Impact of oxygen supplementation on brachial artery hemodynamics and vascular function during ascent to 5,050 m

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Running tittle: O_2 does not improve vascular function during ascent to high altitude

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Abstract

High altitude trekking alters upper limb hemodynamics and reduces brachial artery vascular function in lowlanders. Whether these changes are reversible with the removal of hypoxia is unknown. We investigated the impact of 20 minutes of oxygen supplementation (O_2) on brachial artery hemodynamics, reactive hyperemia (RH; microvascular function) and flow-mediated dilation (FMD; endothelial function). Participants (aged 21-42 years) were examined prior to and with O_2 at 3,440 m (n=7), 4,371 m (n=7), and 5,050 m (n=12) using Duplex ultrasound (days 4,7,10 respectively). At 3,440 m, O_2 decreased brachial artery diameter (–5±5%; P=0.04), baseline blood flow (–44±15%; P<0.001), oxygen delivery (-39±16 P<0.001) and peak RH (–8±8%; P=0.02) but not RH normalized for baseline blood flow. Elevated FMD (P=0.04) with O_2 at 3,440 m was attributed to the reduction in baseline diameter. At 5,050 m, a reduction in brachial artery blood flow (–17±22%; P=0.03), but not oxygen delivery, diameter, RH or FMD occurred with O_2. These findings suggest that during early trekking at high altitude O_2 causes vasoconstriction in the upper limb along the arterial tree (conduit and resistance arteries). With incremental high-altitude exposure, O_2 reduces blood flow without compromising oxygen delivery, RH or FMD, suggesting a differential impact on vascular function modulated by duration and severity of high altitude exposure.

Introduction

Acute hypoxia causes vasodilation to maintain systemic oxygen delivery (Weisbrod and others 2001), while extended exposure to high altitude leads to ventilatory and haematological alterations that progressively restore arterial oxygen content. At high altitude, sympathetic vasomotor outflow to the skeletal muscle vasculature is also increased (Bartsch and Gibbs 2007; Bernardi and others 1998; Busch and others 2018; Hansen and Sander 2003; Simpson and others 2019; Verratti and others 2021). This persistent sympathoexcitation coupled with the removal of hypoxic vasodilation results in vasoconstriction, imposing deleterious arterial shear stress patterns and increasing vascular resistance and decreasing endothelial function (Tremblay and others 2018a; Tremblay and others 2018b; Tymko and others 2020). Additionally, high altitude exposure increases oxidative stress (Lewis and others 2014) and causes elevations in hematocrit and blood viscosity (Hoiland and others 2020; Tremblay and others 2020; Tremblay and others 2018a), which can further disrupt arterial hemodynamics and attenuate endothelial function. Therefore, ascent to high altitude typically reduces vascular function in lowlanders (Tymko and others 2019).

One of the primary regulators of endothelial function is the magnitude and direction of shear stress (Reviewed in Green and others 2017). Acute and chronic disturbances in shear stress patterns can lead to the development of atherosclerotic lesions (Caro and others 1969) and decreased endothelial function (reviewed in Green and others 2017). Blood flow (Dumais and others 2011; Tremblay and others 2018a), diameter (Dumais and others 2011; Tremblay and others 2018a) and mean shear stress decrease, while retrograde shear stress increases under resting conditions during high altitude treks. Further, hypoxic exercise causes pronounced increases in arterial retrograde shear rate in the non-exercising limb (Iwamoto and others 2015). Studies at high altitude have shown reductions in conduit artery (Bakker and others 2015; Lewis and others 2014; Tremblay and others 2018a; Tremblay and others 2018b; Tymko and others 2017a) and resistance artery endothelial function (Stone and others 2021; Tymko and others 2020).

Given that the impaired endothelial function is a common characteristic present in high-altitude diseases (i.e.; high altitude pulmonary edema, chronic mountain sickness) (Berger and others 2005; Rimoldi and others 2012; Tremblay and others 2019; Tymko and others 2020) and is predictive of cardiovascular events (Green and others 2011; Ras and others 2013; Shechter and others 2014; Yeboah and others 2009), understanding whether oxygen supplementation can restore endothelial function is warranted. In Andean highlanders, 1h of hyperoxia (100% O_2) reduces brachial artery diameter and blood flow but does not impact reactive hyperemia (microvascular function). However, hyperoxia only increased FMD in individuals with oxyhemoglobin saturation (SaO_2) below 90% (Rimoldi and others 2012). Conversely, brachial artery diameter, blood flow, reactive hyperemia and FMD are unaltered with oxygen supplementation in Himalayan highlanders (Bruno and others 2014; Erzurum and others 2007). However, whether supplemental oxygen reverses the alterations in brachial artery hemodynamics and function during high-altitude trekking in lowlanders is unknown. The aim of this study was to characterize the impact of supplemental oxygen on upper limb blood flow, shear patterns, micro- and macrovascular function during a high-altitude trek in lowlanders. We hypothesized that supplemental oxygen would decrease brachial artery blood flow and diameter and improve FMD in lowlanders during a high-altitude trek.

