The Thesis Committee for Caitlin Patricia Jarrard
Certifies that this is the approved version of the following Thesis:

Intermittent Hypoxia Attenuates Ischemia-Reperfusion Injury

APPROVED BY

SUPERVISING COMMITTEE:

Sophie Lalande, Supervisor

Hirofumi Tanaka

Intermittent Hypoxia Attenuates Ischemia-Reperfusion Injury

by

Caitlin Patricia Jarrard

Thesis

Presented to the Faculty of the Graduate School of

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Master of Science in Kinesiology

The University of Texas at Austin

May 2020

Dedication

This document is dedicated to my parents, David and Karen. Both of you have given me endless support and have always encouraged me to follow my dreams. I couldn't have gotten to the point I am at today and so I thank both of you. I love both of you so much.

Acknowledgements

Thank you to my advisor Sophie Lalande. I have learned so much being in your lab throughout these last couple years. With your support I have had the ability to grow as a student, teacher, and scientist here at UT. Words do not describe how grateful I am for everything you have given me.

Thank you to Dr. Tanaka for allowing me to collaborate with his lab and always lending a helping hand whenever I have needed it. Thank you to Mercedes Nagel and Sten Stray-Gundersen for assisting me during these studies.

To my friends Mel, Jon, and Anthony. I couldn't have gotten through this program without you three by my side. Through the ups and downs I always knew I could count on any of you. All of you have made my time here unforgettable and I love all of you so much.

Abstract

Intermittent Hypoxia Attenuates Ischemia-Reperfusion Injury

Caitlin Patricia Jarrard, M.S.Kin.

The University of Texas at Austin, 2020

Supervisor: Sophie Lalande

Brief periods of ischemia preceding an ischemia-reperfusion injury, known as ischemia preconditioning, attenuate the reduction in brachial artery endothelial function. It remains unknown whether brief bouts of systemic hypoxemia would similarly mitigate the blunted vasodilatory response induced by an ischemia-reperfusion injury. The purpose of this study was to determine whether intermittent hypoxia protects against an ischemia-reperfusion injury in young healthy adults. Sixteen healthy individuals, 9 men and 7 women (age: 23 ± 3 years, height: 175 ± 9 cm, body weight: 72.9 ± 13.4 kg), participated in the study. Brachial artery endothelial function was assessed by flow-mediated dilation before and after a 20-minute blood flow occlusion. Blood flow occlusion was preceded by either intermittent hypoxia (Hyp) or intermittent normoxia (Norm). Both visits were separated by a period of seven days. Women who had regular menstrual cycles were scheduled in the early follicular phase. Intermittent hypoxia was created by titrating nitrogen into a breathing system to achieve an arterial oxygen saturation of 90%. Intermittent hypoxia consisted of three 4-minute hypoxic cycles separated by 4-minute normoxic cycles. Intermittent hypoxia resulted in a lower arterial oxygen saturation (Hyp: 87 ± 3 vs. Norm: 99 ± 1%, $p < 0.01$), which was equivalent to a lower fraction of inspired oxygen (Hyp: 0.123 ± 0.013, Norm: 0.210 ± 0.003, $p < 0.01$). When preceded by intermittent normoxia, blood flow occlusion resulted in a blunted flow-mediated dilation (Norm: 7.1 ± 2.5 to

4.0 ± 2.4%). In contrast, the reduction in flow-mediated dilation following blood flow occlusion was attenuated by prior exposure to intermittent hypoxia (Hyp: 6.4 ± 1.9 to 4.4 ± 2.3%, $p = 0.048$). Exposure to intermittent hypoxia did not affect mean arterial blood pressure (Hyp: 96 ± 10, Norm: 96 ± 8 mmHg, $p = 0.90$) but significantly increased heart rate (Hyp: 72 ± 7, Norm: 63 ± 7 bpm, $p < 0.01$). In conclusion, exposure to mild levels of intermittent hypoxia attenuates the reduction in flow-mediated dilation induced by blood flow occlusion in young healthy individuals. Thus, intermittent hypoxia represents a potential strategy to mitigate the effect of ischemia-reperfusion injury associated with ischemic cardiovascular events.

Table of Contents

List of Tables

List of Figures

LITERATURE REVIEW

Ischemia-reperfusion injury

The leading cause of mortality in the developed world stems from cardiovascular disease, with ischemic heart disease being the most prevalent (CDC, 2019). Ischemic heart disease refers to an inadequate oxygen supply to the myocardium due to a narrowing or blockage of the coronary arteries caused by a build-up of plaque. Main treatments of ischemic heart disease consist of medications, lifestyle changes, and procedures such as coronary artery bypass grafting or percutaneous coronary interventions (NHLBI, 2020). While restoration of blood flow is necessary, the sudden return of blood flow also paradoxically causes damage to the endothelial cells lining the coronary arteries that contribute to the control of vascular tone. Thus, both the ischemia and the restoration of blood flow to tissues cause damages to the endothelial cells, a phenomenon commonly referred to as ischemia-reperfusion injury (Kharbanda et al., 2001).

Once reperfusion occurs, the oxygen influx contributes to the creation of reactive oxygen species (ROS) (Kalogeris et al., 2012). Reactive oxygen species occur as a byproduct of mitochondrial oxidative phosphorylation resulting in excess ROS that can produced cell and tissue injury (Sies, 1986). ROS accelerate the inactivation of nitric oxide, and therefore reduce nitric oxide bioavailability (Cai & Harrison , 2000; Kalogeris et al., 2012). Nitric oxide is released from endothelial cells and causes relaxation of the vascular smooth muscles therefore a low bioavailability of nitric oxide attenuates vasodilation (Tiefenbacher et al., 1996). Thus, ischemia-reperfusion injury induces an impaired endothelium-dependent vasodilation. It is therefore important to identify strategies to mitigate the effect of ischemia-reperfusion injury associated with blood flow restoration. However, due to the difficulty to assess the endothelial

function of coronary arteries, the effect of an ischemia-reperfusion injury has to be assessed in alternative blood vessels.

