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MRI of cerebral blood flow under hyperbaric conditions in rats

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Abstract

Hyperbaric oxygen (HBO) therapy has a number of clinical applications. However, the effects of acute HBO on basal cerebral blood flow (CBF) and neurovascular coupling are not well understood. This study explored the use of arterial spin labeling MRI to evaluate changes in baseline and forepaw stimulus-evoked CBF responses in rats ($n = 8$) during normobaric air (NB), normobaric oxygen (NBO) (100% O₂), 3 atm absolute (ATA) hyperbaric air (HB) and 3 ATA HBO conditions. T_1 was also measured, and the effects of changes in T_1 caused by increasing oxygen on the CBF calculation were investigated. The major findings were as follows: (i) increased inhaled oxygen concentrations led to a reduced respiration rate; (ii) increased dissolved paramagnetic oxygen had significant effects on blood and tissue T_1 , which affected the CBF calculation using the arterial spin labeling method; (iii) the differences in blood T_1 had a larger effect than the differences in tissue T_1 on CBF calculation; (iv) if oxygen-induced changes in blood and tissue T_1 were not taken into account, CBF was underestimated by 33% at 3 ATA HBO, 10% at NBO and <5% at HB; (v) with correction, CBF values under HBO, HB and NBO were similar ($p > 0.05$) and all were higher than CBF under NB by ~40% ($p < 0.05$), indicating that hypercapnia from the reduced respiration rate masks oxygen-induced vasoconstriction, although blood gas was not measured; and (vi) substantial stimulus-evoked CBF increases were detected under HBO, similar to NB, supporting the notion that activation-induced CBF regulation in the brain does not operate through an oxygen-sensing mechanism. CBF MRI provides valuable insights into the effects of oxygen on basal CBF and neurovascular coupling under hyperbaric conditions.

Keywords

arterial spin labeling; CBF; oxygen therapy; BOLD; magnetic susceptibility

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INTRODUCTION

Hyperbaric oxygen (HBO) therapy has been used in the treatment of decompression sickness, air embolism, chronic wounds, stroke, traumatic brain injury and cerebral palsy, among others (1). The high oxygen content has effects on cardiovascular physiology, such as respiration and vascular tone, which modulate cerebral blood flow (CBF). The elevated inhaled oxygen concentration can substantially reduce the respiration rate (2,3) and increase vasoconstriction (4), which have opposing effects on CBF. Under normobaric oxygen (NBO), hypercapnic effects induced by oxygen inhalation dominate over oxygen-induced vasoconstriction in anesthetized animals, resulting in a net increase in CBF (2,3). Under HBO, oxygen-induced vasoconstriction can potentially be substantially stronger and may dominate the effects on CBF. Two previous studies have reported lower (5) and higher (6) basal CBF under HBO relative to normobaric air (NB).

HBO can also affect neurovascular coupling. Stimulus-evoked increases in neural activity are known to be tightly coupled to local metabolism, tissue oxygenation and CBF under normal conditions. Neurovascular coupling is modulated by the vasoactive products of metabolism (such as H^+ or adenosine) and synaptic signaling (such as nitric oxide) (7). HBO exposure decreases nitric oxide synthase activity, suggesting a possible mechanism of action (5). It has also been suggested that deoxygenated hemoglobin ‘sensors’ may play a role in the regulation of tissue perfusion (8), and thus it is conceivable that dissolved oxygen in the tissue under HBO is sufficient to support a stimulus-evoked increase in oxidative metabolism, obviating the need to increase CBF to meet increased oxygen demand. However, a recent study found, to the contrary, that the stimulus-evoked CBF increase under HBO is not significantly different from that under NB as measured by laser Doppler flowmetry (6). Quantitative CBF measurements during stimulation under HBO could help to provide additional insights into neurovascular coupling under HBO.

