}$. A, having the same origins as β,[31] can be estimated assuming primarily an extravascular BOLD signal and assuming $R_2^*|_{dHb} = R_2'$[32,33]. With an experimentally determined cortical R_2' of approximately $3\,s^{-1}$ at 3 T[34], an average [Hb] of 14 g/dL, a S_vO_2 of 0.6, and a mean CBV_v of 2.5%, from equation (1) we expect a value of $A \approx 14\ s^{-1}g^{-\beta}dL^{\beta}$ at 3 T. *In-vivo* variation in ρ has not been studied directly; we discuss this in the Supplementary Information. We expect ρ to be in the range 2 to 3, assuming a capillary blood volume between 20% to 40% of total blood volume, when the arterial contribution is assumed to be 20% to 30%.[35] Moreover, we expect a value for the oxygen effective permeability k of around 3 μmol/mmHg/ml/min.[22] This value is derived from the literature using a different formalism where oxygen diffusion is assumed to happen at the endothelial wall of capillaries.[36] In equations (9) and (10), we create a practical grouping of A, ρ and k into one multiplicative parameter $A \cdot \rho/k$. At a fixed field strength, all the three parameters are related to tissue structure and vessel geometry, which plausibly affects water and oxygen diffusion in the intravascular and extravascular spaces as well as the volumetric relationship between capillaries, venules and veins. We expect a value of $A \cdot \rho/k$ of the order of $A \cdot \rho/k \approx 10\,s^{-1}g^{-\beta}dL^{\beta}/(\mu mol/mmHg/ml/min)$. The mitochondrial oxygen partial pressure at rest, $P_mO_{2,0}$, must lie between 0 mmHg and the average oxygen tension of the capillary bed. Several *in vivo* studies suggest that oxygen tension at brain mitochondria is small in the healthy brain,[23,37] and this theory is consistent with functional hyperemia in response to increased brain oxygen demand. However, departure from a negligible oxygen tension is plausible in the diseased brain.

In summary, the non-linear mapping in equation (9) permits estimation of OEF_0 from one manipulation of brain physiology. Uncertainty in the mapping is driven by variability in two non-measurable quantities, a proportionality constant $A \cdot \rho/k$, that depends on tissue and micro-vessel structure at a fixed field strength, and $P_mO_{2,0}$. Importantly, these non-measurable quantities affect the non-linear mapping differently. The advantage of the new framework lies in the low variability of these parameters and their diminished influence on the OEF_0 estimation compared to $CBV_{v,0}$.

Simulations

We performed simulations to investigate the ability of hc-fMRI+ and ho-fMRI+ to infer OEF_0. A forward model using equation (9) was implemented to simulate the BOLD signal and was inverted to retrieve OEF_0. In the forward model some variables were fixed (TE, α, β, h ε, φ) (ε is the oxygen plasma solubility, $\varepsilon = 0.0031$ mL/mmHg/dL) while others were simulated based on random sampling from physiologically and physically plausible distributions. When inverting the model, some random variables were unknown and were either fixed a-priori ($A \cdot \rho/k$, $P_mO_{2,0}$), or inferred (OEF_0, $CBV_{cap,0}$ and $MCTT_{,0}$). Firstly, we ran the full forward and inverse analysis without measurement noise as a function of either the value chosen a-priori for the random variables that were fixed during the inversion or other parameters of interest ($P_mO_{2,0}$ and $MCTT_{,0}$). Secondly, we evaluated the effect of measurement noise, which was introduced on measures with lower signal to noise ratio (SNR), namely ASL CBF/CBF_0 and $\Delta BOLD/BOLD_0$. 10^7 simulations per condition were conducted; the non-linear inversions were performed through explicit search of OEF_0 that explained the measures. The explicit search was performed in the full OEF_0 space (between 0 and 1) with a resolution of 0.01. Constant parameters were set to $\alpha = 0.38$, $\beta = 1.3$, $h = 2.8$, $\varepsilon = 0.0031\,mL/mmHg/dL$, $\varphi = 1.34\,mL/g$, $TE = 30\,ms$, whereas random variables were simulated using either normal (N) or gamma (Γ) distributions; additional physiological constraints were applied (please refer to the Table in Supplementary Information for additional information).

Figure 1 reports the distributions of the main random variables used in the forward model simulations. With respect to the parameters that were not measured for the inversion, $A \cdot \rho/k$ was simulated using a normal distribution with an average value of $10\,s^{-1}g^{-\beta}dL^{\beta}/(\mu mol/mmHg/ml/min)$ and a coefficient of variation (CoV) of 0.3, whereas $P_mO_{2,0}$ was simulated using a gamma distribution, allowing variation between zero and $<P_{cap,0}>$ to simulate a large variability in $P_mO_{2,0}$ that might be present in disease.

MRI experiment

Twenty healthy volunteers (13 males, mean age 31.9 ± 6.5 years) were recruited at CUBRIC, Cardiff University, Cardiff, UK. The study was in accordance with the Declaration of Helsinki and was approved by the Cardiff University, School of Psychology Ethics Committee and NHS Research Ethics Committee, Wales, UK. Written consent was obtained from each participant. Data were acquired using a Siemens MAGNETOM Prisma (Siemens Healthcare GmbH, Erlangen) 3 T clinical scanner with a 32-channel receiver head coil (Siemens Healthcare GmbH, Erlangen). A 18 minutes dc-fMRI scan was acquired with interleaved periods of hypercapnia, hyperoxia and medical air being delivered according to the protocol previously proposed.[13,29] 3 periods of hypercapnic gas challenges and 2 periods of hyperoxic gas challenges were performed. CO_2 and O_2 in the lungs were evaluated from the volunteer's facemask using a gas analyzer (AEI Technologies, Pittsburgh, PA, USA).

Calibrated fMRI data were acquired during the gas challenge scheme using a pCASL acquisition with pre-saturation and background suppression[38] and a dual-excitation (DEXI) readout.[39] The labelling duration (τ)

