DOI: 10.1111/bdi.13288

ORIGINAL ARTICLE

BIPOLAR DISORDERS WILEY

Proof-of-concept randomized controlled trial of single-session nitrous oxide treatment for refractory bipolar depression: Focus on cerebrovascular target engagement

William S. H. Kim[1,2] | Mikaela K. Dimick[3,4,5] | Danielle Omrin[3,4] | Rachel H. B. Mitchell[4,5,6] | Daniel Riegert[4,7] | Anthony Levitt[2,4,6] | Ayal Schaffer[2,4,6] | Susan Belo[4,7] | John Iazzetta[4] | Garfield Detzler[4] | Mabel Choi[4,7] | Stephen Choi[4,7] | Nathan Herrmann[2,4,6] | Roger S. McIntyre[5,6,8] | Bradley J. MacIntosh[1,2] | Beverley A. Orser[2,4,7] | Benjamin I. Goldstein[3,4,5,6]

[1]Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada

[2]Hurvitz Brain Sciences Program, Sunnybrook Research Institute, Toronto, Ontario, Canada

[3]Centre for Youth Bipolar Disorder, Centre for Addiction and Mental Health, Toronto, Ontario, Canada

[4]Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada

[5]Department of Pharmacology and Toxicology, University of Toronto, Toronto, Ontario, Canada

[6]Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada

[7]Department of Anesthesiology and Pain Medicine, University of Toronto, Toronto, Ontario, Canada

[8]Mood Disorders Psychopharmacology Unit, University Health Network, Toronto, Ontario, Canada

Correspondence
Benjamin I. Goldstein, Centre for Youth Bipolar Disorder, Child and Youth Psychiatry Division, Centre for Addiction and Mental Health, 100 Stokes Street, M6J 1H4 Toronto, ON, Canada.
Email: benjamin.goldstein@camh.ca

Funding information
Canadian Institutes of Health Research; Fondation Brain Canada; Heart and Stroke Foundation of Canada; National Institute of Mental Health

Abstract

Background: There remain few efficacious treatments for bipolar depression, which dominates the course of bipolar disorder (BD). Despite multiple studies reporting associations between depression and cerebral blood flow (CBF), little is known regarding CBF as a treatment target, or predictor and/or indicator of treatment response, in BD. Nitrous oxide, an anesthetic gas with vasoactive and putative antidepressant properties, has a long history as a neuroimaging probe. We undertook an experimental medicine paradigm, coupling in-scanner single-session nitrous oxide treatment of bipolar depression with repeated measures of CBF.

Methods: In this double-blind randomized controlled trial, 25 adults with BD I/II and current treatment-refractory depression received either: (1) nitrous oxide (20 min at 25% concentration) plus intravenous saline ($n = 12$), or (2) medical air plus intravenous midazolam (2 mg total; $n = 13$). Study outcomes included changes in depression severity (Montgomery-Asberg Depression Rating Scale scores, primary) and changes in CBF (via arterial spin labeling magnetic resonance imaging).

Results: There were no significant between-group differences in 24-h post-treatment MADRS change or treatment response. However, the nitrous oxide group had significantly greater same-day reductions in depression severity. Lower baseline regional CBF predicted greater 24-h post-treatment MADRS reductions with nitrous oxide but not midazolam. In region-of-interest and voxel-wise analyses, there was a pattern of regional CBF reductions following treatment with midazolam versus nitrous oxide.

Conclusions: Present findings, while tentative and based on secondary endpoints, suggest differential associations of nitrous oxide versus midazolam with bipolar depression severity and cerebral hemodynamics. Larger studies integrating neuroimaging targets and repeated nitrous oxide treatment sessions are warranted.

KEYWORDS
arterial spin labeling, bipolar depression, bipolar disorder, cerebral blood flow, midazolam, nitrous oxide

1 | INTRODUCTION

Individuals with bipolar disorder (BD) spend approximately 50% of the time with impairing depression symptoms.[1] Bipolar depression is highly associated with suicidal ideation, self-harm, psychiatric and medical comorbidity, as well as increased mortality.[1–3] In contrast to the numerous approved treatments for mania, only cariprazine, lurasidone, olanzapine/fluoxetine combination, and quetiapine are FDA-approved for the acute treatment of bipolar depression.[4] These approaches often require weeks to reach full effect, and bipolar depression often remains refractory despite the use of these mainstream treatments.[5–7] Taken together, there is a pressing need for rapidly acting novel treatments for refractory bipolar depression.

In the past decade, ketamine, an N-methyl-D-aspartate receptor (NMDAR) antagonist, has come to the fore as a rapidly acting treatment for refractory depression when used in sub-anesthetic doses.[8] Effects of intravenous ketamine are evident within hours and can last for several days, while intranasal esketamine has FDA approval as an add-on for treatment-resistant unipolar depression.[9,10]

Nitrous oxide, also an NMDAR antagonist, has been shown to have rapid-onset antidepressant properties in three small studies of adults with refractory unipolar depression.[11–13] Appealing aspects of nitrous oxide include its ubiquitous availability, low cost, and excellent safety profile with over 60 years of widespread use for various indications such as labor and delivery, dental procedures, and adjunct to general anesthesia.[14,15] In addition to NMDAR-antagonistic effects, nitrous oxide may exude antidepressant effects via other molecular targets including, but not limited to, inhibition of AMPA and kinate glutamate receptors, GABA-C receptors, low voltage-activated calcium channels, and nicotinic acetylcholine receptors. Furthermore, the antidepressant effects of nitrous oxide may be mediated via its effects on the adrenergic neurons in the brainstem or modulation of the endogenous opioid system.[16] Nitrous oxide is a cerebral vasodilator that increases regional cerebral blood flow (CBF), an important index of brain health and metabolism.[17,18] Indeed, nitrous oxide was historically used, in subanesthetic doses, as one of the pioneering methods for quantifying CBF.[19] Vascular pathology is an important aspect of BD,[20–23] as exemplified by reduced frontal CBF in bipolar depression.[24] Furthermore, increases in regional CBF are thought to be associated with improvements in depressive symptoms.[25,26] Therefore, the CBF-modulating effects of nitrous oxide may lead to improvements in depressive symptoms. This proof-of-concept trial tests two novel concepts as they relate to bipolar depression: (1) real-time repeated measures of imaging phenotypes before, during, and immediately after the administration of a rapid-acting pharmacological agent; and (2) the notion that cerebrovascular imaging phenotypes are relevant to nitrous oxide as an antidepressant. This experimental medicine approach integrates measures of target engagement, namely changes in perfusion, which may be related to underlying mechanisms of bipolar depression.[27]

In this study, we examined the acute effects of a single nitrous oxide treatment session versus the active comparator midazolam on depressive symptoms and CBF among adults with treatment-resistant bipolar depression. This approach optimizes the integrity of treatment blinding, as both nitrous oxide and midazolam have psychoactive effects. The primary depression outcome measure was the Montgomery-Åsberg Depression Rating Scale (MADRS[28]) while the primary neurovascular measure was CBF via arterial spin labeling (ASL) magnetic resonance imaging (MRI).[29] Taking together the relevance of CBF to bipolar depression, and the fact that nitrous oxide robustly modulates CBF, we hypothesized that nitrous oxide would significantly improve depressive symptoms relative to midazolam. We further hypothesized that lower frontal CBF at baseline would be associated with greater improvements in depressive symptoms following nitrous oxide treatment and that nitrous oxide treatment would increase frontal CBF.

2 | METHODS

2.1 | Participants

A total of 25 participants between 20 and 60 years of age were recruited from an academic health sciences center and the community via advertisements. All participants provided informed written consent. The local research ethics board approved the study protocol, and it is registered with Health Canada and with ClinicalTrials.gov (NCT02351869). All participants had a primary diagnosis of BD-I or BD-II, assessed via the Structured Clinical Interview for DSM-IV (SCID-IV),[30] and were currently in a major depressive episode (MDE) of at least 4 weeks duration (MADRS score of ≥22).[28] Participants were also required to be treated with at least one anti-manic mood stabilizing medication (i.e., anti-manic anticonvulsant, antipsychotic, and/or lithium). Refractoriness is defined based on the duration of the current MDE in this study.[31] Exclusion criteria were: any new treatments or dose changes within 2 weeks prior to the study; current psychosis; current significant manic symptoms (Young Mania Rating Score (YMRS) score ≥ 12)[32]; current significant suicidality (MADRS item 10 score of ≥4); substance abuse in the past 3 months; any MRI contraindication; active major medical conditions (hepatic, renal, respiratory, or cardio/cerebrovascular disease, diabetes, esophageal reflux, sleep apnea, or B_{12} deficiency/disorders); history of adverse anesthetic reactions; anesthesia American Society of Anesthesiologists (ASA) class ≥2 and scuba diving within the preceding week. Participants with female sex at birth were excluded if they were currently pregnant or breastfeeding and were required to be using a reliable method of contraception.

