Inhaled nitric oxide improves the hepatopulmonary syndrome: a physiologic analysis

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ABSTRACT

The hepatopulmonary syndrome (HPS) is defined by liver dysfunction, intrapulmonary vasodilatation and abnormal oxygenation. Hypoxaemia is progressive and liver transplant is the only effective treatment. Severe hypoxaemia is a life-threatening HPS complication, particularly after transplant. We evaluated gas-exchange and haemodynamic effects of invasive therapies in a consecutive sample of 26 pre-transplant patients. Inhaled nitric oxide significantly improved partial pressure of oxygen (12.4 mm Hg; p=0.001) without deleterious effects on cardiac output. Trendelenburg positioning resulted in a small improvement, and methylene blue did not, though individual responses were variable. Future studies should prospectively evaluate these strategies in severe post-transplant hypoxaemia.

INTRODUCTION

The hepatopulmonary syndrome (HPS) is defined by liver dysfunction, intrapulmonary vasodilatation and abnormal oxygenation.[1] It occurs in up to 32% of people with cirrhosis[1 2] and carries a poor prognosis.[3] Liver transplantation is the only established therapy.[1 4] However, some patients develop refractory hypoxaemia precluding transplant, and severe post-transplant hypoxaemia (requiring 100% fraction of inspired oxygen to maintain oxygen saturation ≥85%) occurs in 12% of patients, carries a 45% mortality and accounts for 68% of post-operative HPS deaths.[5] This complication is thought to be caused by a transient post-transplant exaggeration of underlying HPS pathophysiology.[5] Reports of strategies to manage severe hypoxaemia in HPS are limited to case reports and small case series.[2] We sought to formally evaluate possible strategies in a cohort of stable pre-transplant patients with HPS.

METHODS

We retrospectively analysed consecutive patients from the Canadian HPS Programme (Toronto, Ontario) with moderate to very severe HPS (PaO_2 <70 mm Hg with $AaDO_2$ ≥20 mm Hg)[1] who had physiologic testing between November 2013 and June 2018. The study was approved by St. Michael's Hospital's Research Ethics Board (Toronto).

We inserted an indwelling radial artery catheter and a pulmonary artery catheter and serially evaluated effects of position change, inhaled nitric oxide (iNO), methylene blue (MB) and combinations thereof (figure 1). We waited a minimum of 10 min after each position change and 20 min after any change in FiO_2 and/or administration of iNO before starting measurements. We measured MB effects hourly after completion of the infusion, up to its reported peak effect at 5 hours (used as the main time point for MB effect reporting).[6] Our primary outcome was PaO_2. We also calculated the $AaDO_2$, pulmonary vascular resistance (PVR) and systemic vascular resistance (SVR), and assessed changes in these variables, and in mean systemic arterial pressure, mean pulmonary artery pressure (MPAP), pulmonary capillary wedge pressure (PCWP) and cardiac output (CO).

Additional methodological details are provided in the online supplemental information.

RESULTS

We included 26 participants (15 (58%) female, mean (SD) age 54.6 (10.5) years, PaO_2 46.2 (9.6) mm Hg, see online supplemental table 1 for patient characteristics).

All patients received iNO and 18 patients also received MB. Physiologic measurements at baseline and with each intervention are reported in table 1. Changes in key measurements (oxygenation and CO), and the proportion of patients exhibiting PaO_2 improvements of at least 10% and 20%[2] are shown in table 2 (also see online supplemental figure 1). Significantly higher PaO_2 was seen in the supine compared with the seated position, in the Trendelenburg compared with the supine position, and with iNO (table 2). Addition of MB to iNO did not produce a significant incremental benefit (table 2). There was no significant improvement in PaO_2 5 hours after MB administration (similar results were seen at intervening time points—online supplemental table 2). CO increased in the supine position (as expected due to increased venous return), was unchanged with iNO and increased with MB administered alone, in combination with iNO (compared with no intervention) and when added to iNO (compared with iNO alone) (table 2).

Changes in SVR, PVR and MPAP with each intervention are reported in online supplemental table 3. We found no relationships between baseline characteristics (age, sex, time since HPS diagnosis, PaO_2 (standing), orthodeoxia value, macroaggregated albumin (MAA) shunt fraction or non-invasive shunt fraction) and PaO_2 response to each intervention (online supplemental tables 4, 5).

