Title:
Effect of Body Position and Oxygen Tension on Foramen Ovale Recruitment

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Running Title: Effect of FiO2 and Position on the PFO

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While there is an increased prevalence of stroke at altitude in individuals who are considered to be low-risk for thrombotic events, it is uncertain how venous thrombi reach the brain. The patent foramen ovale (PFO) is a recruitable intracardiac shunt between the right and left atrium. We aimed to determine if body position and oxygen tension affect blood flow through the PFO in healthy adults. We hypothesized that hypoxia and body positions that promote right atrial filling would independently recruit the PFO. Subjects with (n=11) a PFO performed 11 trials, combining four different fractions of inhaled oxygen (FiO₂) (1.0, 0.21, 0.15, and 0.10) and three positions (upright, supine, and 45° head down), with the exception of FiO₂ = 0.10 while 45° head down. After five minutes in each position, breathing the prescribed oxygen tension, saline bubbles were injected into an antecubital vein and a four-chamber echocardiogram was obtained to evaluate PFO recruitment. We observed a high incidence of PFO recruitment in all conditions, with increased recruitment in response to severe hypoxia, and some contribution of body position at moderate levels of hypoxia. We suspect that increased pulmonary vascular pressure, secondary to hypoxia-induced pulmonary vasoconstriction, increased right atrial pressure enough to recruit the PFO. Additionally, we hypothesize that the minor increase in breathing resistance that was added by the mouthpiece, used during experimental trials, affected intrathoracic pressure and venous return sufficiently to recruit the PFO.

Keywords: PFO, hypoxia, altitude, stroke, airway resistance
INTRODUCTION

The foramen ovale is a right-to-left fetal communication that allows blood to be shunted between the right and left atria in utero (4) (3). After birth, decreases in right heart pressure result in reversal of the atrial pressure gradient, favoring higher left atrial pressure and causing functional closure of the patent foramen ovale (PFO). At some future time point, fibrous adhesions can form between the septum secundum and the atrial septum, resulting in anatomical closure of the PFO (4) (3). However, autopsy and echocardiographic studies estimate that anatomical closure remains incomplete in 20-50% of the population, with the incidence of recruitable PFO decreasing as a function of age (16) (15) (28).

Although the majority of individuals with a PFO are asymptomatic, and most cardiologists regard the PFO as a normal cardiac variant, the PFO may be an important contributor to neuropathology. Recruitment of the PFO provides a means for venous thrombi, air, fat, or infectious materials to bypass the lung capillary filter, enter the systemic circulation, and embolize the brain. The prevalence of PFO in cryptogenic stroke patients is higher than both stroke patients with known etiology and stroke-free controls (25) (42) (31) (2) (26). Migraines are a known risk factor for cryptogenic stroke, and a 3.5-fold higher prevalence of PFO exists in patients with migraine and aura than that observed in the general population (5) (17). The speculated etiology of migraine and aura is localized cerebral depolarization caused by small venous emboli or chemical substances that have gained access to the cerebral circulation (21) (33) (13) (44). Supporting this mechanism is a 15-fold higher prevalence of subclinical brain lesions in migraine and aura patients than the general population (21). Emboli gaining access to the arterial circulation through the PFO may explain the high prevalence of both cryptogenic stroke and migraine and aura occurring in individuals with a PFO.
In individuals less than 55 years old, the prevalence of stroke is higher at high altitude than at sea level (11) (37) (6). These individuals are typically at low risk for stroke associated with atherosclerosis and other known stroke risk factors, suggesting that altitude exposure itself is a primary contributor to stroke (45). Hypoxia-induced dehydration and polycythemia may contribute to venous thrombi development at altitude (10) (20), but it is unknown whether intravascular pressure shifts during hypoxia modulate PFO recruitment and facilitate embolization. Increased pulmonary vascular pressure, secondary to hypoxia-induced pulmonary vasoconstriction, may raise right atrial pressure enough to increase the frequency of PFO recruitment (1) (38). Thus, we speculate that recruitment of the PFO under hypoxic conditions may provide a route for venous thrombi to traverse the heart and embolize the brain, potentially explaining the increased risk of stroke at altitude.

