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RESEARCH ARTICLE

Hypoxic preconditioning reduces endothelial ischemia-reperfusion injury in older adults

Sten Stray-Gundersen, Sahar D. Massoudian, Frank Wojan, Hirofumi Tanaka, and Sophie Lalande
Department of Kinesiology and Health Education, The University of Texas at Austin, Austin, Texas

Abstract

Sudden blood flow restoration to an ischemic vessel paradoxically damages endothelial cells. Ischemic preconditioning, caused by repeated bouts of brief ischemia using local or remote cuff inflation before reperfusion, attenuates endothelial dysfunction following an ischemia-reperfusion injury in young adults but does not consistently protect endothelial function in older adults prone to ischemic events. Intermittent exposure to systemic hypoxemia, induced via brief bouts of breathing low levels of oxygen, attenuates endothelial dysfunction following an ischemia-reperfusion injury in young adults. The aim of this study was to determine whether systemic hypoxic preconditioning protects against ischemia-reperfusion injury in older adults. Twelve adults (five women, 57 ± 9 yr) participated in this randomized crossover trial. Endothelium-dependent vasodilation was assessed by brachial artery flow-mediated dilation using a semiautomated diagnostic ultrasound system before and after a 20-min blood flow occlusion that was preceded by either intermittent hypoxia, consisting of three 4-min hypoxic cycles at an oxygen saturation of 80% interspersed with 4-min room air cycles, or intermittent normoxia, consisting of three 4-min normoxic cycles separated by 4-min room air cycles. When preceded by intermittent normoxia, ischemia-reperfusion injury reduced flow-mediated dilation by 4.1 ± 2.6% (6.5 ± 1.7 to 2.4 ± 1.7%). In contrast, flow-mediated dilation was reduced by 2.0 ± 1.5% when ischemia-reperfusion injury was preceded by intermittent hypoxia (5.6 ± 1.7 to 3.6 ± 2.3%). In conclusion, hypoxic preconditioning significantly attenuated the reduction in brachial artery flow-mediated dilation induced by an ischemia-reperfusion injury in older adults at greater risk for ischemic events.

aging; flow-mediated dilation; intermittent hypoxia; ischemia-reperfusion injury

INTRODUCTION

Ischemic heart disease, the most prevalent form of cardiovascular disease and primary cause of death in the United States (1), results from inadequate blood flow and oxygen supply to the coronary arteries. Restoration of blood flow to ischemic blood vessels during surgical treatments such as coronary angioplasty and thrombolytic therapy effectively reestablishes tissue oxygenation. However, reperfusion also paradoxically damages endothelial cells lining the inner surface of blood vessels due to a sudden burst of reactive oxygen species leading to excessive peroxynitrite formation and reduced nitric oxide bioavailability (2, 3). This phenomenon, termed ischemia-reperfusion injury, transiently reduces flow-mediated dilation of the brachial artery, a marker of endothelium-dependent vasodilation (4–9). Interventions are therefore needed to attenuate the endothelial dysfunction associated with ischemia-reperfusion injury.

In young healthy adults, brief repeated periods of local or remote noninjurious ischemia induced by cuff inflation before prolonged ischemia attenuates the reduction in brachial artery flow-mediated dilation induced by an ischemia-reperfusion injury through modulation of inflammatory cell recruitment and activation of ATP-sensitive potassium channels (10, 11). However, this ischemic preconditioning does not appear to consistently protect against endothelial dysfunction induced by an ischemia-reperfusion injury in older adults at risk for ischemic events (7–9, 11). Although Loukogeorgakis et al. (11) first reported that ischemic preconditioning abolished ischemia-reperfusion injury in six individuals with atherosclerosis, this protective effect could not be replicated in older adults or patients with heart failure (7–9). Interestingly, an increase in the volume of tissue exposed to ischemic preconditioning results in a greater degree of protection against ischemia-reperfusion injury in young individuals (11). We previously demonstrated that systemic hypoxic preconditioning, induced by alternating bouts of breathing hypoxic and normoxic air before an ischemia-reperfusion injury, attenuates the reduction in flow-mediated dilation in young adults (12). Thus, the objective of the present study was to determine whether systemic hypoxic preconditioning protects endothelial function against ischemia-reperfusion injury in older adults. We hypothesized that hypoxic preconditioning would attenuate the reduction in brachial artery flow-mediated dilation following an ischemia-reperfusion injury.