2.2 | Study design

Full details on study design and measures are described in Dimick et al. 2020.[33] Depressive and other psychiatric symptoms were assessed via in-person interview 60 min prior to treatment administration and at 60, 120, and 240 ± 20 min post-treatment. Interviews at 240 min, and on days 1, 2, 3, and 7 post-treatment were completed by phone. Depressive symptoms were assessed with the MADRS (primary outcome).[28] The primary endpoint was 24 h post-treatment was selected based on prior studies examining nitrous oxide.[11–13] Additional clinical measures of depression, mania, psychosis, and dissociation are described in Data S1. The modified Patient Rated Inventory of Side Effects (PRISE) was completed at all post-treatment timepoints.[34]

Participants were randomized in a 1:1 ratio to: (1) inhaled nitrous oxide plus intravenous saline, or (2) inhaled medical air plus intravenous midazolam. Treatment was delivered by anesthesiologists in two treatment epochs separated by a 5-min wash-out period: (1) 10% nitrous oxide in oxygen for 10 min with 0.5 ml intravenous saline, and (2) 25% nitrous oxide in oxygen for 20 min with 1.5 ml intravenous saline, followed by 3 min of medical air. The active placebo treatment arm was also delivered in two treatment epochs: (1) intravenous 0.5 mg midazolam, and (2) intravenous 1.5 mg midazolam, followed by 3 min of medical air. These dosing levels were chosen based on previous literature to avoid any adverse events or risks in the MR suite.[9]

Participants, raters, and study psychiatrists were blinded to treatment conditions. Treatment allocation was provided to the anesthesia team in a sealed envelope immediately prior to the scan and the raters and study psychiatrist remained outside of the MRI suite for the duration of the scan. Only the anesthesia team administering treatment and MRI technologist were unblinded.

2.3 | MRI acquisition

The MRI sequences for this study consisted of T1-weighted and pseudo-continuous ASL (pCASL) imaging acquired on a Philips 3 Tesla Achieva MRI system with an 8-channel phased array head coil receiver. Anatomical T1-weighted images were acquired with high-resolution fast-field echo imaging (TR/TE/TI = 9.5/2.3/1400 ms, spatial resolution = 0.63 × 0.63 × 1.2 mm, FOV = 240 mm, slices = 130).

Two series of pCASL images were acquired concurrently with treatment administration (low dose administered at 16 min, full dose administered at 32 min). A single post-label delay sequence was acquired at five timepoints (i) baseline: 0 min; (ii) low dose: 27 min; (iii) full-dose initiation: 32 min; (iv) full-dose cessation: 46 min; (v) 15 min post-treatment: 60 min. A multiple post-label delay sequence was acquired at baseline and 15 min post-treatment timepoints. MRI parameters and processing details are reported in Data S1.

Mean CBF values were extracted from three a priori regions of interest implicated in BD: anterior cingulate cortex, ventral prefrontal cortex, and striatum. Individual regions from the Harvard-Oxford atlas were summed to create masks for the regions of interest.[35] The anterior cingulate cortex included the anterior cingulate gyrus and paracingulate gyrus; the ventral prefrontal cortex was taken as the frontal orbital cortex; the striatum included the caudate, putamen, and nucleus accumbens. The average of the three regions of interest was taken as an omnibus "frontal" region of interest. Data exclusions are summarized in (see Table S1).

2.4 | Statistical analysis

Demographic and clinical characteristics and adverse events were compared between treatment groups using Mann–Whitney *U* tests for continuous variables and chi-square tests for categorical variables. Modified intention-to-treat analyses included all randomized participants with at least one post-baseline measurement. The threshold for statistical significance was set at $p < 0.05$. Adverse events reported at or prior to 120 min post-treatment are presented separately from adverse events reported after 120 min post-treatment.

For the primary study outcome, MADRS score 24 h post-treatment, a repeated measures analysis of covariance (ANCOVA) was designed to test for a treatment effect. We examined the change in MADRS score between baseline and 24 h post-treatment as a function of treatment, controlling for baseline MADRS score. Logistic regression was then used to examine the response rate at 24 h post-treatment (defined as a ≥50% reduction in MADRS) as a function of treatment, controlling for baseline MADRS score. We chose to investigate changes in MADRS score 24 h post-treatment to avoid acute euphoric effects and to align with previous trials.[11,12] As secondary analyses, we modeled MADRS score across five timepoints (pre-treatment, and 60, 120, 240-min, and 24 h post-treatment) using a linear mixed-effects model. Parameters were estimated using restricted maximum likelihood estimation and degrees of freedom were estimated using Satterthwaite's approximation.

Within each treatment group, Pearson's correlation coefficients examined the association between baseline CBF within the frontal region of interest and change in MADRS score 24 h post-treatment. Secondary analyses examined the three regions of interest separately. A comparison of correlations between treatment groups was performed using Fisher's transformation.

A second ANCOVA examined mean change in CBF within the frontal region of interest between baseline and 15 min post-treatment timepoint as a function of treatment, controlling for baseline CBF. Secondary analyses examined the three regions of interest separately. Post hoc linear mixed-effects models were used to examine changes in CBF with treatment, time, and their interaction as fixed effects and participant-specific slopes and intercepts as random effects. Parameters were fit using restricted maximum

likelihood estimation and degrees of freedom were estimated using Satterthwaite's approximation.

Finally, we performed an exploratory whole-brain analysis of voxel-wise change in CBF (see Data S1).

3 | RESULTS

3.1 | Demographic and clinical characteristics

A total of 430 potential participants were screened between August 2015 and March 2020, of which 25 met all eligibility criteria, were randomized, and received study treatment (see Figure S1, Consolidated Standards of Reporting Trials Patient Flowchart). The most common reasons for not meeting eligibility criteria were: not currently in MDE ($n = 123$), the presence of a major medical condition ($n = 59$), not meeting medication criteria ($n = 52$), and the presence of MRI contraindications ($n = 40$). Demographic and clinical characteristics of the nitrous oxide and midazolam groups are presented in Table 1. There were no significant between-group differences in key demographic (i.e., age, sex, race, socioeconomic status) or clinical (i.e., body mass index, baseline blood pressure, lifetime suicidality, psychiatric comorbidities, current medication use) variables. In the overall sample, the mean baseline MADRS score was 28.9 ± 3.5 with no between-group differences (see Table 1). The sample was characterized by a prolonged mean duration of current MDE of 4.3 ± 7.5 years, and 84% were taking first-line (19/25) or second-line (2/25) bipolar depression treatment.[5]

3.2 | Effect of treatment on depressive symptoms

Twelve participants were randomized to nitrous oxide treatment and 13 participants were randomized to midazolam treatment. In the overall sample, there were significant reductions in MADRS from baseline to 24 h post-treatment (primary outcome; $F_{1,23} = 134.81$, $p < 0.001$; Figure 1B). However, there was no significant between-group difference in MADRS change (nitrous oxide group: -16.2 ± 6.1, 95% CI = −20.1 to −12.3; midazolam group: -14.6 ± 7.1, 95% CI = −18.9 to −10.4; $F_{1,23} = 0.34$, $p = 0.56$). Similarly, there were no significant between-group differences in response rate at 24 h post-treatment (nitrous oxide group: 7/12, 58%; midazolam group: 7/13, 54%; OR = 1.1, 95% CI = 0.2 to 5.7, $p = 0.91$).