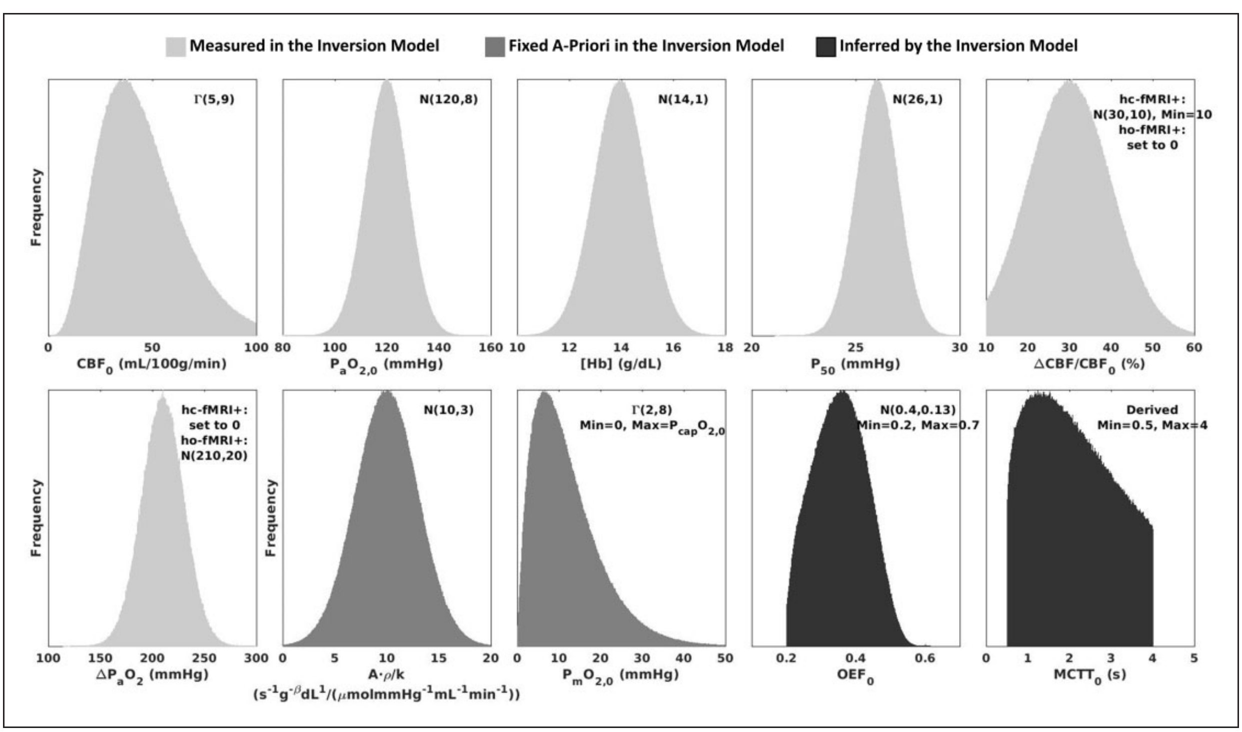

Figure 1. Random variables used to simulate BOLD and ASL signals using a hc-fMRI+ or ho-fMRI+ forward modelling framework. The variables reported in light grey were assumed to be measured for the hc-fMRI+ or ho-fMRI+ inversion model, those reported in medium grey were fixed a-priori in the inversion model and those in dark grey were inferred by the inversion model.

and the Post Label Delay (PLD) were both set to 1.5 s, GRAPPA acceleration (factor = 3) was used with $TE_1 = 10$ ms and $TE_2 = 30$ ms. An effective TR of 4.4 s was used to acquire 15 slices, in-plane resolution 3.4 mm × 3.4 mm and slice thickness 7 mm with a 20% slice gap. A calibration (S_0) image was acquired for ASL quantification with pCASL labelling and background suppression pulses switched off, with TR = 6 s, and TE =10 ms.[13] A high-resolution whole brain structural image, used for GM identification in the fMRI space, was acquired using a 3D Fast Spoiled Gradient-Recalled-Echo T1-weighted acquisition (resolution = 1 × 1 × 1 mm^3, TE = 3.0 ms, TR = 7.8 ms, TI = 450 ms, flip angle = 20°).

For susceptometry-based oximetry, a transverse slice was acquired at approximately 15 mm above the confluence of sinuses (location at which the inferior sagittal, straight, and transverse sinuses join the SSS) using a T2*-weighted spoiled multi-echo gradient-recalled echo (GRE) sequence with: in-plane resolution = 1.6 × 1.6 mm^2, slice thickness = 5 mm, field of view (FOV) = 208 × 208 mm^2, bandwidth= 260 Hz/pixel, three echo times (TEs = 3.92, 7.44, and 10.96 ms), bipolar gradient readout, TR = 35 ms, flip angle = 25°, and acquisition time = 1 min and 7 s. This acquisition was performed in the framework of the OxFlow method, which previous studies have described in detail.[40–42] For vessel identification purposes, two-dimensional T2*-weighted time-of-flight (TOF) images were acquired using a spoiled GRE sequence with: in-plane resolution = 0.86 × 0.86 mm^2, slice thickness = 2 mm, slice gap = 1.34 mm, FOV = 219 × 219 × 234 mm^3, in-plane acceleration factor = 2, bandwidth = 220 Hz/pixel, TE = 4.99 ms, TR = 20 ms, flip angle = 60°. Blood samples were drawn via a finger prick before scanning and were analyzed with the HemoCue Hb 301 System (HemoCue, Ängelholm, Sweden) to calculate [Hb].

fMRI data processing

Gas recordings processing. $P_{ET}CO_2$ and $P_{ET}O_2$ were extracted from CO_2 and O_2 recordings using in-house software in Matlab (Mathworks, Natick, MA). $P_{ET}CO_2$ and $P_{ET}O_2$ points were interpolated (cubic spline function), resampled to match fMRI, and shifted in time to maximally correlate with fMRI signals. $P_{ET}CO_{2,0}$ and $P_{ET}O_{2,0}$, were evaluated at baseline in the first 110 seconds. $P_{ET}O_2$ was assumed equal to PaO_2 for C_aO_2 computation whereas $P_{ET}CO_2$ was assumed equal to $PaCO_2$. P_{50} was inferred from estimates of resting blood pH based on the Henderson-Hasselbalch Equation, assuming $[HCO_3^-]$= 24 mmol/L:[43]

$$pH = 6.1 + \log\left(\frac{[HCO_3^-]}{0.03 \cdot P_aCO_2}\right) \tag{11}$$

and calculating P_{50} according to the linear relation, $P_{50} = 221.87–26.37 \cdot pH$[13].

SaO_2 was calculated from PaO_2 using equation (6) and CaO_2 was inferred using the relation:

$$CaO_2 = \varphi \cdot [Hb] \cdot SO_2 + \varepsilon \cdot PO_2 \tag{12}$$

Finally, to highlight hypercapnic and hyperoxic modulations, $P_{ET}CO_2$ and $P_{ET}O_2$ traces were high-pass filtered with a 4th order Butterworth digital filter and a high-pass frequency of 1/600 Hz.

fMRI processing. Both functional and structural MRIs were processed using FSL[44] and in-house algorithms implemented in Matlab. fMRI timecourses were motion corrected based on 6 degrees of freedom co-registration using MCFLIRT.[45]

High-resolution structural T1-weighted MRIs were skull-stripped using BET[46] and probability maps of Cerebrospinal Fluid (CSF), WM and GM, were computed using FAST.[47] Motion-corrected fMRI timecourses and the skull-stripped T1-weighted MRI, together with tissue probability maps, were coregistered, relying on 12 degrees of freedom affine transformation, to the S_0 image.[45] ASL control-tag difference perfusion data (ΔS) in S_0 space were obtained through surround subtraction of the fMRI timecourses at TE_1, normalized with respect to S_0 and converted to CBF in quantitative units of ml/100g/min through the pCASL single compartment kinetic model of labelled spins and voxelwise signal normalization:[48]

$$CBF = \frac{6000 \cdot \lambda \cdot e^{\frac{PLD}{T1_b}}}{\eta \cdot \ \eta_{inv} \cdot T1_b \cdot \left(1 - e^{-\frac{\tau}{T1_b}}\right)} \cdot \left(\frac{\Delta S}{S_0}\right) \tag{13}$$

where λ is the water partition coefficient ($\lambda = 0.9$ mL/g), $T1_b$ is the T1 relaxation constant of blood, η is the tagging inversion efficiency ($\eta = 0.85$), and η_{inv} is a scaling factor to account for the reduction in tagging efficiency due to background suppression ($\eta_{inv} = 0.88$).[49] The $T1_b$ was calculated from SaO_2 and PaO_2 measures using the experimental relation presented in:[50]

$$T1_b = \frac{1}{1.527 \cdot 10^{-4} \cdot P_aO_2 + 0.1713 \cdot (1 - S_aO_2) + 0.5848} \tag{14}$$

CBF_0 was evaluated in the first 110 seconds. Finally, fractional CBF was high pass filtered with a 4th order Butterworth digital filter with a high-pass frequency of 1/600 Hz. BOLD T2*-weighted time-courses were obtained through surround averaging of the fMRI at TE_2 and they were expressed as relative BOLD changes with respect to the temporal average of the BOLD signal in the first 110 seconds ($BOLD_0$). BOLD relative changes were high pass filtered with a 4th order Butterworth digital filter with a high-pass frequency of 1/600 Hz.