Correspondence: S. Lalande (sophie.lalande@austin.utexas.edu).
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METHODS

Participants

Both men and women were recruited to participate in the study. All participants provided informed written consent for participating in the study. The study was conducted in accordance with the Declaration of Helsinki, registered at ClinicalTrials.gov (NCT05423470), and the protocol was approved by the Institutional Review Board of the University of Texas at Austin (IRB study number 2018110129). Participants were excluded from the study if they had a history of cardiovascular disease, diabetes, or lung disease, had resting blood pressure over 130/80 mmHg, were smokers, were pregnant, or were taking more than one antihypertensive medication.

Study Protocol

This study was a single-blinded, randomized crossover trial. Participants visited the laboratory while fasting on two separate occasions. On both visits, brachial artery flow-mediated dilation was measured before and 15 min after inducing ischemia by inflating a cuff on the upper right arm to 250 mmHg for a period of 20 min (Fig. 1) (5, 6). Ischemia was immediately preceded by either a 20-min intermittent hypoxia protocol or a 20-min intermittent normoxia protocol. Participants were asked to avoid strenuous physical activity on the day before both visits and to maintain a consistent hydration status on the morning of each visit. If applicable, participants took their medication as prescribed on both visits. Since a lasting effect of hypoxic preconditioning on flow-mediated dilation was not observed one week following the stimulus (12), visits were separated by a period of 7 days for men and postmenopausal women. For the premenopausal woman, both visits were performed during the early follicular phase (2 days after the onset of the menstrual cycle) to control for the effect of estrogen on endothelium-dependent flow-mediated dilation of the brachial artery (13).

Intermittent Hypoxia and Intermittent Normoxia

Participants laid in a semirecumbent position throughout the intermittent hypoxia and intermittent normoxia protocols. Intermittent hypoxia consisted of three 4-min hypoxic cycles at a targeted oxygen saturation of 80% interspersed with 4 min of breathing room air. Each 4-min hypoxic cycle began once the participant reached an oxygen saturation of 83%. Hypoxic air was inhaled through a mask connected to a two-way nonrebreathing valve connected to a 5-L nondiffusing bag (Hans Rudolph, Shawnee, KS). The bag was connected to a gas tank of medical grade compressed air (21% oxygen, balance nitrogen) and a gas tank of pure nitrogen. Air was made hypoxic by introducing nitrogen in the breathing circuit. The flow of nitrogen was preset to achieve the targeted oxygen saturation of 80%, and slight manual adjustments were made if oxygen saturation was ±3% from the target. Intermittent normoxia consisted of the same protocol, but nitrogen was not introduced in the breathing circuit.

Brachial Artery Endothelial Function

Heart rate and blood pressure were measured after 15 min of supine rest in a dimly lit room. Endothelium-dependent vasodilation of the brachial artery was assessed by flow-mediated dilation using a semiautomated diagnostic ultrasound system (UNEXEF-38G, UNEX, Corp., Nagoya, Japan) (14). Briefly, this semiautomated diagnostic ultrasound system utilizes B-mode imaging to capture one long-axis and two short-axis images thereby providing a longitudinal and cross-sectional view of the brachial artery. The collated images facilitate the automated probe positioning with continuous correction. Participants extended their right arm 90° away from their body at heart level. Cross-sectional images of the brachial artery were acquired using a high-resolution linear-array transducer positioned 2–8 cm proximal to the antecubital fossa. A cuff was placed ~1 cm distal to the antecubital fossa. Forearm occlusion was induced by rapidly inflating the cuff to a pressure 50 mmHg above the recorded systolic blood pressure for a period of 5 min. After cuff deflation, beat-by-beat blood velocity and brachial artery diameters were recorded for 75–90 s. For each data point, the shear rate was calculated as: shear rate = (blood velocity/diameter) × 8, and blood flow was calculated as: blood flow = blood velocity x brachial artery cross-sectional area. The shear rate area under the curve (AUC) was summed through peak diameter, whereas blood flow AUC was summed over the entire recording.