In exploratory analyses, we examined the response rate between treatment groups at all other post-treatment timepoints (presented in Figure 1A). Response rate at 120 min post-treatment was significantly higher in the nitrous oxide group (11/12; 92%) compared to the midazolam group (5/13; 38%; OR = 16.0, 95% CI = 1.4 to 178.0, $p = 0.02$); there were no between-group differences in response rate at any other post-treatment timepoints. In follow-up analyses, response rates for all three timepoints on the day of treatment (60, 120, and 240 min post-treatment) were summed to create a total response rate on the day of treatment. Participants in the nitrous oxide group showed a response at 2.25 ± 0.87 of the 3 post-treatment assessments on the day of treatment, significantly higher compared to the midazolam group (1.23 ± 1.24; $t = -2.37$, $p = 0.03$).

In secondary analyses modeling MADRS scores across five timepoints, we found no between-group differences in MADRS score at 1-h pre-treatment ($F_{1,29.066} = 2.36$, $p = 0.14$). We observed a significant effect of time ($F_{1,90.815} = 4.23$, $p = 0.04$) such that all participants experienced a reduction in MADRS score. However, the nitrous oxide group did not experience a greater reduction in MADRS score compared to the midazolam group ($F_{1,90.815} = 0.0007$, $p = 0.98$). Overall, these results are in accordance with the ANCOVA results.

3.3 | Association of baseline CBF with change in depression

Within the nitrous oxide group, lower baseline CBF within the omnibus frontal region of interest ($r = 0.66$, $p = 0.04$), as well as the ventral prefrontal cortex ($r = 0.76$, $p = 0.01$), and anterior cingulate cortex ($r = 0.63$, $p = 0.03$), was significantly associated with a greater reduction in MADRS score 24 h post-treatment (Figure 2 and Table S2). Within the midazolam group, there were no significant associations between baseline CBF and change in MADRS score 24 h post-treatment (p-values 0.31–0.90). There were no significant CBF-by-treatment interactions on change in MADRS score 24 h post-treatment.

3.4 | Treatment-related changes in CBF

3.4.1 | Region of interest analysis

Relative to nitrous oxide, midazolam led to significantly greater post-treatment decreases in CBF within the omnibus frontal region of interest ($F_{1,15} = 5.89$, $p = 0.03$) and individually within the anterior cingulate cortex ($F_{1,21} = 4.76$, $p = 0.04$) and ventral prefrontal cortex ($F_{1,15} = 4.58$, $p = 0.05$) (Figure 3 and Table S3). There was no significant between-group difference in CBF change within the striatum. There were significant treatment-by-time effects on the change in CBF within the omnibus frontal region of interest ($F_{1,16.18} = 5.95$, $p = 0.03$) and the anterior cingulate cortex ($F_{1,22.01} = 4.83$, $p = 0.04$), such that midazolam treatment resulted in greater decreases in CBF relative to nitrous oxide (Table S4).

Global grey matter CBF did not differ between groups at baseline ($F_{1,21} = 0.66$, $p = 0.43$). Similarly, the change in global grey matter CBF between baseline and 15 min post-treatment timepoints did not differ ($F_{1,21} = 3.74$, $p = 0.07$). Given the nominal trend, exploratory within-group analyses were conducted, demonstrating that CBF decreased significantly from baseline following midazolam ($t = -3.82$, $p = 0.002$) but not nitrous oxide treatment ($t = -0.46$, $p = 0.66$).

TABLE 1 Demographic and clinical characteristics

	Nitrous oxide ($n = 12$)	Midazolam ($n = 13$)	Test statistic	p
Age (years)	33.6 ± 7.1	34.6 ± 9.7	$U = 75.5$	0.46
Female	6 (50)	7 (54)	$\chi^2 = 0.04$	0.86
Caucasian	9 (75)	7 (54)	$\chi^2 = 0.47$	0.41
Smoking	3 (25)	4 (31)	$\chi^2 = 0.10$	0.75
SES	3.3 ± 1.1	3.3 ± 1.3	$U = 75.0$	0.44
Adjusted BMI (kg/m^2)	29.6 ± 6.6	27.0 ± 5.2	$U = 60.0$	0.17
DBP (mmHg)	83.3 ± 15.3	78.8 ± 6.3	$U = 63.5$	0.22
SBP (mmHg)	120.6 ± 22.4	117.6 ± 10.7	$U = 68.5$	0.31
HR (bpm)	74.7 ± 11.2	78.5 ± 16.8	$U = 70.0$	0.34
Lifetime suicide attempts	4 (33)	3 (23)	$\chi^2 = 0.20$	0.65
Lifetime self-injurious behavior	4 (33)	3 (23)	$\chi^2 = 0.20$	0.65
Lifetime psychosis	6 (50)	5 (39)	$\chi^2 = 0.34$	0.56
Lifetime psychiatric hospitalization	11 (92)	10 (77)	$\chi^2 = 1.01$	0.32
Alcohol use disorder (past)	4 (33)	2 (15)	$\chi^2 = 1.10$	0.29
Substance use disorder (past)	4 (33)	5 (39)	$\chi^2 = 0.07$	0.79
Number of anxiety disorders, lifetime	2.50 ± 1.17	1.92 ± 1.19	$U = 56.0$	0.25
BD I	9 (75)	8 (62)	$\chi^2 = 0.52$	0.47
BD II	3 (25)	5 (39)		
Age of BD onset (years)	22.9 ± 7.0[a]	20.9 ± 6.8	$U = 63.0$	0.65
Current episode duration (years)	3.6 ± 4.7	5.9 ± 9.6	$U = 73.0$	0.40
Current depression symptoms	28.0 ± 3.7	29.8 ± 3.2	$U = 58.5$	0.15
Current mania symptoms	2.2 ± 2.0	1.3 ± 1.0	$U = 60.5$	0.17
Current first-line treatment for bipolar depression	10 (83)	9 (69)	$\chi^2 = 0.68$	0.41
Current second-line treatment for bipolar depression	1 (8)	1 (8)	$\chi^2 = 0.003$	0.95
SGA	4 (33)	7 (54)	$\chi^2 = 1.07$	0.30
Lithium	7 (58)	3 (23)	$\chi^2 = 3.23$	0.07
SSRI antidepressant	1 (8)	0	$\chi^2 = 1.13$	0.29
Non-SSRI antidepressant	2 (16)	1 (8)	$\chi^2 = 0.48$	0.49
Stimulants	1 (8)	2 (15)	$\chi^2 = 0.29$	0.59
Antimanic	9 (75)	9 (69)	$\chi^2 = 0.10$	0.75

Note: Data are presented as mean ± standard deviation, or count (%). p-values were calculated using Mann–Whitney's U test for continuous variables and the chi-square test for categorical variables. Alcohol use disorder includes alcohol abuse and/or alcohol dependence. Substance use disorder includes substance abuse and/or substance dependence.

Abbreviations: BD, bipolar disorder; BMI, body mass index; DBP, diastolic blood pressure; HR, heart rate; NOS, not otherwise specified; SBP, systolic blood pressure; SES, socioeconomic status; SGA, second-generation antipsychotic; SSRI, selective serotonin reuptake inhibitor.

[a]Indicates missing data for one participant.

3.4.2 | Voxel-wise analysis

Voxel-wise analyses are fully reported in Data S1. Briefly, comparing CBF change between groups revealed 8 significant clusters, including the anterior cingulate cortex and insula (Figure 4 and Table S5). All significant clusters reflected significantly decreased CBF relative to baseline within the midazolam group (Figure S2 and Table S6). Similar results at full-dose initiation and

