Effects of Subanesthetic Dose of Nitrous Oxide on Cerebral Blood Flow and Metabolism

A Multimodal Magnetic Resonance Imaging Study in Healthy Volunteers

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ABSTRACT

Background: Nitrous oxide, in a concentration of 50% or more, is a known cerebral vasodilator. This study investigated whether a lower dose (30%) of nitrous oxide would also increase cerebral blood flow. In addition, the authors wished to study whether the increase in cerebral blood flow was accompanied by an increase in cerebral metabolism.

Methods: Multimodal Magnetic Resonance Imaging at 3T was performed, and data were obtained in 17 healthy volunteers during three inhalation conditions: medical air, oxygen-enriched medical air (40% oxygen), and 30% nitrous oxide with oxygen-enriched medical air (40% oxygen). Arterial spin labeling was used to derive the primary tissue specific hemodynamic outcomes: cerebral blood flow, arterial blood volume and arterial transit times. Magnetic Resonance Susceptometry and proton Magnetic Resonance Spectroscopy were used for secondary metabolic outcomes: venous oxygenation, oxygen extraction fraction, cerebral metabolic oxygen rate and prefrontal metabolites.

Results: Nitrous oxide in 40% oxygen, but not 40% oxygen alone, significantly increased gray matter cerebral blood flow (22%; $P < 0.05$) and arterial blood volume (41%; $P < 0.05$). Venous oxygenation increased in both oxygen and nitrous oxide conditions. Compared with medical air inhalation, nitrous oxide condition caused a significantly larger decrease in oxygen extraction fraction than 40% oxygen alone (mean [SD] 11.3 [5.6]% *vs.* 8.3 [5.9]% $P < 0.05$), while global cerebral metabolic rate and prefrontal metabolites remained unchanged.

Conclusions: This study demonstrates that 30% nitrous oxide in oxygen-enriched air (40% oxygen) significantly increases cerebral perfusion, and reduces oxygen extraction fraction, reflecting a strong arterial vasodilatory effect without associated increases in metabolism.

What We Already Know about This Topic

- The effects of nitrous oxide in subanesthetic concentrations to alter cerebral perfusion and metabolism are not clearly described in humans

What This Article Tells Us That Is New

- Thirty percent N_2O in oxygen-enriched air (40% oxygen) significantly increased cerebral perfusion, and reduces the oxygen extraction fraction, suggesting a vasodilatory effect without associated increases in metabolism

NITROUS oxide is known to increase cerebral blood flow (CBF) in animals[1–4]and humans,[3–6] suggesting a vasodilatory effect. However, discrepancies in the results of the studies remain due to different doses, interactions with other anesthetic agents and potential differences between species. With the use of 50% nitrous oxide in isolation, significant increases in cerebral blood flow velocity, and decreases in cerebral vascular tone have been shown.[5,6] The precise mechanism by which nitrous oxide increases CBF remains unclear. In the past, it has been suggested that the increase in CBF could be indirect, due to increases in cerebral metabolism.[7–9] However, the studies using different doses, with and without the presence of other anesthetic agents, support both direct and indirect effects of nitrous oxide on cerebral vasculature.[4,10–12] Recent data suggest that inhalation of 50% nitrous oxide does not induce changes in global cerebral metabolism, despite some regional effects.[11]

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Cerebral vasospasm is common in patients with subarachnoid hemorrhage, and accounts for delayed ischemic neurological deficits.[13] Other causes of clinically relevant vasoconstriction include traumatic brain injury, migraine, catheter manipulation, drug-induced and idiopathic. The treatment of cerebral vasospasm remains challenging, and clinical efficacy has been proven only with nimodipine, a calcium channel blocker. However, nimodipine can cause hypotension, and this can reduce CBF and brain tissue oxygenation.[14] Emerging endovascular approaches with balloon angioplasty, topical papaverine, nicardipine or verapamil remain experimental without proven efficacy.[15] Moreover a recent review of nonaneurysmal cerebral vasoconstrictive syndrome found no beneficial effect of calcium channel blockers on the clinical outcome.[16]

Thus, there is a clear need for improved therapies for cerebral vasoconstrictive syndromes. The ideal cerebral vasodilator would be minimally invasive, easy to deliver, quick to act, and would increase cerebral blood flow without systemic hypotension or other side-effects. Nitrous oxide would meet many of these criteria, and in fact, at 50% concentration, it was reported to increase CBF even during hypocapnia, a surrogate vasoconstrictive state.[9] However, inhalation of 50% nitrous oxide may not be well tolerated by some volunteers.[7,8] Effects, and side-effects, of nitrous oxide are clearly dose-dependent, but most of the existing literature relates to doses of at least 50% nitrous oxide. We wished to explore the cerebral vasodilatory potential of a lower, sub-anesthetic dose of nitrous oxide; in 30% concentration, it has already been suggested to offer cerebral protection.[17] With an overarching aim to explore the therapeutic potential of 30% nitrous oxide in vasoconstrictive syndrome, we foremost need to assess whether this dose increases CBF, without associated metabolic demands.