MRI has recently been applied to investigate the effects of HBO on local magnetic field (B_0), T_2 , T_2^* and T_1 (9,10). Under ambient air, the increased dissolved molecular oxygen – an endogenous paramagnetic relaxation agent – in tissue and plasma water shortens T_2 and T_2^* (11), but is masked by the blood oxygen level-dependent (BOLD) effect. However, the paramagnetic effects of dissolved oxygen on T_2 and T_2^* relaxation under HBO may not be negligible, when even venous hemoglobin is substantially or fully saturated with oxygen (12). The increased hemoglobin saturation under HBO could also obliterate the stimulus-evoked BOLD functional MRI (fMRI) contrasts, but we found strong BOLD fMRI contrasts to forepaw stimulation under HBO (13). Quantitative CBF measurements under HBO could help us to better understand the BOLD fMRI responses and neurovascular coupling under HBO. However, HBO inhalation could shorten water T_1 as a result of dissolved paramagnetic oxygen, affecting the CBF calculation using the arterial spin labeling (ASL) MRI method. NBO can reduce whole-brain water T_1 by 2–7% (14,15) and has been reported to have slight but detectable effects on MRI CBF calculation (16). T_1 is expected to be reduced further under HBO, and thus CBF measurements using ASL under HBO need to take into account the oxygen effects on intravascular and extravascular water T_1 .

The goal of this study was to implement corrections for the ASL technique for the measurement of quantitative CBF under hyperbaric conditions and then to investigate CBF under NB, NBO (100% O₂), 3 atm absolute (ATA) hyperbaric air (HB) and 3 ATA HBO conditions. Experiments were performed under non-stimulated conditions and during forepaw stimulation.

METHODS

Hyperbaric chamber

A custom-made hyperbaric chamber for rodent MRI scanners consisted of an animal cradle placed into a PVC pipe with O-rings on both ends of the cradle to provide a tight seal, as described previously (9). HBO was achieved using ambient air to pressurize the chamber with a separate line to deliver oxygen locally to the nose. This protocol prevents the risk of explosion associated with highly concentrated oxygen. We previously measured the O₂ percentage delivered to the animal with our setup to be 90–93% (9).

Cables for biometric equipment (temperature probe and pulse oximeter), polyethylene tubing for the delivery of intravenous anesthesia and electrodes for forepaw stimulation were passed through a hole in one of the endplates and sealed with silicone sealant. The animal temperature was maintained with circulating warm water through a wound rigid plastic tubing. The chamber was pressurized in 10 min at an average rate of 0.2 atm/min to prevent potential side effects from rapid pressurization. Prolonged or chronic exposure to HBO could cause possible oxygen toxicity, leading to convulsion (17). To minimize potential adverse affects, each trial of HBO exposure was limited to <25 min and interleaved with NB inhalation between HBO trials. For the relatively short duration of acute 3 ATA HBO, we did not observe apparent negative side effects, although this was not vigorously evaluated. Previous studies of similar HBO conditions have reported no oxygen toxicity (5,6).

Animal preparation

Animal experiments were approved by the local Institutional Animal Care and Use Committee. Experiments were performed on male Sprague-Dawley rats (325 ± 50 g, $n = 8$). Rats were initially anesthetized using 5% isoflurane and maintained at 2% isoflurane during preparation. Animals were placed into a head holder with ear and tooth bars and secured into the cradle. The animals were sealed inside the chamber and placed into the MRI scanner. The anesthetic was then switched to α -chloralose (intravenous, 60 mg/kg bolus; followed by 30 mg/kg/h infusion at 45 min post-bolus) because isoflurane concentrations under various pressures could not be readily maintained at a constant level.

The rats were imaged under spontaneous breathing conditions. The respiration rate, heart rate and arterial oxygen saturation were monitored (MouseOx, STARR Life Science Corp., Oakmont, PA, USA), and the rectal temperature was maintained at 37 ± 0.5 °C with a feedback-regulated circulating warm water pad. Needle electrodes were inserted into both forepaws between digits 2 and 4, connected in series. Both forepaws were stimulated simultaneously with 2-mA, 3-Hz, 0.3-ms pulses (2,18) using a paradigm of four repeats of 30-s stimulation and 96-s rest periods.

MRI acquisition

MRI was performed on a 7-T, 30-cm horizontal magnet with 400-mT/m gradients (Biospec, Bruker, Billerica, MA, USA). A circular surface coil (diameter, 2 cm) was used for brain imaging, and a neck coil was used for perfusion labeling (18–20). Coil-to-coil electromagnetic interaction was actively decoupled. Global shimming was only performed once at the beginning of the session.

