J Appl Physiol 130: 846–852, 2021.
First published January 7, 2021; doi:10.1152/japplphysiol.00772.2020

american physiological society | JOURNAL OF APPLIED PHYSIOLOGY

RESEARCH ARTICLE

Hypoxia

Hypoxic preconditioning attenuates ischemia-reperfusion injury in young healthy adults

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Abstract

Ischemic preconditioning attenuates the reduction in brachial artery endothelial function following an ischemia-reperfusion injury. Brief bouts of systemic hypoxemia could similarly mitigate the blunted vasodilatory response induced by an ischemia-reperfusion injury. The aim of the present study was to determine whether an acute bout of intermittent hypoxia protects against an ischemia-reperfusion injury in young healthy individuals. Brachial artery endothelial function was assessed by flow-mediated dilation in 16 young healthy individuals before and after a 20-min upper arm blood flow occlusion to induce ischemia-reperfusion injury. Blood flow occlusion was preceded by either intermittent hypoxia or intermittent normoxia. Intermittent hypoxia consisted of three 4-min hypoxic cycles at an arterial oxygen saturation of 87 ± 3% separated by 4-min normoxic cycles. Intermittent hypoxia resulted in a lower arterial oxygen saturation than intermittent normoxia (hypoxia: 87 ± 3% vs. normoxia: 99 ± 1%, $P < 0.01$), which was equivalent to a lower fraction of inspired oxygen (hypoxia: 0.123 ± 0.013 and normoxia: 0.210 ± 0.003, $P < 0.01$). When preceded by intermittent normoxia, blood flow occlusion resulted in a blunted flow-mediated dilation. In contrast, the reduction in flow-mediated dilation following blood flow occlusion was attenuated by prior exposure to intermittent hypoxia (hypoxia: 6.4 ± 1.9 to 4.4 ± 2.3% and normoxia: 7.1 ± 2.5 to 4.0 ± 2.4%, time × condition interaction $P = 0.048$). Exposure to intermittent hypoxia did not affect mean arterial pressure (hypoxia: 92 ± 9 mmHg and normoxia: 89 ± 8 mmHg, $P = 0.19$) or cardiac output (hypoxia: 5.8 ± 1.1 L·min^{-1} and normoxia: 5.3 ± 1.1 L·min^{-1}, $P = 0.29$). In conclusion, hypoxic preconditioning attenuates the reduction in flow-mediated dilation induced by blood flow occlusion in young healthy individuals. Intermittent hypoxia represents a potential strategy to mitigate the effect of ischemia-reperfusion injury associated with ischemic events.

NEW & NOTEWORTHY Ischemia-reperfusion injury induced by restoration of blood flow following occlusion impairs flow-mediated dilation, a marker of endothelium-dependent vasodilation. In young healthy adults, exposure to intermittent hypoxia, consisting of alternating short bouts of breathing hypoxic and normoxic air, before an ischemia-reperfusion injury significantly attenuated the reduction in flow-mediated dilation. Thus, hypoxic preconditioning represents a potential strategy to mitigate the effect of ischemia-reperfusion injury associated with ischemic events.

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

INTRODUCTION

Ischemic heart disease represents the most common form of cardiovascular disease and the main cause of mortality in the United States (1). Restricted blood flow to arteries, or ischemia, causes a shortage of oxygen needed for cellular metabolism in the surroundings tissues (2). Restoration of blood flow prevents tissue death; however, the sudden reperfusion paradoxically damages endothelial cells lining the blood vessels (2). Indeed, this ischemia-reperfusion injury impairs endothelium-dependent vasodilation, as assessed by flow-mediated dilation of the brachial artery, by as much as 50% in young healthy adults (3–6). An excessive formation of reactive oxygen species and a reduced nitric oxide bioavailability contribute to the endothelial dysfunction associated with ischemia-reperfusion injury (7, 8). In the myocardium, ischemia-reperfusion injury induces coronary endothelial dysfunction, which in turn promotes myocardial infarction. Interventions that attenuate ischemia-reperfusion injury are therefore needed to prevent myocardial infarction in patients with ischemic heart disease.