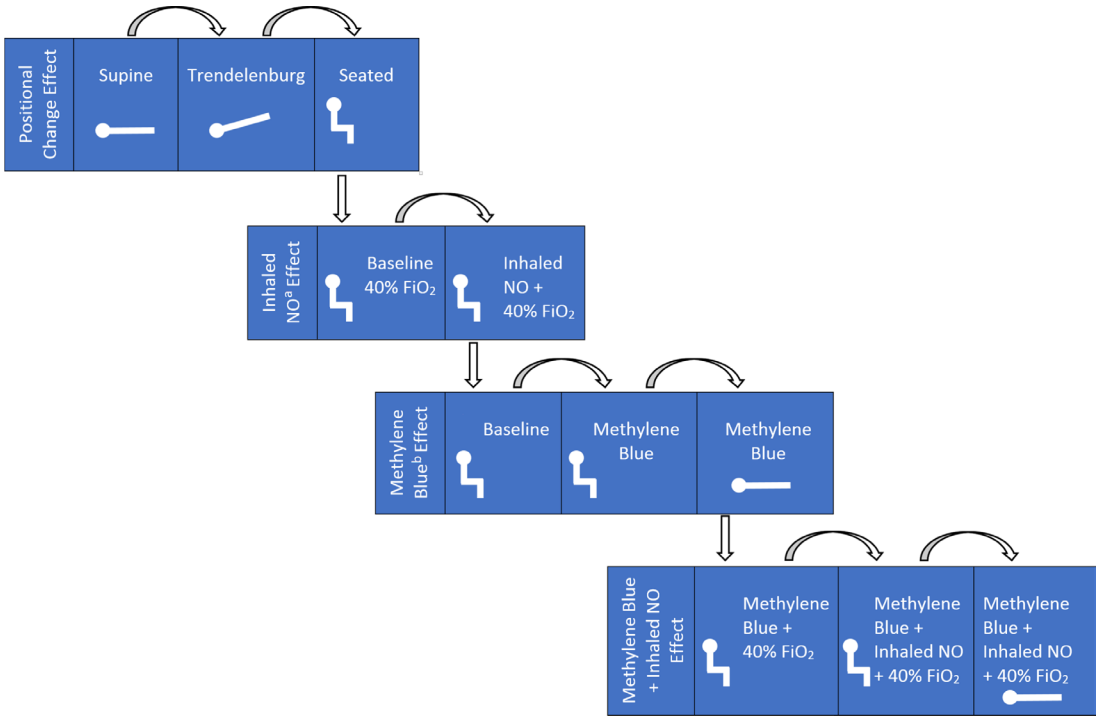

Figure 1 Intervention protocol. Each intervention is shown in order of occurrence. Stick figures represent patient position. Tests were performed on room air unless otherwise specified. [a]Inhaled NO administered at 20 parts per million. [b]Methylene blue effects measured hourly while seated up to 5 hours postintravenous infusion. FiO_2, fraction of inspired oxygen; NO, nitric oxide.

DISCUSSION

In this physiologic study of 26 patients with moderate to very severe HPS, supine positioning, Trendelenburg positioning and iNO significantly improved oxygenation without negative impacts on CO. To our knowledge, this is the largest report of any of these interventions.

The PaO_2 was 3.3 mm Hg higher in the supine compared with the seated position, and 0.9 mm Hg higher in the Trendelenburg position compared with the supine position (table 2). These position changes are theorised to work through diversion of pulmonary blood flow away from pathologically dilated pulmonary vessels at lung bases, reducing shunt and V/Q mismatch.[2 7] Although improvements in PaO_2 were small, it is more relevant to consider the potential impact on tissue oxygen delivery (DO_2). DO_2 is proportional to oxygen saturation, which is dependent on PaO_2 through the sigmoidal oxyhaemoglobin dissociation curve. Patients with severe post-transplant hypoxaemia have saturations of 80%–85% or lower,[5] corresponding to a baseline PaO_2 ≤45 mm Hg. On this steep portion of the oxyhaemoglobin dissociation curve, even small positional PaO_2 improvements could result in clinically significant improvements in saturation and DO_2. These benefits would need to be weighed against the risk of aspiration and ventilator-associated pneumonia. In routine care, Trendelenburg positioning might also be considered in an HPS exercise programme and/or as a sleep positional strategy to reduce overnight oxygen requirements.

Inhaled NO was the most effective intervention, demonstrating a mean PaO_2 improvement of 12.4 mm Hg and responses of ≥10% in over half of patients (table 2). Given that basilar lung vessels may already be maximally dilated,[8] vasodilatory effects of iNO are thought to be more effective in mid and upper lung units, whereby flow is redistributed away from basilar lung units, improving V/Q matching and oxygenation. The same mechanism likely explains observed benefits of garlic in HPS, the active compound of which (allicin) is a potent vasodilator.[9] Unfortunately, we were not able to formally assess regional changes in lung perfusion in order to prove this theory. Overall, our findings suggest that a trial of either iNO or a comparable inhaled vasodilator for severe post-transplant hypoxaemia[2] (and/or for intraoperative hypoxaemia or as a bridge to transplant) would be reasonable. Ambulatory iNO, longer-acting inhaled vasodilators such as inhaled iloprost,[10] and systemic vasodilators might also be evaluated as chronic medical therapies for HPS.

MB infusion did not produce changes in PaO_2 (table 2). As a potent vasoconstrictor, MB is thought to act by directly vasoconstricting dilated vessels at lung bases to improve V/Q matching, and by restoring impaired hypoxic vasoconstriction in poorly ventilated areas.[2] There was a significant increase in MPAP but no significant increase in PVR with MB (table 1, online supplemental table 3). In a previous study of seven subjects, Schenk *et al*[6] reported improvement in oxygenation from 58 mm Hg to 74 mm Hg with MB.[6] Divergence in our results may be attributable to our more severe disease population (baseline PaO_2 46 mm Hg), which may have been less MB responsive due to end-stage vascular remodelling with resulting vasoplegia of dilated HPS vessels.[11] This is supported by Schenk, *et al*'s reported significant PVR increase with MB,[6] compared with a non-significant change in our cohort. Furthermore, this group reported a significant correlation between improvement in oxygenation and increase in PVR.[6] However, both Schenk, *et al*'s report and our own demonstrate variable PaO_2 responses, likely reflective of a heterogeneous population, with some but not all patients having preserved