Methods

Study design

Participants were male lowlanders (n=12; age = 27±7 [mean±SD]) part of the 2016 UBC-Nepal research expedition team (Willie and others 2018). All participants provided written, informed consent, and experimental protocols were approved by the University of British Columbia Clinical Research Ethics Board in adherence with the principles of the Declaration of Helsinki with the exception of registration as a clinical trial. All participants spent 3–9 days in Kathmandu (1400 m) prior to flying to Lukla (2860 m) to begin the hiking ascent to the EV-K2-CNR Pyramid Research laboratory (5050 m). Ascent to the Pyramid Laboratory took place over a 9-day trekking protocol without the use of any acute

mountain sickness prophylactics (e.g., acetazolamide). Participants spent one night in Monjo (2800 m), three nights in Namche Bazaar (3400 m), one night in Deboche (3820 m), and three nights in Pheriche (4371 m) followed by the final trekking day to 5050 m. Testing occurred prior to and during supplemental O_2 administration with a simple face mask (20-minute wash-in) at 3,440 m (day 4 at high altitude; n=7), 4,371 m (day 7; n=7), and 5,050 m (day 10; n=12) (Figure 1). The flow rate of 100% oxygen was titrated in an attempt to mitigate hyperoxia (i.e., we used the minimum flow rate required to maintain at SpO_2 of >98%). More detailed information regarding the ascent profile can be found in the expedition overview (Willie et al., 2018). This study was conducted on a subset of participants from a published study investigating the impact of high-altitude trekking on vascular function (Tremblay and others 2018a).

Brachial artery hemodynamics

Following 20 minutes of supine rest, the brachial artery was imaged using a 10-MHz multifrequency linear array probe (15L4 Smart Mark, Teratech) attached to a high-resolution ultrasound machine (Terason usmart 3300 and Terason t3200, Teratech). One-minute of diameter and blood velocity were recorded to determine blood flow, shear rate and shear patterns. Pulse rate and oxyhemoglobin saturation were measured using a pulse oximeter (Nonin Medical). The 2018 Lake Louise Acute Mountain Sickness score was used to assess acute mountain sickness (Roach and others 2018).

Flow mediated dilation

Reactive hyperemia FMD was measured in the brachial artery (Thijssen and others 2011; Tremblay and others 2018a). A blood pressure cuff (SC5, Hokanson, USA) was placed over the forearm distal to the epicondyles with vessel imaging performed proximal to the cuff. Following a 1-min recording of baseline arterial diameter and blood velocity the cuff was inflated to 250 mmHg for 5-min. Upon cuff deflation, recording resumed for 3-min. Peak diameter was automatically detected using a moving window-smoothing function

(smoothed median across time) (Woodman and others 2001). FMD was calculated as the absolute (mm) and relative (%) change from baseline to peak diameter.

Peak and total reactive hyperemia were acquired as measures of resistance artery function (Limberg and others 2020; Rosenberry and Nelson 2020).

Blood samples

Blood samples were only acquired while breathing room air (i.e., during hypoxia). Approximately 1 ml of arterial blood was withdrawn anaerobically and immediately assessed using an arterial blood gas analyzer to determine hemoglobin concentration ([Hb]) (i-STAT 1, Abbott Point of Care). Venous blood (5 ml) was drawn into a Vacutainer Blood Collection Tube (Becton Dickinson) that contained lithium heparin. Blood viscosity was measured in duplicate within 15 minutes of blood sample acquisition at a shear rate of 225 s^{-1} at 37°C using a cone and plate viscometer (CP-40Z, DV2T Viscometer, Brookfield Amtek, United States) and a circulating water heating bath (TC-150, Brookfield Amtek, United States).

Data analysis

Figure 1

Investigators were blinded to experimental condition and location for all ultrasound analyses. Blood flow was calculated as ([peak envelope blood velocity/2] × [π × (0.5 × diameter)^2] × 60) and shear stress as the product of shear rate ([4 × peak envelope blood velocity]/arterial diameter) and whole blood viscosity at a shear rate of 225 s^{-1} (Tremblay and others 2018a). Antegrade and retrograde shear stress were calculated as shear stress in the positive (forward) and negative (backward) direction, respectively, and mean shear stress as the sum of antegrade and retrograde (time-averaged mean shear stress). The oscillatory shear index (OSI) was calculated as |retrograde shear stress|/(|antegrade shear stress| + |retrograde shear stress|) (Moore and others 1994). The FMD stimulus was quantified as the shear stress area under the curve (SSAUC) from post cuff deflation to peak diameter. Peak reactive hyperemia was calculated as the greatest 3-s post-occlusion blood flow, and total reactive hyperemia was calculated as the blood flow area under the

curve 3-min post cuff deflation. Posteriorly, peak reactive hyperemia was normalized subtracting baseline blood flow values. Oxygen delivery was estimated from [Hb] and pulse oximetry as [(1.34 ml O_2 × [Hb] × SpO_2) × brachial artery blood flow].