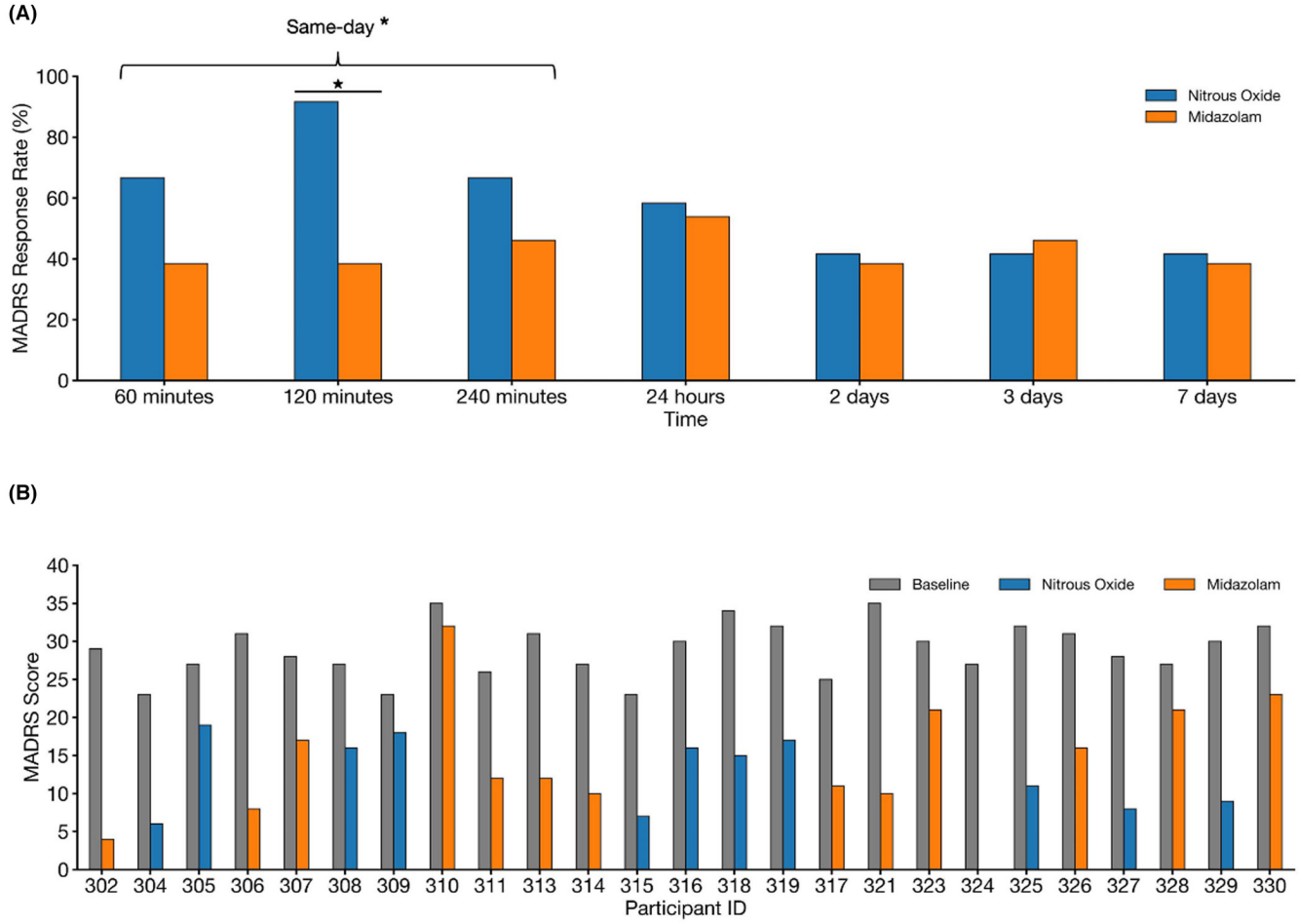

FIGURE 1 Participant MADRS scores. (A) Response rates at each of the post-treatment timepoints. The response rate was defined as >50% reduction in baseline MADRS score. The nitrous oxide group had a significantly higher response rate (92%) compared to the midazolam group (38%) at the 120 min post-treatment, after adjusting for baseline MADRS score ($p < 0.05$). Furthermore, the nitrous oxide group had a significantly higher total response rate on the day of treatment compared to the midazolam group ($p < 0.05$). One participant in the midazolam group was missing data at the 2 and 7 days timepoints. (B) MADRS scores at baseline and post-treatment. Grey boxes indicate baseline scores while blue or orange boxes indicate scores 24 h following nitrous oxide or midazolam treatment, respectively. Participant 324, who received nitrous oxide, recorded a MADRS score of zero at 24 h post-treatment.

cessation timepoints were observed when using single-PLD CBF data (Tables S7 and S8).

3.5 | Safety and quality of blinding

There were no serious adverse events. Tolerability was good, as all participants completed the entire treatment, and side-effect rates were low in both treatment conditions (see Table S9).

Assessment of the quality of blinding, comprising self-reported guesses of treatment allocation, was added to the protocol beginning with the fifth participant and was completed by 18/25 participants (and two raters per participant) at 120 min post-treatment. Of those participants who completed the quality of blind assessment, 8/18 (44.4%) guessed their treatment correctly, and this did not differ significantly by treatment group (nitrous oxide, 3/10 (30%); midazolam, 5/8 (62.5%); $p = 0.17$). The two raters for each participant also completed the quality of blind assessment for each of the 21 participants. The accuracy for both raters was 71.4% (15/21) and concordance between the raters was 61.9% (13/21); these metrics did not differ by treatment group (rater 1: nitrous oxide, 9/11 (81.8%); midazolam, 6/10 (60%); $p = 0.27$; rater 2: nitrous oxide, 7/11 (63.6%); midazolam, 8/10 (80%); $p = 0.41$).

4 | DISCUSSION

In this proof-of-concept clinical trial of nitrous oxide as a treatment for adults with refractory bipolar depression, we investigated the acute effects of nitrous oxide on depressive symptoms and CBF relative to the active comparator midazolam. While there were no significant between-group differences in the primary study outcome, MADRS score 24 h post-treatment, participants in the nitrous oxide group showed significantly higher same-day response rates as