Both processed CBF and BOLD volumes were masked with a GM mask at 50% probability threshold.

Dual-calibrated fMRI analysis. Firstly, OEF_0 maps were obtained with a dc-fMRI analysis. Because of the method's known low SNR, explicit inversion methodologies were avoided and a state-of-the-art method to analyze the data relying on a machine learning approach was used. The machine learning algorithm was fed with fMRI timecourses and, through a time-frequency transformation of fMRI signals to extract features of interest, directly mapped OEF_0 and $CMRO_{2,0}$ relying on a pre-trained model based on simulated data. Please refer to[22] for detailed information.

Single gas calibrated fMRI analysis. Single gas calibrated fMRI analysis was performed on either the hypercapnic (using hc-fMRI+) or the hyperoxic (using ho-fMRI+) modulations within the dc-fMRI experiment. The evaluation of BOLD and ASL changes with physiological manipulations was performed using the general linear model (GLM).[51] $P_{ET}CO_2$ and $P_{ET}O_2$ were concurrently regressed on BOLD and ASL filtered modulations. The GLM β-weight delivered an estimate of BOLD or CBF modulation per unit of mmHg of $P_{ET}CO_2$ and $P_{ET}O_2$. The total modulation was then obtained by multiplying the β-weight with the maximum $P_{ET}CO_2$ or $P_{ET}O_2$ modulation. The SNR of the modulation was estimated by dividing the GLM β-weight by its confidence interval. *In-vivo* data were used to evaluate the between subjects distribution of the unknown parameters of the extended model, namely $A \cdot \rho/k$ and $P_mO_{2,0}$. This analysis was performed by extracting average BOLD and ASL modulations in the GM. These average estimates were used, together with a global estimate of GM OEF_0 obtained with the dc-fMRI analysis, to invert the model and estimate the unknown parameters. The inversion relied on equation (9), which clearly could not be solved for the two unknowns; however, since $A \cdot \rho/k$ and $P_mO_{2,0}$ differently affect the non-linear mapping between BOLD and ASL modulations and OEF_0, we were able to get insight into the average value of both parameters. In particular, we inverted the model assessing the proportionality constant $A \cdot \rho/k$ as a function of the a-priori fixed $P_mO_{2,0}$. We expected the $A \cdot \rho/k$ distribution to have a smaller CoV when $P_mO_{2,0}$ was closer to the correct average value. The non-linear inversion was performed through an explicit search in the range, for $A \cdot \rho/k$, between 0 and $40\,s^{-1}g^{-\beta}dL^{\beta}/(\mu mol/mmHg/ml/min)$ with a resolution of $0.2\,s^{-1}g^{-\beta}dL^{\beta}/(\mu mol/mmHg/ml/min)$ and, for $P_mO_{2,0}$, between 0 and 50 mmHg with a resolution of 1 mmHg. When making predictions with the single gas calibrated models, the unknown parameters were fixed both spatially and between subjects to the optimal values derived in the first step. Using these fixed parameters hc-fMRI+ and ho-fMRI+ inversion models were used for voxelwise estimation of OEF_0 and $CMRO_{2,0}$ and comparison with the estimates derived from the dc-fMRI analysis were made.

OxFlow data processing

For a subset of twelve subjects, GM estimates of OEF_0 using single or dual calibrated fMRI approaches were compared to whole-brain estimates of OEF_0 from SSS derived using the OxFlow procedure. OxFlow images were processed using Matlab and code developed in-house. OEF_0 measurements were obtained based on the normalized difference in signal phase between the first and third TEs ($\Delta\phi/\Delta TE$), with acquisitions having equal gradient polarity.[41] The static background field inhomogeneity was removed using a second-order polynomial fitting.[42] The intravascular phase was measured as the average signal phase in a region of interest centered in the cross-section of SSS relative to the average signal phase in the tissue region surrounding the SSS. The angle (θ) between the SSS and B_0 was evaluated by comparing the slice acquired for OxFlow and the SSS orientation in the slices immediately above and immediately below in the TOF image. Individual measurements of hematocrit (Hct, %) were obtained based on [Hb] assuming a ratio Hct/[Hb]=3 (%dL/g).[52]

OEF_0 was calculated using the infinite cylinder analytical model:[41]

$$OEF_0 = \frac{\frac{2\Delta\phi}{\Delta TE}}{\gamma \cdot \Delta\chi_{do} \cdot Hct \cdot B_0 \cdot \left(\cos^2\theta - \frac{1}{3}\right)} \tag{15}$$

where γ is the proton gyromagnetic ratio ($\gamma = 267.52 \cdot 10^6$ rad/s/T), and $\Delta\chi_{do} = 4\pi \cdot 0.27 \cdot 10^{-6}$ is the magnetic susceptibility difference between fully oxygenated and fully deoxygenated red blood cells.[53]

Statistical analysis

Pearson's correlations and t-tests were performed to assess pairwise associations and biases between the different estimates. Null-hypothesis probabilities (p-values) were calculated using the Student's t distribution (using transformation of correlation for association testing). Normality evaluation was performed prior to statistical inference using the Kolmogorov-Smirnov test.

Results

Simulations

Figure 2 reports the outcome in estimating OEF_0 when using hc-fMRI+ and ho-fMRI+ inversion models with fixed a-priori parameters. Figure 2(a) displays the OEF_0 root mean square error (RMSE) obtained for hc-fMRI+ and ho-fMRI+ with $A \cdot \rho/k = 10\ s^{-1}g^{-\beta}dL^{\beta}/(\mu mol/mmHg/ml/min)$ as a function of $P_mO_{2,0}$. A minimum RMSE of $OEF_0 = 0.039$ was obtained for hc-fMRI+ and a minimum RMSE of $OEF_0 = 0.051$ was obtained for ho-fMRI+, both at $P_mO_{2,0} = 11$ mmHg. Figure 2(b) displays the scatterplots of the simulated OEF_0 vs. the estimated OEF_0 for hc-fMRI+ and ho-fMRI+ when marginalizing the other variables. The scatterplots reported were obtained using a close to optimal $P_mO_{2,0}$, $P_mO_{2,0} = 10$ mmHg, and $P_mO_{2,0} = 0$ mmHg. Figure 2(c) reports the OEF_0 RMSE for the two methods evaluated as a function of two physiological parameters of interest in the forward model, namely $MCTT_0$ and $P_mO_{2,0}$, when fixing a-priori the non-measurable parameters analogous to Figure 2(b). Importantly, when adding noise to BOLD and fractional changes in CBF, the analysis highlighted the stability of the approach with respect to measurement SNR, with the OEF_0 RMSE reaching the OEF_0 RMSE related to model parameters uncertainty at BOLD and ASL SNRs around 4 (refer to Supplementary information for additional information).