Table 1 Physiologic measurements at baseline and with each intervention

	Room air							
		Position change						
	Baseline (upright)* n=26	Supine n=26	P value	Trendelenburg n=21	P value	Baseline (upright)* n=18	MB† (upright) n=18	P value
Oxygenation								
PaO_2 (mm Hg)	48.9 (9.6)‡	52.2 (9.0)‡	0.003	52.4 (9.5)	0.002	49.9 (9.1)	51.0 (9.9)	0.34
$AaDO_2$ (mm Hg)	67.4 (31.6)‡	62.3 (27.9)‡	<0.0001	56.7 (8.3)	<0.0001	62.5 (10.6)	61.7 (12.4)	0.62
Pulmonary haemodynamics§								
CO (L/min)	7.6 (2.1)	8.5 (2.5)	0.0008	8.4 (2.2)	0.005	7.6 (2.3)	8.5 (2.5)	0.01
MPAP (mm Hg)	14.6 (6.5)	17.8 (5.5)	0.02	20.0 (7.0)	0.001	13.6 (5.2)	17.6 (6.9)	0.0002
PCWP¶ (mm Hg)	7.7 (5.4)	10.5 (4.4)	0.01	12.6 (5.1)	0.001	7.5 (5.1)	10.1 (5.6)	0.002
PVR¶ (dyn·s·cm^{-5})	77.6 (48.0)	76.4 (43.4)	0.88	73.9 (35.5)	0.99	68.5 (42.2)	74.0 (47.5)	0.49
Systemic haemodynamics§								
CVP (mm Hg)	6.8 (4.3)	7.1 (3.3)	0.92	8.8 (4.3)	0.14	7.0 (4.0)	8.3 (4.3)	0.01
MAP (mm Hg)	92.1 (12.4)	81.7 (13.5)	0.0002	79.0 (12.3)	<0.0001	93.9 (12.6)	101.9 (13.8)	0.007
SVR (dyn·s·cm^{-5})	977.9 (347.7)	785.9 (344.0)	<0.0001	718.9 (225.8)	<0.0001	999.5 (352.2)	967.2 (332.4)	0.31
	40% FiO_2 Baseline (upright)* n=26	Inhaled NO (upright) n=26		P value	Inhaled NO + MB† (upright) n=18			P value
Oxygenation								
PaO_2 (mm Hg)	85.8 (28.8)	98.2 (39.3)		0.0006	103.0 (30.9)			0.001
$AaDO_2$ (mm Hg)	157.8 (29.6)	145.2 (40.1)		0.0009	140.7 (33.6)			0.0005
Pulmonary haemodynamics§								
CO (L/min)	7.0 (1.9)	6.9 (1.9)		0.71	8.1 (2.6)			0.04
MPAP (mm Hg)	15.4 (5.5)	14.6 (5.6)		0.23	17.3 (5.6)			0.0004
PCWP¶ (mm Hg)	7.8 (4.9)	8.3 (4.9)		0.30	9.8 (5.5)			0.03
PVR¶ (dyn·s·cm^{-5})	103.3 (91.0)	76.9 (43.9)		0.12	81.1 (57.3)			0.41
Systemic haemodynamics§								
CVP (mm Hg)	5.9 (3.9)	5.7 (3.8)		0.65	8.3 (5.3)			0.02
MAP (mm Hg)	93.5 (15.9)	97.5 (11.5)		0.07	107.4 (14.4)			0.001
SVR (dyn·s·cm^{-5})	1093.8 (413.5)	1135.0 (334.2)		0.22	1101.4 (450.1)			0.59

Values reported as mean (SD) and p values calculated using paired t-tests for each intervention; note that some interventions were tested in a smaller number of patients than the baseline comparator; in these cases, only data from patients providing both baseline and intervention data could be compared statistically.
*Although the procedure was started in the supine position, the upright (seated) position is taken as the baseline for each comparison in this table.
†Measured at least 5 hours after MB infusion.
‡Includes one patient on 40% FiO_2 (could not tolerate room air); baseline upright, supine and inhaled NO data points were collected on 40% FiO_2 in this patient; the patient did not undergo Trendelenburg position nor MB testing.
§Two patients did not contribute to haemodynamic data because the pulmonary artery catheter could not be inserted due to recent central venous thrombosis (n=1); and the pulmonary artery catheter malfunctioned (n=1).
¶One additional patient did not contribute pulmonary capillary wedge pressure or PVR data in the Trendelenburg position due to transient catheter malfunction; in an additional two patients, reliable PCWP was not attainable, and we used the diastolic pulmonary artery pressure (PAD) to approximate the PCWP across interventions.
$AaDO_2$, alveolar–arterial oxygen gradient; CO, cardiac output; CVP, central venous pressure; FiO_2, fraction of inhaled oxygen; MAP, mean arterial pressure; MB, methylene blue; MPAP, mean pulmonary artery pressure; NO, nitric oxide; PaO_2, arterial partial pressure of oxygen; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; SVR, systemic vascular resistance.

vasoconstrictor responses. Accordingly, a trial of MB may still be merited in severe post-transplant hypoxaemia, as long as impact on CO (and thus DO_2) is monitored. We also note that adding MB to iNO did not significantly improve PaO_2 compared iNO alone. However, responses were again variable and there was a 13% increase in CO compared with iNO alone, suggesting a DO_2 benefit. Accordingly, we believe that a trial of combined inhaled vasodilator and MB may be merited in severe situations.

Our study has several limitations. The iNO was delivered with FiO_2 of 0.4 as opposed to room air. However, this more closely approximates the real-world scenario in which this salvage therapy would be used, and effects of all interventions were determined based on comparison to a baseline on the same FiO_2. Although much larger than any prior reports, our sample size remains small and may have been underpowered to detect a small MB effect. We did not pursue molecular biomarker testing to confirm or refute the proposed mechanisms of action of reported agents. We also have not prospectively evaluated the effectiveness nor sustainability of these strategies in the post-transplant context.

In summary, we demonstrated that iNO significantly improves oxygenation in severe HPS, without deleterious effects on CO. Although more data are needed, it may be

Table 2 Change in PaO_2 and CO, by intervention

Intervention	N	Change in PaO_2 (mm Hg)	Change in PaO_2 (%)	P value	N (%) with ≥10% PaO_2 increase	N (%) with ≥20% PaO_2 increase	Change in CO (L/min)	Change in CO (%)	P value
Positional effects									
Supine vs seated (orthodeoxia)	26	3.3 (1.3, 5.3)	8 (2.8, 13.1)	0.003	9 (35)	3 (12)	0.8 (0.4, 1.2)	11 (5.5, 16.4)	0.0008
Trendelenburg vs supine	21	0.9 (0.4, 1.5)	2 (0.6, 2.9)	0.02	0	0	0.1 (–0.2, 0.4)	2 (–2.0, 5.0)	0.56
MB									
MB* (seated)	18	1.1 (–1.1, 3.2)	2 (–2.4, 7.1)	0.34	3 (17)†	1 (6)	0.8 (0.3, 1.4)	12 (5.7, 18.5)	0.01
MB* (supine)	18	2.3 (0.1, 4.6)	5 (0.5, 9.3)	0.06	6 (33)†	1 (6)	0.6 (–0.1, 1.4)	8 (–0.4, 17.2)	0.13
Inhaled NO									
Inhaled NO (seated)	26	12.4 (6.2, 18.6)	13 (8.0, 18.7)	0.0006	14 (54)	6 (23)	0.0 (–0.3, 0.2)	0 (–3.6, 3.6)	0.71
Inhaled NO + MB									
Inhaled NO + MB* vs Inhaled NO alone (seated)	18	0.8 (–11.5, 13.1)	6 (–3.8, 15.8)	0.90	7 (39)‡	5 (28)	0.9 (0.2, 1.6)	13 (3.7, 21.4)	0.03