Brief periods of local ischemia performed before a prolonged ischemia offer a protective effect against ischemia-reperfusion injury. Ischemic preconditioning consisting of three 5-min bouts of ischemia separated by 5 min prevents the reduction in flow-mediated dilation in response to an ischemia-reperfusion injury in healthy adults (5, 9). Mechanisms of endothelial preconditioning involve a

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Submitted 8 September 2020 / Revised 24 December 2020 / Accepted 29 December 2020

variety of mediators such as nitric oxide and reactive oxygen species, which activate mitochondrial ATP-sensitive potassium channels (10–12). Ischemic preconditioning performed on the contralateral arm also prevents endothelial dysfunction, implying a systemic protection against ischemia-reperfusion injury (5). Interestingly, three, but not two, cycles of ischemic preconditioning performed on the contralateral arm prevent the reduction in flow-mediated dilation of the brachial artery following ischemia-reperfusion injury (5). However, two cycles of ischemic preconditioning applied to the leg had a protective effect against ischemia-reperfusion injury, suggesting that the volume of tissue exposed to the preconditioning influences the degree of protection (5). Nonetheless, the protective effect of ischemic preconditioning against ischemia-reperfusion injury has not been observed in populations at risk for ischemic events (13, 14).

Intermittent hypoxia represents a potential systemic strategy to prevent the reduction in flow-mediated dilation following ischemia-reperfusion injury. Intermittent hypoxia consists of alternating bouts of breathing hypoxic and normoxic air. Intermittent hypoxemia induces a nitric oxide-dependent vasodilation and an increase in reactive oxygen species, caused by both the hypoxia and the reoxygenation, leading to a protective effect against a subsequent ischemia-reperfusion injury (15, 16). Indeed, hypoxic preconditioning protects against ischemia-reperfusion injury by reducing oxidative stress and stimulating nitric oxide-induced vasodilation during ischemia-reperfusion injury in animals (17). Moreover, systemic hypoxemia induces the same acute absolute forearm blood flow responses in young and older individuals (18, 19), suggesting that hypoxic preconditioning could protect against ischemia-reperfusion injury in populations at risk for ischemic events. Therefore, the aim of this study was to first determine whether hypoxic preconditioning induces a systemic protection against ischemia-reperfusion injury in young healthy adults. We hypothesized that prior exposure to short bouts of intermittent hypoxia would attenuate the reduction in flow-mediated dilation induced by an ischemia-reperfusion injury.

METHODS

Sixteen healthy individuals (7 women and 9 men, age: 23 ± 3 yr, height: 175 ± 9 cm, body weight: 72.9 ± 13.4 kg, body mass index: 23.7 ± 2.7 kg·m^{-2}) participated in the study. All participants provided informed written consent for participating in the study, which was approved by the Institutional Review Board of the University of Texas at Austin. Participants were excluded from the study if they had a history of cardiovascular disease, diabetes or lung disease, had a resting blood pressure over 130/80 mmHg following 15 min of supine rest, took medication affecting the cardiovascular system, were pregnant, or were smokers. Participants visited the laboratory on two occasions (Fig. 1). On both visits, brachial artery endothelial function was assessed before and 15 min after inducing an ischemia-reperfusion injury by inflating a cuff placed on the upper right arm to 250 mmHg for a period of 20 min (3, 4, 9). On *day 1*, a 20-min intermittent hypoxia protocol immediately preceded the blood flow occlusion (hypoxia). On *day 2*, a 20-min intermittent normoxia protocol immediately preceded the blood flow occlusion (normoxia). The visit order was randomized, and participants were blinded to the condition. Participants avoided intense physical activity on the day before both visits and reported to the laboratory in the morning after fasting for at least 10 h. Visits were separated by a period of 7 days for men and women using contraceptives. In women not using contraceptives, visits were separated by 1 mo and took place in the early follicular phase of the menstrual cycle.