In-Vivo evaluation of gas, CBF and BOLD modulations

Figure 3 reports the processing steps, in an exemplar subject, that were used to derive the hypercapnic and the hyperoxic CBF and BOLD modulations. Figure 3(a) shows O_2 signals acquired through the gas analyzer with the estimated $P_{ET}O_2$ traces whereas Figure 3(b) depicts CO_2 signals and $P_{ET}CO_2$ traces. Figure 3(c) shows example of the ASL CBF/CBF_0 and the filtered and fitted $P_{ET}CO_2$. Figure 3(d) shows the relative

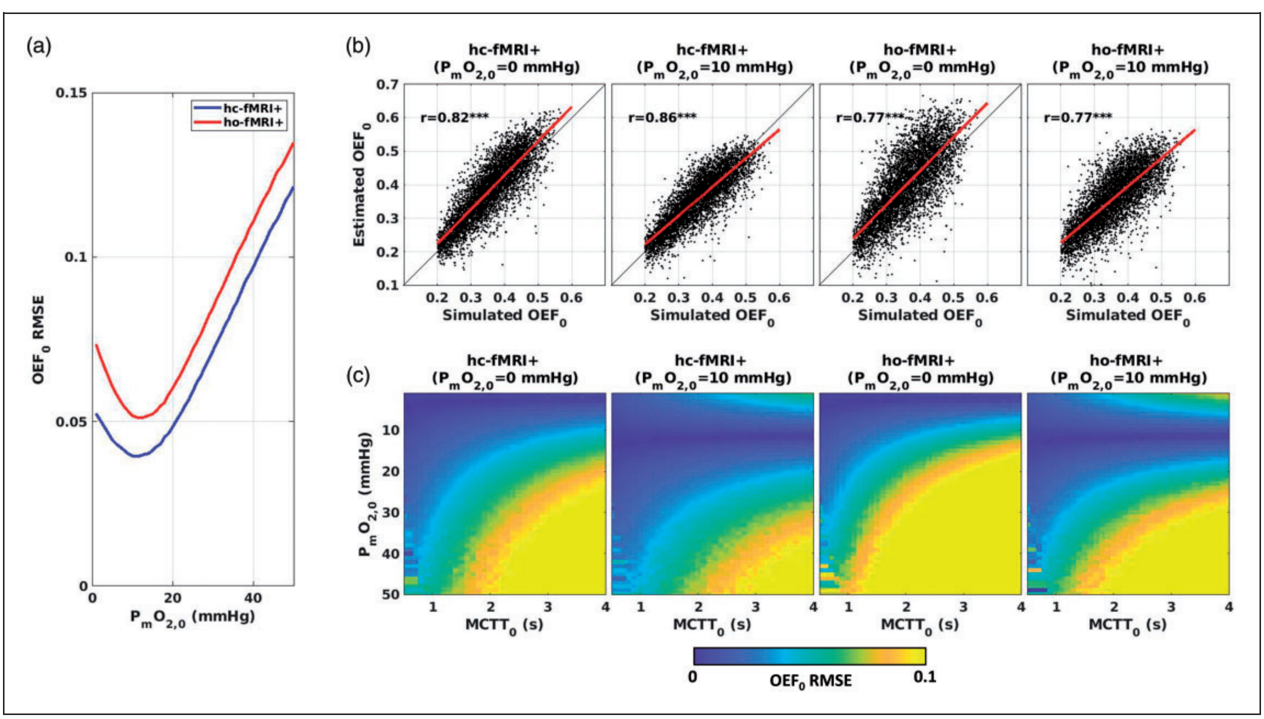

Figure 2. (a) RMSE in OEF_0 for hc-fMRI+ and ho-fMRI+ inversion models with $A \cdot \rho/k = 10\ s^{-1}g^{-\beta}dL^{\beta}/(\mu mol/mmHg/ml/min)$ as a function of $P_mO_{2,0}$. (b) Scatterplots of the simulated and estimated OEF_0 for hc-fMRI+ and ho-fMRI+ inversion models assuming either $P_mO_{2,0} = 0$ mmHg or $P_mO_{2,0} = 10$ mmHg; (c) RMSE in OEF_0 as a function of the forward model $MCTT_0$ and $P_mO_{2,0}$ for hc-fMRI+ and ho-fMRI+ inversion models assuming either $P_mO_{2,0} = 0$ mmHg or $P_mO_{2,0} = 10$ mmHg. ***$p < 10^{-3}$.

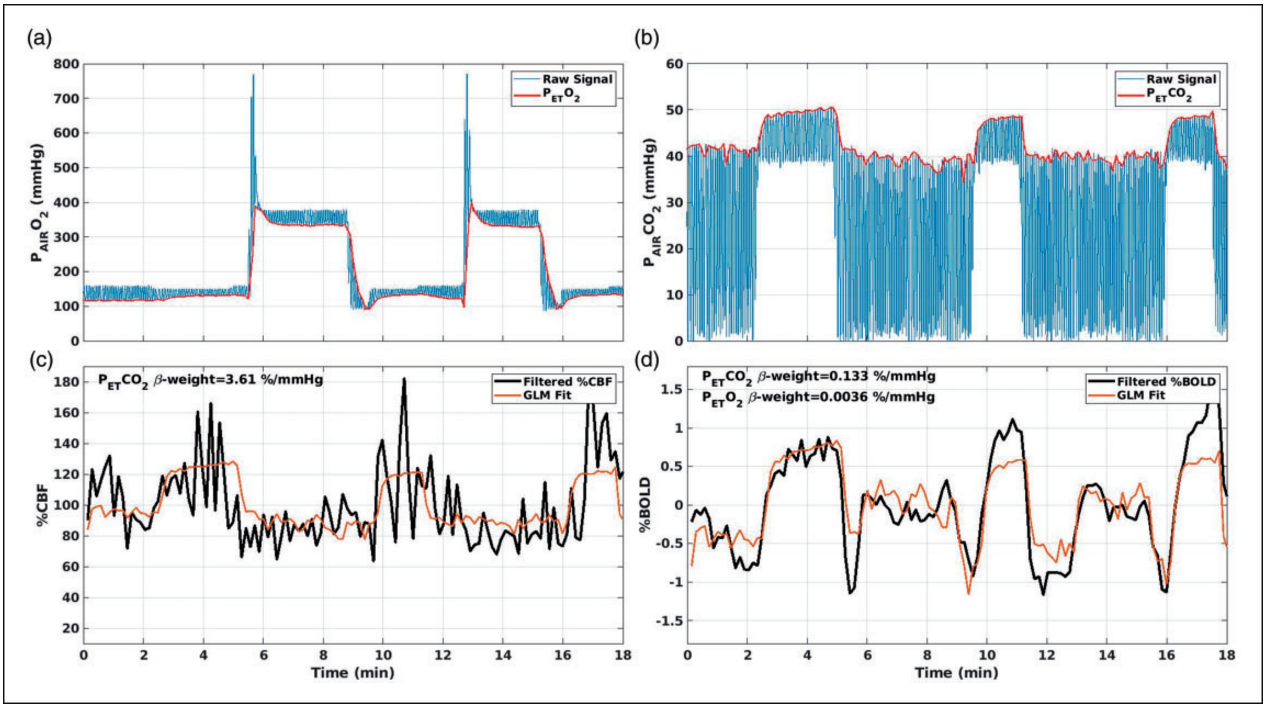

Figure 3. Example of: (a) O_2 and estimated $P_{ET}O_2$ traces; (b) CO_2 and estimated $P_{ET}CO_2$ traces; (c) GM CBF/CBF_0 and fitted $P_{ET}CO_2$ trace. The β-weight of the GLM fit, with units of a CVR, CBF/CBF_0/mmHg, was multiplied by the maximum modulation $\Delta P_{ET}CO_2$ to obtain the hypercapnic CBF/CBF_0. (d) GM average $\Delta BOLD/BOLD_0$ and fitted $P_{ET}CO_2$ and $P_{ET}O_2$ traces. The β-weights, with units of %BOLD/mmHg of $P_{ET}CO_2$ and $P_{ET}O_2$, were multiplied by the maximum modulation $\Delta P_{ET}O_2$ and $\Delta P_{ET}O_2$ to obtain the hypercapnic and the hyperoxic $\Delta BOLD/BOLD_0$.