In this volunteer study, we aimed to investigate the effects of 30% nitrous oxide on CBF and cerebral metabolic demand using multimodal magnetic resonance imaging at 3T. We used arterial spin labeling (ASL), susceptometry and proton spectroscopy to measure tissue specific CBF, arterial blood volume (aBV), arterial transit time, global venous oxygenation, relative oxygen extraction fraction (OEF), metabolic rate of oxygen ($CMRO_2$) and prefrontal metabolite levels during three experimental conditions–that is, inhalation of medical air, 40% oxygen, and 30% nitrous oxide in 40% oxygen.

Materials and Methods

Participants

Twenty two un-medicated, nonsmoker healthy volunteers (9 men and 13 women; age range of 22–35 yr) were recruited for this study. The study was approved by the local Research Ethics Committee and the Nottingham University Hospital Trust's Research and Development Department (Nottingham, England). All subjects gave written informed consent. Participants had no history of any systemic illness. All subjects received modest financial compensation for the inconvenience of participation in the study. The final data set for analysis was available on 17 subjects (seven men, mean age of 24.7 yr). Of the five subjects who were excluded, one felt uncomfortable being in the scanner with a facemask and hence did not undergo the study, two subjects could not complete the study due to technical problems related to the scanning sequence, and the data on the other two could not be analyzed due to excessive head motion artifacts.

Experimental Design and Procedure

Participants were scanned in a single session during three conditions in a fixed order that was concealed to the participants, but necessary to avoid confounding carry-over effects from nitrous oxide: breathing medical air (baseline), oxygen-enriched medical air (40% oxygen) or 30% nitrous oxide with oxygen-enriched medical air (40% oxygen). Each experimental condition was maintained for 10 min before scanning to allow for equilibration. The whole scanning session lasted for 1 h and 35 min including an initial 6-min anatomical scan, 23-min scans for each condition, and 20 min of equilibration time. Medical gas deliveries and physiological monitoring were conducted with an anesthesia machine compatible with magnetic environment Aestiva (GE Healthcare, General Electric Company, Madison, WI). Medical gases were delivered using a tight-fitting face mask. The monitoring included noninvasive blood pressure, heart rate, end-tidal carbon dioxide, and peripheral oxygen saturation.

Data Acquisition

All experiments were carried out using a 3T Achieva Magnetic Resonance Imaging scanner (Philips, Eindhoven, Netherlands) and an 8-channel phased array head coil. The scanning protocol included an anatomical scan, a three dimensional Magnetization Prepared Rapid Gradient Echo sequence with echo time = 3.8 ms, repetition time = 8.3 ms, field of view of $256 \times 160\,mm^2$; pixel size, 1×1 mm and slice thickness, 1 mm without gaps. For the ASL scan, we used Quantitative Signal Targeting by Alternating Radiofrequency Pulses Labeling of Arterial Regions to allow measurements of CBF, aBV and arterial transit time.[18] The scan parameters of Quantitative Signal Targeting by Alternating Radiofrequency Pulses Labeling of Arterial Regions ASL were kept identical to a previous multi-centre stability trial[19]: repetition time/echo time/Δ inversion time/inversion time1 = 4000/23/300/40 ms, 13 inversion times (40–3640 ms), 64×64 matrix, 7 slices, slice thickness = 6 mm, 2 mm gap, field of view = 240×240, flip-angle = 35/11.7°, sensitivity encoding = 2.5, 84 averages.

A flow-compensated gradient echo sequence was acquired (slice thickness of 2 mm, field of view $224 \times 224\,mm^2$ and repetitive time/echo time of 32.8/23.5 ms) that was shown to allow estimation of venous oxygenation,[18,19] and together with CBF derived from ASL to determine the global

cerebral metabolic oxygen rate ($CMRO_2$) based on Fick's principle.[20,21]

A single-voxel proton Magnetic Resonance Spectroscopy was acquired using a point resolved spectroscopy sequence with two acquisitions consisting of 64 averages, 8 phase cycles, echo time = 80 ms and repetitive time = 2000 ms from a voxel ($35 \times 15 \times 15\ mm^3$) in the prefrontal/anterior cingulate cortex (fig. 1). Second order pencil beam shimming method on a manually selected volume-of-interest and water suppression by excitation prior to each acquisition were applied as implemented on the Philips platform.[22] To apply water scaling further, 16 averages without water suppression were acquired.

Data Processing

ASL Data Analysis. Postprocessing was carried out using Easy Magnetic Resonance Imaging software[18] after the images were exported to a personal computer running Interactive Data Language 6.1 (ITT Visual Information Solutions, Boulder, CO). Strong motion artifact pairs were excluded as described previously by Petersen *et al.*[23] Maps denoting CBF, aBV, arterial transit time and spin-lattice relaxation time were estimated as described previously.[17] The calculated spin-lattice relaxation time maps from the Look-Locker saturation recovery data were used for the segmentation of gray matter using Functional Magnetic Resonance Imaging of the Brain Centre's Automated Segmentation Tool as part of analytic tools of Functional Magnetic Resonance Imaging of the Brain Centre's Software Library (Functional Magnetic Resonance Imaging of the Brain Centre, Oxford, United Kingdom). In addition, thalamus region-of-interest (ROI) binary masks were drawn manually using tools of Functional Magnetic Resonance Imaging of the Brain Centre's Software Library viewer. Binary gray matter masks were multiplied with hemodynamic maps to extract tissue and region-specific hemodynamic values, whereas the whole brain CBF was obtained after applying an inverse cerebro-spinal fluid mask to the CBF map.