Statistics

All statistical analyses were conducted in RStudio (version 1.1.423, RStudio: Integrated Development for R. RStudio, Inc., Boston, MA, URL: http://www.rstudio.com/). Paired t-tests were used to test for statistical differences from room air and supplemental O_2 at each altitude with coupled effect size calculations (Cohen's d). Normality was assessed using the Shapiro-Wilk's test. Nonnormally-distributed data were assessed using Wilcoxon signed rank tests with the Wilcoxon effect size calculated (r-value where 0.1 - < 0.3 (small effect), 0.3 - < 0.5 (moderate effect) and ≥ 0.5 (large effect)). Linear mixed models were used to account for differences in the SSAUC and diameter in the assessment of FMD using the R package lme4 (Bates and others 2015) with eta-squared calculated to determine the effect size. FMD was analyzed with and without SSAUC included as a covariate. To account for changes in baseline diameter, allometric scaling of FMD was performed with a linear mixed model with the difference between the natural log of peak and baseline diameters as the dependent variable with condition and time as factors and the natural log of baseline diameter as the covariate (Atkinson and Batterham 2013). Linear mixed-model estimated means were back transformed [(e^estimated means – 1) × 100] to obtain allometrically-scaled FMD and standard deviations [(e^standard error – 1) × 100] × √n. Data are presented as mean±SD.

Results

Participant characteristics and hemodynamics (Table 1 and 2)

Descriptive characteristics of the volunteers tested at each elevation are summarized in Table 1. Only one participant exhibited mild acute mountain sickness (2018 Lake Louise Acute Mountain Sickness Score = 4) at 3,440 m elevation. The supplemental oxygen flow rate was 3.3±1.6 L/min at 3,440 m, 3.7±1.1 L/min at 4,371 m and 5.7±1.2 L/min at 5,050 m (5,050 m). By design, O_2 increased SpO_2 at each elevation (Table 2). Heart rate tended to decrease during O_2 at 3,440 m (effect size = 1.13, p=0.058) and at 5,050 m (effect size = 0.53, p=0.09) but was unchanged in 4,371 m.

Baseline flow, shear patterns and diameter (Table 2, Figure 2)

Brachial artery hemodynamics are presented in Table 2. Baseline blood flow (Figure 2B) decreased during O_2 at 3,440 m ($p<0.001$) and 5,050 m (p=0.03), but not at 4,371 m (p=0.21). Mean shear stress decreased at 3,440 m (effect size= 1.27, p=0.02) and 5,050 m (effect size= –0.792, p=0.03). Antegrade shear stress was reduced (effect size= 1.11 p=0.03) when assessed at 4,371 m and at 5,050 m (effect size= 0.686, p=0.04). Finally, OSI nearly doubled with O_2 at 3,440 m (p=0.04). Brachial artery diameter (Figure 2A) was reduced by 5.3±5.3% during O_2 in 3,440 m (p=0.04) but was not different during O_2 in 4,371 m (p=0.57) or at 5,050 m (p=0.30). Estimated oxygen delivery decreased by 39±16% during O_2 at 3,440 m ($p<0.001$).

Reactive hyperemia and flow-mediated dilation (Table 2, Figure 3)

The O_2 supplementation increased FMD (Figure 3B, p=0.034) and SSAUC-adjusted FMD (p=0.0493) at 3,440 m. However, upon accounting for the reductions in baseline diameter, allometrically-scaled FMD was not different during O_2 supplementation at 3,440 m (p=0.145). Moreover, FMD was not different during O_2 supplementation at 4,371 m or at 5,050 m. The O_2 supplementation decreased peak RH (p=0.016) (Figure 3A) at 3,440 m but not at 4,371 m nor at 5,050 m. No differences were found on peak RH after normalization for baseline flow (Table 2).

Discussion

Main findings

This study looked to assess whether O_2 supplementation alters brachial artery hemodynamics and vascular function in lowlanders during ascent to 5,050 m. The main finding was that oxygen supplementation at high altitude generally results in peripheral vasoconstriction but does not improve FMD or reactive hyperemia. Taken together, these results show that oxygen supplementation does not improve conduit artery or microvascular function, patterns of shear stress, or improve peripheral oxygen delivery in healthy male trekkers at high altitude.

Brachial artery hemodynamics at high altitude

High-altitude trekking tends to (Dumais and others 2011; Tremblay and others 2018a), but does not always (Bakker and others 2015), reduce brachial artery diameter and blood flow compared to pre-trekking measures. This reduction in peripheral blood flow suggests that unlike during laboratory-based hypoxia interventions (Berger and others 2005; Blitzer and others 1996; Casey and others 2010), peripheral vasoconstriction outcompetes hypoxic vasodilation at high altitude. Previous studies have determined that this net vasoconstriction may be caused by factors that increase vascular resistance, for example, hypoxia-increased sympathetic nerve activity (Hansen and Sander 2003; Simpson and others 2019; Tymko and others 2017b), increased blood viscosity, decreased NO bioavailability and/or increased oxidative stress (Lewis and others 2014). Although previous studies have assessed the impact of oxygen supplementation on conduit vascular function of highlanders (Bruno and others 2014; Rimoldi and others 2012), only one group found an improvement in a subgroup of Andean highlanders with low oxygen saturation (Rimoldi and others 2012). Our data show that, similar to what was found in Andeans (Rimoldi and others 2012),

oxygen supplementation reduced baseline diameter, baseline blood flow and O_2 delivery at 3,440 m and reduced blood flow at 5,050 m. This influence of oxygen supplementation likely reflects the removal of the masked but persistent hypoxic vasodilation and, possibly, hyperoxia-mediated (Smit and others 2018) or enhanced sympathetic nerve activity (Hansen and Sander 2003; Tymko and others 2020). Indeed, severe hyperoxia increases oxidative stress and reactive oxygen species production (Fratantonio and others 2021), which could modulate vascular tone. However, the short duration and titration of O_2 in our study should have mitigated these influences. The reduction in blood flow, but not diameter or oxygen delivery observed at 5,050 m in our protocol, suggests less hypoxic vasodilation and/or hyperoxic vasoconstriction sensitivity with longer duration and more severe hypoxia. The reductions in blood flow observed during oxygen supplementation in this experiment likely induced detrimental shear stress patterns (table 2), reducing mean and anterograde shear stress, which, over time, may contribute to reductions in endothelial function.