Intermittent Hypoxia and Intermittent Normoxia

The intermittent hypoxia protocol consisted of three 4-min hypoxic cycles interspersed with 4-min normoxic cycles. Participants were in a semirecumbent position during the breathing protocol. Hypoxic air was inhaled through a mask connected to a two-way non-rebreathing valve, which was connected to a 5-L non-diffusing gas bag (Hans Rudolph, Shawnee, KS). The non-diffusing bag was connected to a gas tank of compressed air. Air was made hypoxic by titrating nitrogen into the breathing circuit. The flow of nitrogen was controlled to achieve an arterial oxygen saturation of 90%, as measured by pulse oximetry. Due to the high individual variability in hypoxic ventilatory responses, a fixed fraction of inspired oxygen can result in a wide range of arterial oxygen saturation across individuals. Therefore, intermittent hypoxia was not performed at a fixed oxygen level but at a targeted arterial oxygen saturation in order to induce the same level of hypoxemia in all participants. This intermittent hypoxia protocol is mild as it represents less than 10 cycles of hypoxia lasting between 15 s and 4 min at a fraction of inspired oxygen between 0.10 and 0.14 (20). Unlike chronic hypoxic exposure, risks associated with an acute hypoxic exposure of three 4-min cycles are minimal (18, 19). Moreover, five bouts of 6 min of intermittent hypoxia reducing arterial oxygen saturation to 67% do not cause any discomfort (21). Intermittent normoxia consisted of the same protocol, but nitrogen was not added to the breathing circuit.

Brachial Artery Endothelial Function

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) equipped with a high-resolution linear-array transducer (22). Heart rate and blood pressure were obtained following 15 min of supine rest in a dimly lit laboratory room. Participants extended their right arm 90° away

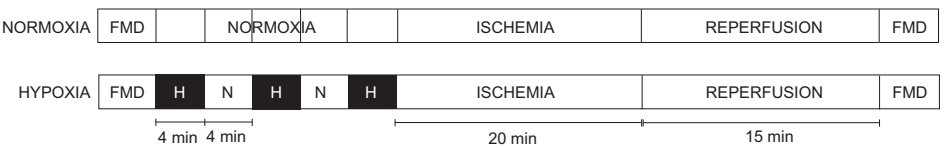

Figure 1. Study protocol. FMD, flow-mediated dilation; H, hypoxia; N, normoxia.

from their body at heart level. A transducer was positioned over the brachial artery 2–8 cm proximally to the antecubital fossa and a cuff was placed 0.5–2.0 cm distal to the antecubital fossa. Cross-sectional images of the artery were acquired utilizing the automated transducer, which self-adjusted to provide a clear longitudinal image of the artery. Forearm occlusion was induced with a cuff inflator to a supra-systolic pressure for 5 min. Beat-by-beat blood velocity and brachial artery diameters were recorded for ~80 s following cuff release. Baseline brachial artery diastolic diameter was determined semiautomatically, and changes in diameter were tracked automatically by the ultrasound system following cuff release. Blood flow was calculated by multiplying the cross-sectional area of the brachial artery by blood velocity, and blood flow area under the curve (AUC) was summed through the duration of the recording. Shear rate was calculated by multiplying 8 by the quotient of blood flow velocity and brachial artery diameter. Shear rate AUC was summed through peak diameter.

Pulmonary Gas Exchange and Hemodynamics

Breath-by-breath measures of pulmonary gas exchange were collected using a metabolic cart calibrated with standardized gas and room air (Ultima Cardio2, MGC Diagnostics, MN), and analyzed every 10 s throughout both the intermittent hypoxia and intermittent normoxia protocol. The pneumotachometer was mounted between the mask and the nonrebreathing valve of the breathing circuit. Hemodynamics and arterial oxygen saturation were continuously monitored by finger plethysmography and pulse oximetry throughout the protocol (NOVA, Finapres Medical Systems, Amsterdam, The Netherlands). Brachial arterial blood pressure, heart rate, stroke volume, cardiac output, and total peripheral resistance were derived from an arterial waveform obtained by finger plethysmography, a method validated against invasive measures (23). Level correction and an upper arm cuff return-to-flow measurement were used to reconstruct the intrabrachial arterial pressure from the finger arterial pressure (24). All data were recorded in LabChart for later analyses (Powerlab, ADInstruments).

Data and Statistical Analyses

Average values for pulmonary gas exchange and hemodynamic variables were calculated for the last minute of each hypoxic cycle of the intermittent hypoxia protocol and for the last minute of each normoxic cycle of the intermittent normoxia protocol. A one-way repeated-measures analysis of variance was used to evaluate the effect of each cycle of intermittent hypoxia and intermittent normoxia on physiological variables. A paired t test was used to evaluate the effect of condition (normoxia vs. hypoxia) on pulmonary gas exchange, hemodynamics, arterial oxygen saturation, and change in flow-mediated dilation. Two-way repeated-measures ANOVA was used to evaluate the effect of time (pre- vs. post-blood flow occlusion) and condition (normoxia vs. hypoxia) on measures of vasodilator function. When main effects or a time and condition interaction were significant, post hoc analyses were performed using Tukey's test. With a power of 0.80 and an α level of 0.05, a minimum sample size of 12 individuals was needed to detect a 2.0% difference in the post-ischemia-reperfusion flow-mediated dilation between conditions with a standard deviation of 2.2% (25). Using the reduction in flow-mediated dilation induced by the ischemia-reperfusion injury in both conditions in our 16 individuals resulted in a power of 0.72. $P < 0.05$ was considered statistically significant. All values are reported as means ± standard deviation.