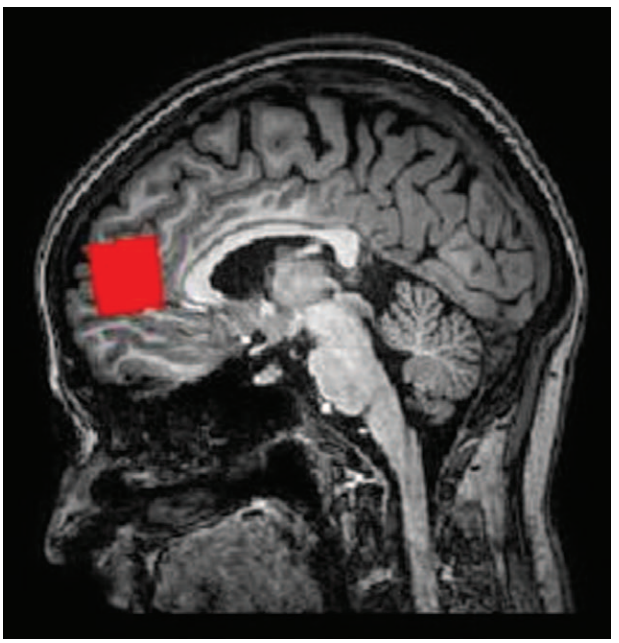

Fig. 1. Point resolved magnetic resonance spectroscopy voxel positioned over the prefrontal/anterior cingulate cortex.

Phase-image Processing, Venous Oxygenation, OEF and $CMRO_2$ Calculations

Image analysis and Fick's principle based $CMRO_2$ calculations were carried out as described previously using in-house software.[18–20] Phase images were unwrapped using the Phase Region Expanding Labeller for Unwrapping Discrete Estimates algorithm in Functional Magnetic Resonance Imaging of the Brain Centre's Software Library (Oxford, United Kingdom).[24] The resulting phase maps were high-pass filtered to remove background field inhomogeneities that could lead to low-frequency spatial differences.[25,26] Venous oxygenation and OEF were estimated in the distal aspect of the superior sagittal sinus from the phase images using Magnetic Resonance Susceptometry according to recently reported and validated protocols.[18,19,27] To more specifically explore metabolic changes in the deep brain structures, specifically thalami, we also evaluated the internal cerebral veins. The ROIs were manually drawn using Functional Magnetic Resonance Imaging of the Brain Centre's Software Library viewer over the superior sagittal sinus, internal cerebral vein, and its surrounding tissue. To avoid any contamination from the surrounding tissue as previously suggested,[19] venous ROIs were thresholded at a signal intensitiy of 1.5 SD or more above the mean value in the tissue ROI. Phase values were averaged over the venous pixels in the ROI (φV) and the pixels assigned to tissue (φT).[19] Blood oxygen saturation was estimated from the measured phase difference between vein and tissue ($\Delta\varphi = \varphi V - \varphi T$) in an infinite cylinder approximation[19]:

$$\Delta\varphi = 2\pi\gamma B_0 \Delta\chi \left(\cos^2\theta - \frac{1}{3}\right) TE \qquad (1)$$

where γ is the gyromagnetic ratio of the proton, B_0 is the main magnet field, θ is the angle between the cylinder and the B_0 and TE is the echo time.[19] The susceptibility difference $\Delta\chi$ in turn can be expressed using oxygen saturation as:

$$\Delta\chi = \Delta\chi_{do} Hct (1 - Y_v) \qquad (2)$$

where $\Delta\chi_{do}$, which was measured to be 1.8×10^{-7}, is the susceptibility difference per unit hematocrit between fully deoxygenated blood and fully oxygenated blood.[28] Using normative values of hematocrit and assuming parallel orientation of distal superior sagittal sinus to the main magnetic field, venous oxygenation reflecting the drainage of the majority of the supratentorial brain tissue can be derived.[20] Moreover, as neither the position nor the angle of sagittal sinus changes between the three experimental conditions

in one scanning session, the difference in OEF (ΔOEF) between two conditions can be calculated as:

$$\Delta OEF = \frac{0.45(\Delta\varphi_1 - \Delta\varphi_0)}{\Delta\varphi_0} \quad (3)$$

CBF derived for supratentorial brain tissue (gray and white matter) to match the venous territory of the distal superior sagittal sinus, and venous oxygenation parameters were used to estimate the $CMRO_2$ based on the Fick's principle[20,27]:

$$CMRO_2 = C_a CBF(Y_a - Y_v) \quad (4)$$

where *Ya* is the measured arterial oxygen saturation (table 1), *Yv* is venous oxygen saturation, and *Ca* is blood oxygen carrying capacity determined to be 834 μmol O_2/100 ml blood for the normative hematocrit of 0.44. For the arterial oxygen saturation, we used the monitored values for each subject.