We hypothesized that hypoxia would increase the frequency of PFO recruitment in normal, healthy humans with a previously discovered PFO. Considering changes in body position affect right atrial preload (11) (37) (6), we additionally hypothesized that body positions promoting right atrial filling may also affect the frequency of PFO recruitment, individually or in combination with hypoxia. Foramen ovale recruitment was evaluated using saline contrast echocardiography in eleven adults while breathing four different fractions of inhaled oxygen (FiO₂, 1.0, 0.21, 0.15, and 0.10) in three different body positions (upright, supine, and 45° head down tilt).
METHODS

Subjects. Eleven healthy, non-smoking adults were recruited from another study conducted by our laboratory that excluded participants for the presence of PFO. Exclusion criteria for this study included current pregnancy or breastfeeding, previous diagnosis of cardiopulmonary disease, intra-atrial or ventricular defect, intrapulmonary arteriovenous malformation or other significant cardiac anomaly, daily medication use other than hormonal birth control, and any neurological or motor deficit that would prevent participation. The study received approval from the University of Wisconsin School of Medicine and Public Health’s Institutional Review Board. Each subject gave written, informed consent prior to participating.

Saline Contrast Echocardiogram. Four of eleven participants were studied on the same day their PFO was identified. The remaining seven participants returned to the laboratory after the discovery of their PFO for study on a subsequent day. In all participants, the presence of a PFO was reevaluated immediately prior to the experimental protocol.

Saline contrast echocardiography, with and without a Valsalva maneuver, was used to identify the PFO. A 22-gauge catheter was inserted in an antecubital vein and externally connected to two, three-way stopcocks attached in series. A 10 mL syringe was attached to each stopcock. Bubbles were created by manually flushing 4 mL of sterile saline and 1 mL of air between the two syringes. The Valsalva maneuver was standardized such that each participant wore nose clips and exhaled against an occluded mouthpiece to generate +40 cmH₂O mouth pressure, coinciding with the injection of agitated saline contrast. After 10-15 seconds, pressure was released and the transeptal passage of bubbles was assessed. A saline contrast echocardiogram without Valsalva was performed to ensure that participants did not have atrial or ventricular septal defects, or resting intrapulmonary arteriovenous malformations.
**Experimental Protocol.** Subjects performed trials combining four different FIO$_2$ levels (1.0, 0.21, 0.15, 0.10, balance N$_2$) with three body positions (upright, supine, and 45° head down tilt) in a randomized order, except head down tilt with FIO$_2$ = 0.10 was not performed, since preliminary trials showed that this oxygen-position combination often resulted in SpO$_2$ dropping to <70%. Therefore, this yielded 11 independent trials for the assessment of PFO recruitment. Subjects breathed through a low resistance mouthpiece fitted to a two-way non-rebreathing valve (model 2700, Hans Rudolph, Shawnee, KS). A gas blender combining 100% O$_2$ and N$_2$ was used to create the desired FIO$_2$, which was validated with a commercially available oxygen analyzer (MiniOx, Ohio Medical Corp, Gurnee, IL) and ventilation and mixed expired CO$_2$ and O$_2$ were continuously sampled (Ultima PFX, Medgraphics, St. Paul, MN). Peripheral oxygen saturation (SpO$_2$) was continuously assessed by finger sensor pulse oximetry (Nexfin, BMEYE, Amsterdam, NL) and heart rate was continuously measured by a three lead ECG.

After five minutes in each condition, an agitated saline contrast injection was performed. An apical four-chamber echocardiogram was acquired and the transeptal passage of contrast was evaluated (GE Vivid Ultrasound, Tirat Carmel, Israel). The foramen ovale was considered patent when contrast appeared in the left atrium within three cardiac cycles following complete opacification of the right atrium (43). Only considering the first three cardiac cycles allowed for the exclusion of bubbles that may have passed through inducible intrapulmonary arteriovenous anastomoses. These pathways are transiently recruitable by hypoxia and allow for the right-to-left passage of bubbles in ≥5 cardiac cycles (7, 24, 27).

Between trials, participants rested for five minutes in the supine position, breathing room air without a mouthpiece. The tricuspid regurgitation peak velocity was then measured in the left lateral position in order to estimate pulmonary artery systolic pressure using the modified Bernoulli equation (46) (19) (32). This was done to ensure that pulmonary artery systolic
pressure, which may have been elevated in response to hypoxia, was normal before the commencement of a new trial. The experimental positions did not allow for accurate transthoracic echocardiographic assessment of the left ventricular outflow tract and the tricuspid regurgitation peak velocity. Therefore, these were only evaluated while participants rested between trials, and not during the trials.