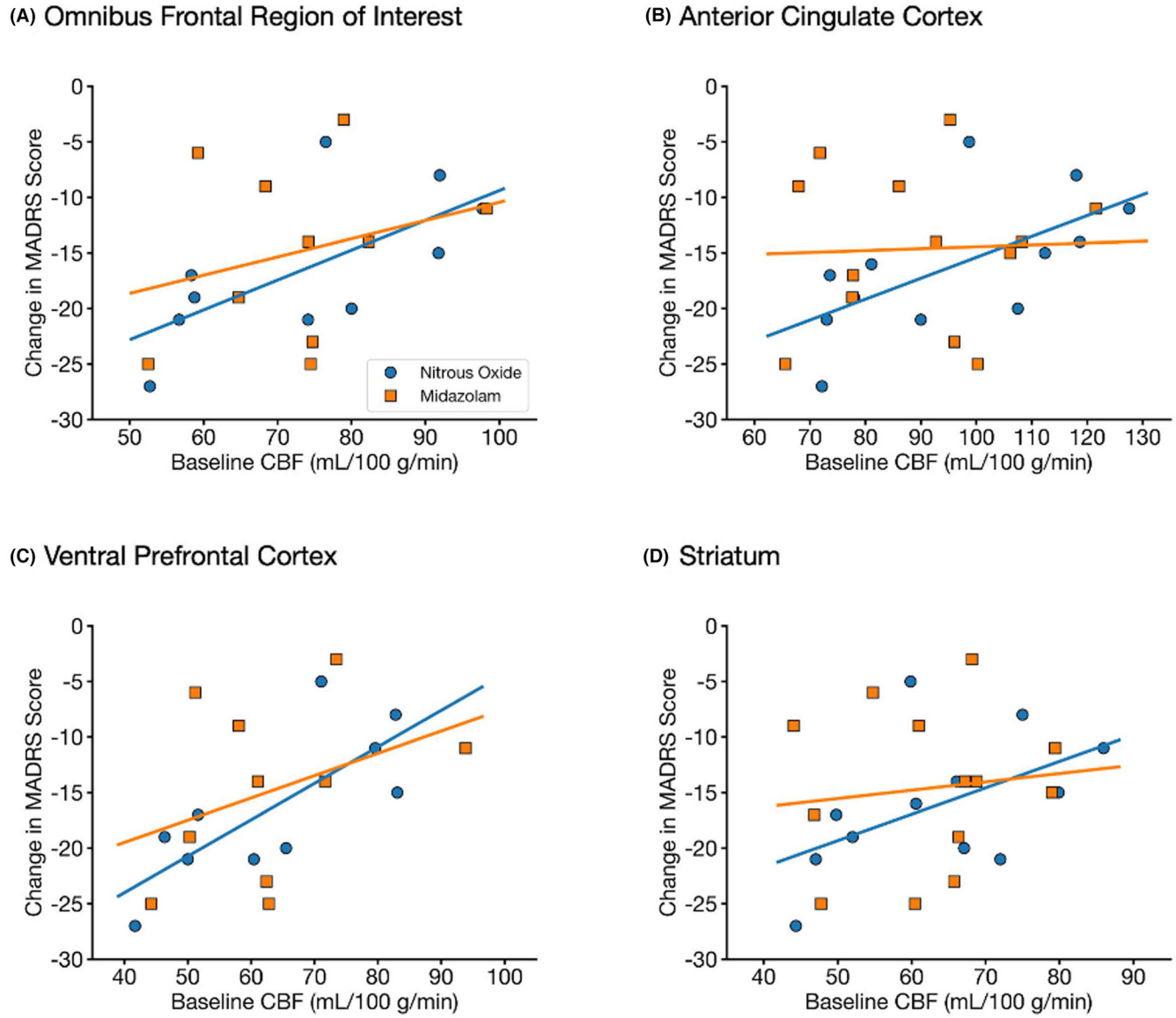

FIGURE 2 Associations between baseline cerebral blood flow within frontal regions of interest and change in MADRS score 24 h following nitrous oxide or midazolam treatment. Baseline cerebral blood flow within the (A) omnibus frontal region of interest, (B) anterior cingulate cortex, and (C) ventral prefrontal cortex was significantly associated with a change in MADRS score 24 h following nitrous oxide, but not midazolam treatment. No significant associations were observed within the (D) striatum. The nitrous oxide group is depicted by blue circles while the midazolam group is depicted by orange squares.

compared to the midazolam group. Our imaging hypothesis investigating CBF as a predictor of treatment response was supported: lower baseline CBF was associated with greater improvements in depressive symptoms 24 h following nitrous oxide treatment, but not following midazolam treatment. Findings relating to our second imaging hypothesis showed midazolam-related decreases, rather than the anticipated nitrous oxide-related increases, in CBF. These findings provide useful information regarding the potential efficacy of nitrous oxide as a treatment for bipolar depression and demonstrate the potential utility of neurovascular phenotypes as predictors of nitrous oxide response.

Improvement in dimensional depressive severity and response rate 24 h following nitrous oxide treatment is similar to previous trials of nitrous oxide and ketamine in adults with unipolar or bipolar depression.[11,12,36] Whereas, response to midazolam in the current study (change in MADRS score = −14.6, response rate = 54%) was higher than reported in 4 midazolam-controlled ketamine studies of bipolar or unipolar depression.[36] This aligns with the low accuracy of participants' guesses regarding treatment conditions. The robust response to midazolam in the current study may relate to differences in dosing, study design (e.g., multiple supportive study staff on the day of treatment, see Figure S3), and/or study population. Given the high prevalence of comorbid anxiety disorders in this cohort, which is a known predictor of greater placebo response,[37] anxiolytic effects of benzodiazepines on overlapping depressive symptoms may also be contributory.[38] We speculate that our response rate was

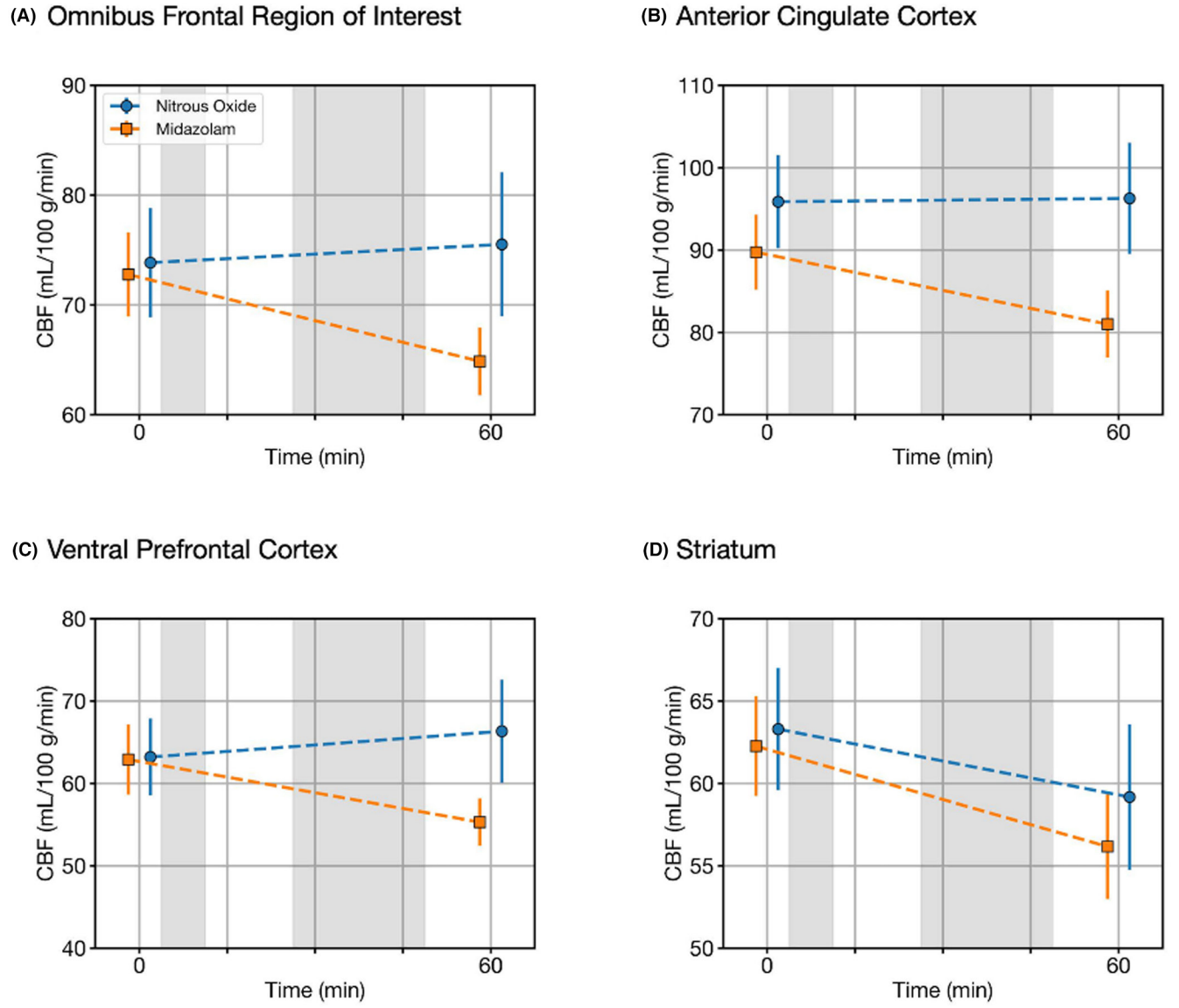

FIGURE 3 Mean cerebral blood flow change within regions of interest between baseline and 15 min post-treatment ASL timepoints. Relative to nitrous oxide, midazolam led to significantly greater post-treatment decreases in CBF within the omnibus frontal region of interest (nitrous oxide: −0.24 ± 6.65 ml/100 g/min vs. midazolam: −7.92 ± 6.94 ml/100 g/min), anterior cingulate cortex (nitrous oxide: −1.62 ± 8.56 ml/100 g/min; midazolam: −8.74 ± 7.51 ml/100 g/min), and ventral prefrontal cortex (nitrous oxide: 1.33 ± 10.00 ml/100 g/min; midazolam: −7.60 ± 9.99 ml/100 g/min). There was no significant between-group difference in CBF in the striatum (nitrous oxide: −5.20 ± 5.90 ml/100 g/min; midazolam: −6.10 ± 4.84 ml/100 g/min). The nitrous oxide group is depicted by blue circles while the midazolam group is depicted by orange squares. Shaded regions indicate treatment administrations while in-scanner. Error bars represent standard errors of the mean. ASL, arterial spin labeling; CBF, cerebral blood flow.

similar to ketamine but the midazolam control group has a higher response which may have attenuated signal detection in the nitrous oxide group.

Importantly, nitrous oxide dosing in the current study (10% × 10 min, 25% concentration × 20 min) was less than one-quarter the dose of other nitrous oxide studies in the depression literature (i.e., 50% concentration × 60 min).[11–13] We opted for a conservative dosing approach, due to concerns about possible precipitation of mania and/or psychosis, alongside concerns about vomiting while positioned supine within the MRI bore. In exploratory analyses, we found a significantly greater response rate at 120 min post-treatment, and across all same-day timepoints, in the nitrous oxide group as compared to the midazolam group. These are tentatively promising indicators in a study, conceived prior to the publication of other nitrous oxide studies and informed by MRI-related risk management considerations, that have shown a signal despite the low treatment dose. We speculate that a longer inhalation session, aligning with other nitrous oxide studies,[11–13] may result in longer-lasting antidepressant effects.