Magnetic Resonance Spectrometry Data Analysis

Cerebral metabolite concentrations were estimated using a linear combination model.[29,30] An appropriate basis-data set was used and water signal served as internal standard for absolute quantification. The quality of metabolite quantification was assessed by the Cramér-Rao lower bounds, expressed as the percentage of standard deviation with a recommended standard threshold of Cramér-Rao lower bounds less than 20% for good quality fits. Glutamate, Glutamine, *N*-acetylaspartate and total creatine were chosen as metabolites of main interest to index the total metabolic glutamate pool known to correlate with glucose metabolism[31] and the neurotransmitter pool.[32]

Statistical Analysis

All statistical analysis was performed using Statistical Package for Social Sciences (SPSS v17, SPSS Inc, Chicago, IL).

The primary outcomes in this study were hemodynamic metrics, especially gray matter and whole brain CBF. Site-specific reproducibility data from participation in a large multi-center study[23] revealed a less than 10% coefficient of variation for gray matter CBF. We calculated that a sample size of 15 participants will be required to determine a 15% increase in CBF with a power of 0.95 using three-level one-way ANOVA at a significance of 0.05, and allowing for a 33% drop out rate due to challenging set-up and unpredictable movement artifacts.

Initially, explorative analysis was done for all data using Shapiro-Wilk's test and the skewed data (oxygen saturation, end-tidal carbon dioxide concentration and arterial blood volume) were log transformed.

Physiological monitoring data, and experimental data (except OEF changes, and global $CMRO_2$), were analyzed using repeated measures ANOVA with the experimental condition as within subject factor. For the comparisons of cerebral blood flow and blood volume, end-tidal carbon dioxide was taken as a covariate. Comparisons between different experimental conditions were made by using the Tukey *post-hoc* test. Comparisons of changes in oxygen extraction fractions during 40% oxygen and during 30% nitrous oxide (in 40% oxygen) from medical air conditions, and the values of global $CMRO_2$ during 40% oxygen and 30% nitrous oxide (in 40% oxygen), were made using paired *t* tests. Statistical significance for all tests was set at $P < 0.05$ and all results are presented as mean ± SD, unless stated otherwise.

Results

Of the 22 volunteers, one did not undergo the study and the other 21 underwent all three experimental conditions. None of the volunteers found inhalation of 30% N_2O unpleasant or intolerable. For the reasons given above, magnetic resonance imaging data from only 17 subjects were analyzed.

The effect of Nitrous Oxide On Systemic Physiological Parameters

Following nitrous oxide inhalation, a very small but statistically significant increase in the systolic blood pressure and peripheral oxygen saturation was found. In addition, there was a small decrease in end-tidal carbon dioxide noted, but all values remained within normal physiological range. No other change was observed (table 1).

Table 1. Physiological Parameters During Different Gas Inhalation Conditions (n = 17)

	Medical air	40% Oxygen	40% Oxygen + 30% Nitrous oxide
Systolic blood pressure (mmHg)	123 ± 9	123 ± 10	127 ± 12*
Diastolic blood pressure (mmHg)	65 ± 5	65 ± 7	68 ± 7
Heart rate (bpm)	64 ± 10	62 ± 8	61 ± 8
Peripheral oxygen saturation (%)	99.2 ± 0.7	99.8 ± 0.2*	99.8 ± 0.3*
Respiratory rate (bpm)	11 ± 2	11 ± 2	10 ± 3
End-tidal carbon dioxide (%)	5.4 ± 0.3	5.4 ± 0.4	5.2 ± 0.4*

All values are represented as mean ± SD.

*Statistical significance when compared with values obtained during inhalation of medical air.

Table 2. Tissue Specific Cerebral Blood Flow, Arterial Blood Volume and Arterial Transit Time for All Three Experimental Conditions

Experimental Conditions	Gray Matter CBF (ml/100 g/min)	Gray Matter aBV (ml/100 g)	Gray Matter ATT (seconds)
Medical air	39.5 ± 2.11	0.53 ± 0.02	0.41 ± 0.01
Oxygen	40.5 ± 2.87	0.64 ± 0.07	0.42 ± 0.01
Nitrous oxide	48.4 ± 4.1*	0.75 ± 0.07*	0.42 ± 0.01

Values are represented as mean ± SD.

* $P < 0.05$, when compared with the values obtained during inhalation of medical air or oxygen.

aBV = arterial blood volume; ATT = arterial transit time; CBF = cerebral blood flow.

The Effect of Nitrous Oxide On Cerebral Hemodynamics

There was an approximate 22.5% increase in global gray matter CBF while breathing 30% nitrous oxide with 40% oxygen compared to medical air ($P = 0.035$, table 2, fig. 2). Also significant increases occurred in the whole brain CBF and thalami ($P = 0.001$, table 3). There were no changes detected in gray matter or whole brain CBF measurements following 40% oxygen inhalation conditions compared with medical air (table 2).

Global gray matter aBV increased following nitrous oxide conditions compared with the medical air conditions (41%, $P = 0.014$, table 2, fig. 3). No changes were detected during 40% oxygen conditions compared with medical air inhalation. The arterial transit times remained unchanged during all three conditions (table 2).