The magnitude of foramen ovale patency in response to Valsalva was scored according to the degree of contrast passage from right atrium to left as: [0 = no bubbles in left heart; 1 = passage of 1-3 bubbles; 2 = passage of 4-12 bubbles; 3 = > 12 bubbles entering the left ventricle as a bolus; 4 = > 12 bubbles filling the left ventricle, with a density less than the right ventricle; 5 = > 12 bubbles filling the left ventricle, with a density equal to the right ventricle] [33]. All scores were based off the single cardiac cycle with the greatest density of contrast, out of the first three cardiac cycles following contrast appearance in the right heart. A licensed cardiac and vascular sonographer, blinded to the study’s hypothesis and the experimental conditions, independently scored the echocardiograms.

**Data Analysis.** Descriptive and physiological data are presented as means ± SD. A multi-level generalized linear mixed effects model (version 9.3, SAS Institute Inc., Cary NC) was used to analyze the association between position, FiO₂ and PFO response (defined as an opening of the PFO). The main effects of position and breathing level, as well as the interaction between position and breathing, on the frequency of PFO recruitment was evaluated. Pairwise comparisons between position, FiO₂, and the interaction between position and FiO₂ were performed using both unadjusted (for multiple comparisons) and adjusted analysis (Tukey’s HSD method for multiple comparisons). All p-values are two-sided and p<0.05 indicates statistical significance.

**RESULTS**
Anthropometric data and initial PFO evaluation. The anthropometric characteristics of the participants are shown in Table 1. Two individuals (10 & 11) with a Valsalva-induced PFO score of 1 from our earlier evaluation did not have PFO recruitment in response to the Valsalva performed on the day of the experiment (score = 0) (Table 2), or in response to any of the experimental trials. However, because they had a previously-proven PFO, observed in our laboratory, their data are included in our data set.

Ventilation, heart rate, and SpO2. Ventilation trended toward being inversely correlated with FiO2. As expected, heart rate was independently affected by both position (p < 0.001) and SpO2 (p<0.001) was affected by FiO2 (p < 0.001) but not position (p = 0.70) (Table 3).

Main and interaction effects of position and FiO2 on PFO recruitment. There was a high occurrence of PFO recruitment in response to all trials. There was a significant main effect of FiO2 on the frequency of PFO recruitment (p = 0.02), where an FiO2 = 0.10 had greater incidences of PFO recruitment than FiO2 = 1.0 (p < 0.01) (Figure 1). There was no effect of position alone on the frequency of PFO recruitment (p = 0.30) (Figure 1). At an FiO2 of 0.15, there was a combined effect of position and O2 level, where greater occurrences of PFO recruitment were observed in the supine position compared to the upright position (p < 0.05) (Figure 2). Pairwise comparisons within each FiO2 category and within each position category are shown Table 4.

Initial PFO evaluation and frequency of PFO recruitment. As a post hoc analysis, low (score 0-2) versus high (score 3-5) bubble passage in response to the initial Valsalva-induced PFO evaluation was correlated with frequency of recruitment during experimental trials (Mann-Whitney non-parametric U test) and trended toward, but was not significant (p=0.051) (Figure 3). Data are reported as the average of the absolute bubble scores.
DISCUSSION.

We were motivated to perform this study by the increased prevalence of stroke at altitude, particularly in individuals who are considered to be low-risk for thrombotic events (i.e. <55 years) [17] [18] [19]. We observed a high incidence of PFO recruitment in all conditions, with increased recruitment in response to severe hypoxia, and some contribution of body position at moderate levels of hypoxia.

Overall high frequency of PFO recruitment. During screening, no participant had a right-to-left intracardiac shunt without a Valsalva. Unexpectedly, 45% of participants recruited their PFO while supine, during normoxic quiet breathing through the mouthpiece. We identify two differences between the experimental condition and our screening – 1) a difference in position (supine versus left lateral) and 2) the addition of the mouthpiece, which provides a small amount of extrathoracic airway resistance.