RESULTS

Participants had a resting heart rate of 58 ± 8 beats/min and a resting systolic and diastolic blood pressure of 118 ± 11 and 66 ± 6 mmHg, respectively, as measured following 15 min of supine rest on the normoxia day. There were no significant differences in any hemodynamic and pulmonary gas exchange variables across the three hypoxic cycles of the intermittent hypoxia protocol, therefore, an average value was calculated for each variable. Similarly, there were no differences in any variables across the three normoxic cycles of the intermittent normoxia protocol, and average values were calculated for each variable. By design, intermittent hypoxia induced a reduction in arterial oxygen saturation, end-tidal O_2, and fraction of inspired oxygen compared with intermittent normoxia (Table 1). When compared with intermittent normoxia, there was no effect of intermittent hypoxia on respiratory rate, tidal volume, minute ventilation, and end-tidal CO_2 (Table 1). Similarly, intermittent hypoxia did not significantly affect systolic blood pressure, diastolic blood pressure, mean arterial pressure, cardiac output, stroke volume, or total peripheral resistance. However, intermittent hypoxia significantly increased heart rate compared with intermittent normoxia (Table 1).

Hypoxic preconditioning attenuated the reduction in flow-mediated dilation following blood flow occlusion (Table 2). Indeed, the reduction in flow-mediated dilation was significantly lower with intermittent hypoxia compared with intermittent normoxia (Fig. 2). Brachial artery baseline diameters and peak diameters were greater following blood flow occlusion in both conditions (Table 2). However, the

Table 1. *Hemodynamic and pulmonary gas exchange variables during intermittent normoxia and intermittent hypoxia*

Variable	Normoxia	Hypoxia
Systolic blood pressure, mmHg	121 ± 11	123 ± 11
Diastolic blood pressure, mmHg	74 ± 9	76 ± 10
Mean arterial pressure, mmHg	89 ± 8	92 ± 9
Heart rate, beats/min	62 ± 7	71 ± 6*
Stroke volume, mL	87 ± 19	82 ± 17
Arterial oxygen saturation, %	99 ± 1	87 ± 3*
Cardiac output, L·min^{-1}	5.3 ± 1.1	5.8 ± 1.1
Total peripheral resistance, mmHg·L^{-1}·min^{-1}	17.8 ± 3.6	16.8 ± 3.1
Respiratory rate, breaths·min^{-1}	13 ± 4	13 ± 3
Tidal volume, mL	632 ± 259	608 ± 191
Minute ventilation, L·min^{-1}	7.33 ± 2.50	7.17 ± 1.37
End-tidal CO_2, mmHg	37.1 ± 3.3	35.3 ± 3.4
End-tidal O_2, mmHg	103.5 ± 4.7	53.6 ± 8.4*
Fraction of inspired oxygen, %	21.0 ± 0.3	12.3 ± 1.3*

A paired t test was used to evaluate the effect of condition on pulmonary gas exchange and hemodynamics; n = 16. *$P < 0.05$ vs. normoxia.

Table 2. *Brachial artery characteristics pre- and post-blood flow occlusion with intermittent normoxia and intermittent hypoxia*

Variable	Normoxia		Hypoxia		Time	Condition	Interaction
	Pre	Post	Pre	Post			
Flow-mediated dilation, %	7.1 ± 2.5	4.0 ± 2.4	6.4 ± 1.9	4.4 ± 2.3	<0.01	0.64	0.048
Baseline diameter, mm	3.94 ± 0.70	4.19 ± 0.83	3.98 ± 0.71	4.21 ± 0.81	<0.01	0.63	0.88
Peak diameter, mm	4.20 ± 0.68	4.34 ± 0.80	4.23 ± 0.73	4.39 ± 0.80	0.03	0.64	0.85
Change in diameter, mm	0.27 ± 0.07	0.15 ± 0.08	0.25 ± 0.06	0.17 ± 0.08	<0.01	0.93	0.07
Time to peak diameter, s	55 ± 12	52 ± 14	60 ± 11	56 ± 12	0.45	0.09	0.88
Shear rate, AUC	13,188 ± 10,794	8,157 ± 7,084	13,636 ± 11,606	9,182 ± 6,922	0.01	0.62	0.79
Blood flow, AUC	8,128 ± 9,437	6,626 ± 7,782	8,110 ± 8,061	6,749 ± 6,732	0.12	0.96	0.88