Vascular function at high altitude

The myriad of stressors encountered in high altitude environments have been shown to reduce conduit artery endothelial function in lowlanders. Studies have generally found that reductions in FMD are mainly observed in studies that involved active ascent to altitude (Bakker and others 2015; Lewis and others 2014; Tremblay and others 2018b; Tymko and others 2017a; Tymko and others 2017b). While O_2 increased FMD at 3,440 m, this was attributed to a reduction in baseline diameter and thus does not indicate that O_2 improved endothelial function. This suggests that in healthy male lowlanders trekking to high altitude, O_2 does not increase endothelial-dependent vasodilation in the brachial artery. This highlights the importance of including these corrected measurements under similar conditions (i.e., SSAUC-adjusted FMD and allometrically scaled FMD), when analyzing FMD data as previously suggested (Atkinson and Batterham 2013). This is especially relevant in studies at high altitude and hypoxia, which are known to influence blood

viscosity, vascular tone and conduit artery diameters (Dumais and others 2011; Theunissen and others 2022; Tremblay and others 2020; Tremblay and others 2018a).

Hypoxia-mediated increased sympathetic activity (Tymko and others 2020), and oxidative stress (Stone and others 2021) appear to contribute to reduced resistance artery endothelial function at high altitude. Interestingly, Stone et al (2021) also found that lower oxygen saturations are related to greater reductions in endothelium-mediated vasodilation, and forearm blood flow, leading us to speculate that oxygen supplementation would improve the blunted dilatory response. However, our results show that oxygen supplementation does not improve resistance artery function, and that in fact, oxygen supplementation does initially reduce peak reactive hyperemia. This latter point suggests vasoconstriction but not a reduction in resistance artery function since the baseline blood flow (Figure 2B) is also reduced. As shown in Table 2, no changes were detected in total reactive hyperemia after normalization by baseline blood flow, suggesting that microvascular function was not reduced by O_2, although the latter may have elicited vasoconstriction. A possible reason for this reduction in baseline diameter at 3,440 m (Figure 2A), is the removal of the hypoxic stimulus. As it has been described before, the withdrawal of the hypoxic stimulus reverses the hypoxia-mediated vasodilation (Blitzer and others 1996; Crawford and others 1959; Ross and others 1962). It is also important to mention that our study found no changes in total reactive hyperemia and SSAUC that might suggest a change in hyperemic stimulus (Table 2).

Methodological considerations:

Limitations:

This study was conducted during an expedition organized by the University of British Columbia to Nepal in 2016 with exploratory purposes in a young, healthy male population. The lack of female participants hindered a further analysis for sex differences in our study, making difficult to extrapolate these findings to female populations. Nonetheless, we are aware that sex could be a potential factor to account for when assessing endothelial

function (Black and others 2009; Green and others 2016; Moreau and others 2012). Indeed, in Tibetan highlanders, oxygen supplementation reduced forearm blood flow in females but not in males, suggesting a possible sex-related differential blood flow response upon oxygen supplementation (Erzurum and others 2007).

Our study did not include blood pressure post O_2 supplementation, and this can be a potential major limitation for our study. Despite, as reported previously by our group, the increase in blood pressure elicited by sustained hypoxia remains unchanged during the rest of the active ascent (Hoiland and others 2019; Tremblay and others 2018b), previous findings in lowlanders at high altitude have shown an increase in mean arterial pressure and no change in muscle sympathetic nerve activity after oxygen supplementation (Simpson and others 2019). The lack of change in muscle sympathetic nerve activity makes us speculate that the increase in mean arterial pressure is likely due to the removal of hypoxic vasodilation (i.e., net vasoconstriction). This latter observation might indicate that oxygen supplementation may contribute to increased mean arterial pressure in lowlanders.

Prolonged endurance exercise can influence vascular function, though this is typically evident in the active limb and immediately postexercise (Dawson and others 2008; King and others 2021). We did not test immediately upon arrival after trekking, waiting until the next day to minimize the influence of exercise on our vascular measures. However, whether O_2 influences vascular function during immediate recovery from high altitude trekking, or during inactive ascent to high altitude, is unclear.

Conclusion:

In summary, our findings show that during the first days of active ascent at high altitude O_2 supplementation causes vasoconstriction in the upper limb, as evidenced by the reduction in brachial artery diameter and blood flow, limiting oxygen delivery and reactive hyperemia. With prolonged, more severe high-altitude exposure, O_2 supplementation reduces blood

flow in the upper limb without compromising oxygen delivery, reactive hyperemia, or FMD. This suggests that supplemental oxygen has a differential impact on vascular function which is modulated by duration and/or severity of high-altitude exposure.