Pulmonary Gas Exchange and Hemodynamics

Pulmonary gas exchange was assessed using a metabolic cart calibrated with standardized gas and room air (Ultima Cardio2, MGC Diagnostics, MN), and averaged every 10 s throughout both protocols. The pneumotachometer was mounted between the mask and the nonrebreathing valve of the breathing circuit. Hemodynamics and peripheral oxygen saturation were continuously recorded using finger plethysmography and pulse oximetry, respectively (NOVA, Finapres Medical Systems, Amsterdam, The Netherlands). Brachial arterial blood pressure, heart rate, stroke volume, cardiac output, and total peripheral resistance were derived from the arterial waveform obtained by finger plethysmography, a method validated against invasive measures (15). All data were recorded using LabChart software for later analyses (PowerLab, ADInstruments, Inc., Colorado Springs, CO).

Statistical Analyses

A two-way repeated-measures analysis of variance was performed to evaluate the effect of time (pre- vs. postischemia-reperfusion injury) and condition (intermittent hypoxia vs. intermittent normoxia) on measures of vascular endothelial function. When main effects or interactions

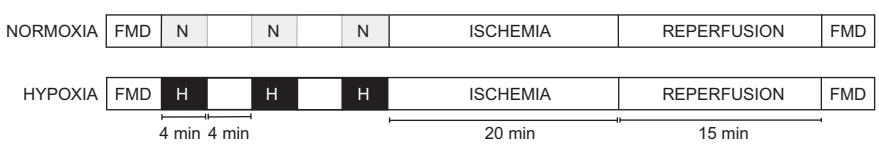

Figure 1. Study protocol. Hypoxic and normoxic cycles were interspersed with 4 min of breathing room air. FMD, flow-mediated dilation; H, hypoxia; N, normoxia.

were significant, post hoc analyses were performed using Bonferroni's test. Baseline values for each hemodynamic and pulmonary gas exchange variable consisted of the 1-min average preceding the start of the intermittent normoxia and intermittent hypoxia protocols. Average values for hemodynamic and pulmonary gas exchange variables were calculated from each 4-min hypoxic cycle of the intermittent hypoxia protocol and for each 4-min normoxic cycle of the intermittent normoxia protocol. A paired *t* test was used to evaluate the effect of the condition (intermittent hypoxia vs. intermittent normoxia) on pulmonary gas exchange, hemodynamics, oxygen saturation, and change in flow-mediated dilation. A post hoc power analysis performed using the difference in the changes in flow-mediated dilation induced by ischemia-reperfusion injury in both conditions in our 12 participants resulted in a power of 0.61. $P < 0.05$ was considered statistically significant. All values are reported as means ± standard deviation.

RESULTS

Participants' characteristics are presented in Table 1. There were no differences in baseline pulmonary gas exchange and hemodynamics variables between conditions. As targeted, intermittent hypoxia induced a reduction in oxygen saturation corresponding to a lower fraction of inspired oxygen and end-tidal oxygen levels compared with intermittent normoxia (Table 2, Fig. 2). There was no effect of intermittent hypoxia on respiratory rate, tidal volume, minute ventilation, or end-tidal carbon dioxide compared with intermittent normoxia (Table 2). Moreover, intermittent hypoxia did not influence cardiac output, stroke volume, total peripheral resistance, and systolic and diastolic blood pressure (Table 2). Intermittent hypoxia resulted in a greater heart rate compared with intermittent normoxia (Table 2).