AUC, area under the curve. Two-way repeated-measures ANOVA was used to evaluate the effect of time and condition on measures of vasodilator function; $n = 16$.

change in diameter and shear rate AUC were smaller following blood flow occlusion in both conditions. Time to peak diameter was not affected by blood flow occlusion in both conditions. Both intermittent hypoxia and blood flow occlusion were well tolerated by the participants.

DISCUSSION

The aim of this study was to determine whether short exposure to intermittent hypoxia prevents the reduction in flow-mediated dilation induced by an ischemia-reperfusion injury in young healthy adults. Under normoxic conditions, blood flow occlusion induced a 43% reduction in flow-mediated dilation, similar to the usually reported 36%–50% decreases in flow-mediated dilation following an ischemia-reperfusion injury in healthy individuals (3–6). As hypothesized, exposure to three 4-min cycles of mild levels of hypoxia acutely attenuated the reduction in flow-mediated dilation measured 15 min following blood flow occlusion in young healthy individuals. Intermittent normoxia and intermittent hypoxia resulted in a 3.1% and 2.0% reduction in flow-mediated dilation following blood flow occlusion, respectively.

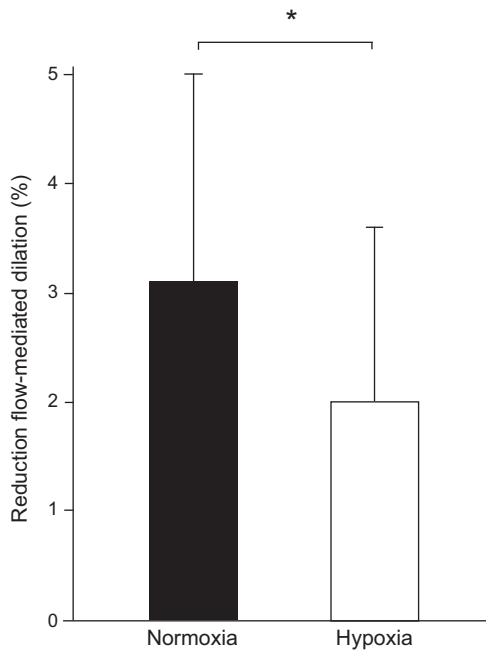

Figure 2. Reduction in brachial artery flow-mediated dilation induced by upper arm blood flow occlusion with intermittent normoxia and intermittent hypoxia. *$P < 0.05$ vs. normoxia. A paired t test was used to evaluate the effect of condition; $n = 16$.

Brief periods of hypoxic preconditioning presumably protect against endothelial dysfunction induced by an ischemia-reperfusion injury through acute changes in the balance between reactive oxygen species and nitric oxide levels (17, 26). The reduced arterial oxygen content associated with intermittent hypoxia results in an increased production of reactive oxygen species (15, 16, 27). Although excessive reactive oxygen species production causes lipid peroxidation leading to endothelial dysfunction (28), low levels of reactive oxygen species generated by mild intermittent hypoxia appear to have a protective effect against ischemia-reperfusion injury (29–32). Deoxyhemoglobin produces nitric oxide, which induces an endothelium-dependent vasodilation during hypoxic conditions (15, 33). Nitric oxide also plays an ambiguous role on vascular function as small increases in nitric oxide production act as a protective mechanism, whereas large increases in nitric oxide production lead to the formation of additional reactive oxygen species upon reperfusion, further contributing to endothelial dysfunction (17, 34). Based on these previous findings in animal models, it is suggested that short-cyclic bouts of hypoxia and normoxia trigger both acute and chronic changes in the balance between reactive oxygen species and nitric oxide levels, and thereby provide a protective effect against ischemia-reperfusion injury in humans. In humans, adenosine receptor activation reportedly plays an important role in initiating remote ischemic preconditioning through the release of circulating cardioprotective factor(s) (35). Systemic hypoxia increases skeletal muscle interstitial adenosine levels (36), therefore, adenosine may also be involved in the protective effect of intermittent hypoxia against ischemia-reperfusion injury. Although not performed in the present study, assessment of nitric oxide bioavailability, reactive oxygen species production, and adenosine receptor activation would clarify the mechanisms by which an acute bout of intermittent hypoxia protects endothelial function against ischemia-reperfusion injury in humans.