Values reported as mean (95% CI) and p values calculated using paired t-tests for each value.
All comparisons are to no intervention, in the same position, on the same fraction of inspired oxygen, unless stated otherwise.
*Measured 5 hours after MB infusion.
†One patient had a ≥10% drop in PaO_2 with each of the following interventions: 17% drop in PaO_2 with MB (seated), 11% drop in PaO_2 with MB (supine), 13% drop in PaO_2 with inhaled NO + MB (seated) (a different patient experienced each of these drops across interventions).
‡Four patients had a ≥10% drop in PaO_2, as follows: drop in PaO_2 of 10%, 13%, 33%, 36%.
CO, cardiac output; MB, methylene blue; NO, nitric oxide; PaO_2, arterial partial pressure of oxygen.

considered early in the management of severe hypoxaemia, whether as a bridge to transplant, during transplant or after. Longer-acting vasodilators require study as possible maintenance therapy for HPS. Small but significant effects were seen with Trendelenburg positioning, but not with MB. However, observed individual variability in effects suggests that pre-transplant testing of all agents in high-risk patients may be helpful in guiding post-transplant management in the event of severe hypoxaemia. Future studies are required to prospectively evaluate the effect of iNO in patients with severe post-transplant hypoxaemia and measure correlations between pre-transplant and post-transplant responses.

Contributors SG and AA-H designed the study and acquired the data. SG and RT analysed the data and drafted the work. All authors revised the work for important intellectual content and approved the final version.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The study was approved by St. Michael's Hospital's Research Ethics Board (Toronto).

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REFERENCES

1 Krowka MJ, Fallon MB, Kawut SM, *et al*. International liver transplant Society practice guidelines: diagnosis and management of hepatopulmonary syndrome and Portopulmonary hypertension. *Transplantation* 2016;100:1440–52.
2 Nayyar D, Man HSJ, Granton J, *et al*. Proposed management algorithm for severe hypoxemia after liver transplantation in the hepatopulmonary syndrome. *Am J Transplant* 2015;15:903–13.
3 Fallon MB, Krowka MJ, Brown RS, *et al*. Impact of hepatopulmonary syndrome on quality of life and survival in liver transplant candidates. *Gastroenterology* 2008;135:1168–75.
4 Iyer VN, Swanson KL, Cartin-Ceba R, *et al*. Hepatopulmonary syndrome: favorable outcomes in the MELD exception era. *Hepatology* 2013;57:2427–35.
5 Nayyar D, Man HSJ, Granton J, *et al*. Defining and characterizing severe hypoxemia after liver transplantation in hepatopulmonary syndrome. *Liver Transpl* 2014;20:182–90.
6 Schenk P, Madl C, Rezaie-Majd S, *et al*. Methylene blue improves the hepatopulmonary syndrome. *Ann Intern Med* 2000;133:701–6.
7 Gómez FP, Martínez-Pallí G, Barberà JA, *et al*. Gas exchange mechanism of orthodeoxia in hepatopulmonary syndrome. *Hepatology* 2004;40:660–6.
8 Fallon MB. Hepatopulmonary syndrome: more than just a matter of tone? *Hepatology* 2006;43:912–4.
9 Abrams GA, Fallon MB. Treatment of hepatopulmonary syndrome with Allium sativum L. (garlic): a pilot trial. *J Clin Gastroenterol* 1998;27:232–5.
10 Krug S, Seyfarth H-J, Hagendorff A, *et al*. Inhaled iloprost for hepatopulmonary syndrome: improvement of hypoxemia. *Eur J Gastroenterol Hepatol* 2007;19:1140–3.
11 Hughes JMB. The hepatopulmonary syndrome: no way out? *Eur Respir J* 2005;25:211–2.

Supplementary Information

Supplementary Methods

Study Population

We included consecutive patients from the Canadian HPS Program (Toronto, Ontario) with moderate to very severe HPS, defined as: liver disease; evidence of intrapulmonary vascular dilatation on contrast echocardiography (microbubbles appearing in the left heart ≥ 3 cardiac cycles after appearing in the right heart, following injection of 10 mL of agitated saline in a peripheral arm vein); and standing room air PaO_2 < 70 mmHg [with alveolar-arterial oxygen gradient ($AaDO_2$) ≥ 20 mmHg],[1] who had physiologic testing as per a pre-defined clinical protocol between November 2013 to June 2018. The study was approved by St. Michael's Hospital's Research Ethics Board (Toronto) prior to data collection (all patients consented to have their data included in a database for analysis).

Interventions

Each patient was electively admitted to the cardiac intensive care unit at St. Michael's Hospital (Toronto). We inserted an indwelling radial artery catheter (Becton Dickinson, Franklin Lakes, USA) to measure repeated arterial blood gases (ABGs) and mean systemic arterial pressure (MAP), and an internal jugular venous Cordis sheath through which a balloon-tipped pulmonary artery catheter (Edwards Lifesciences, Irvine, USA) was floated to measure haemodynamics. Placement of the pulmonary artery catheter was confirmed by chest radiograph.