CBF measurements were made using the continuous ASL technique with single-shot, gradient-echo, echo planar imaging acquisition (18–20). Paired images were acquired alternately -one with ASL and the other without. The labeling pulse was a 2-s square pulse. MRI parameters were as follows: TR = 3 s; nominal flip angle, 90°; TE = 20 ms; field of view, 25.6 × 25.6 mm²; matrix, 96 × 96; seven 1.5-mm-thick slices.

T_1 was measured using inversion-recovery, gradient-echo, echo planar imaging with a field of view of 25.6 × 25.6 mm², matrix of 96 × 96, TR = 12 s, seven 1.5-mm-thick slices, TE = 9.9 ms, ten inversion times from 23 to 3623 ms, equally spaced by 400 ms, two-thirds partial Fourier acquisition and three averages.

Data analysis

Image analyses were performed using Matlab (MathWorks, Natick, MA, USA), Statistical Parametric Mapping 5 (SPM5, University College London, London, UK), Mango (UT Health Science Center, San Antonio, TX, USA) and STIMULATE (University of Minnesota, Minneapolis, MN, USA). CBF was calculated using (21,22):

$$CBF = \frac{\lambda}{T_1} \cdot \frac{S_{NL} - S_L}{2S_{NL} \alpha \cdot e^{(\tau - \delta)/T_1} \cdot e^{-\tau/T_{1a}}} \quad [1]$$

where S_{NL} and S_L are the signal amplitudes of the non-labeled and labeled images, respectively, λ is the brain-blood partition [a value of 0.9 was used (23)], δ is the post-labeling delay [a value of 250 ms was used] and τ is the arterial transit time [a value of 250 ms was used (24)]. T_1 and T_{1a} are the relaxation times for tissue and arterial blood, respectively. During the transit time from the labeling plane at the neck to the local capillaries, the label efficiency is reduced as a result of T_1 recovery of the labeled blood as (25):

$$\alpha = \alpha_0 e^{-\tau/T_{1a}} \quad [2]$$

where α is the effective label efficiency at the tissue and α_0 is the efficiency of the labeling pulse at the neck.

To evaluate the effects of oxygen on arterial blood T_{1a} , we calculated T_{1a} from the arterial pO_2 using the linear relationship between $1/T_1$ and dissolved pO_2 as $T_{1a}^{-1} = p_a O_2 \cdot r_1 + T_{10}^{-1}$, where the relaxivity of oxygen in blood (r_1) is $2.5 \times 10^{-4} \text{ s}^{-1}/\text{mmHg}$ (26). A value of T_{1a} at NB of 2.2 s at 7 T (27) was used to calculate T_{10} , the T_1 value of deoxygenated blood. The

arterial pO_2 (p_aO_2) was related to the inspired pO_2 (p_iO_2) based on published data (12,28–30), which could be readily fitted to a linear function: $p_aO_2 = 0.9039p_iO_2 - 61.91$. Arterial blood T_{1a} values were plotted as a function of inspired pO_2 .

The labeling efficiencies were calculated using Equation [2] and the calculated T_{1a} values for different arterial transit times at different inspired pO_2 . An α value at NB of 0.75 was used (20), from which α_0 and α at different inhaled oxygen concentrations were calculated. Labeling efficiencies were normalized with respect to that of the NB condition for comparison. The arterial T_1 values and the normalized labeling efficiencies were plotted as a function of inhaled pO_2 .

T_1 maps of the brain tissue were calculated using a three-parameter, non-linear fit of the inversion recovery data to $M = M_0 - B \cdot M_0 \cdot e^{(-T_1/T_1)}$, where M is the signal intensity at a given inversion time, M_0 is the equilibrium signal and B is the efficiency of the inversion pulse. Whole-brain T_1 values (from all seven slices) were measured and plotted as a function of inhaled oxygen. The percentage error contributions to the CBF calculation as a result of arterial T_1 and tissue T_1 differences were determined.

The measured CBF was then corrected to account for the differences in tissue and arterial blood T_1 under different inhaled oxygen concentrations. It should be noted that tissue T_1 was measured for 4 ATA HB and 4 ATA HBO, but CBF and evoked CBF responses were not measured (see Fig. 2b, later). Tissue T_1 was not measured under the 3 ATA HB condition, and so its value was interpolated based on the T_1 values from the other three conditions for CBF correction at 3 ATA HB. CBF values of the whole brain (from all seven slices) and a few major brain regions were also tabulated.