BOLD change and the filtered $P_{ET}CO_2$ and $P_{ET}O_2$ traces fitted onto $\Delta BOLD/BOLD_0$. The GLM β-weight (in units of cerebrovascular reactivity, CVR, or in units of signal per mmHg of $P_{ET}O_2$) were multiplied by the maximum gas modulation to obtain the hypercapnic CBF/CBF_0 and the hypercapnic as well as hyperoxic $\Delta BOLD/BOLD_0$ modulations. Additional information on gas and signal modulations are reported in the Supplementary Information. The GLM analysis delivered an SNR (evaluated as the statistical relevance of the β-weight) of $SNR_{ASL} = 6.1$ (SD = 5.4), hypercapnic $SNR_{BOLD} = 16$ (SD = 12.7) and hyperoxic $SNR_{BOLD} = 8.6$ (SD = 7.52).

In-Vivo estimation of modeling parameters

Figure 4 reports the analysis performed *in-vivo* to evaluate the modelling parameters. Figure 4(a) reports the subjects' average value (and standard error, SE) of $A \cdot \rho/k$ as a function of $P_mO_{2,0}$. The value is reported for both hc-fMRI+ and ho-fMRI+. In agreement with equation (9), for higher $P_mO_{2,0}$ the estimate of $A \cdot \rho/k$ decreased. We obtained, for a $P_mO_{2,0} = 0$, an average value of $A \cdot \rho/k = 8.85\, s^{-1}g^{-\beta}dL^{\beta}/(\mu mol/mmHg/ml/min)$ (SE = 0.58 $s^{-1}g^{-\beta}dL^{\beta}/(\mu mol/mmHg/ml/min)$) for hc-fMRI+ and $A \cdot \rho/k = 6.03$ $s^{-1}g^{-\beta}dL^{\beta}/(\mu mol/mmHg/ml/min)$ (SE = 0.41 $s^{-1}g^{-\beta}dL^{\beta}/(\mu mol/mmHg/ml/min)$) for ho-fMRI+. Figure 4(b) reports the CoV of $A \cdot \rho/k$ for hc-fMRI+ and ho-fMRI+ as a function of $P_mO_{2,0}$. The smallest CoV was obtained with a $P_mO_{2,0} \approx 0$ for both hc-fMRI+ (CoV = 0.29) and ho-fMRI+ (CoV = 0.31) with a monotonic CoV increase at increasing $P_mO_{2,0}$.

Figure 4(c) reports the comparison between hc-fMRI+ and ho-fMRI+ estimates of $A \cdot \rho/k$ for each subject, when fixing the $P_mO_{2,0}$ at the value of $P_mO_{2,0} = 0$ mmHg . A good correlation was obtained with a r = 0.71, df = 18, $p = 4.2 \cdot 10^{-4}$, with a smaller hyperoxic estimate.

In-vivo estimation of oxygen extraction fraction: Calibrated fMRI vs. dual-calibrated fMRI (section 3). Figure 5(a) reports exemplar OEF_0 and $CMRO_{2,0}$ maps obtained with dc-fMRI, hc-fMRI+ and ho-fMRI+.

Notably, subjects' average spatial variabilities (estimated as standard deviation, SD) in the GM OEF_0 of SD = 0.17 (SE = 0.003), SD = 0.13 (SE = 0.002) and SD = 0.15 (SE = 0.002) were obtained for dc-fMRI, hc-fMRI+ and ho-fMRI+, respectively.

Figure 5(b) reports the scatterplots and the Bland-Altmann plots comparing the average OEF_0 in the GM between dc-fMRI and the single calibration approaches. Average global GM OEF_0 (mean±SD)

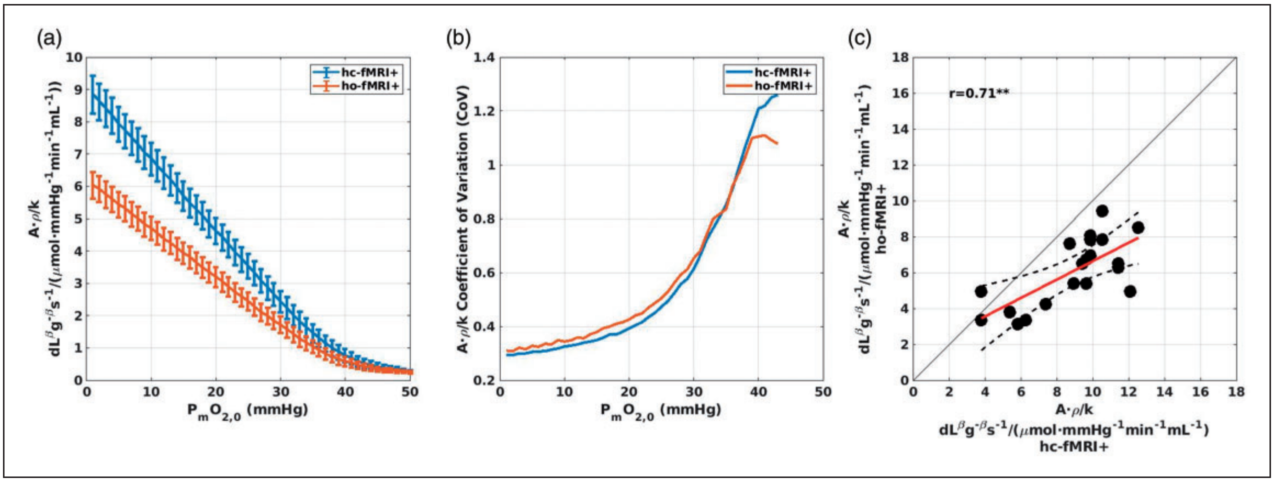

Figure 4. Results of the analysis evaluating the modelling unknown parameters that used hc-fMRI+ or ho-fMRI+ and the OEF_0 derived from the dc-fMRI analysis. (a) Subjects' average (and SE) estimate of the scaling parameter A·ρ/k of the model as a function of the $P_mO_{2,0}$ assumed. (b) Subjects' CoV of the scaling parameter A·ρ/k as a function of $P_mO_{2,0}$ assumed. (c) Comparison between hc-fMRI+ and ho-fMRI+ estimates of A·ρ/k for each subject, assuming a $P_mO_{2,0} = 0$ mmHg. $^{**}p < 0.01$.

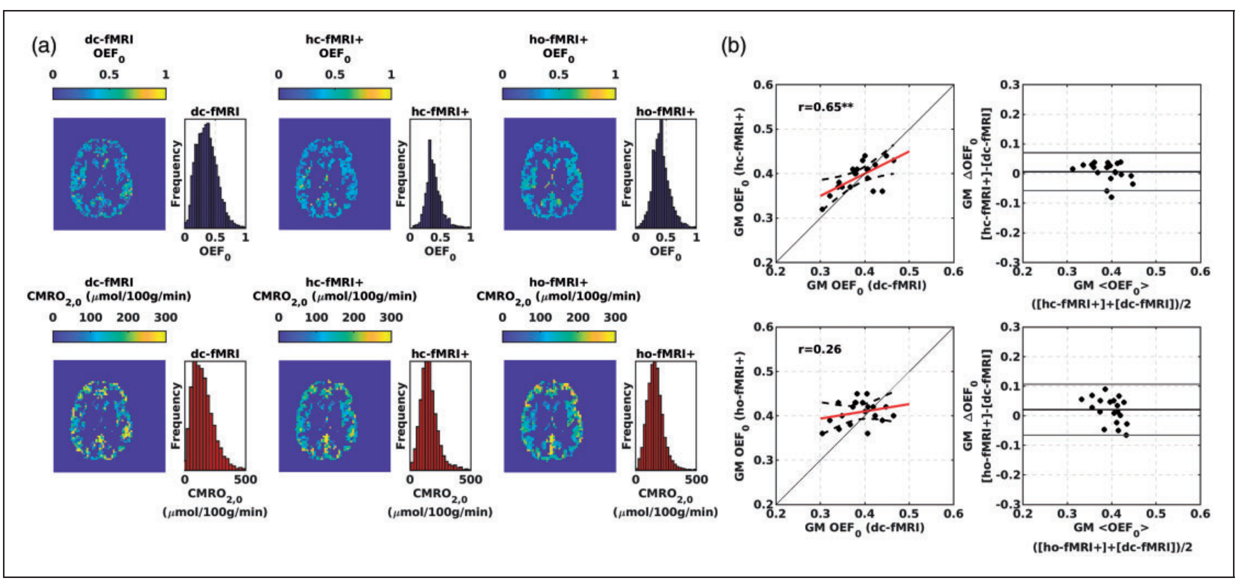

Figure 5. (a) Exemplar GM OEF_0 and $CMRO_{2,0}$ maps for a participant of the study obtained with dc-fMRI (left colum), hc-fMRI+ (central column) and ho-fMRI+ (right column). (b) Scatterplots and Bland-Altmann plots comparing the average OEF_0 in the GM between the hc-fMRI+ (upper row) and ho-fmri+ (lower row) and dc-fMRI. $^{**}p < 0.01$; $^{***}p < 10^{-3}$.