The Effect of Nitrous Oxide On Venous Oxygenation, OEF and $CMRO_2$

Venous oxymetry based on medical resonance susceptometry showed increased oxygenation of the superior sagittal sinus when breathing 40% oxygen, and 30% nitrous oxide in 40% oxygen, compared to the medical air inhalation (table 3). The reduction in OEF was significantly larger in low-dose nitrous oxide conditions compared with 40% oxygen, as estimated in superior sagittal sinus and internal cerebral vein drainage areas. (table 3).

Estimates of global $CMRO_2$ showed no significant change between 40% oxygen alone, and 30% nitrous oxide in 40% oxygen inhalation (table 3).

The Effect of Nitrous Oxide On Prefrontal Metabolic Profile

High quality metabolic fits were achieved for all spectra and all metabolites of interest such as Glutamate and glutamine, and reference metabolites, N-acetyl-asparate and total creatine (table 4). No differences were observed for any of these metabolites between the three conditions.

Discussion

We have shown that low-dose (30%) nitrous oxide caused a ~20% increase in the whole brain and gray matter CBF explained by concomitant arterial vasodilation. Importantly, this increase in CBF was associated with increased venous oxygenation and decreased OEF, with no change detected in global $CMRO_2$.

In line with previous research,[2,4–8] we found a 22% increase in the global gray matter CBF and 20% in gray and white matter supratentorial CBF during nitrous oxide inhalation. The observed extent of the increase in CBF with

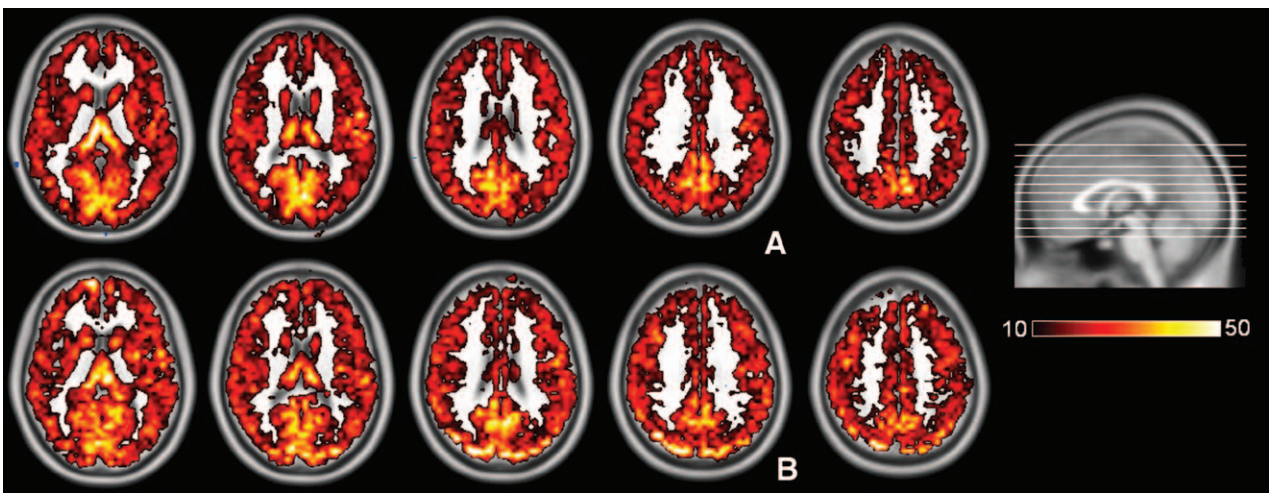

Fig. 2. Group averaged cerebral blood flow maps. (*A*) Averaged map for the medical air inhalation. (*B*) Averaged map for the 30% nitrous oxide in a 40% oxygen and medical air mixture condition. Color coding shown – cerebral blood flow in ml/100 g/min.

Table 3. Cerebral Blood Flow in Superior Sagittal Sinus and Thalamus Area, Oxygenation in Superior Sagittal Sinus Drainage Area, Changes in Oxygen Extraction Fraction During Oxygen and Nitrous Oxide Conditions from Medical Air Condition in Superior Sagittal Sinus and Internal Cerebral Veins Drainage Area, and Calculated Cerebral Oxygen Consumption During Oxygen and Nitrous Oxide Condition

Experimental Conditions	SSS Drainage Area CBF (ml/100 g/min)	Thalamus CBF (ml/100 g/min)	Y_v SSS Drainage Area (%)	ΔOEF (SSS) (%)	ΔOEF (ICV) (%)	Global $CMRO_2$ (μmol/100 g/min)
Medical air	31.0 ± 4.7	43.4 ± 6.6	0.71 ± 0.03			
Oxygen	33.4 ± 6.5	44.1 ± 8.3	0.76 ± 0.04*	−8.3 ± 5.9	−9.7 ± 5.4	64.9 ± 18.6
Nitrous oxide	37.1 ± 7.2*†	48.2 ± 10.8*†	0.78 ± 0.05*†	−11.3 ± 5.6†	−11.4 ± 5.9†	67.1 ± 20.7

Values are given as mean ± SD.

* and † indicate $P < 0.05$ when compared with values obtained during medical air and oxygen, respectively.