Flow-mediated dilation

Measures of brachial artery endothelial function act as a surrogate measure of coronary artery endothelial function (Anderson et al., 1995). Assessment of endothelial function of the brachial artery consists in measuring the dilation of the brachial artery induced by an increase in blood flow following a short period of occlusion, known as flow-mediated dilation. Briefly, this method consists in measuring the increase in brachial artery diameter following a 5-minute upper arm occlusion induced by inflating a blood pressure cuff to supra-systolic levels (Kharbanda et al., 2001). Flow-mediated dilation occurs when the vessel wall becomes exposed to a frictional force known as shear stress. Blood flow determines shear stress, a vector parallel to the longitudinal axis of the blood vessel (Niebauer & Cooke, 1996). The endothelial cells lining the blood vessel sense this increase in force, thus releasing a variety of vasodilators such as nitric oxide to allow the vascular smooth muscle to relax, consequently decreasing shear stress (Wilson et al., 2016). Therefore, when the blood vessel becomes reperfused following a period of occlusion, the increased blood flow causes an increase in shear stress which activates nitric oxide release. Thus, flow-mediated dilation represents endothelium-dependent vasodilation.

The effect of an ischemia-reperfusion injury can be assessed by determining the effect of blood flow occlusion and reperfusion on brachial artery flow-mediated dilation. An ischemia-reperfusion injury to the brachial artery occurs by occluding blood flow to the arm by inflating a cuff to supra-systolic levels for a period of 20 minutes, which significantly decreases flow-

mediated dilation. Flow-mediated dilation was blunted by 27% following an ischemia-reperfusion injury in young healthy, recreationally active individuals (Brunt et al., 2016). Similarly, flow-mediated dilation was decreased by 50% following an ischemia-reperfusion injury in young sedentary individuals (Figure 1) (Loukogeorgakis et al., 2007). In addition, others (Aboo Bakkar et al., 2018) demonstrated a reduction in flow-mediated dilation of 56% in fourteen physically inactive males.

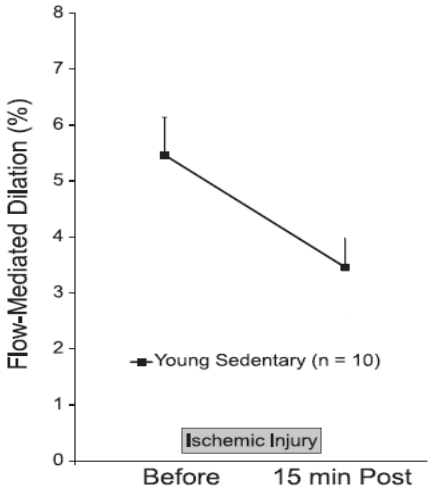

Figure 1. Flow-mediated dilation before and 15 minutes after ischemia-reperfusion injury (adapted from (DeVan et al., 2011). Used with permission from American Physiological Society.

Ischemic preconditioning

Previous studies investigated different interventions to diminish impairment in endothelial function following an ischemia-reperfusion injury. One of these interventions, ischemic preconditioning, consists in a few short bouts of ischemia induced by occluding blood flow to the arm by inflating a cuff to supra-systolic levels prior to the ischemia-reperfusion injury. Ischemic preconditioning largely attenuates the reduction in flow-mediated dilation induced by ischemia-reperfusion injury. Indeed, three 5-minute cycles of alternating ischemia

and reperfusion lessened the attenuation in flow-mediated dilation to 4 - 17% in healthy volunteers (Kharbanda et al., 2001; Loukogeorgakis et al., 2007). Kharbanda *et al.* (2001) preconditioned the vessel three times for 5 minutes at a cuff inflation of 200 mmHg 5-minutes prior to inducing an ischemia-reperfusion injury. Flow-mediated dilation of the radial artery was attenuated by 17% in the preconditioning trial compared to the reduction of 54% without ischemic preconditioning (Figure 2). Using a similar protocol on the contralateral arm, Loukogeorgakis *et al.* (2007) reported a 4% reduction in brachial artery flow-mediated dilation in comparison to a 50% reduction in flow-mediated when there was no ischemic preconditioning.

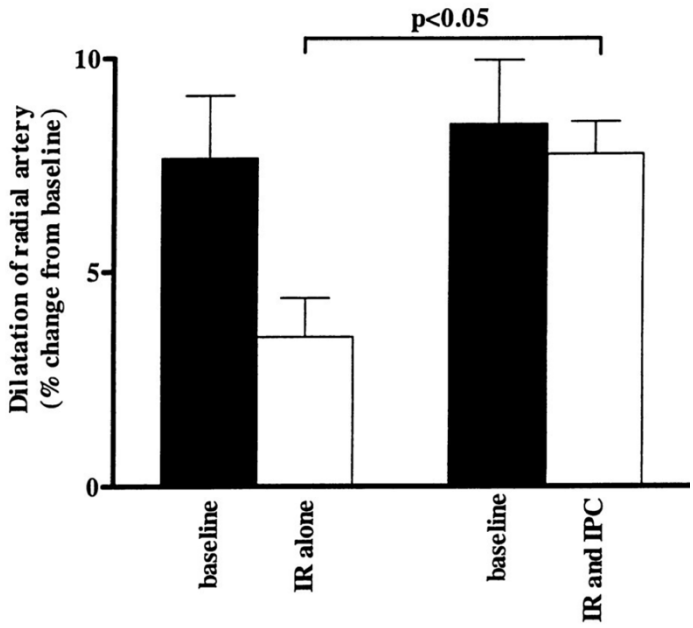

Figure 2. Dilation of the radial artery at baseline, after ischemia-reperfusion (IR) alone, and after IR preceded by ischemic preconditioning (IR and IPC) (Kharbanda et al., 2001). Used with permission from Wolters Kluwer.

Ischemic preconditioning generates ROS allowing for a cardioprotective effect. Excessive ROS formation during ischemia can contribute to the reperfusion injury, but ROS are important in triggering the cardioprotective mechanism in preconditioning (Pagliaro & Penna, 2015). This protective effect stems from the opening of mitochondrial ATP-sensitive potassium channels which have been shown to protect against ischemia-reperfusion through allowing an influx of K+ into the matrix, causing an increase in ROS (Andrukhiv et al., 2006; Loukogeorgakiset al., 2007). Ischemic preconditioning also inhibits the opening of the mitochondrial permeability transition pores which contributes greatly to the cardioprotection of the vessel (Andrukhiv et al., 2006; Garlid et al., 2009; Hausenloy et al., 2009). The closing of these vessels avoids ROS-induced ROS release and redox stress which can cause further damage to the vessel (Andrukhiv et al., 2006).