We conducted the following series of interventions in order to measure the effects position change, inhaled nitric oxide (iNO), methylene blue (MB), and combinations thereof on oxygenation and haemodynamics (Figure 1):

1. Supine position (room air)
2. Trendelenburg position (-20°) (room air)
3. Seated position (room air)
4. Seated position (40% FiO_2)
5. Seated position [40% FiO_2 + 20 parts per million (ppm) iNO]
6. Seated position (room air)
7. Seated position (room air + MB) (at one, two, three, four, and five hours after MB infusion)*
8. Supine position (room air + MB)
9. Seated position (40% FiO_2 + MB)
10. Seated position (40% FiO_2 + MB + 20 ppm iNO)
11. Supine position (40% FiO_2 + MB + 20 ppm iNO)

* All patients (n=26) received iNO. Eight patients did not receive MB due to current use of SSRIs (n=4), TCAs (n=1), or both an SSRI and a TCA (n=1); and inability to tolerate full day testing (n=2), leaving 18 patients for MB administration.

After each intervention, we drew an ABG, measured MAP through the radial arterial catheter, and measured the following variables through the pulmonary artery catheter: central venous pressure (CVP), mean pulmonary artery pressure (MPAP), pulmonary capillary wedge pressure (PCWP), and cardiac output (CO) (measured by thermodilution, using triplicate values within ≤ 10% of one another). After each position change, we ensured that both pulmonary artery catheter and arterial line transducers were maintained at the phlebostatic axis. For all seated measurements, participants sat at the edge of

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the bed with their legs over the side of the bed. We waited a minimum of ten minutes after each position change and twenty minutes after any change in FiO_2 and/or administration of iNO before starting measurements. Nitric oxide was delivered with oxygen (FiO_2 at 40%) through the INOmax DSIR (IKARIA Inc., Seattle, USA). We maintained FiO_2 at 40% with use of an oxygen blender, adjusted in accordance with readings from a continuous sampling line near the participant mask. To isolate the effect of iNO, we placed participants on oxygen at 40% FiO_2, with 0 ppm iNO through the INOmax DSIR, measured all variables, then added iNO at 20 ppm to the same circuit and re-measured. MB was infused at a dose of 3mg/kg over 15 minutes, and we measured MB effects hourly after completion of the infusion, up to five hours, which was previously reported to be the peak MB effect time in HPS (and thus set a priori as the main time point for MB effect reporting).[2] All other interventions after MB infusion (interventions eight to eleven above) were completed serially after the five-hour post-infusion mark. Given the risk of serotonin toxicity, participants using selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors (MAOIs) or tricyclic antidepressants (TCAs) were not given MB, and completed interventions one to five only.

Outcomes

Our primary outcome was PaO_2 from ABG analysis. We also calculated the $AaDO_2$, using the following equation:

$$AaDO_2 = [F_iO_2(P_{atm} - P_{H2O}) - (PaCO_2/0.8)] - P_aO_2$$

Where $AaDO_2$ represents the alveolar-arterial oxygen gradient; FiO_2 represents the fraction of inspired oxygen; P_{atm} represents the atmospheric pressure (760 mmHg); P_{H2O} represents the partial pressure of water vapour (47 mmHg); $PaCO_2$ represents the arterial partial pressure of CO_2; and PaO_2 represents the arterial partial pressure of O_2.

We also assessed changes in CO, MAP, MPAP, PCWP, pulmonary vascular resistance (PVR), and systemic vascular resistance (SVR), calculated as follows:

$$PVR = [80 \times (MPAP - PCWP)]/CO$$

$$SVR = [80 \times (MAP - CVP)]/CO$$

Where MPAP represents mean pulmonary artery pressure; PCWP represents pulmonary capillary wedge pressure; MAP represents mean arterial pressure; and CO represents cardiac output.

Statistical Analyses

We provide descriptive statistics (means with standard deviations and/or proportions) for baseline patient characteristics, and oxygenation (PaO_2, $AaDO_2$) and haemodynamic variables with each intervention. We used paired t-tests to compare the mean baseline value to the mean value following each intervention, for PaO_2 and key haemodynamic variables. In an exploratory analysis, we measured Pearson's correlation coefficient for change in PaO_2 with each intervention and the following baseline characteristics: age; time since HPS diagnosis; standing PaO_2; orthodeoxia value; macroaggregated albumin shunt fraction (MAA – a nuclear study used to estimate anatomic shunt, measured with Abrams' technique, which exclusively compares brain and lung technetium counts to estimate shunt as opposed to total body counts which can be influenced by decreases in renal blood flow caused by cirrhosis);[3] and non-invasive shunt fraction (calculated based on an ABG on 100% FiO_2). We used two-sample t-tests to compare change in PaO_2 with each intervention between the following clinically

relevant categories: male vs female; baseline (standing) PaO_2 > 50 mmHg vs ≤ 50 mmHg;[4] baseline orthodeoxia > 4 mmHg vs ≤ 4 mmHg;[1,5] and MAA shunt fraction ≥20% vs < 20%.[4,6] All analyses were performed using Stata/MP 15 (StataCorp LLC, College Station, USA); a p-value of < 0.05 was considered significant.

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Supplementary Tables

Supplementary Table 1. Characteristics of Study Participants

Participant	Etiology of Liver Disease	Child-Pugh Score	MELD Score	Standing PaO_2 (mmHg)	Standing $AaDO_2$ (mmHg)	Orthodeoxia[a] (mmHg)	MAA Shunt[d] (%)	Oximetric Shunt[b] (%)	DLCO[c] (% predicted)
1	Alcohol	B7	11	48	66	1	13	18	72
2	Autoimmune hepatitis	B8	12	53	66	5	1	12	25
3	Alcohol	B9	10	53	60	12	15	7	62
4	NASH	C10	18	44	70	21	25	11	55
5	Hepatitis C	C10	12	64	44	9	8	6	50
6	NASH	B7	9	47	57	22	17	26	50
7	Hepatitis C	B7	15	40	73	17	27	25	50
8	Hepatitis C	B7	9	39	75	13	31	8	27
9	Cryptogenic cirrhosis	B8	17	33	88	30	30	27	29
10	NASH	A6	11	38	85	15	18	21	52
11	Hepatitis C/ alcohol	A6	8	52	58	2	18	21	52
12	NASH/ alcohol	B9	17	54	66	24	11	15	54
13	Hepatitis C/ alcohol	C10	20	56	58	15	15	13	54
14	NASH	B8	13	48	68	10	14	10	57
15	Hepatitis C	A6	9	59	25	4	19	15	53
16	Hepatitis C	A6	10	35	79	6	44	10	40
17	NASH	B7	10	62	47	7	19	5	61
18	NASH	B8	22	58	41	-2	NA	9	NA
19	Alcohol	A5	13	35	83	11	51	18	61
20	Sickle cell hepatopathy	B9	15	45	70	16	24	12	47
21	NASH	C10	15	38	70	11	35	30	63