Prior research on vascular subtypes of depression has focused on structural cerebrovascular imaging phenotypes (i.e., white matter hyperintensities) in late life, whereas functional cerebrovascular

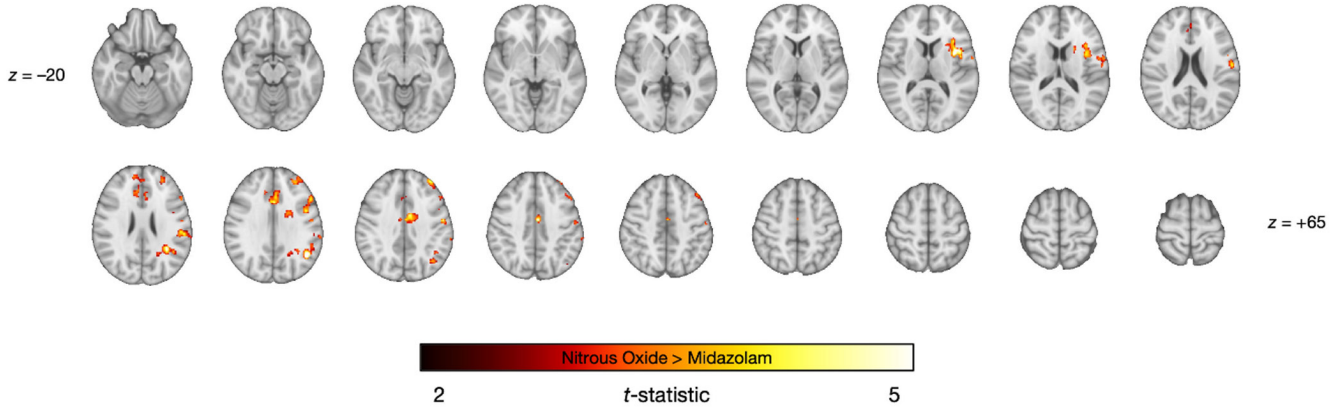

FIGURE 4 Between-group voxel-wise analyses of multi-PLD cerebral blood flow change. Relative to nitrous oxide, midazolam led to greater decreases in multi-PLD cerebral blood flow, covarying for age and sex. The cluster-level inference was applied using a cluster-forming threshold of $p < 0.005$ (uncorrected) and a cluster-extent threshold of $p < 0.05$. Statistical maps are presented in radiological convention. CBF, cerebral blood flow; multi-PLD, multiple post-label delays.

imaging phenotypes may offer unique advantages and be relevant across the lifespan.[39] We found that lower baseline CBF within the anterior cingulate and ventral prefrontal cortices predicted greater reductions in MADRS scores 24 h following nitrous oxide, but not midazolam treatment. Others have reported similar associations, with lower baseline CBF acting as a predictor of subsequent mood changes.[25,40,41] Furthermore, baseline CBF differed between treatment responders and non-responders,[42] supporting the concept of CBF as a potential predictor of treatment response.[43]

The use of repeated real-time CBF measurements is novel. Prior studies evaluating CBF in relation to antidepressant treatment in the major depressive disorder have either measured CBF at pre-treatment only or at 1 day to 6 months following treatment.[25,41,44,45] A recent study examining CBF at baseline and 24 h following ketamine treatment for a major depressive disorder found CBF increased following treatment with ketamine, and lower CBF at baseline was associated with greater increases in CBF and greater reduction of depressive severity.[41] Imaging findings from the current study highlight acute CBF changes within brain regions and networks known to be implicated in depression. Primary and secondary region of interest analyses converged with exploratory whole-brain analyses, yielding findings in the anterior cingulate cortex, as well as regions including the posterior cingulate gyrus, precuneus, and angular gyrus. These are regions in the default mode network, highlighted in previous ketamine studies as being related to antidepressant response.[41,46,47] For instance, a recent study reported increased CBF within the cingulate cortex alongside reductions in depression 24 h following ketamine infusion among adults with treatment-resistant depression.[25] At the full-dose cessation timepoint, we observed between-group differences in CBF change within reward-related regions, such as the anterior cingulate cortex, insula, and thalamus.[48] Together, these findings provide evidence of dynamic network activation throughout nitrous oxide administration, which may be related to subsequent changes in depressive symptoms. Indeed, prior evidence that psychiatric treatments (including pharmacological and brain stimulation approaches) are associated with changes in CBF, often proportional with symptomatic changes, in similar regions to those identified in the current study, supports the potential value of CBF metrics as indicators of treatment response.[45,49–52]

There were no serious adverse events observed following either nitrous oxide or midazolam treatment in the current study. In contrast to ketamine, nitrous oxide does not appear to precipitate increases in blood pressure or psychotic symptoms. Given the higher predisposition to psychosis among individuals with BD relative to major depressive disorder, nitrous oxide may be particularly well suited for the treatment of bipolar depression.[16] Based on prior trials, higher concentration and longer duration of nitrous oxide are related to increased side effects, especially nausea/vomiting.[11,12] In the current study, one participant in the nitrous oxide group (8%), and no participants in the midazolam group, experienced nausea within 2 h post-treatment; there were no incidences of vomiting or regurgitation during inhalation. Taking these findings together with the efficacy findings, we speculate that a full 1-h session of 25% nitrous oxide may optimize the balance between efficacy and tolerability.

There are several study limitations. First, this proof-of-concept study had a limited sample size. While enrolment was ultimately closed due to the onset of the COVID-19 pandemic, recruitment had been ongoing for nearly 5 years. Reasons for this were multifactorial, including MRI-related exclusions, and operational challenges in securing concurrent MRI availability, availability of the multiple study team members, and participant availability. Second, the nitrous oxide dosing was approximately one-quarter of that used in other depression studies. Third, we did not have restrictive medication exclusions, instead choosing to enroll a clinically heterogeneous sample. This approach adds variability, but bolsters ecological validity, as BD is characterized by substantial clinical and pharmacologic heterogeneity. Fourth, there was no non-psychoactive control group (i.e., intravenous saline plus inhaled room air) which limits our ability to interpret whether the reduction in depressive symptoms was due to treatment or placebo effect related to non-specific psychoactive effects of nitrous oxide and midazolam. Aligning with most acute

ketamine studies, we opted for a psychoactive control to improve the integrity of the blind. We acknowledge that psychoactive comparators may have had effects on CBF and suggest future studies include both psychoactive and non-psychoactive controls (i.e., saline plus room air). Lastly, study interviewers correctly guessed treatment allocation meaningfully better than chance, whereas participant guess accuracy was less than chance. This may be due to specific symptom reporting during post-treatment interviews (e.g., attributing fatigue to midazolam, or attributing spontaneous reports of odor detection to nitrous oxide). In contrast, participant guess accuracy was 44%, less than chance, which suggests the integrity of the blind.

In conclusion, this proof-of-concept study provides preliminary insights into the use of nitrous oxide for the acute treatment of refractory bipolar depression. Although we found no significant difference in depression symptoms between treatment groups for the primary endpoint, we found tentative evidence of greater improvements in depressive symptoms following nitrous oxide versus midazolam in secondary analyses. Furthermore, lower baseline CBF predicted greater reductions in depression severity within the nitrous oxide group. Present findings provide support for the integration of cerebrovascular neuroimaging phenotypes in future psychiatric treatment studies using experimental medicine paradigms.[53] Future studies of nitrous oxide as a treatment for bipolar depression are warranted to further evaluate the effect of nitrous oxide concentration and duration on efficacy, tolerability, and cerebral hemodynamics.

ACKNOWLEDGMENTS

The authors wish to thank all study participants for donating their time to this study. This study was supported by philanthropic donations to Sunnybrook Health Sciences Centre, including the Brenda Smith Fund.