Preconditioning through systemic hypoxia

Since brief bouts of ischemia prevents the reduction in flow-mediated dilation induced by an ischemia-reperfusion injury, it is hypothesized that systemic hypoxia may also protect endothelial function against ischemia-reperfusion injury. Normal air contains an oxygen concentration of 20.9%. Breathing low levels of oxygen, induced by reducing the fraction of inspired oxygen, leads to systemic hypoxemia, a lower concentration of oxygen in the blood. In healthy adults, the net effect of systemic hypoxemia is skeletal muscle vasodilation (Dinenno et al., 2003). Nitric oxide appears to be a major contributor to the compensatory dilator responses observed during hypoxia. Moreover, the nitrate-nitrite-nitric oxide pathway, operating in parallel to the classic L-arginine-nitric oxide synthase pathway to generate nitric oxide, is greatly enhanced under hypoxic conditions (Kim-Shapiro et al., 2006). Hypoxia increases nitric oxide

production through the activation of hypoxia-inducible factor-1 (HIF-1 α). HIF-1α stimulates the expression of inducible nitric oxide synthase (iNOS) which is directly responsible for increasing the bioavailability of nitric oxide, thus inducing vasodilation (Jung Frank et al., 2000; Wilson et al., 2016).

Intermittent hypoxia consists in alternating short bouts of breathing hypoxic and normoxic air. Intermittent hypoxia protected against ischemia-reperfusion injury in animals. Chronic intermittent hypobaric hypoxia has been seen to protect against ischemia-reperfusion injury induced in the rat heart (Bu et al., 2015). Intermittent hypobaric hypoxia lasting 28 or 42 days at an altitude of 3000 m protected the heart against injury in developing rats through the recovery of the left ventricle and diminished ultrastructural damage to the myocardium (Bu et al., 2015). Intermittent hypoxia, consisting of 5 alternating bouts of 5-6 minutes of hypoxia at a fraction of inspired oxygen of 0.06 and normoxia, induced a cardioprotective effect in response to ischemia-reperfusion injury in mice (Caiet al., 2003). Indeed, infarct size was significantly reduced in hearts from mice exposed to this intermittent hypoxia protocol 24 hours before inducing an ischemia-reperfusion injury to the coronary arteries. It was conclude that hypoxic preconditioning acted as a protective mechanism due to the expression of HIF-1 (Cai et al., 2003). Indeed, mice with the reduced HIF-1 protein showed no protective effect from intermittent hypoxia, suggesting that cardioprotection depends on HIF-1α expression (Cai et al., 2003). In addition to increasing nitric oxide bioavailability, HfIF-1α regulates expression of genes involved in maintaining oxygen homeostasis in response to a change in concentration of oxygen, such as hypoxia, as well as shifting the metabolism to anaerobic metabolism (Yoon et al., 2006; Ziello et al., 2007). HIF-1α coordinates this shift by inducing glycolytic enzymes and

glucose transporters to allow the cells to produce energy in a hypoxic environment (Ziello et al., 2007). Therefore, expression of HIF-1α prior to the ischemia-reperfusion injury protects the heart against damage caused by the ischemia and reperfusion. Intermittent hypoxia may therefore induce endothelial function protection via a mechanism distinct from the local protective response induced by brief ischemic preconditioning.

In humans, the ability of intermittent hypoxia to prevent the reduction in flow-mediated dilation following ischemia-reperfusion injury remains unknown. Therefore, the aim of this project was to determine whether exposure to intermittent hypoxia protects against ischemia-reperfusion injury in young healthy adults. We hypothesized that intermittent hypoxia will attenuate the reduction in flow-mediated dilation following ischemia-reperfusion injury in young healthy adults.

MANUSCRIPT

INTRODUCTION

The leading cause of mortality in the developed world stems from cardiovascular disease, with 17.9 million individuals dying each year worldwide (CDC, 2019). One in every four deaths result from the most common form of cardiovascular disease, ischemic heart disease. Characteristics of ischemic heart disease include a narrowing of the coronary arteries due to a plaque build-up on the artery wall, causing an inadequate blood flow and damages to the endothelial cells lining the blood vessels. While the process of unblocking the coronary arteries allows an influx of oxygen to the previously ischemic area, the sudden restoration of blood flow simultaneously damages the endothelial cells, known as ischemia-reperfusion injury. This injury results from the excessive production and formation of reactive oxygen species during ischemia and reperfusion, which causes a reduction in the bioavailability of nitric oxide, thus reducing endothelium-dependent vasodilation (Kharbandaet al., 2001).

Brachial artery endothelial function strongly correlates with coronary artery endothelial function (Takse et al. 1998). Therefore, measures of brachial endothelial function, such as flow-mediated dilation, can act as a surrogate measure of coronary artery endothelial function (Anderson et al., 1995). Flow-mediated dilation, an indicator of nitric oxide-dependent endothelial function, represents the dilation of the brachial artery following increases in blood flow induced by a transient period of ischemia. An ischemia-reperfusion injury similar to that observed in the coronary arteries can be achieved by inflating a cuff on the arm for a period of 20 minutes before rapidly deflating the cuff, which causes a sudden restoration of blood flow to the arm. Following an ischemia-reperfusion injury, decreases in flow-mediated dilation ranges

between 36% to 50% in sedentary and young healthy individuals respectively (DeVan et al., 2011; Loukogeorgakis et al., 2007)

Ischemic preconditioning, consisting in few short bouts of local ischemia induced by occluding blood flow to the arm, attenuates the reduction in flow-mediated dilation induced by ischemia-reperfusion injury, possibly due to the opening of the mitochondrial ATP-sensitive potassium channels and the inhibition of the opening of the mitochondrial permeability transition pores (Garlid et al., 2009; Kharbanda et al., 2001; Loukogeorgakis et al., 2007). These two mechanisms allow an influx of K+ into the matrix increasing ROS and avoiding ROS-induced ROS release and redox stress respectively (Andrukhiv et al., 2006).