CBF = cerebral blood flow; $CMRO_2$ = cerebral oxygen consumption; ICV = internal cerebral veins; OEF = oxygen traction fraction; SSS = superior sagittal sinus; Y_v = venous oxygenation.

30% nitrous oxide in this study is significant when seen in the context of an 8% increase in CBF caused by a clinically relevant vasodilatory dose of nimodipine (30micrograms/kg/h) in healthy volunteers.[33] We also showed that the CBF increase was linked to a vasodilatory effect demonstrating a large (>40%) increase of gray matter aBV, similar to the previous studies.[34,35] However, one study failed to detect a change in global cerebral blood volume following 42% nitrous oxide in healthy human subjects as measured by single photon emission computed tomography.[35] This discrepancy may be explained by the fact that ASL, as used in our experiment, uniquely allows to measure arterial blood volumes while single photon emission computed tomography measures total cerebral blood volume, and unchanged or even reduced volumes in the venous compartment may have confounded that study. From a clinical perspective, it is the arterial vasodilatatory effect that is aimed for to achieve improvement in CBF during cerebral vasoconstriction syndrome. In this study, we were unable to detect any changes in arterial transit time, probably because the increase in CBF was matched by associated vasodilatation.

We showed that venous oxygenation increased significantly while subjects were breathing 40% oxygen, and 30% N_2O in 40% oxygen. Venous oxygenation was estimated based on magnetic resonance susceptometry using a recently validated method,[18,36] and our findings concur with previous studies.[20,21,37–39] Importantly, we found an 11% reduction in the oxygen extraction fraction between 30% nitrous oxide and medical air that was significantly larger than the change observed between oxygen *versus* medical air. To derive a change in the oxygen extraction fraction, we used a recently developed method[19] that has previously been validated in healthy volunteers undergoing hyperventilation-induced hypocapnia.[19] In contrast to the known detrimental effects of the hypocapnia state, we could demonstrate that low-dose nitrous oxide has an opposite beneficial effect with increase in CBF and decrease in OEF fractions indexing an improved oxygen extraction capacity.

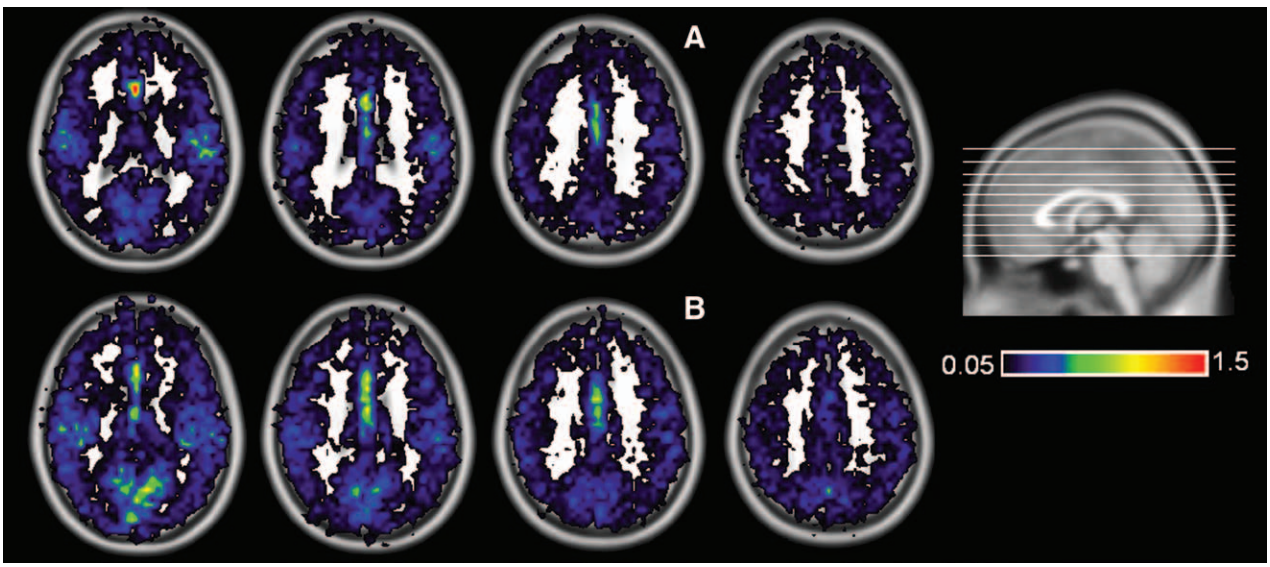

Fig. 3. Group averaged arterial blood volume maps. (*A*) Averaged map for the medical air inhalation. (*B*) Averaged map for the 30% nitrous oxide in a 40% oxygen and medical air. Color coding shown – arterial blood volume in ml/100 g.