CONFLICT OF INTEREST

S. Choi collaborates on clinical studies supported by in-kind software donations from Cogstate Ltd. (USA). B. Goldstein acknowledges research grant support from Brain Canada, the Canadian Institutes of Health Research, the Heart and Stroke Foundation, the National Institute of Mental Health, and the Departments of Psychiatry at the University of Toronto and Sunnybrook Health Sciences Centre. Dr. Goldstein also acknowledges his position as RBC Investments Chair in Children's Mental Health and Developmental Psychopathology at CAMH, a joint Hospital-University Chair between the University of Toronto, CAMH, and the CAMH Foundation. R. McIntyre has received research grant support from CIHR/GACD/Chinese National Natural Research Foundation, speaker/consultation fees from Lundbeck, Janssen, Alkermes, Mitsubishi Tanabe, Purdue, Pfizer, Otsuka, Takeda, Neurocrine, Sunovion, Bausch Health, Novo Nordisk, Kris, Sanofi, Eisai, Intra-Cellular, NewBridge Pharmaceuticals, Abbvie, and is a CEO of Braxia Scientific Corp. R. Mitchell has received an honorarium from Medscape for appearing in a documentary on bipolar depression, and research salary support from the University of Toronto's Department of Psychiatry, Sunnybrook Health Sciences Centre's Department of Psychiatry, and the Sunnybrook Foundation. B. Orser serves on the Board of Trustees of the International Anesthesia Research Society (San Francisco, California) and is a co-director of the Perioperative Brain Health Center (Toronto, Ontario, Canada; http://www.perioperativebrainhealth.com). She is a named inventor on a Canadian patent (2,852,978) and two U.S. patents (9,517,265 and 10,981,954). The new methods identified in the patents aim to prevent and treat delirium and persistent neurocognitive deficits after anesthesia and surgery, as well as to treat mood disorders. She collaborates on clinical studies supported by in-kind software donations from Cogstate Ltd. (USA). D. Riegert is a consultant for Profound Medical. All other authors report no financial relationships with commercial interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID

William S. H. Kim https://orcid.org/0000-0003-1257-1348
Mikaela K. Dimick https://orcid.org/0000-0001-8371-1585
Danielle Omrin https://orcid.org/0000-0002-7384-5747
Rachel H. B. Mitchell https://orcid.org/0000-0002-6361-0469
Ayal Schaffer https://orcid.org/0000-0001-6220-5042
Garfield Detzler https://orcid.org/0000-0002-0542-6524
Stephen Choi https://orcid.org/0000-0003-3662-9073
Roger S. McIntyre https://orcid.org/0000-0003-4733-2523
Bradley J. MacIntosh https://orcid.org/0000-0001-7300-2355
Beverley A. Orser https://orcid.org/0000-0001-7292-2926
Benjamin I. Goldstein https://orcid.org/0000-0003-0340-349X

REFERENCES

1. Baldessarini RJ, Vieta E, Calabrese JR, Tohen M, Bowden CL. Bipolar depression: overview and commentary. *Harvard Rev Psychiatry*. 2010;18(3):143-157. doi:10.3109/10673221003747955
2. Judd LL, Akiskal HS, Schettler PJ, et al. Psychosocial disability in the course of bipolar I and II disorders: a prospective, comparative, longitudinal study. *Arch Gen Psychiatry*. 2005;62(12):1322-1330. doi:10.1001/archpsyc.62.12.1322
3. Post RM. The impact of bipolar depression. *J Clin Psychiatry*. 2005;66(Suppl 5):5-10.
4. Butler M, Urosevic S, Desai P, et al. *Treatment for Bipolar Disorder in Adults: A Systematic Review*. Vol. 208. Agency for Healthcare Research and Quality; 2018.
5. Yatham LN, Kennedy SH, Parikh SV, et al. Canadian network for mood and anxiety treatments (CANMAT) and International Society for Bipolar Disorders (ISBD) 2018 guidelines for the management of patients with bipolar disorder. *Bipolar Disord*. 2018;20(2):97-170. doi:10.1111/bdi.12609
6. Thase ME, Macfadden W, Weisler RH, et al. Efficacy of quetiapine monotherapy in bipolar I and II depression: a double-blind, placebo-controlled study (the BOLDER II study). *J Clin Psychopharmacol*. 2006;26(6):600-609. doi:10.1097/01.jcp.0000248603.76231.b7
7. Nierenberg AA, Ostacher MJ, Calabrese JR, et al. Treatment-resistant bipolar depression: a STEP-BD equipoise randomized

effectiveness trial of antidepressant augmentation with lamotrigine, inositol, or risperidone. *Am J Psychiatry*. 2006;163(2):210-216. doi:10.1176/appi.ajp.163.2.210