22	Autoimmune hepatitis	B7	11	29		87	NA	54	NA	51	
23	Cryptogenic cirrhosis	A5	8	47		57	NA	25	NA	75	
24	Alcohol	B7	16	48		68	22	13	15	56	
25	NASH	B8	14	41		70	9	38	17	40	
26	Portal vein thrombosis	B7	9	34		77	2	77	19	NA	
Mean (SD) or Proportion	NASH 31% Hepatitis C 19% Alcohol 15% Autoimmune Hepatitis 8% Other 27%	A 23% B 62% C 15%	12.8 (3.8)	46.2	(9.6)	65.6 (14.9)	11.8 (8.1)	26 (17)	15 (7)	51	(13)

Age and sex were omitted from the table to protect patient identities

[a] Measured as the difference in PaO_2 between supine and standing position; 19/24 (79%) fulfilled published criteria for orthodeoxia (> 4 mmHg PaO_2 drop with supine to upright position change)[1]

[b] Calculated by comparing arterial blood gas results on room air versus 100% FiO_2 (normal value ≤ 6%)

[c] Values were adjusted for haemoglobin, where available

[d] Normal value ≤ 6%

CP denotes Child-Pugh; MELD denotes model of end-stage liver disease; PaO_2 denotes arterial partial pressure of O_2; MAA denotes macroaggregated albumin; FiO_2 denotes fraction of inspired O_2; DLCO denotes diffusing capacity of the lung for carbon monoxide; COPD denotes chronic obstructive pulmonary disease; NASH denotes non-alcoholic steatohepatitis; ILD denotes interstitial lung disease

Supplementary Table 2. Hourly Changes in Oxygenation Following Methylene Blue Administration (n=18)

Time after Methylene Blue Infusion	Change in PaO_2 (mmHg)	Change in PaO_2 (%)	p-value	N (%) with ≥10% improvement in PaO_2	N (%) with ≥20% improvement in PaO_2
1 hour (Seated)	0.2 (3.0)	0 (6)	0.76	0	0
2 hours (Seated)	0.4 (3.7)	1 (8)	0.66	2 (11)	0
3 hours (Seated)	1.4 (3.5)	3 (7)	0.11	4 (22)	0
4 hours (Seated)	1.2 (3.8)	3 (8)	0.19	5 (28)	0
5 hours (Seated)	1.1 (4.6)	2 (10)	0.34	3 (17)	1 (6)
5 hours (Supine)	2.3 (4.8)	5 (10)	0.06	6 (33)	1 (6)

Values reported as mean (SD)

All comparisons are to no intervention, in the same position, on the same FiO_2

PaO_2 denotes arterial partial pressure of oxygen

Supplementary Table 3. Changes in Key Haemodynamic Variables, by Intervention

Intervention	N	Systemic Vascular Resistance Change (dyn·sec·cm^{-5})	Change (%)	p-value	Pulmonary Vascular Resistance Change (dyn·sec·cm^{-5})	Change (%)	p-value	Mean Pulmonary Artery Pressure Change (mmHg)	Change (%)	p-value
Supine vs Seated (Orthodeoxia)	24	-192.0 (156.6)	-20 (15)	<0.001	-1.2 (40.3)	13 (57)	0.88	3.2 (6.2)	55 (102)	0.02
Trendelenburg vs Supine	21	-30.3 (86.8)	-3 (14)	0.13	8.1 (22.1)	21 (54)	0.12	2.7 (3.5)	15 (17)	0.002
Methylene Blue[a] (Seated)	18	-32.3 (131.0)	-3 (12)	0.31	5.1 (29.7)	21 (72)	0.49	3.9 (3.5)	28 (31)	<0.001
Methylene Blue[a] (Supine)	18	33.0 (236.2)	7 (33)	0.56	13.1 (47.4)	78 (299)	0.26	2.7 (4.9)	21 (34)	0.03
Inhaled NO (Seated)	24	41.3 (159.4)	9 (26)	0.22	-26.4 (79.8)	-8 (49)	0.12	-0.8 (3.3)	-4 (26)	0.23
Inhaled NO + Methylene Blue[a] (Seated)	18	31.4 (242.2)	8 (35)	0.59	-7.8 (39.3)	12 (67)	0.41	2.5 (2.4)	18 (24)	<0.001
Inhaled NO + Methylene Blue[a] vs Inhaled NO Alone (Seated)	18	-9.4 (245.1)	-1 (23)	0.87	15.9 (57.1)	53 (102)	0.25	3.6 (3.5)	29 (29)	<0.001

Values reported as mean (SD)

All comparisons are to no intervention, in the same position, on the same FiO_2, unless stated otherwise

[a] Measured five hrs after methylene blue infusion

NO denotes nitric oxide

Supplementary Table 4. Pearson's Correlations Between Baseline Patient Characteristics and Change in PaO_2, by Intervention