Group CBF percentage change activation maps between control and stimulation periods were calculated. Magnitude and percentage changes in CBF as a result of forepaw stimulation were tabulated for 6×6 -pixel regions of interest (ROIs) encompassing the forepaw primary somatosensory cortices (S1).

Statistical analysis

Group-average data are expressed as the mean $\pm$ standard error of the mean (SEM). Paired t -tests with Bonferroni–Holm corrections were used for comparison across oxygen conditions. $p < 0.05$ was taken to be statistically significant.

RESULTS

Physiological parameters

Table 1 summarizes the respiration rate, heart rate and arterial blood oxygen saturation under NB, NBO, HB and HBO. Respiration rates were significantly lower under NBO, HB and HBO compared with NB ($p < 0.05$), significantly lower under HB and HBO compared with NBO ($p < 0.05$), but not significantly different between HB and HBO ($p > 0.05$). Heart rate was not significantly different amongst groups ($p > 0.05$). Arterial blood oxygen saturations were significantly higher under NBO, HB and HBO compared with NB ($p < 0.05$), but not significantly different amongst NBO, HB and HBO ($p > 0.05$).

CBF correction for the blood and tissue T_1 effects from dissolved O_2

To evaluate the possible effects of arterial blood T_1 differences from dissolved oxygen on CBF calculation, arterial T_1 was determined as a function of inspired O_2 based on literature data. Arterial T_1 decreased with increasing inspired pO_2 as expected (Figure. 1) (whereas $1/T_1$ versus inspired pO_2 would be linear). An arterial T_1 of 2.2 s at NB (27) would be reduced to 1.1 s at 3 ATA HBO. Reduced arterial T_1 from increased dissolved oxygen gave rise to reduced effective labeling efficiency.

Figure 2a shows the calculated relative labeling efficiency for three arterial transit times ($\tau = 0.1, 0.2$ and 0.3 s). The labeling efficiency decreased with increasing inspired pO_2 as a result of decreasing arterial T_1 , with the labeling efficiency being reduced more strongly at longer arterial transit times as expected. To evaluate the possible effects of HBO on tissue T_1 and thus on CBF calculation, tissue T_1 was determined as a function of inspired pO_2 (Figure. 2b). Tissue T_1 decreased as a result of higher dissolved O_2 with increasing inhaled oxygen concentrations. This relation was approximately linear. If the effects of O_2 on T_1 are not taken into account, CBF will be underestimated.

The combined blood and tissue T_1 effects on CBF calculation under different oxygen inhalation concentrations are shown in Figure. 3. The tissue T_1 effect on CBF was weaker than the blood T_1 effect. If the effects of O_2 on T_1 are not taken into account, CBF will be underestimated by ~33% at 3 ATA HBO, <10% at NBO and <5% at HB.

Effects of hyperbaric conditions on CBF

Figure 4 shows the uncorrected and corrected CBF under NB, NBO, HB and HBO from the whole brain. Prior to correction, CBF under HBO was significantly lower than at HB and NBO ($p < 0.05$), whereas CBF after correction was not significantly different between NBO, HB and HBO ($p > 0.05$). After correction, CBF values at NBO, HB and HBO were all significantly higher than at NB by $40 \pm 6\%$, $34 \pm 11\%$ and $40 \pm 15\%$, respectively ($p < 0.05$ for all), suggesting that oxygen-induced hypercapnia overcomes potential oxygen-induced vasoconstriction. Subsequent analysis employed CBF with corrections. CBF from three different brain regions showed similar patterns to the whole brain for NB, NBO, HB and HBO conditions (Figure. 5), indicating that the effects are global.

CBF fMRI maps on forepaw stimulation and the absolute and relative evoked CBF changes under different inhaled oxygen concentrations are shown in Figure. 6. The basal CBF values of the S1 cortices are similar to those of the whole brain. Stimulation evoked robust CBF responses in the somatosensory cortex under all conditions. The stimulus-evoked CBF magnitude increases were not significantly different across NB, NBO, HB and HBO conditions ($p > 0.05$). The stimulus-evoked CBF percentage increases were also not significantly different across the four conditions ($p > 0.05$).