The mouthpiece used during trials increased breathing resistance by 1.5 cmH$_2$O/L/s. While this increase is minimal, the slight elevation in resistance may have been sufficient to affect intrathoracic pressure and cause cyclic elevations in venous return, thus causing PFO recruitment. Indeed, during normal breathing, fluctuations in venous return are respiratory cycle dependent [42]. During inspiration, intrathoracic pressure becomes negative, widening the pressure gradient between the right atrium and peripheral veins and facilitating venous return. The drop in intrathoracic pressure causes expansion of the right heart and vena cava, further facilitating venous return. Additionally, right heart expansion may stretch the atrial septum and facilitate ease of PFO recruitment [42].

Augmented venous return, compounded with a direct effect of position on the atrial septum may explain why we observed the highest frequency of PFO recruitment in response to the upright
position in normoxia. A decrease in preload caused by the gravitational effects of being upright can place additional stretch on the atrial septum (47) (39). Patients with Platypnoea-Orthodeoxia Syndrome experience right-to-left shunting through the PFO while upright that can be alleviated by reclining supine (47) (39) (36) (29). While the etiology of this condition is often multifactorial, one contributing factor is that the additional stretch placed on the atrial septum when upright causes venous return to preferentially flow towards the atrial septal wall (47) (39). This can cause PFO recruitment in the absence of an elevated mean right atrial pressure (29) (18). Enhanced atrial stretch induced by the upright position combined with the effects of breathing resistance on right heart expansion may similarly directly venous return towards the foramen ovale, facilitating PFO recruitment during under our experimental conditions.

Despite a plausible mechanism, whether breathing resistance plays a causal role in PFO recruitment remains unknown, but could have important implications for specific populations. Divers who have had decompression illness and individuals susceptible to high altitude pulmonary edema have a higher prevalence of PFO (1) (40). Interestingly, both populations commonly experience increased breathing resistance. The regulator of self-contained underwater breathing apparatus increases both inspiratory and expiratory resistance (41), and individuals at altitude commonly adopt pursed lip breathing, which served to improve gas exchange at the expense of increased expiratory resistance (30). It is possible that breathing resistance may play a role in pathophysiology of decompression illness and high altitude pulmonary edema.

**Enhanced PFO recruitment during hypoxic conditions.** We found enhanced PFO recruitment in response to severe hypoxia (FiO₂ = 0.10). We saw no effect of position at FiO₂ = 0.10, but we were unable to study individuals breathing a FiO₂ = 0.10 while in the head down position. Therefore it is unknown if a synergist effect of hypoxia and increased right atrial filling while in the head down position would have had a significant effect on PFO recruitment. Additionally, we
observed a combined effect of hypoxia and position during FiO₂ = 0.15 trials, where both the supine and head down positions had greater frequencies of PFO recruitment than the upright position. We speculate that the increase in PFO recruitment during our hypoxic conditions was largely a result of hypoxic pulmonary vasoconstriction, increasing pulmonary vascular resistance and, consequently, pulmonary artery and right heart pressures. Fractions of inspired oxygen of 0.10 and 0.15 are equivalent to PO₂ values at 15,000 and 8,000 feet altitude respectively. Thus, our findings may have implications for high altitude travelers and inhabitants with a PFO, as well as aircraft passengers, since cabin pressure is equivalent to 8,000 feet (9). Considering air travel is an independent risk factor for deep vein thrombosis, cause for concern may be warranted for aircraft passengers with a PFO who are on long duration flights (9) (8) (23).

However, it is important to note that our findings were observed at sea level, with normobaric hypoxia. Recruitment of the PFO may be different when hypoxia occurs in the context of low barometric pressure, potentially by altering airway and pulmonary vascular resistance,

Hypobaric hypoxia elicits a more profound arterial hypoxemia than normobaric hypoxia (34), presumably inducing a stronger hypoxic pulmonary vasoconstrictor response. Indeed, pulmonary artery pressure is higher at 3810 m altitude (12,500 feet) as compared to normobaric hypoxia of the same P/O2 (14), consistent with a stronger hypoxic pulmonary vasoconstrictor response. Thus, it is possible that our results underestimate the extent PFO recruitment occurs in response to hypobaric hypoxia. Alternatively, gas viscosity is lower at lower barometric pressures which would decrease airway resistance (12), potentially decreasing the frequency of PFO recruitment. Future studies in hypobaric conditions are needed to address the relative importance of these factors.