were 0.39 ± 0.04, 0.39 ± 0.03, and 0.40 ± 0.03 for dc-fMRI, hc-fMRI+ and ho-fMRI+, respectively. hc-fMRI+ OEF_0 was significantly correlated with that of dc-fMRI ($r = 0.65$, $df = 18$, $p = 2 \cdot 10^{-3}$, OEF_0 RMSE $= 0.033$) whereas that of ho-fMRI+ was not ($r = 0.26$, $df = 18$, $p = 0.27$, OEF_0 RMSE $= 0.044$). No significant bias between the different approaches was found, but this was dependent on the proportionality constant calibration using dc-fMRI. A significant correlation, with no bias, was obtained between hc-fMRI+ and ho-fMRI+ ($r = 0.50$, $df = 18$, $p = 0.02$).

In-vivo estimation of oxygen extraction fraction: Calibrated fMRI vs. OxFlow (section 4). Figure 6 reports the scatterplots and the Bland-Altmann plots comparing the global OEF_0 of the fMRI approaches to the OEF_0 estimated in the SSS using OxFlow in a subset of 12 subjects. Figure 6(a) shows, for one representative subject, the magnitude image and the processed phase image used

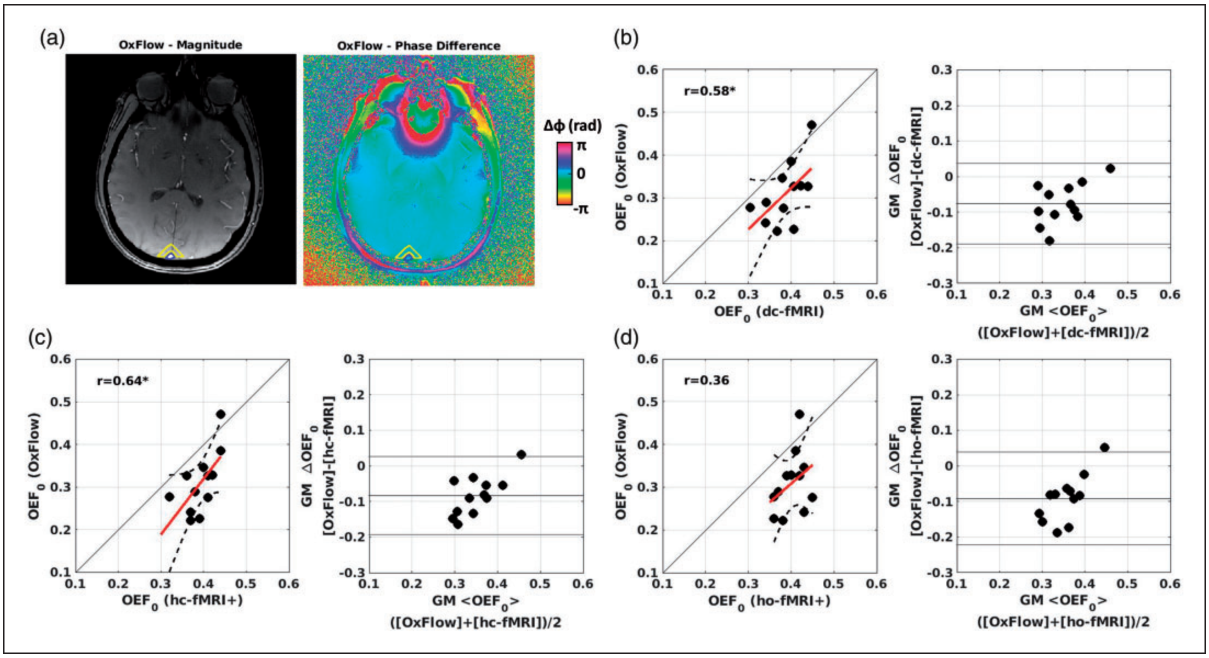

Figure 6. Scatterplots and Bland-Altmann plots comparing the OEF_0 of the calibrated fMRI approaches and OxFlow in a subset of subjects. (a) Example of magnitude (arbitrary units) and processed phase images used to estimate OEF_0 in the SSS within the OxFlow method. SSS and the reference region are outlined in blue and yellow, respectively. OxFlow vs. (b) dc-fMRI; (c) hc-fMRI+; (d) ho-fMRI+. $^*p < 0.05$.

to estimate OEF_0 in the SSS within OxFlow. Average SSS OEF_0 estimated using OxFlow was 0.31 ± 0.07. Significant associations of the average OEF_0 in the GM using a fMRI approach with whole-brain OEF_0 retrieved using OxFlow were obtained for dc-fMRI ($r = 0.58$, $df = 10$, $p = 0.048$, $RMSE = 0.034$, Figure 6(b)) and hc-fMRI+ ($r = 0.64$, $df = 10$, $p = 0.025$, Figure 6(c), $RMSE = 0.041$). No significant association was obtained using ho-fMRI+ ($r = 0.36$, $df = 10$, $p = 0.24$, Figure 6(d), $RMSE = 0.066$). A systematic bias was obtained with the OxFlow underestimating the OEF_0 with respect to fMRI. For the two fMRI approaches that delivered a significant association with OxFlow, an absolute difference between the dc-fMRI and OxFlow of $\Delta OEF_0 = 0.077$ with a $t = 4.57$, $df = 11$, $p = 8 \cdot 10^{-4}$, and an absolute difference between hc-fMRI+ and OxFlow OEF_0 of $\Delta OEF_0 = 0.083$ with a $t = 5.13$, $df = 11$, $p = 3.2 \cdot 10^{-4}$ were obtained.

Discussion

We introduced a framework for mapping OEF_0 and $CMRO_{2,0}$ using single gas calibrated fMRI. The method integrates a flow-diffusion model of oxygen transport[24] with the steady-state BOLD signal model.[16] Simulations suggest the approach to be valid over a wide range of brain physiology. The new approach, when applied to hypercapnia, compared well with dc-fMRI and whole-brain OEF_0 assessed in the SSS using OxFlow. Compared to dc-fMRI, the novel method permits a simpler stimulation paradigm based on a single exogenous gas challenge[27] or, presumably, on an endogenous challenge such as breath hold,[54] and makes the approach robust to measurement noise.