It was therefore hypothesized that exposure to systemic hypoxia may represent another strategy to prevent or attenuate ischemia-reperfusion injury. In healthy adults, the net effect of systemic hypoxemia is a skeletal muscle vasodilation mediated by a release of nitric oxide from the endothelium (Dinenno et al., 2003). Intermittent hypoxia consists of alternating between breathing short bouts of hypoxic air and normoxic air. Intermittent hypoxia increases nitric oxide production through the activation of HIF-1α, which is directly responsible for increasing the bioavailability of nitric oxide (Jung Frank et al., 2000). Notably, when mice heterozygous for a knockout allele at the locus encoding HIF-1α were exposed to intermittent hypoxia, cardiac protection was lost indicating that cardioprotection through intermittent hypoxia is reliant on the HIF-1α gene (Cai et al., 2003). Upon the activation of HIF-1α, nitric oxide increases in response, thus inducing vasodilation in the vessel (Wilson et al., 2016).

Whether intermittent hypoxia prevents the reduction in flow-mediated dilation following an ischemia-reperfusion injury in humans remains unknown. Therefore, the aim of this study was to determine whether intermittent hypoxia protects against ischemia-reperfusion injury in young healthy adults. We hypothesized that intermittent hypoxia would attenuate the reduction in flow-mediated dilation following ischemia-reperfusion injury in young healthy adults.

METHODS

Sixteen healthy individuals, 9 men and 7 women, participated in the study. 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, took medication affecting the cardiovascular system, were pregnant, or were smokers. Participants visited the Clinical Exercise Physiology Laboratory on two occasions. On one visit, participants were exposed to intermittent normoxia and on the other visit, the same participants were exposed to intermittent hypoxia. The visit order was randomized, and participants were blinded to the condition. On Visit 1 (intermittent normoxia), endothelium-dependent vasodilation of the brachial artery was assessed by flow-mediated dilation before and 15 minutes after an ischemia-reperfusion injury caused by 20 minutes of blood flow occlusion of the right arm (Figure 3). On Visit 2 (intermittent hypoxia), a 20-minute intermittent hypoxia protocol immediately preceded the ischemia-reperfusion injury, with measures of flow-mediated dilation taking place before intermittent hypoxia and 15 minutes after the ischemia-reperfusion injury. Intermittent hypoxia consisted of alternating short periods of breathing hypoxic and normoxic air through a breathing circuit. Participants avoided intense physical activity on the day prior to both visits and reported to the laboratory in the morning after fasting for at least 10 hours. In men and women using contraceptives, both visits were separated

by a period of seven days. One woman was not using contraceptives, therefore, both visits took place in the early follicular phase of the menstrual cycle.

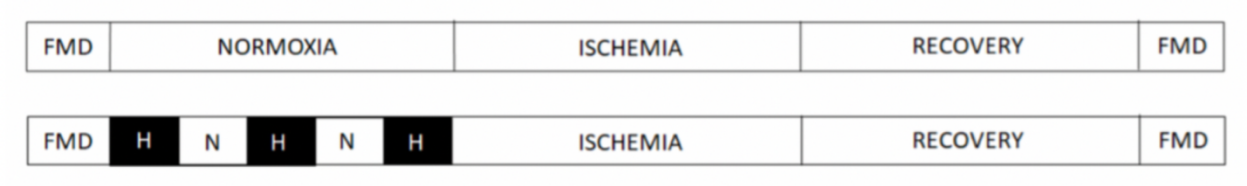

Figure 3. Study protocol. FMD: Flow-mediated dilation. H: intermittent hypoxia bout lasting 4 minutes. N: normoxia bout lasting 4 minutes. Ischemia: blood flow occlusion lasting 20-minutes. Recovery: reperfusion of blood flow for 15 minutes.

Flow-mediated dilation

Participants first rested in a supine, dimly lit room, for 15 minutes. Brachial blood pressure and heart rate was measured three times following the 15 minutes of rest. Endothelium-dependent vasodilation of the brachial artery was assessed by flow-mediated dilation before and 15 minutes after the ischemia-reperfusion injury using a semi-automated ultrasound machine (UNEXEF-38G, UNEX Corp., Japan) equipped with a high-resolution linear-array transducer positioned over the brachial artery. Participants extended their arm 90 degrees away from their body at heart level. A high-resolution linear-array transducer was positioned 2-8 cm proximally to the antecubital fossa and a cuff was placed 0.5-2.0 cm distal to the antecubital fossa. Longitudinal images of baseline brachial artery diameters were recorded for an average of 90 seconds, blood velocities were measured at an insonation angle of <60° for 30 seconds prior to forearm occlusion. Forearm occlusion was induced with a rapid cuff inflator to a supra-systolic pressure for 5 minutes. Beat-by-beat blood velocity and brachial artery diameters were recorded for approximately 90 seconds following cuff release. Measures of baseline diameter, peak

diameter and time to peak diameter were automatically calculated. Shear stress was estimated using shear rate, which was calculated by multiplying 8 by the quotient of blood flow velocity and brachial artery diameter. Shear rate area under the curve (AUC) was summed through peak diameter.

Ischemia-reperfusion injury

To induce ischemia-reperfusion injury, a blood pressure cuff placed on the upper arm was inflated to 250 mmHg for a period of 20 minutes before deflating the cuff to allow reperfusion of the blood into the arm for a period of 15 minutes.

Intermittent hypoxia

The intermittent hypoxia protocol consisted of three 4 minutes hypoxic cycles at an arterial oxygen saturation of 90% interspersed with 4 minutes normoxic cycles. Participants laid down during the entire breathing protocol, which lasted 20 minutes with a total of 12 minutes of hypoxic exposure. Hypoxic air was inhaled through a mask connected to a 2-way non-rebreathing valve (Hans Rudolph, Inc, USA), which was connected to a 5-liter non-diffusing gas bag (Hans Rudolph, Inc, USA). The rebreathing bag was connected to a gas tank of compressed air. Air was made hypoxic by introducing nitrogen in the breathing circuit. The flow of nitrogen was controlled to achieve an arterial oxygen saturation of 90%, as measured by pulse oximetry. Intermittent normoxia consisted of the same protocol but without adding nitrogen to the breathing circuit.