Blood flow occlusion was well tolerated by all participants. Ischemia-reperfusion injury reduced flow-mediated dilation in both conditions. However, the reduction in flow-mediated dilation was attenuated with intermittent hypoxia in comparison to intermittent normoxia (Table 3, Fig. 3). Figure 4 shows individual brachial artery flow-mediated dilation before and after ischemia-reperfusion injury with intermittent normoxia and intermittent hypoxia. Brachial artery baseline diameters were greater following ischemia-reperfusion injury in both conditions, with a greater increase in baseline brachial artery diameter observed with intermittent normoxia than intermittent hypoxia (Table 3). Because of these different changes in baseline brachial artery diameter between conditions, the presence of inadequate scaling was verified by examining the slope of the relation between logarithmically transformed baseline and peak diameter, and flow-mediated dilation was reassessed using the allometric modeling solution (16, 17). Analyzing the flow-mediated dilation responses using baseline brachial artery diameter as a covariate did not alter our results (interaction effect: $P = 0.04$). Peak diameter, time to peak diameter, shear rate AUC, blood flow AUC, and peak blood flow were not affected by ischemia-reperfusion injury or condition (Table 3).

Table 1. *Participants' characteristics*

Variables	Means ± SD
n, women	12 (5)
Age, yr	57 ± 9
Height, cm	173 ± 8
Body weight, kg	75.8 ± 13.4
Body mass index, kg/m^2	25.4 ± 4.4
Systolic blood pressure, mmHg	109 ± 10
Diastolic blood pressure, mmHg	72 ± 9
Heart rate, beats/min	59 ± 10
Physical activity levels, h/week	3.9 ± 1.9
Medication use, *n*	
Angiotensin II blocker	1
Diuretic	1
Statin	2
Proton-pump inhibitor	1
Estrogen and progesterone	1
Levothyroxine	1

Table 2. *Hemodynamics and pulmonary gas exchange during intermittent normoxia and intermittent hypoxia*

Variables	Normoxia	Hypoxia
Arterial oxygen saturation, %	97 ± 1	80 ± 2*
Systolic blood pressure, mmHg	121 ± 6	122 ± 10
Diastolic blood pressure, mmHg	76 ± 9	78 ± 9
Mean arterial pressure, mmHg	91 ± 8	92 ± 9
Heart rate, beats/min	60 ± 10	68 ± 10*
Stroke volume, mL	83 ± 22	83 ± 24
Cardiac output, L/min	5.1 ± 1.4	5.8 ± 1.8
Total peripheral resistance, mmHg/L/min	19.7 ± 5.9	18.5 ± 8.3
Fraction of inspired oxygen, %	21.0 ± 0.3	11.4 ± 0.7*
Respiratory rate, breaths/min	12.6 ± 4.2	12.6 ± 2.5
Tidal volume, mL	475 ± 107	656 ± 208
Minute ventilation, L/min	5.7 ± 2.3	8.0 ± 3.3
End-tidal CO_2, mmHg	37.3 ± 4.0	36.2 ± 3.1
End-tidal oxygen, mmHg	102 ± 7	45 ± 4*

A paired *t* test was used to evaluate the effect of condition on pulmonary gas exchange and hemodynamic variables; $n = 12$, 5 women. *$P < 0.05$ between intermittent hypoxia and intermittent normoxia.

DISCUSSION

The present study sought to determine whether exposure to intermittent hypoxia attenuates ischemia-reperfusion injury in older adults. In accordance with our hypothesis, three 4-min hypoxic cycles at a targeted oxygen saturation

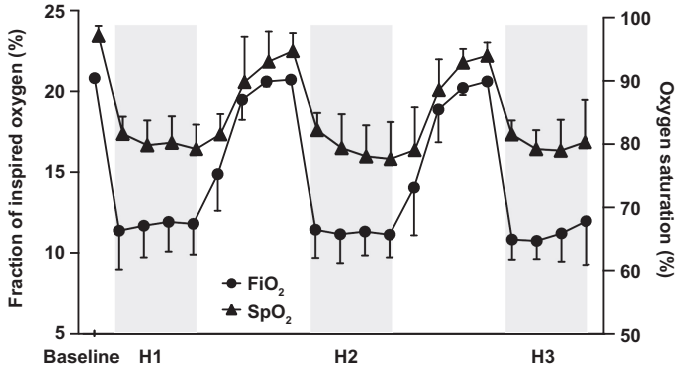

Figure 2. Peripheral oxygen saturation (Sp_{O_2}) and fraction of inspired oxygen (FI_{O2}) responses to three 4-min hypoxic cycles at a targeted oxygen saturation of 80% (H1 to H3) interspersed with four min of breathing room air. $n = 12$, 5 women.