Table 4. Metabolite Levels (Institutional Units) in the Prefrontal Cortex for All Three Conditions, and Respective Fitting Accuracies

	Medical Air	Oxygen	Nitrous Oxide
N-acetyl-aspartate	8.48 ± 0.85	8.34 ± 0.70	8.19 ± 0.97
CRLB	2.82 ± 0.53	2.88 ± 0.60	3.06 ± 0.66
Glutamate	6.66 ± 0.36	6.99 ± 0.79	6.83 ± 0.94
CRLB	5.65 ± 0.86	6.29 ± 1.26	7.53 ± 3.56
Glutamine	1.45 ± 0.30	1.54 ± 0.49	1.39 ± 0.45
CRLB	15.41 ± 2.96	16.94 ± 5.02	18.63 ± 4.16
Total creatine	7.21 ± 0.7	7.36 ± 0.46	7.29 ± 0.51
CRLB	8.5 ± 1.81	8.8 ± 1.36	8.6 ± 1.98

CRLB = Cramer Rao lower bound.

To further confirm the beneficial hemodynamic alteration induced by low-dose nitrous oxide, we sought to exclude an increased metabolic demand that could offset the potentially neuroprotective CBF increase. Hence, we derived estimates of global $CMRO_2$ following the standard Fick's principle based on venous oxygenation[27] and whole brain CBF. It may be noted that the value of $CMRO_2$ as calculated using this method is sensitive to the changes in venous oxygen saturation. Since cerebral venous oxygen saturation has been shown to increase with increases in partial pressure of oxygen in arterial blood,[37–39] and the nitrous oxide condition involved inhaling 30% nitrous oxide in 40% oxygen, we took the 40% oxygen condition as the control condition to interpret changes in $CMRO_2$ induced by nitrous oxide (table 3). We found no change in $CMRO_2$ during nitrous oxide inhalation. These results are consistent with a previous study, which also did not show any global change in $CMRO_2$ with 50% N_2O[11]; however, a regional variation in distribution of $CMRO_2$, in particular increased $CMRO_2$ in subcortical regions, was seen. Accordingly, we separately assessed changes in blood flow and the oxygen extraction fraction in the thalami and the internal cerebral veins as the main draining veins of the thalami. We found a similar increase in CBF and decrease in OEF in the internal cerebral vein drainage area as for the global assessment suggesting that 30% nitrous oxide did not have a differential metabolic effect on the deep brain structures. We acknowledge that there are no published test–retest studies available for the $CMRO_2$ estimation method which we used in our study. Nevertheless, taken together with our findings of increased venous oxygenation, and decreased OEF, it may be reasonable to conclude that inhalation of 30% N_2O does not increase $CMRO_2$. However, it will be prudent to prove this with a direct measure of $CMRO_2$.

We used normative, rather than individual, values (0.44) of hematocrit for calculating the oxygen extraction fraction and $CMRO_2$. This could have added to the between individual variability in the calculated values of OEF and $CMRO_2$. However, this variability is unlikely to influence the subject changes which we report here. Our estimated $CMRO_2$ values are lower than those previously reported.[20,21] This can be explained by the choice of CBF for both gray and white matter, rather than gray matter CBF only as in previous work.[27] In fact, when using gray matter CBF for $CMRO_2$ estimates, close agreement with $CMRO_2$ values were achieved.[20,21,27,40,41] Both methods are imprecise, as none accurately reflects the drainage territory of the superior sagittal sinus. We adopted a conservative approach to be over-inclusive of subcortical structures, as previous reports suggested mainly subcortical metabolic changes.[20] The resulting underestimation bias of our chosen approach is, however, unlikely to affect our reported findings as we are mainly interested in the relative changes between conditions.

Nitrous oxide is an N-methyl D-aspartate receptor antagonist, and may thus alter glutamate signaling. In addition, high dose nitrous oxide inhalation has been reported to increase the neural activity in the anterior cingulate cortex,[8] a key structure in emotional processing, antinociception and anxiolysis. Hence, we profiled metabolites from the anterior cingulate cortex, and the observation of unchanged glutamate or glutamine pools would suggest that low-dose nitrous oxide does not significantly upregulate glutamatergic activity in this region. In line with a previous study,[42] we found a slight increase in systolic blood pressure and a decrease in end-tidal carbon dioxide concentration with low-dose nitrous oxide, but the values remained within the normal physiological range.

We utilized noninvasive ASL perfusion measurements and the variability of this particular method was shown to be around 15%, previously.[23] Quantitative accuracy of arterial spin labeling and its comparison with other measurements (such as Positron Emission Tomography, Computed Tomography) and Xenon based methods has been discussed extensively elsewhere. Overall, ASL is thought to estimate the gray matter blood flow correctly.[23,42–44] In contrast, white matter perfusion cannot be reliably estimated using this technique.[45,46] Hence, we have refrained from reporting white matter hemodynamic effects, but instead used whole brain tissue perfusion to derive appropriate $CMRO_2$ estimates.