**Day to day variability in PFO recruitment.** We took care to rigorously standardize the methodology for conducting Valsalva-induced saline contrast echocardiography. The mouth pressure (+40 cmH₂O) and the length of the breath-hold (15 seconds) was consistent in all
participants and we took care to note bowing of the atrial septum after release of the Valsalva.

Surprisingly, two individuals with a previously discovered PFO via Valsalva did not have PFO recruitment on the day of study. The reason for this finding is not clear. The prevalence of PFO decreases with age (16), and although only four to eleven months passed between the previous and current study, it is possible that complete anatomical closure of the PFO occurred in these individuals. Another explanation is that variations in central venous pressure affect PFO recruitment. Variations in central venous pressure would presumably affect the pressure gradient that is created during a Valsalva maneuver. The high intrathoracic pressure generated during the breath-hold phase of a Valsalva maneuver impedes venous return, thus creating a pressure gradient between the falling right atrial pressure and venous pressure proximal to the impedance (22). Upon release of the Valsalva, this gradient drives venous return and raises right atrial pressure. Lower baseline values of central venous pressure would attenuate the pressure gradient, thus the driving force for venous return upon Valsalva release. Therefore, daily variations in hydration status or other factors affecting central venous pressure may produce false-negative PFO evaluation results. Additionally, a correlation exists between PFO size and pathological events. Patients with neurological events, particularly multiple cerebrovascular events, have significantly larger PFOs than controls (35). We investigated whether low (score 0-2) versus high (score 3-5) bubble passage during the initial Valsalva-induced PFO evaluation was correlated with frequency of recruitment during experimental trials. Although not statistically significant (p =0.051) there was a trend of lower incidences of PFO recruitment occurring in individuals with low bubble passage in response to Valsalva.

Applicability and limitations of our findings. While there is an increased risk of stroke at altitude, it remains debated how venous thrombi get to the brain. We do not know if the PFO is a contributing factor or a risk modifier for stroke at altitude, but our results show an enhanced frequency of PFO recruitment during severe hypoxia, and that body position affects the frequency
of PFO recruitment with moderate hypoxia. We suspect that increased pulmonary vascular pressure, secondary to hypoxia-induced pulmonary vasoconstriction, raises right atrial pressure sufficiently to increase the frequency of PFO recruitment. Although there was an effect of FIO₂ on PFO recruitment, there was a high frequency of PFO recruitment in response to all trials. We hypothesize that this was due to the minor increase in respiratory resistance provided by the mouthpiece. Nonetheless, this condition was imposed on all participants during all trials, suggesting that the effects of hypoxia and body position on PFO recruitment exist in addition to any effect of respiratory resistance. Furthermore, whether a high Valsalva PFO score translates to higher blood across the PFO at altitude, and whether PFO closure is warranted in these individuals, remains an important question worthy of further study.

Conclusions. We show that severe hypoxia affects PFO recruitment, and that positions promoting right atrial filling affect PFO recruitment during moderate hypoxia. These findings may suggest a possible mechanism to explain the incidence of ischemic neurological events at altitude in otherwise low-risk individuals. Furthermore this study suggests that a minimal amount of respiratory load may affect PFO recruitment.

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FIGURE LEGENDS

Figure 1. Overall frequency of PFO recruitment in response to position and FIO2. There was no effect of position alone on PFO recruitment. There was a main effect of FIO2 on PFO recruitment. Specifically, the frequency of recruitment during FIO2 trials of 1.0 was significantly less than the frequency of recruitment during FIO2 trials of 0.10 (p < 0.01).

Figure 2. Interaction of position and FIO2 on PFO recruitment. At FIO2=0.15, a higher frequency of PFO recruitment was observed in the supine and 45° head down positions, compared to the upright position (p < 0.05).