	Correlation (R) with Change in PaO_2, by Intervention (95% Confidence Interval)					
	Trendelenburg vs Supine	Supine vs Upright (Orthodeoxia)	iNO (Seated)	Methylene Blue (Seated)	Methylene Blue (Supine)	iNO + Methylene Blue (Seated)
Age (years)	-0.13 (-0.53, 0.32) p=0.56	-0.02 (-0.40, 0.37) p=0.91	-0.04 (-0.42, 0.35) p=0.85	-0.03 (-0.49, 0.44) p=0.91	-0.19 (-0.60, 0.30) p=0.45	0.14 (-0.35, 0.57) p=0.57
Time since HPS Diagnosis (years)	-0.21 (-0.59, 0.24) p=0.36	0.25 (-0.15, 0.58) p=0.24	-0.24 (-0.57, 0.16) p=0.23	0.19 (-0.30, 0.60) p=0.44	-0.14 (-0.67, 0.35) p=0.58	0.19 (-0.30, 0.60) p=0.46
PaO_2 (standing, mmHg)	0.07 (-0.37, 0.49) p=0.77	-0.29 (-0.61, 0.11) p=0.16	-0.06 (-0.44, 0.34) p=0.78	0.11 (-0.38, 0.55) p=0.68	-0.35 (-0.70, 0.14) p=0.15	-0.11 (-0.55, 0.38) p=0.68
Orthodeoxia (mmHg)	-0.31 (-0.67, 0.17) p=0.19	NA	-0.05 (-0.44, 0.36) p=0.80	0.21 (-0.30, 0.63) p=0.41	0.12 (-0.38, 0.57) p=0.64	-0.11 (-0.56, 0.39) p=0.68
^{99m}Tc-MAA (%)	0.25 (-0.22, 0.62) p=0.30	0.02 (-0.38, 0.41) p=0.92	-0.05 (-0.44, 0.35) p=0.82	-0.31 (-0.69, 0.20) p=0.23	0.36 (-0.15, 0.72) p=0.16	-0.00 (-0.48, 0.48) p=1.00
Non-invasive shunt fraction (%)	0.01 (-0.45, 0.46) p=0.96	0.13 (-0.29, 0.51) p=0.54	-0.27 (-0.61, 0.15) p=0.21	0.31 (-0.20, 0.69) p=0.23	0.10 (-0.40, 0.55) p=0.71	0.06 (-0.43, 0.53) p=0.81

All comparisons are to no intervention, in the same position, on the same fraction of inspired oxygen, unless stated otherwise

Effect of methylene blue interventions (both seated and supine) were measured at five hours after intravenous administration

PaO_2 denotes arterial partial pressure of oxygen, ^{99m}Tc-MAA denotes ^{99m}Tc macroaggregated albumin, iNO denotes inhaled nitric oxide

Supplementary Table 5. Differences in PaO_2 Response to Each Intervention, by Baseline Patient Characteristics

	Mean Change in PaO_2 by Category (mmHg), by Intervention (95% confidence Interval and p-value[a] for difference in change)					
	Trendelenburg vs Supine	Supine vs Seated (Orthodeoxia)	iNO Seated	Methylene Blue Seated	Methylene Blue Supine	iNO + Methylene Blue Seated
Sex M (n=11) F (n=15)	M 1.0, F 2.4 Difference: 1.4 (-3.0, 5.8) p=0.25	M 3.7, F 1.9 Difference: -1.8 (-7.8, 4.2) p=0.27	M 18.2, F 9.8 Difference: -8.4 (-28.2, 11.4) p=0.13	M 3.9, F 0.7 Difference: -3.2 (-22.6, 16.2) p=0.53	M 6.6, F 3.2 Difference: -3.4 (-21.1, 14.3) p=0.47	M 20.0, F 18.2 Difference: -1.8 (-42.4, 38.8) p=0.86
PaO_2 while standing >50mmHg[1] Yes (n= 9) No (n=17)	No 1.4, Yes 2.4 Difference: 1.0 (-3.98, 5.98) p=0.45	No 3.1, Yes 2.0 Difference: -0.9 (-6.64, 4.84) p=0.55	No 13.2, Yes 13.6 Difference: 0.4 (-21.0, 21.8) p=0.94	No 2.4, Yes 2.0 Difference: -0.4 (-21.8, 21.0) p=0.94	No 7.0, Yes -0.5 Difference: -7.5 (-25.6, 10.6) p=0.14	No 22.5, Yes 10.2 Difference: -12.3 (-50.4, 25.8) p=0.24
Known Orthodeoxia (>4mmHg)[1] Yes (n=19) No (n= 5)	No 3.1, Yes 1.5 Difference: -1.6 (-8.5, 5.3) p=0.39	NA	No 15.6, Yes 14.3 Difference: -1.3 (-30.6, 28.0) p=0.86	No -3.6, Yes 4.4 Difference: 8.0 (-16.2, 32.2) p=0.23	No -0.4, Yes 6.5 Difference: 6.9 (-16.5, 30.3) p=0.28	No 25.0, Yes 19.5 Difference: -5.5 (-55.5, 44.5) p=0.67
^{99m}Tc-MAA (≥20%)[4] Yes (n=12) No (n=13)	No 0.8, Yes 2.5 Difference: 1.7 (-2.9, 6.3) p=0.18	No 2.7, Yes 2.8 Difference: 0.1 (-5.2, 5.4) p=0.94	No 11.6, Yes 16.1 Difference: 4.5 (-17.4, 26.4) p=0.44	No 3.2, Yes 2.0 Difference: -0.8 (-14.7, 13.1) p=0.82	No 0.2, Yes 8.1 Difference: 7.9 (-8.5, 24.3) p=0.09	No 19.4, Yes 19.7 Difference: 0.3 (-32.0, 32.6) p=0.97