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Author contributions:

Gustavo Vizcardo-Galindo: Formal Analysis (lead), Writing – Original Draft (lead), writing-review and editing (lead).

Connor Howe: Writing – Original draft (support), writing – review and editing (equal), Data curation (support).

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Dr. Ainslie: Funding acquisition (lead), Conceptualization (equal), Resources (lead), Writing-original draft (support), Writing – review and editing (equal).

Dr. Tremblay: Conceptualization (equal), Writing-original draft (support), Writing – review and editing (equal), Data analysis (support), Investigation (lead).

Conflict of Interest:

The authors have no conflicts of interest.

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Abbreviations:

FMD: flow-mediated dilation

SS: shear stress

SSAUC: shear stress area under the curve

RH: reactive hyperemia

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LEGENDS

Table 1. Participant characteristics. Data are presented as mean ± standard deviation.

Table 2. Variables measured while breathing room air and during oxygen supplementation. Data are presented as mean ± standard deviation. *=p<0.05; **p<0.01; ***p<0.001 for parametric tests and α = p<0.05 for non-parametric tests.

Figure 1. Schematic of the ascent protocol. After spending 3–9 days in Kathmandu, participants were flown to Lukla from where the trekking ascent started. The testing was done on days 4, 7, and 10 at high altitude.

Figure 2. Hemodynamic parameters. The present figure shows median and interquartile range – SD for both brachial artery baseline diameter and brachial artery blood flow pre- and during O2 supplementation at each altitude (A, B= 3,440 m, C, D = 4,371 m, and E, F = 5,050 m), where *p < 0.05; ***p < 0.001. SD, standard deviation.

Figure 3. Conduit and resistance artery function. Peak RH (A, C, and E) and relative FMD (B, D, and F) at each altitude, before and during O2 supplementation. (A, B) = 3,440 m, (C, D) = 4,371 m, and (E, F) = 5,050 m). Box plots show the median value and quartile percentiles; where *p < 0.05. FMD, flow-mediated dilation.

Figure 1.

552 Table 1. Participant Characteristics. Data are presented as mean ± standard deviation.

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	Location		
	Namche (3,440 m)	**Pheriche (4,371 m)**	**Pyramid (5,050 m)**
Participants (n)	7	7	12
Age (years)	27±7	27±7	27±6
Height (m)	1.80±0.07	1.80±0.07	1.79±0.07
Mass (kg)	77±9	77±9	77±8
Body mass index (kg / m^2)	23.8±2.4	23.8±2.4	24.2±2.1
Blood viscosity at a shear rate of 225 s^{-1} (mPa·S)	4.30±0.28	4.39±0.21	4.85±0.31
Hemoglobin concentration (g/dl)	14.77±0.82	14.37±0.63	14.44±0.86
Lake Louise Score	1±1	0±1	1±1

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Table 2. Variables measured while breathing room air and during oxygen supplementation. Data are presented as mean ± standard deviation.

	Namche (3440 m)				Pheriche (4,371 m)				Pyramid (5,050 m)			
	Room air	**O_2**	**Effect size**	**P-value**	**Room air**	**O_2**	**Effect size**	**P-value**	**Room air**	**O_2**	**Effect size**	**P-value**
Oxygen flow (l/min)		3.3±1.6				3.7±1.1				5.7±1.2		
SpO_2 (%)	92±2	100±1	3.2	8.63e-05**	87±4	99±1	3.55	8.30e-05**	83±3	99±1	5.40	1.08e-09**
HR (min^{-1})	63±16	56±13	–0.084	0.05788	61±13	58±12	–0.180	0.6512	70±22	61±16	–0.531	0.0928
Baseline diameter (mm)	4.40±0.33	4.16±0.29	–0.998	0.0385*	4.37±0.47	4.40±0.39	0.114	0.774	4.38±0.38	4.36±0.33	–0.073	0.8051
Peak diameter (mm)	4.60±0.30	4.46±0.30	–0.481	0.2506	4.61±0.42	4.65±0.37	0.196	0.6219	4.52±0.38	4.57±0.30	0.164	0.5824
Absolute FMD (mm)	0.20±0.14	0.30±0.054	0.914	0.05199	0.25±0.1	0.26±0.12	0.077	0.8448	0.14±0.099	0.20±0.69	0.517	0.1009
Relative FMD (%)	4.67±3.45	7.26±1.34	1.03	0.034*	5.87±3.04	5.92±2.72	0.0167	0.9661	3.29±2.38	4.73±1.78	0.539	0.08877
SSAUC-adjusted FMD (%)	4.82±2.57	7.11±2.57	0.756	0.0493*	5.90±2.67	5.90±2.67	0.0258	0.9979	3.29±2.15	4.74±2.15	0.940	0.08746
Allometrically scaled FMD (%)	4.96±2.51	6.91±2.51	0.655	0.145	5.75±2.20	5.98±2.20	0.009	0.78	3.28±1.92	4.70±1.92	0.455	0.11
Time to peak diameter (s)	47±10	52±12	0.330	0.4156	39±7	38±10	–0.037	0.925	47±21	45±13	–0.119	0.6878
SSAUC	864±206	930±227	0.317	0.4343	696±155	702±235	0.026	0.9479	808±326	825±225	0.0564	0.8487
Baseline blood flow (ml/min)	35±9	20±7	–2.63	0.0004**	29±9	25±11	–0.530	0.21	38±25	32±24	-0.700	0.03379*
Baseline oxygen delivery (ml/min)	6.38±1.66	3.87±1.35	–2.42	0.01563$^{\alpha}$	4.87±1.62	4.78±2.13	–0.073	0.8527	5.96±3.58	5.98±4.21	0.0153	0.9588
Normalized peak RH (ml/min)	299±76.7	287±58.9	–0.401	0.3291	288±71.6	292±93.6	0.0781	0.843	284±95.6	276±100	–0.092	0.7557
Peak RH (ml/min)	335±77	307±59	–0.825	0.01563$^{\alpha}$	317±79	317±103	0.004	0.9918	323±103	308±99	–0.158	0.5958
Total RH (ml)	263±119	218±76	–0.592	0.1684	221±88	230±120	0.154	0.6977	217±129	221±97	0.0635	0.8298
Mean SS (dyn cm^{-2})	3.04±0.97	2.02±1.05	–1.27	0.01532*	2.52±0.74	2.06±0.75	–0.621	0.1516	3.62±2	3.06±2.17	–0.792	0.01912*
Antegrade SS (dyn cm^{-2})	3.59±1.12	2.94±1.66	–0.632	0.1458	3.60±1.16	2.88±0.79	–1.11	0.02621*	4.56±1.96	3.96±2.07	–0.686	0.03664*
Retrograde SS (dyn cm^{-2})	–0.55±0.36	–0.92±0.64	–0.593	0.1677	–1.09±0.51	–0.82±0.64	0.646	0.1382	–0.94±0.83	-0.90±0.81	0.0584	0.8435
OSI (au)	0.12±0.07	0.23±0.05	1.01	0.03756*	0.22±0.05	0.20±0.11	–0.224	0.5748	0.16±0.12	0.19±0.11	0.135	0.2982