Simulations

The simulations relied on a forward model assuming the new framework to be correct. When inverting the model, the unknown random variables were: (i) $A \cdot \rho/k$, a lumped parameter dependent on field strength, tissue structure and vessel geometry and (ii) the mitochondrial oxygen pressure at rest, $P_mO_{2,0}$. Variability in $A \cdot \rho/k$ ($CoV = 0.3$) was based on in-vivo data (Figure 4), whereas the $P_mO_{2,0}$ was simulated in the range 0-$<P_{cap}O_2>$ (Figure 1). When inverting the forward model with these parameters fixed, we obtained low OEF_0 RMSE (around 0.05) for both hc-fMRI+ and ho-fMRI+ when marginalizing all other variables, with slightly better performance for hc-fMRI+ (Figure 2(a) and (b)). This highlighted the unknown parameters' reduced effect on the mapping between the measurable variables and OEF_0. In fact, when considering OEF_0 RMSE as a function of a wide range of two interesting physiological variables, $P_mO_{2,0}$ and $MCTT_0$, the OEF_0 RMSE was small. Only with very high $P_mO_{2,0}$ (>25 mmHg) and long $MCTT_0$ (>2.5 s) the

Table 1. Main variables and abbreviations used in the study, reported in alphabetical order.

Abbreviation	Meaning	Units	Abbreviation	Meaning	Units
0	As subscript defines the physiological variable at baseline	/	[Hb]	Concentration of hemoglobin in blood	g/dL
α	Grubb exponent relating fractional change in CBV_v to fractional change in CBF	Dimensionless	β	Field strength and vessel geometry dependent exponent within the steady-state BOLD signal model	Dimensionless
β-weight	Coefficient of the GLM	/	γ	Gyromagnetic ratio of the proton	rad/s/T
$\Delta\chi_{do}$	Magnetic susceptibility difference between fully oxygenated and fully deoxygenated blood	Relative	$\Delta BOLD/BOLD_0$	Relative change in BOLD signal	Relative
ΔS	Tag-Control ASL image	Not defined	ε	Oxygen plasma solubility	mL/mmHg/dL
η	Tagging inversion efficiency of PCASL	Dimensionless	η_{INV}	Scaling factor accounting for reduction in tagging efficiency due to back-ground suppression	Dimensionless
λ	Water partition coefficient of the tissue	mL/g	ρ	$CBV_{v,0}/CBV_{cap,0}$	Relative
φ	Oxygen binding capacity of hemoglobin	mL/g	Φ	Phase MRI image	rad
τ	Labelling duration of PCASL	s	A	Field strength and vessel geometry dependent proportionality constant within the steady-state BOLD signal model	$s^{-1}g^{-\beta}dL^{\beta}$
ASL	Arterial spin labelling	/	B_0	Static magnetic field	T
BOLD	Blood oxygen level dependent	Not Defined	C_aO_2	Concentration of oxygen in arteries	mL/dL
CBF	Cerebral blood flow	mL/100 g/min	CBF/CBF_0	Fractional change in CBF	Relative
CBV_{cap}	Capillary blood volume	Relative (or ml/100 g)	CBV_v	dHb-sensitive blood volume	Relative (or ml/100 g)
$CMRO_2$	Cerebral metabolic rate of oxygen	μmol/100 g/min	$CBV_v/CBV_{v,0}$	Fractional change in CBV_v	Relative
CSF	Cerebrospinal fluid	/	CTT	Capillary transit time	s
CoV	Coefficient of variation	Relative	CVR	Cerebrovascular reactivity (CBF/CBF_0/mmHg of CO_2)	%/mmHg
dc-fMRI	Dual-Calibrated functional MRI	/	dHb	Deoxy-hemoglobin concentration in tissue	g/100 g
GLM	General Linear Model	/	GM	Grey matter	/
GRE	Gradient Echo Sequence	/	k	Effective permeability to oxygen of the capillary endothelium and brain tissue	μmol/mmHg/mL/min
h	Hill constant involved in the non-linear relationship between oxygen partial pressure and hemoglobin saturation in blood	Dimensionless	hc-fMRI+	Single gas calibrated fMRI using a hypercapnic modulation and the steady-state BOLD signal model extended with the proposed flow-diffusion analytical framework of oxygen transport	/

(continued)

Table 1. Continued.

Abbreviation	Meaning	Units	Abbreviation	Meaning	Units
hc-fMRI	Single gas calibrated fMRI using a hypercapnic modulation and the steady-state BOLD signal model	/	Hct	Hematocrit	%
ho-fMRI+	Single gas calibrated fMRI using a hyperoxic modulation and the steady-state BOLD signal model extended with the proposed flow-diffusion analytical framework of oxygen transport		ho-fMRI	Single gas calibrated fMRI using a hyperoxic modulation and the steady-state BOLD signal model	/
MCTT	Mean capillary transit time	s	M	Maximum BOLD modulation	Relative
OxFlow	Validated macrovascular global measure of OEF_0, inferred through phase measures of the magnetic susceptibility of blood in the sagittal sinus relative to surrounding tissue	/	OEF	Oxygen Extraction Fraction	Relative
P_{50}	Oxygen partial pressure when half of hemoglobin is saturated with oxygen	mmHg	P_aO_2	Partial pressure of oxygen in arteries	mmHg
P_aCO_2	Partial pressure of carbon dioxide in arteries	mmHg	$P_{cap}O_2$	Partial pressure of oxygen in the capillary	mmHg
PCASL	Pseudo-continuous ASL	/	$P_{ET}O_2$	End-tidal partial pressure of oxygen	mmHg
$P_{ET}CO_2$	End-tidal partial pressure of carbon dioxide	mmHg	P_mO_2	Partial pressure of oxygen at the mitochondria	mmHg
PLD	Post label delay of PCASL	s	$R_2^*\|_{dHb}$	Rate of free induction decay due to dHb	1/s
RMSE	Root mean square error	Variable	S_0	Proton Density Image for ASL normalization	Not defined
$S_{cap}O_2$	Capillary oxygen saturation of hemoglobin	Relative	S_aO_2	Arterial oxygen saturation of hemoglobin	Relative
SD	Standard deviation	Variable	SE	Standard error (standard deviation of the mean)	Variable
S_vO_2	Venous oxygen saturation of hemoglobin	Relative	SNR	Signal to Noise Ratio	Dimensionless
SSS	Superior Sagittal Sinus	/	$T1_b$	MRI longitudinal relaxation time constant of blood	s
TE	Time of echo of the MRI sequence	s	TOF	Time of flight MRI	/
TR	Time of repetition of the MRI sequence		WM	White matter	/

RMSE increased significantly. Very high $P_mO_{2,0}$ and long $MCTT_0$, associated with low CBF_0, are expected only in diseases that heavily alter oxygen supply, vasculature and mitochondrial function.

The simulations revealed the effect of BOLD and ASL measurement noise. For both BOLD and ASL modulations, the OEF_0 RMSE quickly reached the value caused by uncertainty in physiology at an $SNR \approx 4$. This is the SNR of the modulation estimate, not the temporal SNR of the raw signals. For example, when the modulation is estimated within a GLM framework regressing $P_{ET}O_2$ and $P_{ET}CO_2$ onto BOLD and ASL modulations (Figure 3), the SNR is the GLM β-weight divided by its confidence interval. Average voxel SNRs were between 6 and 16 *in vivo* for both signals and gas challenges. The robustness to noise of the approach is advantageous compared to dc-fMRI, that often relies on constrained inversion algorithms, trading off accuracy for higher stability.[22]