Hemodynamics

Arterial oxygen saturation was continuously monitored and recorded by pulse oximetry (NOVA, Finapres Medical Systems, Amsterdam, Netherlands) throughout the intermittent hypoxia and normoxia protocol. Resting hemodynamics were monitored during the intermittent hypoxia and normoxia protocol. An arterial waveform obtained by finger plethysmography from the middle finger of the left had was continuously recorded (NOVA, Finapres Medical Systems, Amsterdam, Netherlands). Brachial arterial blood pressure, heart rate, stroke volume, cardiac output and total peripheral resistance was derived from the arterial waveform, a method which has been validated against invasive measures (Wesseling et al., 1993). All data were recorded in LabChart (Powerlab, ADInstruments Inc., CO, USA) for later analysis.

Pulmonary Gas Exchange

Breath-by-breath measures of pulmonary gas exchange such as volume of oxygen and carbon dioxide, respiratory rate, tidal volume, and minute ventilation were determined from a pneumotachometer (Ultima Cardio2, MGC Diagnostics, MN, USA) throughout the intermittent hypoxia and normoxia protocol. The pneumotachometer was mounted between the mask and the non-rebreathing valve of the breathing circuit.

Statistical Analysis

A one-way repeated measures analysis of variance was used to evaluate whether the last minute of each cycle of intermittent hypoxia or intermittent normoxia triggered the same physiological responses. A one-way repeated measures analysis of variance was used to evaluate the effect of conditions (intermittent normoxia and intermittent hypoxia) on pulmonary gas

exchange, hemodynamics, and arterial oxygen saturation. A two-way repeated measures analysis of variance was used to evaluate the effect of time (pre vs. post-blood flow occlusion) and condition (intermittent normoxia vs. intermittent hypoxia) on measures of vasodilator function. When appropriate, post hoc analyses were performed using Tukey's test. $P<0.05$ was considered significant.

RESULTS

Participants' characteristics are shown in Table 1. Participants had a resting heart rate of 58 ± 8 bpm and a resting systolic and diastolic blood pressure of 118 ± 11 and 66 ± 6 mmHg, respectively. There was no significant difference in any hemodynamic and pulmonary gas exchange variables across hypoxic cycles, therefore, an average value was calculated for each variable. Similarly, there was no difference in any variables across normoxic cycles, and average values were calculated for each variable. Intermittent hypoxia resulted in a lower fraction of inspired oxygen, which resulted in a lower arterial oxygen saturation in comparison to intermittent hypoxia (Table 2). There was no effect of intermittent hypoxia on oxygen consumption, respiratory rate, tidal volume, minute ventilation, and end-tidal CO_2 (Table 2). Similarly, intermittent hypoxia did not significantly affect systolic, diastolic, mean arterial pressure, cardiac output, stroke volume, and total peripheral resistance. However, intermittent hypoxia significantly increased heart rate (Table 2).

Table 1. Participants' characteristics

Age (years)	23 ± 3
Height (cm)	175 ± 9
Weight (kg)	72.9 ± 13.4
BMI (kg/m^2)	23.7 ± 2.7

Table 2. Hemodynamic and pulmonary gas exchange variables during intermittent hypoxia and intermittent normoxia.

	Normoxia	Hypoxia
Systolic Blood Pressure (mmHg)	121 ± 11	123 ± 11
Diastolic Blood Pressure (mmHg)	74 ± 9	76 ± 10
Mean Arterial Blood Pressure (mmHg)	89 ± 8	92 ± 9
Heart Rate (bpm)	62 ± 7	71 ± 6 *
Stroke Volume (ml)	87 ± 19	82 ± 17
Arterial Oxygen Saturation (%)	99 ± 1	87 ± 3 *
Cardiac Output (L/min)	5.3 ± 1.1	5.8 ± 1.1
Total Peripheral Resistance (mmHg/L/min)	17.8 ± 3.6	16.8 ± 3.1
Oxygen Consumption (ml/kg/min)	3.46 ± 1.30	2.45 ± 0.81
Respiratory Rate (breaths/minute)	13 ± 4	13 ± 3
Tidal Volume (ml)	632 ± 259	608 ± 191
Minute Ventilation (L/min)	7.33 ± 2.50	7.17 ± 1.37
End-Tidal Carbon Dioxide (mmHg)	37.1 ± 3.3	35.3 ± 3.4
Fraction of Inspired Oxygen (%)	21.0 ± 0.3	12.3 ± 1.29 *

*$p < 0.05$ between Hypoxia and Normoxia

When preceded by intermittent normoxia, blood flow occlusion resulted in a blunted flow-mediated dilation (Figure 4). In contrast, the reduction in flow mediated dilation following blood flow occlusion was attenuated by prior exposure to intermittent hypoxia (Figure 4). Brachial artery baseline and peak diameters were greater following blood flow occlusion during both intermittent normoxia and intermittent hypoxia (Table 3). However, the change in diameter and shear rate AUC were smaller following blood flow occlusion during intermittent normoxia and intermittent hypoxia (Table 3). Time to peak diameter was not affected by blood flow occlusion in both conditions (Table 3)