Figure

Pulse oximetry saturation (SpO_2), Heart rate (HR), Flow-mediated dilation (FMD), Shear stress area under the curve (SSAUC), Reactive hyperemia (RH), Shear stress (SS), Oscillatory shear index (OSI). *=$p<0.05$; **$p<0.001$ for parametric tests and $\alpha = p<0.05$ for non-parametric tests.

Figure 1

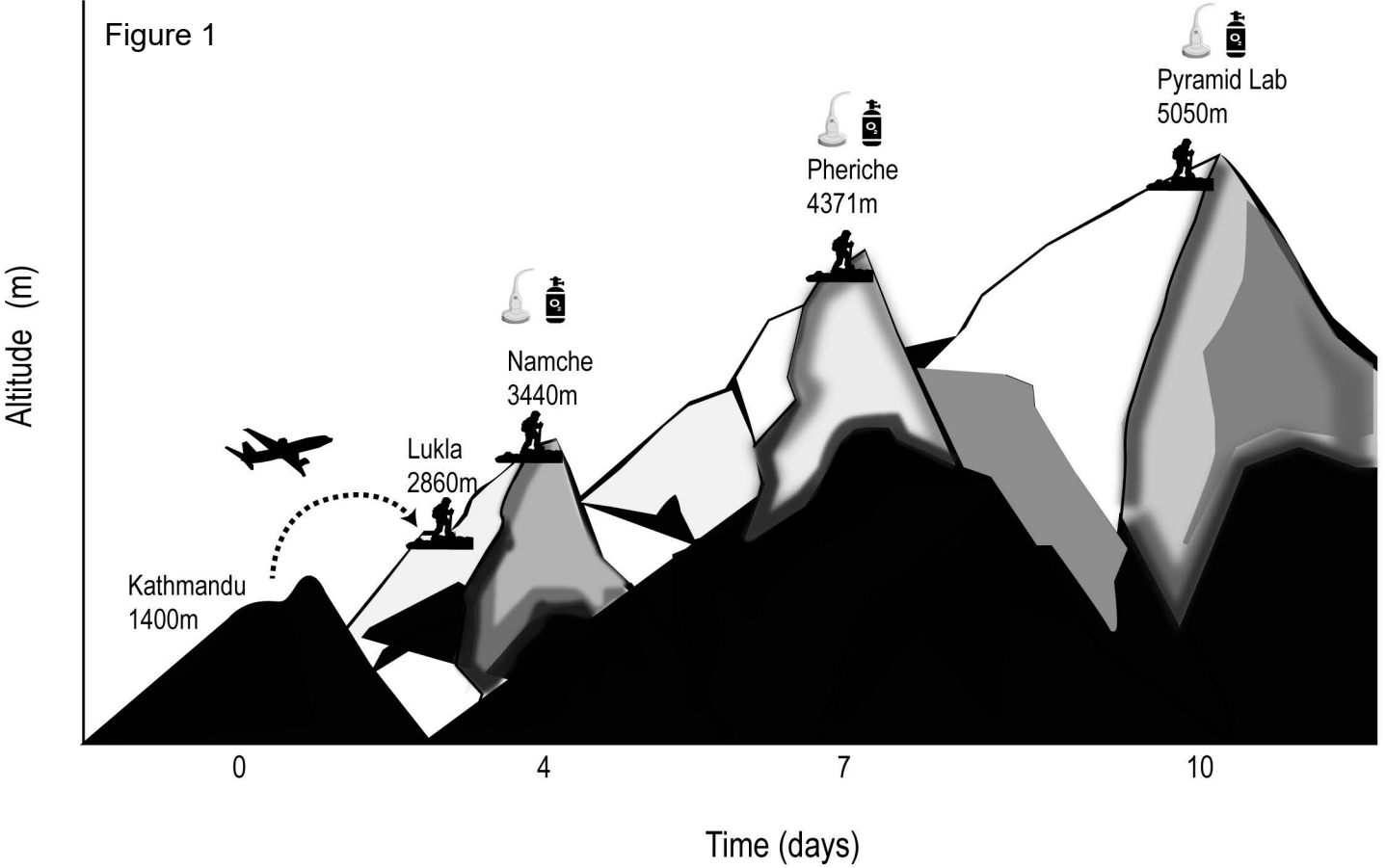

Pyramid Lab
5050m
Pheriche
4371m
Namche
3440m
Lukla
2860m
Kathmandu
1400m
Altitude (m)
0
4
7
10
Time (days)

Figure 2

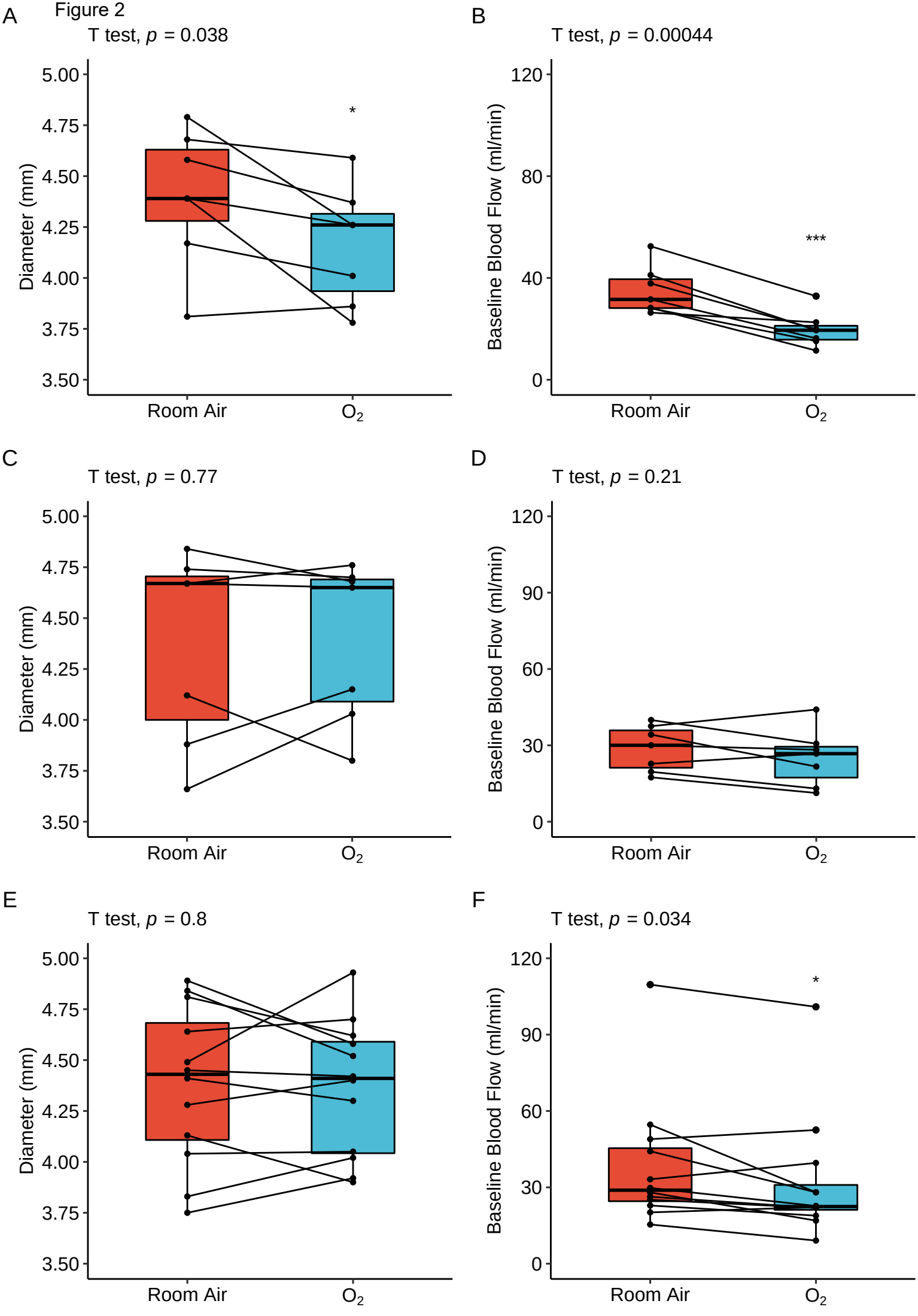
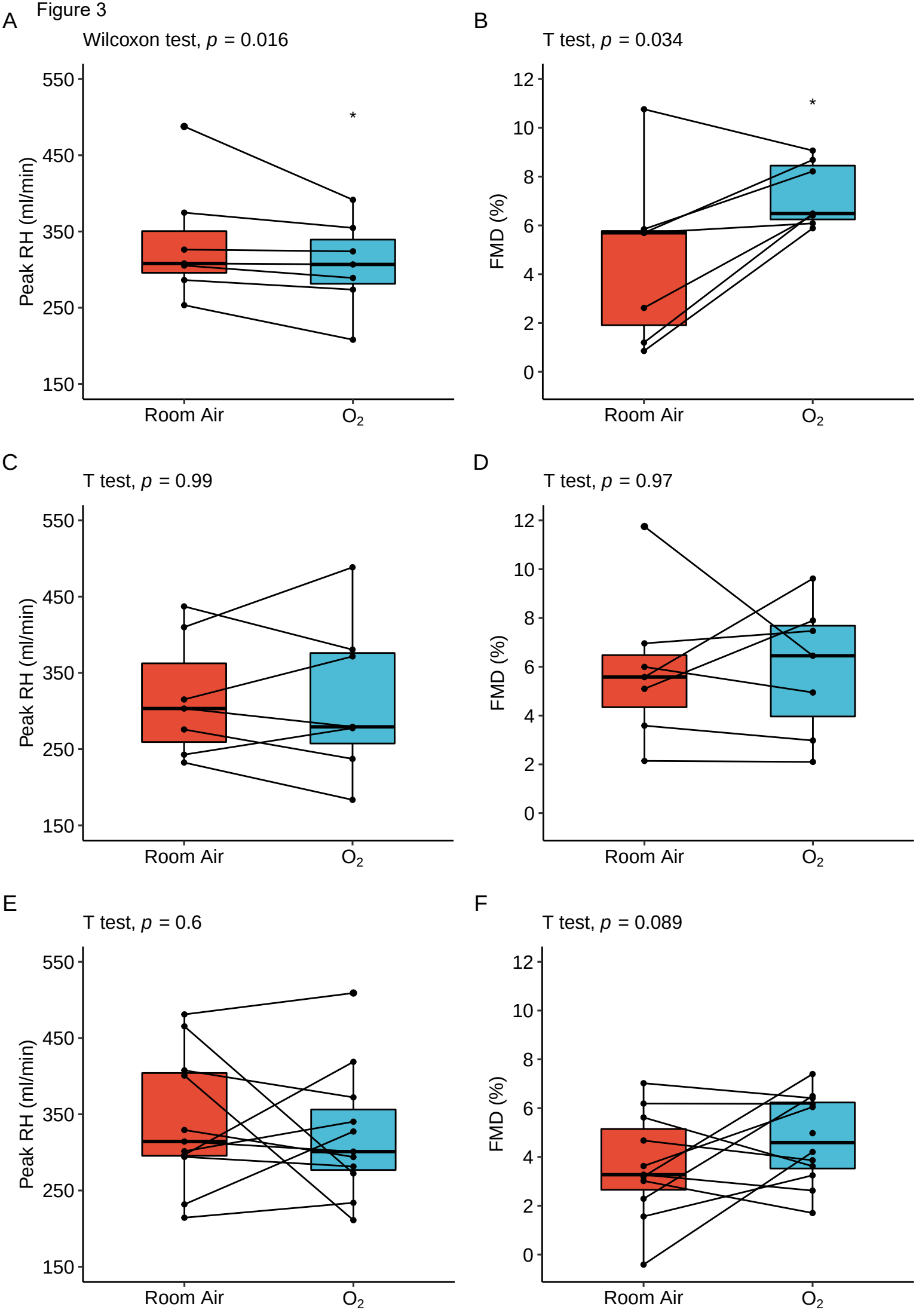