DISCUSSION

This study investigated the effects of dissolved oxygen under hyperbaric conditions on intravascular and extravascular T_1 in the calculation of CBF using the ASL technique and implemented corrections. This approach was used to evaluate the physiological effects of

HBO on basal CBF and CBF regulation. The major findings were as follows: (i) increased inhaled oxygen concentrations lead to a reduced respiration rate; (ii) if oxygen-induced changes in blood and tissue T_1 are not taken into account in the quantification, CBF is underestimated by 33% at 3 ATA HBO, 10% at NBO and <5% at HB; (iii) increasing dissolved oxygen concentrations reduce blood and tissue water T_1 , which affect ASL CBF calculation, with the blood T_1 contribution having a larger effect than the tissue T_1 contribution; (iv) with correction, CBF amongst HBO, HB and NBO conditions is not significantly different, but CBF under HBO, HB and NBO is about 40% higher than under NB, indicating that oxygen-induced hypercapnia overcomes vasoconstriction; and (v) robust CBF increases are detected under HBO, supporting the notion that activation-induced CBF regulation in the brain does not operate through an oxygen-sensing mechanism.

Effects of HBO on blood and tissue T_1

The combined oxygen-induced changes in blood and tissue T_1 lead to an underestimated CBF if the effect is not taken into account. The exact extent of underestimation may depend on the ASL CBF model used for the calculation. Brain tissue T_1 was measured, which is a weighted average of brain and blood T_1 . The blood volume is small (typically <5%) and thus the blood T_1 contribution to the whole-brain T_1 is negligible. We estimated the blood T_1 from literature data to correct for oxygen-induced effects on the ASL calculation. Alternatively, the arterial labeling efficiency could also be measured directly (31). It is also possible to measure blood T_1 *in vivo* (32) to account for the difference in arterial labeling efficiency, although it is challenging to do so for fast-flowing arterial blood and small vessels in rats. A recent white paper providing the recommended implementation of ASL gives a more detailed description of the effects of arterial T_1 on ASL MRI (25).

Effects of HBO on respiration and basal CBF

Respiration rates were lower under NBO, HB and HBO compared with NB, consistent with rat respiration data under NBO (2). However, the respiration rates did not scale with the inhaled oxygen concentrations. NBO had five times, whereas HB at 3 ATA had three times, the inhaled $[O_2]$ compared with NB, but the respiration rate at NBO was higher than that at HB. Similarly, the respiration rate under 3 ATA HBO did not further decrease relative to HB, despite the inhaled $[O_2]$ differing by an additional factor of five. These findings suggest that there are direct or indirect hyperbaric effects on respiration. There are several possible explanations: (i) there is increased turbulent flow near the trachea during expiration under hyperbaric pressure, which could arise from increased gas density (33); increased turbulence means increased work required to expire the same amount of gas, which could thus attenuate the ventilation response; (ii) the respiratory tidal volume could differ between normobaric and hyperbaric conditions without a change in respiration rate; (iii) the respiration rate could not be further reduced from HB to HBO without compromising homeostasis; (iv) the relationship between respiration rate and arterial pCO_2 , and/or the relationship between CBF and arterial pCO_2 , is not linear; indeed, CBF *versus* arterial pCO_2 levels off when pCO_2 reaches 45 mmHg (3). pCO_2 under HBO is expected to be at the higher end (ca. 45 mmHg) of the normal physiological range because of the reduced respiration based on animal NBO blood-gas data (2). Further studies are needed to evaluate these and other possible explanations.

A reduced respiration rate leads to hypercapnia, which increases basal CBF as observed, consistent with a previous CBF study under NBO (2). CBF values at NBO, HB and HBO were all significantly higher than CBF at NB by about 40%, suggesting that oxygen-induced hypercapnia overcomes oxygen-induced vasoconstriction. However, CBF values under NBO, HB and HBO were not significantly different from each other. This is not surprising because of the weak relationship between respiration rate and arterial $p\text{CO}_2$ as described above.

Dissolved oxygen also has vasoconstrictive effects which oppose the effect of respiration-induced hypercapnia. Oxygen-induced vasoconstriction caused by oxygen inhalation has been reported to reduce CBF by 10–13% in humans during NBO (4). Under HBO, nitric oxide synthase activity was reduced relative to NB, which results in reduced nitric oxide, suggesting a possible cause of reduced CBF under HBO (5). Moreover, when respiration and CO_2 are maintained at the same levels during HBO through mechanical ventilation, CBF is reduced under HBO compared with NB, demonstrating oxygen inhalation-induced vasoconstriction (5). Our study was performed under free-breathing conditions, and we found that the effects of oxygen-induced vasoconstriction on CBF are probably masked by oxygen-induced hypercapnia, even at 3 ATA HBO, resulting in a net increase in CBF. This finding is consistent with a previous basal CBF study under NBO (2).