Figure 3. Initial PFO evaluation and frequency of PFO recruitment. There was trend toward an increased frequency of PFO recruitment in individuals with a higher baseline, Valsalva-induced PFO score (p = 0.051).
REFERENCES


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<th>Table 1. Anthropometric characteristics.</th>
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<tbody>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Height (cm)</td>
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<tr>
<td>Weight (kg)</td>
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<tr>
<td>Body Mass Index (kg/m²)</td>
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</table>
A 0-5 scale quantifying the spatial distribution and density of bubbles in left heart was used to score Valsalva-induced PFO recruitment. ND = No data, and pertains to the individuals who were studied on the day their PFO was identified (subjects 1-5). Previous scores are included for participants who had a PFO evaluated in one of our previous studies (subjects 6-11) (See Methods).
<table>
<thead>
<tr>
<th>Position</th>
<th>FlO2</th>
<th>V_e (L/min)</th>
<th>Heart Rate (bpm)</th>
<th>SpO2 (%)</th>
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<tr>
<td>Upright</td>
<td>1.00</td>
<td>11.4 ± 1.9</td>
<td>64 ± 15</td>
<td>100 ± 1</td>
</tr>
<tr>
<td></td>
<td>0.21</td>
<td>12.6 ± 2.4</td>
<td>67 ± 9</td>
<td>98 ± 1</td>
</tr>
<tr>
<td></td>
<td>0.15</td>
<td>12.8 ± 1.5</td>
<td>70 ± 10</td>
<td>90 ± 1</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>14.3 ± 1.6</td>
<td>81 ± 12</td>
<td>73 ± 1</td>
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<tr>
<td>Supine</td>
<td>1.00</td>
<td>11.2 ± 1.0</td>
<td>56 ± 10</td>
<td>100 ± 1</td>
</tr>
<tr>
<td></td>
<td>0.21</td>
<td>10.0 ± 1.2</td>
<td>54 ± 8</td>
<td>98 ± 1</td>
</tr>
<tr>
<td></td>
<td>0.15</td>
<td>10.9 ± 2.1</td>
<td>62 ± 9</td>
<td>91 ± 3</td>
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<tr>
<td></td>
<td>0.10</td>
<td>12.4 ± 1.2</td>
<td>75 ± 9</td>
<td>71 ± 9</td>
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<tr>
<td>Head Down Tilt</td>
<td>1.00</td>
<td>10.1 ± 0.7</td>
<td>56 ± 8</td>
<td>100 ± 1</td>
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<tr>
<td></td>
<td>0.21</td>
<td>11.0 ± 0.9</td>
<td>60 ± 8</td>
<td>99 ± 1</td>
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<tr>
<td></td>
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<td>11.6 ± 2.7</td>
<td>62 ± 7</td>
<td>88 ± 8</td>
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Data collected during experimental trials. FlO2, fraction inspired oxygen; V_e, exhaled minute ventilation BTPS; bpm, beats per minute; SpO2, peripheral oxygen saturation. Data represent mean ± SD.
Table 4. Pairwise comparisons within each FIO₂ category.

<table>
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<th>FIO₂</th>
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<td>0.15</td>
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<td>&gt;0.999</td>
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<td>0.15*</td>
<td>HDT vs. Upright</td>
<td>0.047</td>
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<tr>
<td>0.15*</td>
<td>Supine vs. Upright</td>
<td>0.047</td>
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<td>HDT vs. Upright</td>
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<td>0.21</td>
<td>Supine vs. Upright</td>
<td>0.439</td>
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<td>1.00</td>
<td>HDT vs. Supine</td>
<td>0.051</td>
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<td>1.00</td>
<td>HDT vs. Upright</td>
<td>0.401</td>
</tr>
<tr>
<td>1.00</td>
<td>Supine vs. Upright</td>
<td>0.332</td>
</tr>
</tbody>
</table>

p-values adjusted for multiple comparisons. HDT, head down tilt position. * indicates p < 0.05.
Table 5. Pairwise comparisons within each position category.

<table>
<thead>
<tr>
<th>Position</th>
<th>FIO\textsubscript{2} Comparisons</th>
<th>p-value</th>
</tr>
</thead>
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<td>HDT</td>
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<td>0.15 vs. 1.00</td>
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<td>HDT</td>
<td>0.21 vs. 1.00</td>
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<tr>
<td>Supine</td>
<td>0.15 vs. 0.21</td>
<td>0.431</td>
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<td>0.15 vs. 1.00</td>
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<td>0.15 vs. 0.21</td>
<td>0.045</td>
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p-values adjusted for multiple comparisons. HDT, head down tilt position. * indicates p < 0.05.