8. Rosenblat JD, Carvalho AF, Li M, Lee Y, Subramanieapillai M, McIntyre RS. Oral ketamine for depression: a systematic review. *J Clin Psychiatry*. 2019;80(3). doi:10.4088/JCP.18r12475
9. Fava M, Freeman MP, Flynn M, et al. Double-blind, placebo-controlled, dose-ranging trial of intravenous ketamine as adjunctive therapy in treatment-resistant depression (TRD). *Mol Psychiatry*. 2018;25:1592-1603. doi:10.1038/s41380-018-0256-5
10. Kraus C, Wasserman D, Henter ID, Acevedo-Diaz E, Kadriu B, Zarate CA Jr. The influence of ketamine on drug discovery in depression. *Drug Discov Today*. 2019;24(10):2033-2043. doi:10.1016/j.drudis.2019.07.007
11. Nagele P, Palanca BJ, Gott B, et al. A phase 2 trial of inhaled nitrous oxide for treatment-resistant major depression. *Sci Transl Med*. 2021;13(597). doi:10.1126/scitranslmed.abe1376
12. Nagele P, Duma A, Kopec M, et al. Nitrous oxide for treatment-resistant major depression: a proof-of-concept trial. *Biol Psychiatry*. 2015;78(1):10-18. doi:10.1016/j.biopsych.2014.11.016
13. Guimarães MC, Guimarães TM, Hallak JE, Abrão J, Machado-de-Sousa JP. Nitrous oxide as an adjunctive therapy in major depressive disorder: a randomized controlled double-blind pilot trial. *Braz J Psychiatry*. 2021. doi:10.1590/1516-4446-2020-1543
14. Becker DE, Rosenberg M. Nitrous oxide and the inhalation anesthetics. *Anesth Prog Winter*. 2008;55(4):124-130; quiz 131–2. doi:10.2344/0003-3006-55.4.124
15. Onody P, Gil P, Hennequin M. Safety of inhalation of a 50% nitrous oxide/oxygen premix: a prospective survey of 35,828 administrations. *Drug Saf*. 2006;29(7):633-640. doi:10.2165/00002018-200629070-00008
16. Kalmoe MC, Janski AM, Zorumski CF, Nagele P, Palanca BJ, Conway CR. Ketamine and nitrous oxide: the evolution of NMDA receptor antagonists as antidepressant agents. *J Neurol Sci*. 2020;412:116778. doi:10.1016/j.jns.2020.116778
17. Dashdorj N, Corrie K, Napolitano A, Petersen E, Mahajan RP, Auer DP. Effects of subanesthetic dose of nitrous oxide on cerebral blood flow and metabolism: a multimodal magnetic resonance imaging study in healthy volunteers. *Anesthesiology*. 2013;118(3):577-586.
18. Deutsch G, Samra SK. Effects of nitrous oxide on global and regional cortical blood flow. *Stroke*. 1990;21(9):1293-1298.
19. Kety SS, Schmidt CF. The nitrous oxide method for the quantitative determination of cerebral blood flow IN man: theory, procedure and normal values. *J Clin Invest*. 1948;27(4):476-483. doi:10.1172/jci101994
20. Goldstein BI, Schaffer A, Wang S, Blanco C. Excessive and premature new-onset cardiovascular disease among adults with bipolar disorder in the US NESARC cohort. *J Clin Psychiatry*. 2015;76(2):163-169. doi:10.4088/JCP.14m09300
21. Goldstein BI. Bipolar disorder and the vascular system: mechanisms and new prevention opportunities. *Can J Cardiol*. 2017;33(12):1565-1576. doi:10.1016/j.cjca.2017.10.006
22. Goldstein BI, Carnethon MR, Matthews KA, et al. Major depressive disorder and bipolar disorder predispose youth to accelerated atherosclerosis and early cardiovascular disease: a scientific Statement from the American Heart Association. *Circulation*. 2015;132(10):965-986. doi:10.1161/CIR.0000000000000229
23. Crump C, Sundquist K, Winkleby MA, Sundquist J. Comorbidities and mortality in bipolar disorder: a swedish national cohort study-comorbidities and mortality in bipolar disordercomorbidities and mortality in bipolar disorder. *JAMA Psychiat*. 2013;70(9):931-939.
24. Toma S, MacIntosh BJ, Swardfager W, Goldstein BI. Cerebral blood flow in bipolar disorder: a systematic review. *J Affect Disord*. 2018;241:505-513. doi:10.1016/j.jad.2018.08.040
25. Sahib AK, Loureiro JRA, Vasavada MM, et al. Single and repeated ketamine treatment induces perfusion changes in sensory and limbic networks in major depressive disorder. *Eur Neuropsychopharmacol*. 2020;33:89-100. doi:10.1016/j.euroneuro.2020.01.017
26. Wei W, Karim HT, Lin C, et al. Trajectories in cerebral blood flow following antidepressant treatment in late-life depression: support for the vascular depression hypothesis. *J Clin Psychiatry*. 2018;79(6). doi:10.4088/JCP.18m12106
27. Insel TR. The NIMH experimental medicine initiative. *World Psychiatry*. 2015;14(2):151-153. doi:10.1002/wps.20227
28. Montgomery SA, Asberg M. A new depression scale designed to be sensitive to change. *Br J Psychiatry*. 1979;134:382-389. doi:10.1192/bjp.134.4.382
29. Alsop DC, Detre JA, Golay X, et al. Recommended implementation of arterial spin-labeled perfusion MRI for clinical applications: a consensus of the ISMRM perfusion study group and the European consortium for ASL in dementia. *Magn Reson Med*. 2015;73(1):102-116. doi:10.1002/mrm.25197
30. First MB, Spitzer RL, Gibbon M, Williams JB. *User's Guide for the Structured Clinical Interview for DSM-IV Axis I Disorders SCID-I: Clinician Version*. American Psychiatric Pub; 1997.
31. Fountoulakis KN. Refractoriness in bipolar disorder: definitions and evidence-based treatment. *CNS Neurosci Ther*. 2012;18(3):227-237. doi:10.1111/j.1755-5949.2011.00259.x
32. Young RC, Biggs JT, Ziegler VE, Meyer DA. A rating scale for mania: reliability, validity and sensitivity. *Br J Psychiatry*. 1978;133:429-435. doi:10.1192/bjp.133.5.429
33. Dimick MK, Omrin D, MacIntosh BJ, et al. Nitrous oxide as a putative novel dual-mechanism treatment for bipolar depression: proof-of-concept study design and methodology. *Contemp Clin Trials Commun*. 2020;19:100600. doi:10.1016/j.conctc.2020.100600
34. Wisniewski SR, Rush AJ, Balasubramani GK, Trivedi MH, Nierenberg AA. Self-rated global measure of the frequency, intensity, and burden of side effects. *J Psychiatr Pract*. 2006;12(2):71-79.
35. Fischl B, van der Kouwe A, Destrieux C, et al. Automatically parcellating the human cerebral cortex. *Cereb Cortex*. 2004;14(1):11-22. doi:10.1093/cercor/bhg087
36. Wilkinson ST, Farmer C, Ballard ED, et al. Impact of midazolam vs. saline on effect size estimates in controlled trials of ketamine as a rapid-acting antidepressant. *Neuropsychopharmacology*. 2019;44(7):1233-1238. doi:10.1038/s41386-019-0317-8
37. Stein DJ, Baldwin DS, Dolberg OT, Despiegel N, Bandelow B. Which factors predict placebo response in anxiety disorders and major depression? An analysis of placebo-controlled studies of escitalopram. *J Clin Psychiatry*. 2006;67(11):1741-1746. doi:10.4088/jcp.v67n1111
38. Petty F, Trivedi MH, Fulton M, Rush AJ. Benzodiazepines as antidepressants: does GABA play a role in depression? *Biol Psychiatry*. 1995;38(9):578-591. doi:10.1016/0006-3223(95)00049-7
39. Alexopoulos GS. The vascular depression hypothesis: 10 years later. *Biol Psychiatry*. 2006;60(12):1304-1305. doi:10.1016/j.biopsych.2006.09.006
40. Leaver AM, Vasavada M, Joshi SH, et al. Mechanisms of antidepressant response to electroconvulsive therapy studied with perfusion magnetic resonance imaging. *Biol Psychiatry*. 2019;85(6):466-476. doi:10.1016/j.biopsych.2018.09.021
41. Gärtner M, de Rover M, Václavů L, Scheidegger M, van Osch MJP, Grimm S. Increase in thalamic cerebral blood flow is associated with antidepressant effects of ketamine in major depressive disorder. *World J Biol Psychiatry*. 2022;1-15. doi:10.1080/15622975.2021.2020900
42. Berggren Å, Gustafson L, Höglund P, Johanson A. A long-term follow-up of clinical response and regional cerebral blood flow changes in depressed patients treated with ECT. *J Affect Disord*. 2014;167:235-243. doi:10.1016/j.jad.2014.06.005
43. Wang DJ, Chen Y, Fernández-Seara MA, Detre JA. Potentials and challenges for arterial spin labeling in pharmacological magnetic resonance imaging. *J Pharmacol Exp Ther*. 2011;337(2):359-366. doi:10.1124/jpet.110.172577

44. Kaichi Y, Okada G, Takamura M, et al. Changes in the regional cerebral blood flow detected by arterial spin labeling after 6-week escitalopram treatment for major depressive disorder. *J Affect Disord*. 2016;194:135-143. doi:10.1016/j.jad.2015.12.062
45. Joe AY, Tielmann T, Bucerius J, et al. Response-dependent differences in regional cerebral blood flow changes with citalopram in treatment of major depression. *J Nucl Med*. 2006;47(8):1319-1325.
46. Evans JW, Szczepanik J, Brutsché N, Park LT, Nugent AC, Zarate CA Jr. Default mode connectivity in major depressive disorder measured up to 10 days after ketamine administration. *Biol Psychiatry*. 2018;84(8):582-590. doi:10.1016/j.biopsych.2018.01.027
47. Sahib AK, Loureiro JR, Vasavada M, et al. Modulation of the functional connectome in major depressive disorder by ketamine therapy. *Psychol Med*. 2020;3:1-10. doi:10.1017/s0033291720004560
48. Haber SN, Knutson B. The reward circuit: linking primate anatomy and human imaging. *Neuropsychopharmacology*. 2010;35(1):4-26. doi:10.1038/npp.2009.129
49. Khalili-Mahani N, Niesters M, van Osch MJ, et al. Ketamine interactions with biomarkers of stress: a randomized placebo-controlled repeated measures resting-state fMRI and PCASL pilot study in healthy men. *Neuroimage*. 2015;108:396-409. doi:10.1016/j.neuroimage.2014.12.050
50. Loo CK, Sachdev PS, Haindl W, et al. High (15 Hz) and low (1 Hz) frequency transcranial magnetic stimulation have different acute effects on regional cerebral blood flow in depressed patients. *Psychol Med*. 2003;33(6):997-1006. doi:10.1017/s0033291703007955
51. Kito S, Fujita K, Koga Y. Regional cerebral blood flow changes after low-frequency transcranial magnetic stimulation of the right dorsolateral prefrontal cortex in treatment-resistant depression. *Neuropsychobiology*. 2008;58(1):29-36. doi:10.1159/000154477
52. Navarro V, Gastó C, Lomeña F, et al. Frontal cerebral perfusion after antidepressant drug treatment versus ECT in elderly patients with major depression: a 12-month follow-up control study. *J Clin Psychiatry*. 2004;65(5):656-661. doi:10.4088/jcp.v65n0509
53. Goldstein BI, MacIntosh BJ. The unrealized promise of cerebrovascular magnetic resonance imaging in psychiatric research across the lifespan. *Eur Neuropsychopharmacol*. 2021;55:11-13. doi:10.1016/j.euroneuro.2021.10.862

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Kim WSH, Dimick MK, Omrin D, et al. Proof-of-concept randomized controlled trial of single-session nitrous oxide treatment for refractory bipolar depression: Focus on cerebrovascular target engagement. *Bipolar Disord*. 2023;25:221-232. doi:10.1111/bdi.13288