Basal electrophysiological activity

There is another possible, albeit unlikely, explanation for the increased basal CBF under HBO relative to NB. Neural activity could be higher under NBO and HBO relative to NB, thus leading to an elevated basal CBF. This is unlikely to be the case because basal spontaneous electrophysiological activity was slightly reduced under HBO (13). Spontaneous activities of the theta, alpha, beta and gamma bands decreased under HBO compared with NB, whereas the delta band showed a decreasing trend, but did not reach statistical significance. Previous studies have reported reduced oxygen consumption in brain slices under HBO (34) and reduced glucose consumption in pig brain *in vivo* (35), but no changes in delta and theta band activity in humans (36) under HBO compared with NB. In summary, the available data do not support higher neural activity under HBO or NBO relative to NB under basal conditions, and thus this factor cannot explain the elevated basal CBF observed under HBO and NBO conditions.

Evoked CBF responses

Strong stimulus-evoked CBF fMRI responses were detected under HB and HBO, but their percentage changes were not significantly different from those under NB and NBO. This finding is consistent with a previous study investigating whisker stimulation under HBO using laser Doppler flowmetry (6), which measured relative CBF changes. Based on relative CBF changes alone, the lack of difference in evoked percentage changes between conditions could be because non-stimulated and stimulated conditions both changed by the same extent (i.e. both numerator and denominator decrease or increase by the same amount). ASL MRI (in contrast with laser Doppler flowmetry) also provides magnitude CBF changes, enabling the resolution of the above ambiguity. Indeed, we found that CBF values under non-stimulated conditions for HB, NBO and HBO were higher than that for NB. Stimulus-

evoked magnitude CBF changes, however, were not significantly different amongst the four different conditions.

Together, our data and those of Lindauer *et al.* (6) show that activation-induced CBF regulation in the brain does not operate through the release of vasoactive mediators based on hemoglobin deoxygenation or through a tissue-based oxygen-sensing mechanism. It is possible, albeit unlikely, that the strong stimulus-evoked CBF response to HBO is a result of elevated evoked neural activity under HBO. To the contrary, stimulus-evoked electrical activity was significantly lower under HBO compared with NB (13), and the evoked change in oxygen consumption was mildly reduced under NBO in humans compared with NB (37). Thus, the available data do not support the notion that evoked neural activity under HBO, HB or NBO is higher compared with that under NB, and thus this factor cannot explain the evoked CBF responses observed under HB conditions.

Limitations and future perspectives

There are a few limitations of this study. One limitation is that we did not perform independent validation of CBF under HBO, which is not trivial under hyperbaric conditions. Only the respiration rate was measured, but the respiratory tidal volume could differ amongst different oxygen inhalation conditions. Blood gas measurements would be informative, but it is not trivial to sample blood gas under hyperbaric conditions because the withdrawn blood would have to be measured under atmospheric pressure. Arterial labeling efficiency should be measured to confirm the reduced labeling efficiency caused by T_1 effects of dissolved oxygen on CBF.

CONCLUSIONS

Increasing dissolved paramagnetic oxygen concentrations reduce blood T_1 and extravascular T_1 , resulting in the underestimation of CBF by the ASL technique. HBO reduces the respiration rate, resulting in elevated CBF, as do NBO and HB to a similar extent. Robust stimulation evokes CBF increases even under HBO, supporting the notion that activation-induced CBF regulation in the brain does not operate through an oxygen-sensing mechanism. This work provides insights into the HBO effects on ASL CBF measurements, physiological parameters, basal CBF and neurovascular coupling under HBO.

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Abbreviations used

ASL	arterial spin labeling
ATA	atmospheres absolute
BOLD	blood oxygen level dependent
CBF	cerebral blood flow

fMRI	functional MRI
HB	hyperbaric air
HBO	hyperbaric oxygen
NB	normobaric air
NBO	normobaric oxygen
ROI	region of interest
S1	primary somatosensory cortex
SEM	standard error of the mean

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Figure 1.