Table 3. *Brachial artery characteristics before and after ischemia-reperfusion during intermittent normoxia or intermittent hypoxia*

Variables	Normoxia		Hypoxia		Time	Condition	Interaction
	Pre	Post	Pre	Post			
Flow-mediated dilation, %	6.5 ± 1.7	2.4 ± 1.7*	5.6 ± 1.7	3.6 ± 2.3†	<0.01	0.83	0.03
Baseline diameter, mm	3.74 ± 0.61	4.01 ± 0.79*	3.75 ± 0.62	3.88 ± 0.77‡	0.01	0.03	0.01
Peak diameter, mm	3.98 ± 0.61	4.11 ± 0.82	3.95 ± 0.62	4.01 ± 0.75	0.18	0.07	0.21
Change in diameter, mm	0.24 ± 0.05	0.10 ± 0.07	0.20 ± 0.05	0.13 ± 0.07	<0.01	0.91	0.05
Time to peak diameter, s	50 ± 13	48 ± 21	47 ± 12	49 ± 12	0.99	0.89	0.45
Shear rate AUC	6,478 ± 3,098	5,657 ± 1,511	7,180 ± 5,333	7,516 ± 5,051	0.74	0.38	0.40
Blood flow AUC	3,225 ± 1,751	3,548 ± 2,281	3,682 ± 2,665	4,090 ± 3,176	0.45	0.31	0.87
Peak blood flow, mL/min	69 ± 38	106 ± 75	81 ± 58	113 ± 99	0.09	0.48	0.80

A two-way repeated-measures analysis of variance was used to evaluate the effect of time and condition on measures of vascular function; n = 12 (5 women) for all measures beside n = 11 (4 women) for time to peak diameter, shear rate AUC, blood flow AUC, and peak blood flow. AUC, area under the curve. *Different from normoxia pre, †different from hypoxia pre, ‡different from normoxia post.

of 80% attenuated the reduction in flow-mediated dilation induced by an ischemia-reperfusion injury in older adults. Specifically, hypoxic preconditioning halved the reduction in flow-mediated dilation after an ischemia-reperfusion injury, with decreases of 4.1 ± 2.6% and 2.0 ± 1.5% corresponding to 95% confidence intervals of [2.7, 5.6] and [1.1, 2.8] observed with intermittent normoxia and intermittent hypoxia, respectively. It is hypothesized that the systemic nature of the intervention, which results in a greater volume of tissue exposed to reduced oxygen levels, explains the efficacy of hypoxic preconditioning in attenuating ischemia-reperfusion injury in older adults.

Short intermittent ischemic bouts precondition the endothelium to subsequent prolonged ischemia. Indeed, intermittent ischemic bouts induce nitric oxide and reactive oxygen species formation, which activates ATP-sensitive potassium channels and upregulates antioxidant defenses by suppressing platelet aggregation and leukocyte adherence to the endothelium (3, 18, 19). Other interventions that stimulate reactive oxygen species and enhance nitric oxide bioavailability may also be effective against ischemia-reperfusion injury. In this context, mild levels of intermittent hypoxemia, induced by breathing low levels of oxygen, stimulate both reactive oxygen species production and nitric oxide synthesis (20–23). We previously demonstrated that exposure to three 4-min hypoxic cycles at a targeted oxygen saturation of 90% attenuates by a third the reduction in flow-mediated dilation following an ischemia-reperfusion injury in young adults (12). Based on these previous findings, we hypothesized that exposure to a greater hypoxic severity may further protect against ischemia-reperfusion injury. Moreover, hypoxia at an oxygen saturation of ~82% was