A
T test, p = 0.038
Diameter (mm)
5.00
4.75
4.50
4.25
4.00
3.75
3.50
*
Room Air
O2
B
T test, p = 0.00044
Baseline Blood Flow (ml/min)
120
80
40
0
***
Room Air
O2
C
T test, p = 0.77
Diameter (mm)
5.00
4.75
4.50
4.25
4.00
3.75
3.50
Room Air
O2
D
T test, p = 0.21
Baseline Blood Flow (ml/min)
120
90
60
30
0
Room Air
O2
E
T test, p = 0.8
Diameter (mm)
5.00
4.75
4.50
4.25
4.00
3.75
3.50
Room Air
O2
F
T test, p = 0.034
Baseline Blood Flow (ml/min)
120
90
60
30
0
*
Room Air
O2

Figure 3


A
Wilcoxon test, p = 0.016
Peak RH (ml/min)
550
450
350
250
150
*
Room Air
O2
B
T test, p = 0.034
FMD (%)
12
10
8
6
4
2
0
*
Room Air
O2
C
T test, p = 0.99
Peak RH (ml/min)
550
450
350
250
150
Room Air
O2
D
T test, p = 0.97
FMD (%)
12
10
8
6
4
2
0
Room Air
O2
E
T test, p = 0.6
Peak RH (ml/min)
550
450
350
250
150
Room Air
O2
F
T test, p = 0.089
FMD (%)
12
10
8
6
4
2
0
Room Air
O2