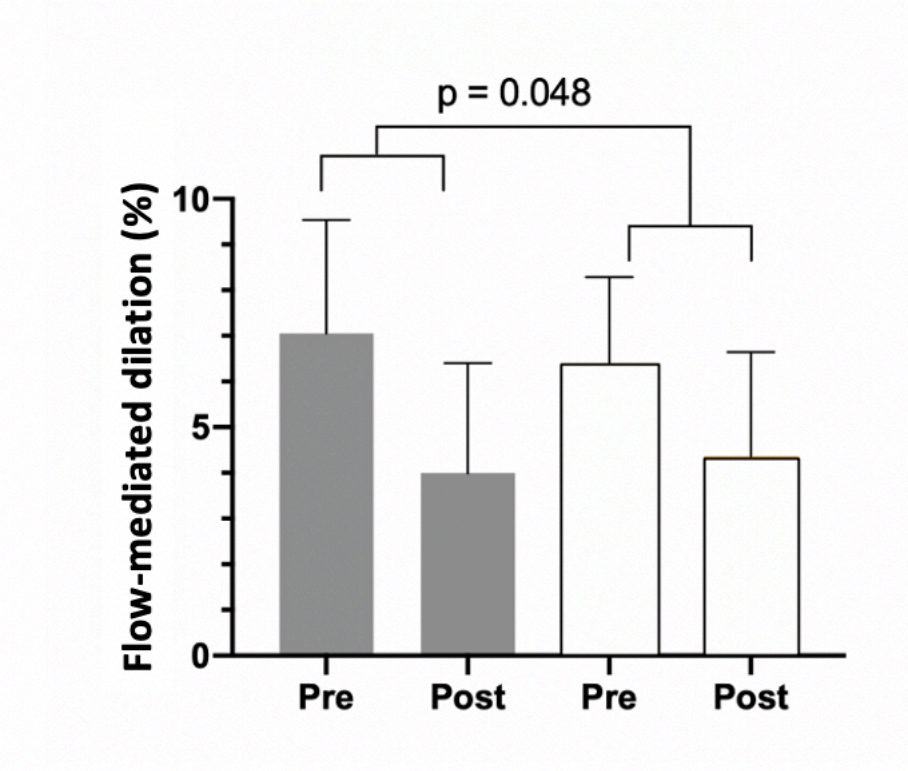

Figure 4. Vasodilation rate pre-and post-blood flow occlusion during intermittent normoxia (grey) and intermittent hypoxia (white). Time and condition interaction, p = 0.048.

Table 3. Vasodilator function pre- and post-blood flow occlusion.

	Normoxia		Hypoxia	
	Pre	Post	Pre	Post
Baseline Diameter (mm)	3.94 ± 0.70 *	4.19 ± 0.83	3.98 ± 0.71	4.21 ± 0.81
Peak Diameter (mm)	4.20 ± 0.68 *	4.34 ± 0.80	4.23 ± 0.73	4.39 ± 0.80
Change in Diameter (mm)	0.27 ± 0.07 *	0.15 ± 0.08	0.25 ± 0.06	0.17 ± 0.08
Time to Peak Diameter (s)	55 ± 12	52 ± 14	60 ± 11	55 ± 13
Shear Rate (AUC)	13188 ± 10794 *	8157 ± 7084	13172 ± 11224	8741 ± 6388

* Main effect for time

DISCUSSION

The aim of this research project was to determine whether exposure to intermittent hypoxia attenuates the reduction in flow-mediated induced by an ischemia-reperfusion injury in young healthy adults. It was observed that exposure to mild levels of intermittent hypoxia attenuates the reduction in flow-mediated dilation induced by blood flow occlusion in young healthy individuals. During intermittent normoxia, blood flow occlusion induced a 43% reduction in flow-mediated dilation, which is similar to the previously reported reductions in flow-mediated dilation ranging between 27-50% induced by the same blood flow occlusion protocol in young individuals (Brunt et al., 2016; DeVan et al., 2011; Loukogeorgakis et al., 2007). Following a short exposure to mild levels of intermittent hypoxia, blood flow occlusion induced a 32% reduction in flow-mediated dilation. Thus, intermittent hypoxia represents a potential strategy to mitigate the effect of ischemia-reperfusion injury.

Effect of ischemic preconditioning on flow-mediated dilation

Previous studies reported that ischemic preconditioning mitigates the effects of an ischemia-reperfusion injury (Kharbanda et al., 2001; Loukogeorgakiset al., 2007). Ischemic preconditioning consists of brief bouts of cuff inflation prior to the 20 minute blood flow occlusion. Indeed, three 5-minute bouts of ischemic preconditioning significantly reduced the flow-mediated dilation by 17% in the radial artery compared to a 54% decrease no ischemic-preconditioning at all (Kharbanda et al., 2001). Whereas three short bouts of ischemic preconditioning in the contralateral arm similarly blunted the decrease in flow-mediated dilation by 4% in young healthy adults compared to 54% without ischemic preconditioning (Loukogeorgakis et al., 2007). Ischemic preconditioning opens up mitochondrial K_{ATP} channels and inhibits the opening of the mitochondrial permeability transition pores, which have been shown to protect against an ischemia-reperfusion injury (Gross & Auchampach, 1992).

Effect of intermittent hypoxia on flow-mediated-dilation

Few animal studies showed that intermittent hypoxia protects against ischemia-reperfusion injury induced in the heart (Bu et al., 2015; Cai et al., 2003). Bu *et al.* (2015) found that chronic intermittent hypobaric hypoxia lasting 28 and 42 days, 5 hours each day, at an altitude of 3000 m protects the heart against an ischemia-reperfusion injury in developing rats, as seen through recovery of left ventricle function and diminished ultrastructural damage to the myocardium. A mechanism that seems necessary to the cardioprotective effect of hypoxia exposure is the hypoxia-inducible factor 1 alpha (HIF-1α). Intermittent hypoxia reduced infarct size in mice (Cai et al., 2003). Reducing the expression of HIF-1α protein completely abolished the protective effects induced by intermittent hypoxia (Cai et al., 2003). These protective effects

stem from an increase in nitric oxide bioavailability, regulating genes involved in maintaining oxygen homeostasis, and shifting the metabolism to anaerobic metabolism (Yoon et al., 2006; Ziello et al., 2007). This shift occurs by HIF-1α regulating glycolytic enzymes and glucose transports allowing the cell to produce energy, despite the hypoxic environment (Ziello et al., 2007). Expression of HIF-1α in the gene is essential for cardioprotection against an ischemia-reperfusion injury. In humans, intermittent hypoxia reduced brachial flow-mediated dilation by 32%, whereas normoxia decreased flow-mediated dilation by 43%. Although intermittent hypoxia did attenuate flow-mediated dilation, the mechanism remains unknown in humans.