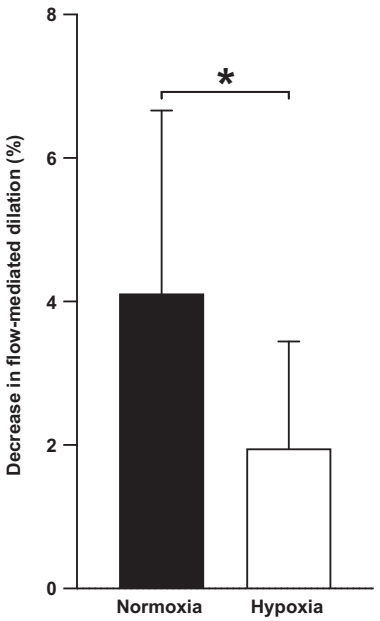

Figure 3. Average change in brachial artery flow-mediated dilation induced by ischemia-reperfusion injury with intermittent normoxia and intermittent hypoxia. A paired t test was used to evaluate the effect of the condition. *$P < 0.05$ between intermittent hypoxia and intermittent normoxia. n = 12, 5 women.

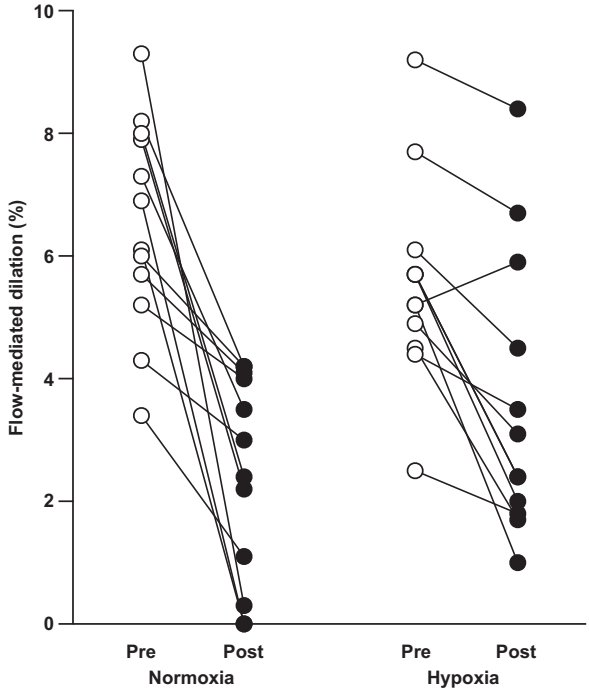

Figure 4. Individual brachial artery flow-mediated dilation before and after ischemia-reperfusion injury with intermittent normoxia and intermittent hypoxia. A two-way analysis of variance was used to evaluate the effect of time and condition. n = 12, 5 women.

reported to increase forearm vasodilation in a population with reduced nitric oxide bioavailability (24). The present study showed that the same number of cycles at a greater hypoxic severity triggering an oxygen saturation of 80% further attenuated the reduction in flow-mediated dilation following ischemia-reperfusion injury in older adults. Since small bursts of reactive oxygen species and nitric oxide activate neuroprotective pathways against a subsequent ischemia-reperfusion injury (3, 18, 19), future studies should assess nitric oxide metabolism and oxidative stress to clarify the mechanisms by which hypoxic preconditioning protects against endothelial dysfunction.

The reduction in brachial artery diameter assessed by flow-mediated dilation following ischemia-reperfusion injury was smaller with intermittent hypoxia than with intermittent normoxia. Of note, baseline brachial artery diameters increased following ischemia-reperfusion injury with both intermittent hypoxia and intermittent normoxia, which is consistent with the previously reported sustained dilation of the brachial artery 15 min after an ischemia-reperfusion injury (4, 7, 12, 25) due to endothelium-independent vasodilation caused by the prolonged blood flow occlusion (26). However, the magnitude of the increase in baseline brachial artery diameter was greater with intermittent normoxia than with intermittent hypoxia. Because of the inverse relation between baseline diameter and flow-mediated dilation (27), the greater increase in baseline brachial artery diameter following intermittent normoxia may relate to the greater decrease in flow-mediated dilation observed in this condition. Analyzing the flow-mediated dilation responses using baseline brachial artery diameter as a covariate did not alter our results, confirming that there was an attenuated reduction in flow-mediated dilation after ischemia-reperfusion injury with intermittent hypoxia when compared with intermittent normoxia.