One of the limitations of this study is that we examined only one dose of nitrous oxide. A dose-response study is required to ascertain the optimal dose for cerebral vasodilating

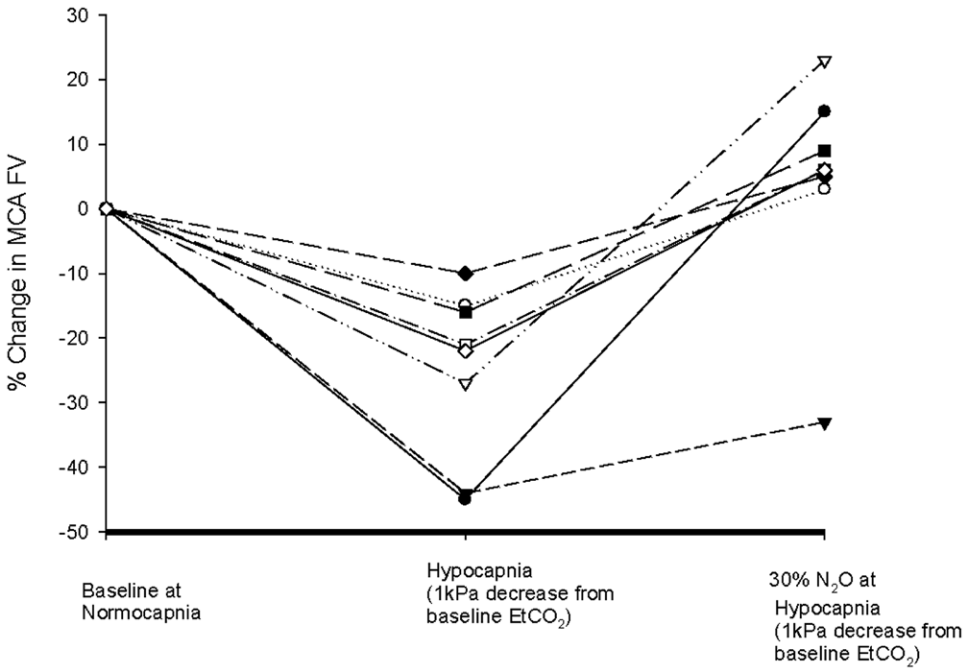

Fig. 4. Middle cerebral artery flow velocity, expressed as percentage change from the baseline value, in healthy volunteers (n = 8) during hypocania and inhalation of 30% nitrous oxide while maintaining hypocapnia. The changes have been normalized for 1 kPa decrease in end-tidal carbon dioxide. MCA FV = middle cerebral artery blood flow velocity; N_2O = Nitrous oxide; $EtCO_2$ = end tidal carbon dioxide; kPa = kilopascal.

effects. Also, we did not randomize the study conditions. This was to keep nitrous oxide for the last in the sequence as, in absence of any psychometric data, we wished to minimize the time which volunteers spent in the scanner after having inhaled nitrous oxide. Future work should look at the psychometric effects, if any, of the low dose of nitrous oxide. In this study, the nitrous oxide condition was maintained for 23 min after equilibration. More work will be required to determine whether or not the nitrous oxide-induced changes in CBF are sustained over a longer period of time. In addition, more work will be required to establish the effects of the low dose of nitrous oxide in vasoconstrictive syndromes. Previous work has already shown that during hypocapnia, a surrogate cerebral vasoconstrictive state, with an addition of 50% N_2O to the inhaled gases causes significant increases in CBF compared to hypocapnia alone.[9] At this time, we have an ongoing transcranial Doppler study in this Department on the cerebral hemodynamic effects of nitrous oxide. The protocol, in part, involves healthy volunteers maintaining hypocapnia for 5 min, and then inhaling 30% nitrous oxide while maintaining hypocapnia for a further 5 min. In this surrogate vasoconstrictive condition induced by hypocapnia (~1 kPa reduction in end-tidal carbon dioxide), preliminary data so far collected from 8 participants show that, taking the average, 1 kPa reduction in end-tidal carbon dioxide caused a 25% decrease in middle cerebral artery blood flow velocity, and the inhalation of 30% nitrous oxide in 40% oxygen tended to return it back to the baseline (fig. 4). While these data provide some evidence of the effectiveness of low-dose nitrous oxide in reversing 'physiological' cerebral vasoconstriction, further work will be required to determine its effectiveness in 'pathological' cerebral vasoconstriction. In addition, the effects of low-dose nitrous oxide on cerebral autoregulation and intracranial pressure in patients with compromised intracranial compliance will need to be determined, before the results of the present study can be translated into first proof of concept studies in patients with vasoconstrictive symptoms.

In conclusion, the inhalation of 30% N_2O in 40% oxygen showed promising potential for future therapeutic trials in vasoconstrictive syndrome. This study demonstrated significant arterial vasodilation resulting in increased cerebral perfusion and cerebral venous oxygenation, and reduced the oxygen extraction fraction in healthy volunteers.

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ANESTHESIOLOGY REFLECTIONS FROM THE WOOD LIBRARY-MUSEUM

Wood-swinging Paul Wood, M.D.: "Tee or Tea?"

Like many of the relatively flat Midwestern states, Indiana hosts a large number of public and private golf courses. The cartoon (*above*) features the Hoosier founder of the Wood Library-Museum, Paul Wood, as an overly deliberate duffer whose shouldered golf club has become a bird's perch near the golf course's tea stand. Wood's caddy appears to be falling asleep (anesthetized?) while carrying the anesthesiologist's golf bag—a bag which is relaying a mythical patient's vital signs from the operating room. Perhaps the cartoonist was encouraging Paul Wood to abandon the golf tee and to stick with sipping tea…. (Copyright © the American Society of Anesthesiologists, Inc.)

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