Shear rate

Wall shear stress directly correlates with flow-mediated dilation, indicating that the greater the shear stress, the higher the flow-mediated dilation (Gnasso et al., 2001). It was hypothesized that the reduction in shear rate area under the curve (AUC) following blood flow occlusion would be attenuated with intermittent hypoxia since hypoxia induces vasodilation. Breathing low amounts of oxygen allows a cascade of events starting with HIF-1α and ending with an increase in nitric oxide, inducing vasodilation (Wilson et al., 2016). The 20 minute blood flow occlusion decreased shear rate AUC, but the decrease in both the intermittent hypoxia and normoxia protocols were the same. This decrease in shear rate AUC is what is responsible for the lower flow-mediated dilation after blood flow occlusion because flow-mediated dilation and shear rate are directly correlated (Gnasso et al., 2001).

The present study showed that a very short exposure to mild levels of intermittent hypoxia was sufficient to attenuate the reduction in brachial artery endothelial function following an ischemia-reperfusion injury. The intermittent hypoxia protocol consisted of three 4 minutes

hypoxic cycles at a targeted arterial oxygen saturation of 90%. Nonetheless, short exposure to mild levels of intermittent hypoxia attenuated the reduction in flow-mediated dilation induced by ischemia-reperfusion injury in young heathy individuals. It remains to be determined whether a longer hypoxic exposure duration to a lower arterial oxygen saturation results in a greater protective effect against ischemia-reperfusion injury. Moreover, administering the intermittent hypoxia protocol during the period of blood flow occlusion could also potentially attenuate the reduction in flow-mediated dilation. This technique, known as remote postconditioning, is defined as "an ischemic conditioning stimulus applied concurrently with an injurious ischemic episode but at a remote site" (Kerendi et al., 2005). Remote postconditioning in the leg has been seen to reduce flow-mediated dilation in the brachial artery by 12%, a similar degree of protection to ischemic preconditioning due to the protection from the activation of the mitochondria K_{ATP} channels (Loukogeorgakis et al., 2007).

CONCLUSION

Exposure to mild levels of intermittent hypoxia attenuated the reduction in flow-mediated dilation induced by blood flow occlusion in young healthy individuals. Thus, intermittent hypoxia represents a potentially safe, noninvasive strategy to mitigate the effect of ischemia-reperfusion injury in patients with ischemic cardiovascular events.

REFERENCES

Aboo Bakkar, Z., Fulford, J., Gates, P. E., Jackman, S. R., Jones, A. M., Bond, B., & Bowtell, J. L. (2018). Prolonged forearm ischemia attenuates endothelium-dependent vasodilatation and plasma nitric oxide metabolites in overweight middle-aged men. *European Journal of Applied Physiology*, *118*(8), 1565–1572. https://doi.org/10.1007/s00421-018-3886-z

Anderson, T. J., Uehata, A., Gerhard, M. D., Meredith, I. T., Knab, S., Delagrange, D., Lieberman, E. H., Ganz, P., Creager, M. A., & Yeung, A. C. (1995). Close relation of endothelial function in the human coronary and peripheral circulations. *Journal of the American College of Cardiology*, *26*(5), 1235–1241. https://doi.org/10.1016/0735-1097(95)00327-4

Bertuglia, S. (2008). Intermittent hypoxia modulates nitric oxide-dependent vasodilation and capillary perfusion during ischemia-reperfusion-induced damage. *American Journal of Physiology-Heart and Circulatory Physiology*, *294*(4), H1914–H1922. https://doi.org/10.1152/ajpheart.01371.2007

Brunt, V. E., Jeckell, A. T., Ely, B. R., Howard, M. J., Thijssen, D. H. J., & Minson, C. T. (2016). Acute hot water immersion is protective against impaired vascular function following forearm ischemia-reperfusion in young healthy humans. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, *311*(6), R1060–R1067. https://doi.org/10.1152/ajpregu.00301.2016

Bu, H., Yang, C., Wang, M., Ma, H., Sun, H., & Zhang, Y. (2015). KATP channels and MPTP are involved in the cardioprotection bestowed by chronic intermittent hypobaric hypoxia in the developing rat. *The Journal of Physiological Sciences*, *65*(4), 367–376. https://doi.org/10.1007/s12576-015-0376-5

Cai Hua, & Harrison David G. (2000). Endothelial Dysfunction in Cardiovascular Diseases: The Role of Oxidant Stress. *Circulation Research*, *87*(10), 840–844. https://doi.org/10.1161/01.RES.87.10.840

Cai Zheqing, Manalo Dominador J., Wei Guo, Rodriguez E. Rene, Fox-Talbot Karen, Lu Huasheng, Zweier Jay L., & Semenza Gregg L. (2003). Hearts From Rodents Exposed to Intermittent Hypoxia or Erythropoietin Are Protected Against Ischemia-Reperfusion Injury. *Circulation*, *108*(1), 79–85. https://doi.org/10.1161/01.CIR.0000078635.89229.8A

CDC. (2019, December 9). *Coronary Artery Disease | cdc.gov*. Centers for Disease Control and Prevention. https://www.cdc.gov/heartdisease/coronary_ad.htm

Chen, J., Ye, Z., Wang, X., Chang, J., Yang, M., Zhong, H., Hong, F., & Yang, S. (2018). Nitric oxide bioavailability dysfunction involves in atherosclerosis. *Biomedicine & Pharmacotherapy*, *97*, 423–428. https://doi.org/10.1016/j.biopha.2017.10.122

Das, D. K., Maulik, N., Sato, M., & Ray, P. S. (1999). Reactive oxygen species function as second messenger during ischemic preconditioning of heart. In D. K. Das (Ed.), *Stress Adaptation, Prophylaxis and Treatment* (pp. 59–67). Springer US. https://doi.org/10.1007/978-1-4615-5097-6_7

DeVan, A. E., Umpierre, D., Harrison, M. L., Lin, H.-F., Tarumi, T., Renzi, C. P., Dhindsa, M., Hunter, S. D., & Tanaka, H. (2011). Endothelial ischemia-reperfusion injury in humans: Association with age and habitual exercise. *American Journal of Physiology-Heart and Circulatory Physiology*, *300*(3), H813–H819. https://doi.org/10.1152/ajpheart.00845.2010

Dinenno, F. A., Joyner, M. J., & Halliwill, J. R. (2003). Failure of systemic hypoxia to blunt α-adrenergic vasoconstriction in the human forearm. *The Journal of Physiology*, *549*(Pt 3), 985–994. https://doi.org/10.1113/jphysiol.2003.042507