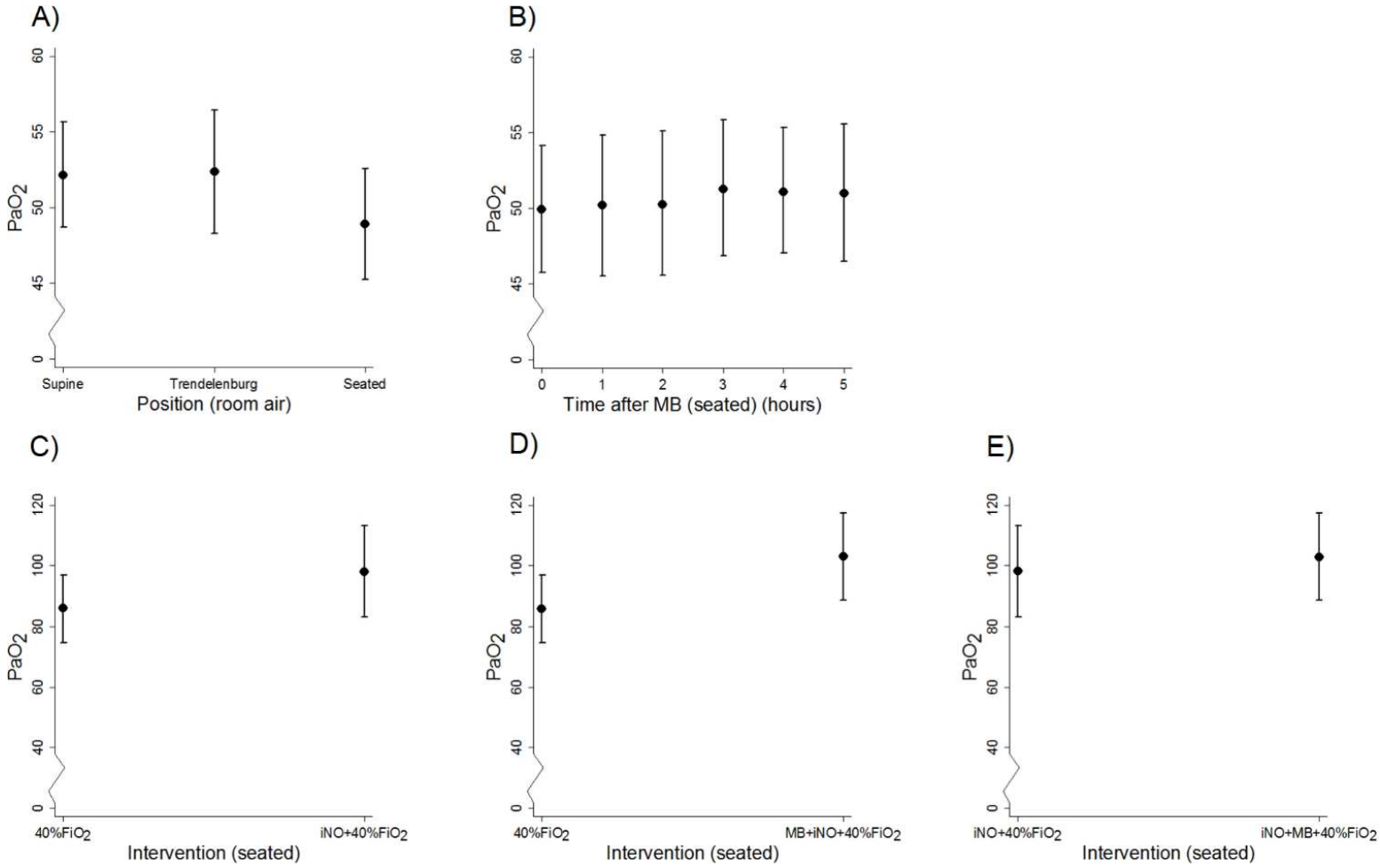
All comparisons are to no intervention, in the same position, on the same fraction of inspired oxygen, unless stated otherwise

Effect of methylene blue interventions (both seated and supine) were measured at five hours after intravenous administration

[a] Two-sample t-test

PaO_2 denotes arterial partial pressure of oxygen, ^{99m}Tc-MAA denotes ^{99m}Tc macroaggregated albumin, iNO denotes inhaled nitric oxide

Supplementary Figure 1. Effect of Various Interventions on Partial Pressure of Oxygen (PaO_2)



The mean PaO_2 with each intervention is represented by a data point, and whiskers represent the 95% confidence intervals. P-values for changes between states (panels A,C,D,E) are provided in Table 2 and p-values for changes at each time point after methylene blue infusion (panel B) are provided in Supplementary Table 3. A) Effect of position changes (n=21). B) Effect of methylene blue at five hours (n=18). C) Effect of inhaled nitric oxide (n=26). D) Effect of inhaled nitric oxide combined with methylene blue at five hours (n=18). E) Effect of inhaled nitric oxide combined with methylene blue at five hours compared to inhaled nitric oxide alone (n=18).

PaO_2 denotes the arterial partial pressure of oxygen, MB denotes methylene blue, iNO denotes inhaled nitric oxide, FiO_2 denotes fraction of inspired oxygen

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References

1. Krowka, M.J., et al. International Liver Transplant Society Practice Guidelines: Diagnosis and Management of Hepatopulmonary Syndrome and Portopulmonary Hypertension. Transplantation **100**, 1440-1452 (2016).
2. Schenk, P., et al. Hepatopulmonary syndrome: prevalence and predictive value of various cut offs for arterial oxygenation and their clinical consequences. Gut **51**, 853-859 (2002).
3. Abrams, G.A., Jaffe, C.C., Hoffer, P.B., Binder, H.J., Fallon, M.B. Diagnostic utility of contrast echocardiography and lung perfusion scan in patients with hepatopulmonary syndrome. Gastroenterology **109**, 1283–1288 (1995).
4. Nayyar, D., Man, H.S., Granton, J. & Gupta, S. Defining and characterizing severe hypoxemia after liver transplantation in hepatopulmonary syndrome. Liver Transpl **20**, 182-190 (2014).
5. Krowka, M.J. & Cortese, D.A. Hepatopulmonary syndrome. Current concepts in diagnostic and therapeutic considerations. Chest **105**, 1528-1537 (1994).
6. Nayyar, D., Man, H.S., Granton, J., Lilly, L.B. & Gupta, S. Proposed management algorithm for severe hypoxemia after liver transplantation in the hepatopulmonary syndrome. Am J Transplant **15**, 903-913 (2015).