Modeling parameters

Investigation of model parameters suggested an average value of $A \cdot \rho/k$ of the order of $10\,s^{-1}g^{-\beta}dL^{\beta}/(\mu mol/mmHg/ml/min)$ when using hc-fMRI+, and an average value of $P_mO_{2,0}$ in the healthy population close to 0 for both hc-fMRI+ and ho-fMRI+ (Figure 4) both results agreed with expectations.[36,55] In fact, the estimate of $A \cdot \rho/k$ decreased beyond expectations at increasing $P_mO_{2,0}$ and increased its CoV as a function of the assumed $P_mO_{2,0}$, with a rapid increase above 20 mmHg. This work indeed suggests a particularly low average $PmO2_{,0}$ in the healthy brain. However, it should be stressed that a strong increase in CoV of $A \cdot \rho/k$ was only observed at $PmO_{2,0}$ above 20 mmHg. The confidence interval of the estimate still cannot provide a definitive answer on the average $PmO_{2,0}$ within the range 0-20 mmHg. There is work suggesting the mitochondrial $PmO_{2,0}$ is about $\sim$12 mmHg[56,57] which goes against the common assumption of $PmO_{2,0}$ being near zero in the healthy brain[36] and, indeed, this is still an open debate. Nonetheless, the simulations of the study clearly demonstrate that the approach estimating OEF_0 has limited sensitivity to the value of $PmO_{2,0}$ if $PmO_{2,0}$ and $MCTT_0$ are not both very high. The good correlation between the hypercapnic and the hyperoxic estimates indicated consistency. However, we obtained a value of $A \cdot \rho/k$ for ho-fMRI+ around 30% smaller than expected. This result might be a cross-talk effect of the hypercapnic on the hyperoxic BOLD modulations in the dc-fMRI experiment, or an overestimation of ΔPaO_2.

Comparison with dc-fMRI and OxFlow. Spatial homogeneity of OEF_0 in healthy subjects is often taken as an indicator of successful OEF_0 mapping. hc-fMRI+ and ho-fMRI+ decreased OEF_0 spatial variability in GM compared to dc-fmri. The lower variability of hc-fMRI+ and ho-fMRI+ suggests a greater robustness, with respect to measurement SNR, compared to the dc-fMRI. Comparison of GM OEF_0 estimates suggests that hc-fMRI+ is a valid alternative to dc-fMRI (Figure 5(b)). In addition, when comparing GM OEF_0 of the different fMRI approaches with global OEF_0 in the SSS through OxFlow, clear associations with the OxFlow OEF_0 were obtained for both dc-fMRI and hc-fMRI+ (Figure 6). We identified a bias between the OxFlow and the fMRI estimates. OxFlow yielded a lower global OEF_0 with values around 0.31 and 0.39 for Oxflow and fMRI, respectively. This difference might be explained by previous work, where Oxflow using analytical modelling gave high estimates of venous saturation.[58] Systematic differences in the global OEF_0 estimated using heterogeneous MRI or non MRI techniques are established in the literature. Work performed using measures of T2 relaxation in venous blood, delivered global OEF_0 between 0.36 and 0.38.[59,60] These values were close to global OEF_0 estimated using the gold standard ^{15}O positron emission tomography (^{15}O PET)[60,61] and are more compatible with the OEF_0 obtained with calibrated fMRI approaches. Nonetheless, once a good correlation between modalities is established, biases can be corrected using data-driven approaches or through better models of the underlying physics and physiology. The low performance of ho-fMRI+ is indeed a negative result of the study. Hyperoxia is generally better tolerated than hypercapnia[19] and it would be more easily applicable in clinical settings. The lower performance of hyperoxia is plausibly related to the noisier estimate of M. Moreover, hyperoxic BOLD modulation is primarily sensitive to $CBV_{v,0}$ and largely insensitive to OEF_0; in fact, hyperoxia can be used to estimate $CBV_{v,0}$.[62] The oxygen saturation change due to hyperoxia stimulus is independent of the baseline oxygen saturation over most of the physiological range. In contrast, the oxygen saturation change to a hypercapnic challenge is linearly related to the resting saturation. The sensitivity pattern of the hyperoxic modulation makes the estimation of OEF_0 with the new framework completely reliant on the flow-diffusion model approximations that link $CBV_{v,0}$ to OEF_0. The model approximations are indeed less influential with hypercapnia, which has a larger sensitivity to OEF_0 with respect to $CBV_{v,0}$, making the hypercapnia approach less noisy and biased.

Limitations of the method. The main limitations of the new method are mostly shared with dc-fMRI.[14] The approach using hypercapnia relies on a local CBF increase, a vascular reserve, which may be absent in diseases such ischemic stroke, where vessels may be maximally dilated in an attempt to maintain perfusion. In addition, the method might be vulnerable to larger than expected changes in ρ or k, which are probably not independent. Although large changes in these parameters appear unlikely in many brain diseases, we might expect relevant tissue and vascular remodeling in some disease, such as brain tumors.[63] The limitations of the approach in diseases with concurrent very high $P_mO_{2,0}$ and long $MCTT_0$ are noted earlier and should be assessed in future studies.

Study limitations

The main limitation of the simulation study lies in the assumption of an exact analytical model with the error in the estimate of OEF_0 being introduced only by the limited number of measurable variables. The main simplifying assumption of the model was the replacement of the CTT in one straight capillary with the MCTT in the voxel capillary bed. This is an approximation, since, due to the non-linear mapping between CTT, OEF and oxygen diffusion between capillary and tissue, the complete CTT distribution within a capillary bed affects the macroscopic OEF.[64] Without changing MCTT, OEF can be increased through homogenization of the CTT among capillaries. Future extension of the model might include the CTT heterogeneity (CTTH), a measure of the second moment of the CTT distribution within the capillary bed.[65]

With respect to the *in-vivo* validation using dc-fMRI, a limiting factor was related to the investigation of the proposed model proportionality constant $A \cdot \rho/k$ and $P_mO_{2,0}$. These estimates were evaluated assuming the OEF_0 derived from the dc-fMRI machine learning analysis[22] to be exact. In fact, noise in the dc-fMRI OEF_0 limited our investigation of the model parameters to global evaluation within the GM. Moreover, another limitation was the problem of having two unknowns and one equation. By exploiting the different effects of these parameters on the non-linear mapping between variables, we were able to get insight into both parameters, however only at a between subjects' average level. Alternative approaches should be used to investigate the different physiological parameters (e.g., ρ and k) contributing to the proportionality constant, which cannot be separately investigated using standard fMRI approaches. Comparison against non-MRI technology would be essential for definitive validation of the approach. Future validation is also necessary beyond the healthy controls involved in the study, to populations affected by diseases that might alter brain metabolism and for which the proposed model's validity might reduce.

Conclusion

We introduced a novel single gas calibrated fMRI framework integrating a steady-state flow-diffusion model of oxygen transport into the BOLD signal model. Uncertainty in the integrated model is driven by variability in a proportionality constant $A \cdot \rho/k$, that depends on tissue and microvascular structure at a fixed field strength, and $PmO_{2,0}$. The advantage of the new framework lies in the limited influence of these parameters on OEF_0. Even by fixing them to plausible values, the simulations showed the OEF_0 RMSE was below 0.05 over a wide range of physiology meaning that the method may reliably identify between-subjects OEF_0 differences greater than approximately 10%. Only with concurrently very high $PmO_{2,0}$ (>25 mmHg) and long $MCTT_0$ (>2.5 s) did the RMSE increase significantly suggesting that the method should work in diseases not drastically altering brain physiology. Importantly, the approach was highly robust to measurement noise. The method, when using hypercapnia, compared well with dc-fMRI and with whole-brain OEF_0 derived using the OxFlow method. Lack of positive results when using hyperoxia may be related to the method being primarily sensitive to $CBV_{v,0}$. The simplified calibrated fMRI method using hypercapnia has potential for application in clinical settings.

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Declaration of conflicting interests

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Authors' contributions

AMC and MG developed imaging and analysis methods, and analyzed and interpreted the data. AMC, MG and RGW drafted the manuscript. FWW, RGW, KM and VT conceived the project. MG, HLC, RCS, EP, NS, SK, SJ, CF and KM set up and executed the experiment. DM, EB, AERS and EE contributed to data analysis. All authors revised and approved the final submission.

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Supplemental material

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