There was no prolonged lasting effect of hypoxic preconditioning on baseline flow-mediated dilation. In our previous study in young adults (12), conditions were separated by a period of 7 days in men and women using contraceptives, with half of the participants being first exposed to intermittent hypoxia and the other half to intermittent normoxia. We reported that baseline flow-mediated dilation was similar between conditions when intermittent hypoxia was performed before intermittent normoxia. In the present study, we similarly observed that baseline flow-mediated was not different between conditions when intermittent hypoxia was performed before intermittent normoxia (hypoxia: 6.1 ± 1.2% and normoxia: 6.9 ± 1.8%, P = 0.22), suggesting that a single acute bout of intermittent hypoxia does not induce a prolonged protective effect on endothelial function.

We and others previously reported that repetitive hypoxic bouts, consisting of 4–6 min at a fraction of inspired oxygen of 10%–12%, do not affect blood pressure in young healthy individuals (12, 28, 29). In the present study, we demonstrated that short hypoxic bouts at a fraction of inspired oxygen of 11% also do not affect blood pressure in older adults. Exposure to intermittent hypoxia usually increases minute ventilation. Indeed, five 6-min hypoxic bouts at oxygen levels of 10% or at a targeted oxygen saturation of 80% and seven 5-min hypoxic bouts at an oxygen saturation of 70%–80% increased minute ventilation (30–32). In contrast, our intermittent hypoxia protocol of three 4-min hypoxic cycles at a fraction of inspired oxygen of 11% did not significantly affect minute ventilation, which is consistent with our previous findings that exposure to five or eight 4-min cycles at a fraction of inspired oxygen of 10% does not affect minute ventilation (33). Thus, hypoxic preconditioning consisting of three 4-min hypoxic cycles at a targeted oxygen saturation of 80% has minimal impact on hemodynamics and pulmonary gas exchange in older adults.

Of note, three participants taking medications that affect flow-mediated dilation and the response to ischemia-reperfusion injury were included in this crossover study (34–36). Interestingly, baseline flow-mediation dilation was similar between these three participants and the group (6.1 ± 2.1% vs. 6.0 ± 1.7%). Moreover, hypoxic preconditioning similarly halved the reduction in flow-mediated dilation following an ischemia-reperfusion injury in these participants, with decreases of 3.2 ± 2.2% and 1.7 ± 1.0% observed with intermittent normoxia and intermittent hypoxia, respectively.

Perspectives and Significance

Intermittent hypoxia attenuates endothelial dysfunction following ischemia-reperfusion injury in older adults. Thus, hypoxic preconditioning represents a well-tolerated and effective intervention to immediately protect against ischemia-reperfusion injury. The systemic nature of the intervention appears to provide greater protection than local or remote ischemic preconditioning against ischemia-reperfusion injury in older adults at greater risk for ischemic events. Hypoxic preconditioning could therefore be used as an adjunct intervention to current reperfusion strategies to further protect against ischemia-reperfusion injury in patients undergoing non-emergency therapeutic reperfusion. Future studies should determine whether additional hypoxic cycles provide greater protection against ischemia-reperfusion injury and determine the influence of cardiovascular risk factors such as hypercholesteremia, diabetes, and hypertension on the efficacy of hypoxic preconditioning in older adults.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

H.T. and S.L. conceived and designed research; S.S.-G., S.D.M., and F.W. performed experiments; S.S.-G. analyzed data; S.S.-G. and S.L. interpreted results of experiments; S.S.-G. prepared figures; S.S.-G. drafted manuscript; S.D.M., F.W., H.T., and S.L. edited and revised manuscript; S.S.-G., S.D.M., F.W., H.T., and S.L. approved final version of manuscript.

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