Short bouts of poikilocapnic intermittent hypoxia at an average arterial oxygen saturation of 87% equivalent to an average fraction of inspired oxygen of 0.12 did not affect pulmonary gas exchange or blood pressure in young healthy

individuals. The lack of effect on ventilation may result from the titration of nitrogen into the breathing circuit to a targeted arterial oxygen saturation, and to the short duration of the hypoxic cycles. The observed lack of effect of intermittent hypoxia on blood pressure is in agreement with previous findings that repetitive bouts of normobaric, poikilocapnic hypoxia consisting of 4–6 min at a fraction of inspired oxygen of 0.10–0.12 did not affect arterial blood pressure in young healthy individuals (37, 38). Because hypercapnia is responsible for the sustained sympathoexcitation associated with longer exposure to hypoxia (39), a maintained end-tidal CO_2 during short-hypoxic bouts likely contributes to the lack of increase in blood pressure observed with intermittent hypoxia.

In men and women using contraceptives, visits were separated by a period of 7 days. However, the duration of the beneficial effect of an acute bout of intermittent hypoxia on flow-mediated dilation remains unknown. The protective effect of seven consecutive days of remote ischemic preconditioning on flow-mediated dilation was observed to persist for up to 8 days after cessation of the intervention (40). In the present study, the order of the conditions was randomized, with half of the participants being first exposed to intermittent hypoxia and the other half to intermittent normoxia. Moreover, baseline flow-mediated dilation was similar between conditions (normoxia: 8.3 ± 2.5% and hypoxia: 7.5 ± 2.4%, $P = 0.13$) for the individuals who performed the intermittent hypoxia protocol before the intermittent normoxia protocol, suggesting that a single acute bout of intermittent hypoxia does not induce a prolonged lasting protective effect on endothelial function. Similarly, there was no significant difference in baseline flow-mediated dilation for all participants between conditions (normoxia: 7.1 ± 2.5% and hypoxia: 6.4 ± 1.9%, $P = 0.08$). However, it is possible that a tendency for a greater baseline flow-mediated dilation with intermittent normoxia contributed to the greater reduction in flow-mediated dilation following ischemia-reperfusion injury.

Although ischemic preconditioning prevents the decrease in flow-mediated dilation in young healthy individuals (5, 9), the protective effect of ischemic preconditioning against ischemia-reperfusion injury has not been observed in older healthy individuals (14) or patients with heart failure (13). Thus, ischemic preconditioning may provide limited protection against ischemia-reperfusion injury in populations at risk for ischemic events. The present study shows that short exposure to mild levels of intermittent hypoxia was sufficient to attenuate the reduction in flow-mediated dilation induced by an ischemia-reperfusion injury in young healthy individuals. Most importantly, systemic hypoxemia induces the same acute absolute forearm blood flow responses in young and older individuals (18, 19), and patients with type 2 diabetes demonstrate an even greater hypoxic vasodilation than age-matched healthy individuals (41), suggesting that hypoxic preconditioning could protect against ischemia-reperfusion injury in populations at risk for ischemic events. In addition, there are no safety concerns associated with exposure to intermittent hypoxia. On the contrary, repeated exposure to intermittent hypoxia reduced systolic blood pressure in obese individuals and patients with hypertension (42, 43), improved endothelial function in patients with stable angina (44), and enhanced exercise tolerance in patients with coronary artery disease (45). Moreover, chronic exposure to hypoxia reduces mortality from ischemic heart disease and arteriosclerotic disease in individuals residing at altitude (46, 47). Thus, hypoxic preconditioning represents a promising alternative to ischemic preconditioning in clinical populations. It remains to be determined whether exposure to a greater hypoxic severity could fully prevent ischemia-reperfusion injury, and whether hypoxic preconditioning prevents ischemia-reperfusion injury in individuals at greater risk for ischemic events such as older individuals and patients with type 2 diabetes.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

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

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