Engelland, R. E., Hemingway, H. W., Tomasco, O. G., Olivencia-Yurvati, A. H., & Romero, S. A. (2020). Acute lower leg hot water immersion protects macrovascular dilator function following ischaemia–reperfusion injury in humans. *Experimental Physiology*, *105*(2), 302–311. https://doi.org/10.1113/EP088154

Garlid, A. O., Jaburek, M., Jacobs, J. P., & Garlid, K. D. (2013). Mitochondrial reactive oxygen species: Which ROS signals cardioprotection? *American Journal of Physiology - Heart and Circulatory Physiology*, *305*(7), H960–H968. https://doi.org/10.1152/ajpheart.00858.2012

Garlid, K. D., Costa, A. D. T., Quinlan, C. L., Pierre, S. V., & Santos, P. D. (2009). Cardioprotective Signaling to Mitochondria. *Journal of Molecular and Cellular Cardiology*, *46*(6), 858–866. https://doi.org/10.1016/j.yjmcc.2008.11.019

Gnasso, A., Carallo, C., Irace, C., De Franceschi, M. S., Mattioli, P. L., Motti, C., & Cortese, C. (2001). Association between wall shear stress and flow-mediated vasodilation in healthy men. *Atherosclerosis*, *156*(1), 171–176. https://doi.org/10.1016/S0021-9150(00)00617-1

Green Daniel J., Dawson Ellen A., Groenewoud Hans M.M., Jones Helen, & Thijssen Dick H.J. (2014). Is Flow-Mediated Dilation Nitric Oxide Mediated? *Hypertension*, *63*(2), 376–382. https://doi.org/10.1161/HYPERTENSIONAHA.113.02044

Gross G J, & Auchampach J A. (1992). Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs. *Circulation Research*, *70*(2), 223–233. https://doi.org/10.1161/01.RES.70.2.223

Jung Frank, Palmer Lisa A., Zhou Nan, & Johns Roger A. (2000). Hypoxic Regulation of Inducible Nitric Oxide Synthase via Hypoxia Inducible Factor-1 in Cardiac Myocytes. *Circulation Research*, *86*(3), 319–325. https://doi.org/10.1161/01.RES.86.3.319

Kalogeris, T., Baines, C. P., Krenz, M., & Korthuis, R. J. (2012). Cell Biology of Ischemia/Reperfusion Injury. *International Review of Cell and Molecular Biology*, *298*, 229–317. https://doi.org/10.1016/B978-0-12-394309-5.00006-7

Kerendi, F., Kin, H., Halkos, M. E., Jiang, R., Zatta, A. J., Zhao, Z.-Q., Guyton, R. A., & Vinten-Johansen, J. (2005). Remote postconditioning. Brief renal ischemia and reperfusion applied before coronary artery reperfusion reduces myocardial infarct size via endogenous activation of adenosine receptors. *Basic Research in Cardiology*, *100*(5), 404–412. https://doi.org/10.1007/s00395-005-0539-2

Kharbanda Rajesh K., Peters Mark, Walton Benjamin, Kattenhorn Mia, Mullen Michael, Klein Nigel, Vallance Patrick, Deanfield John, & MacAllister Raymond. (2001). Ischemic Preconditioning Prevents Endothelial Injury and Systemic Neutrophil Activation During Ischemia-Reperfusion in Humans In Vivo. *Circulation*, *103*(12), 1624–1630. https://doi.org/10.1161/01.CIR.103.12.1624

Kim-Shapiro, D. B., Schechter, A. N., & Gladwin, M. T. (2006). Unraveling the Reactions of Nitric Oxide, Nitrite, and Hemoglobin in Physiology and Therapeutics. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *26*(4), 697–705. https://doi.org/10.1161/01.ATV.0000204350.44226.9a

Loukogeorgakis Stavros P., Williams Rupert, Panagiotidou Anna T., Kolvekar Shyamsunder K., Donald Ann, Cole Tim J., Yellon Derek M., Deanfield John E., & MacAllister Raymond J. (2007). Transient Limb Ischemia Induces Remote Preconditioning and Remote

Postconditioning in Humans by a KATP Channel–Dependent Mechanism. *Circulation*, *116*(12), 1386–1395. https://doi.org/10.1161/CIRCULATIONAHA.106.653782

Luscher, T. F., & Vanhoutte, P. M. (1990). *The Endothelium*. CRC Press.

Niebauer, J., & Cooke, J. P. (1996). Cardiovascular effects of exercise: Role of endothelial shear stress. *Journal of the American College of Cardiology*, *28*(7), 1652–1660. https://doi.org/10.1016/S0735-1097(96)00393-2

Paszkowiak, J. J., & Dardik, A. (2003). Arterial Wall Shear Stress: Observations from the Bench to the Bedside. *Vascular and Endovascular Surgery*, *37*(1), 47–57. https://doi.org/10.1177/153857440303700107

Pierini, D., & Bryan, N. S. (2015). Nitric Oxide Availability as a Marker of Oxidative Stress. In D. Armstrong (Ed.), *Advanced Protocols in Oxidative Stress III* (pp. 63–71). Springer. https://doi.org/10.1007/978-1-4939-1441-8_5

Wilson, C., Lee, M. D., & McCarron, J. G. (2016). Acetylcholine released by endothelial cells facilitates flow-mediated dilatation. *The Journal of Physiology*, *594*(24), 7267–7307. https://doi.org/10.1113/JP272927

Yoon, D., Pastore, Y. D., Divoky, V., Liu, E., Mlodnicka, A. E., Rainey, K., Ponka, P., Semenza, G. L., Schumacher, A., & Prchal, J. T. (2006). Hypoxia-inducible factor-1 deficiency results in dysregulated erythropoiesis signaling and iron homeostasis in mouse development. *The Journal of Biological Chemistry*, *281*(35), 25703–25711. https://doi.org/10.1074/jbc.M602329200

Ziello, J. E., Jovin, I. S., & Huang, Y. (2007). Hypoxia-Inducible Factor (HIF)-1 Regulatory Pathway and its Potential for Therapeutic Intervention in Malignancy and Ischemia. *The Yale Journal of Biology and Medicine*, *80*(2), 51–60.