Estimated arterial T_1 (T_{1a}) versus inspired pO_2 . T_{1a} values were calculated from the arterial pO_2 using the linear relationship between $1/T_1$ and dissolved pO_2 as $T_{1a}^{-1} = p_a O_2 \cdot r_1 + T_{10}^{-1}$, where the relaxivity of oxygen in blood (r_1) is $2.5 \times 10^{-4} \text{ s}^{-1}/\text{mmHg}$ (26). A value of T_{1a} at NB of 2.2 s at 7 T (27) was used to calculate T_{10} , the T_1 value of deoxygenated blood. The arterial pO_2 ($p_a O_2$) was related to inspired pO_2 ($p_i O_2$) based on published data (12,28–30). HB, hyperbaric air; HBO, hyperbaric oxygen; NB, normobaric air; NBO, normobaric oxygen.

Figure 2.

(a) Effective labeling efficiency (normalized to NB) *versus* inspired pO_2 at three different arterial transit times. (b) Brain tissue T_1 *versus* inspired pO_2 . Data were normalized with respect to the NB condition for each animal. HB, hyperbaric air; HBO, hyperbaric oxygen; NB, normobaric air; NBO, normobaric oxygen.

Figure 3.

Relative underestimation of cerebral blood flow (CBF) caused by T_1 effects *versus* inspired pO_2 . Error contributions arise from the use of NB T_1 values in the CBF calculation [Equation [1]] while blood and tissue T_1 are changing, as shown in Figs 1, 2b. HB, hyperbaric air; HBO, hyperbaric oxygen; NB, normobaric air; NBO, normobaric oxygen.

Figure 4.

Uncorrected (a) and corrected (b) whole-brain cerebral blood flow (CBF) values under different inhaled pO_2 conditions. $n = 8$, error bars are standard error of the mean (SEM). $*p < 0.05$ from NB. The whole-brain region of interest (ROI) included the entire brain for the seven image slices. HB, hyperbaric air; HBO, hyperbaric oxygen; NB, normobaric air; NBO, normobaric oxygen.

Figure 5.

Regional calculated cerebral blood flow (CBF) using corrected CBF analysis. $n = 8$, error bars are standard error of the mean (SEM). * $p < 0.05$ from NB. The inset shows representative regions of interest (ROIs) (shown only on one hemisphere) used for regional CBF analysis for the cortex, caudate-putamen (CPu) and cingulate. HB, hyperbaric air; HBO, hyperbaric oxygen; NB, normobaric air; NBO, normobaric oxygen.

Figure 6.

Left: cerebral blood flow (CBF) functional MRI (fMRI) activation maps overlaid on basal CBF images. Gray scale bar, basal CBF from 0 to 0.85 mL/g/min. Yellow–red color scale bar, CBF percentage changes from 50% to 200%. The green regions of interest (ROIs) indicate where the data were obtained from the primary somatosensory cortex (S1). Right: absolute CBF during baseline (shaded bars) and forepaw stimulation (white bars) under different inhaled oxygen conditions. The evoked CBF percentage changes are shown numerically. Data were obtained from the green ROIs shown. $n = 8$, error bars are $\pm$ standard error of the mean (SEM). * $p < 0.05$ comparing basal CBF values. HB, hyperbaric air; HBO, hyperbaric oxygen; NB, normobaric air; NBO, normobaric oxygen.

Table 1

Respiration rate, heart rate and arterial oxygen saturation (S_pO_2) under normobaric air (NB), normobaric oxygen (NBO), 3 atm absolute (ATA) hyperbaric air (HB) and 3 ATA hyperbaric oxygen (HBO)

	NB	NBO	HB	HBO
Respiration rate (bpm)	78.2 ± 2.2	63.6 ± 4.3 ¹	57.1 ± 3.9 ^{1,2}	53.5 ± 3.3 ^{1,2}
Heart rate (bpm)	385 ± 16.4	375 ± 17.1	355 ± 17.3	367 ± 16.9
S_pO_2 (%)	94.7 ± 0.9	99.1 ± 0.2 ¹	99.1 ± 0.2 ¹	99.2 ± 0.2 ¹

$n = 6$, ± standard error of the mean (SEM).

¹ Difference from NB ($p < 0.05$).

² Difference from NBO ($p < 0.05$).