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RESEARCH ARTICLE

Don't Deny Your Inner Environmental Physiologist: Investigating Physiology with Environmental Stimuli

Seven days of ischemic preconditioning augments hypoxic exercise ventilation and muscle oxygenation in recreationally trained males

Afton D. Seeley,[1,2,3] Aaron R. Caldwell,[2,3] Lawrence P. Cahalin,[4] Soyeon Ahn,[5] Arlette C. Perry,[1] Brian Arwari,[1] and Kevin A. Jacobs[1]

[1]*Department of Kinesiology and Sport Sciences, School of Education and Human Development, University of Miami, Coral Gables, Florida;* [2]*Thermal and Mountain Medicine Division, United States Army Research Institute of Environmental Medicine, Natick, Massachusetts;* [3]*Oak Ridge Institute of Science and Education, Oak Ridge, Tennessee;* [4]*Department of Physical Therapy, University of Miami Miller School of Medicine, Coral Gables, Florida; and* [5]*Department of Educational and Psychological Studies, School of Education and Human Development, University of Miami, Coral Gables, Florida*

Abstract

This investigation sought to assess whether single or repeated bouts of ischemic preconditioning (IPC) could improve oxyhemoglobin saturation (Sp_{O_2}) and/or attenuate reductions in muscle tissue saturation index (TSI) during submaximal hypoxic exercise. Fifteen healthy young men completed submaximal graded exercise under four experimental conditions: *1*) normoxia (NORM), *2*) hypoxia (HYP) [oxygen fraction of inspired air ($F_{I_{O_2}}$) = 0.14, ~3,200 m], *3*) hypoxia preceded by a single session of IPC (IPC1-HYP), and *4*) hypoxia preceded by seven sessions of IPC, one a day for 7 consecutive days (IPC7-HYP). IPC7-HYP heightened minute ventilation ($\dot{V}_E$) at 80% HYP peak cycling power output (W_{peak}) ($+10.47 \pm 3.35$ L·min^{-1}, $P = 0.006$), compared with HYP, as a function of increased breathing frequency. Both IPC1-HYP ($+0.17 \pm 0.04$ L·min^{-1}, $P < 0.001$) and IPC7-HYP ($+0.16 \pm 0.04$ L·min^{-1}, $P < 0.001$) elicited greater oxygen consumption ($\dot{V}O_2$) across exercise intensities compared with NORM, whereas $\dot{V}O_2$ was unchanged with HYP alone. Sp_{O_2} was unchanged by either IPC condition at any exercise intensity, yet the reduction of muscle TSI during resting hypoxic exposure was attenuated by IPC7-HYP ($+9.9 \pm 3.6\%$, $P = 0.040$) compared with HYP, likely as a function of reduced local oxygen extraction. Considering all exercise intensities, IPC7-HYP attenuated reductions of TSI with HYP ($+6.4 \pm 1.8\%$, $P = 0.001$). Seven days of IPC heightens ventilation, posing a threat to ventilatory efficiency, during high-intensity submaximal hypoxic exercise and attenuates reductions in hypoxic resting and exercise muscle oxygenation in healthy young men. A single session of IPC may be capable of modulating hypoxic ventilation; however, our present population was unable to demonstrate this with certainty.

blood flow; breathing frequency; $S_{p_{O_2}}$; tissue saturation index

INTRODUCTION

Hypoxic environments induce a marked decrement in circulating oxygen availability and limit the performance of physical work in a severity-proportional manner (1). At submaximal intensities, the human body compensates for even substantial decreases in arterial oxygen content by balancing elevations of ventilation, cardiac output, and skeletal muscle blood flow to maintain oxygen delivery (2). These adjustments equate to a given absolute workload requiring greater physiological adjustment in hypoxia compared with normoxia and therefore meaningful reductions in sustainable hypoxic workload (3). With the added burden of hypobaria, military service members who may need to ascend, at times rapidly, to terrestrial hypoxic environments where they will engage in sustained submaximal physical work are consequently impaired (4). At ~3,000 m, sustained physical tasks may take as much as 12% longer to perform, which is the product of an ~291% increase in submaximal task duration for every 1-km increase in altitude (5).

Ischemic preconditioning (IPC) involves the induction of localized ischemia with 5-min occlusion and 5-min reperfusion cycles applied repeatedly, typically three or four times, to the proximal upper or lower limbs. IPC can provide remote endogenous protection from ischemia-reperfusion injury in multiple organ systems thought to be mediated by several different signaling mediators (6), including nitric oxide and G protein cell surface-coupled receptors (e.g., adenosine, bradykinin, angiotensin) (7). These factors have considerable influence on the vasculature, to include that of the lungs. In healthy

Correspondence: A. D. Seeley (afton.d.seeley.ctr@health.mil).
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volunteers, IPC appears capable of attenuating the typical rise in pulmonary arterial systolic pressure, a measure of hypoxic pulm-onary vasoconstriction (HPV), induced by both normobaric (8, 9) and hypobaric (10) hypoxia. An inverse relationship bet-ween hypoxic ventilatory responsiveness and HPV at a consistent level of arterial oxygenation (11) suggests that IPC may be capable of influencing hypoxic ventilation (12). Kim and colleagues (9) have recently suggested that a single session of IPC improves pulmonary markers related to breathing efficiency as well as ventilation-perfusion matching during low-intensity exercise. With the imposition of 5 consecutive days of IPC, improvements in arterial oxygen saturation during an exercise challenge have also been demonstrated (10).

Time trial performance at moderate altitude (~2,200–2,400 m) is enhanced when preceded by a single IPC bout (13, 14). Enhanced physical performance with IPC is linked to improved oxygen extraction (13–15), enhanced skeletal muscle blood volume (14), and improved arterial oxygen saturation (13). Furthermore, IPC applied for 7 days has elicited reductions in muscle deoxygenated hemoglobin/myoglobin (12–30%) and heightened delta cycling efficiency (~3%) during steady-state submaximal exercise in normoxia (16, 17). Similarly, a 7-day IPC procedure enhanced delta cycling efficiency (~5%) during incremental exercise in severe [oxygen fraction of inspired air (FI_{O_2}) = 0.103] hypoxia along with improvements in muscle oxygen delivery utilization matching during hypoxic exercise onset (18). Improvements in mitochondrial efficiency induced by IPC have been proposed to be mediated by VEGF/Akt3/peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) (19, 20) or reactive oxygen species activation of AMP-activated protein kinase (AMPK) pathways (21).

Existent literature suggests that IPC may be capable of modulating local and systemic oxygenation adjustments to submaximal exercise at moderate/severe hypoxia. Seven days of IPC appears sufficient to significantly attenuate the use of oxygen within exercising skeletal muscle (16–18), whereas preliminary evidence suggests that single or repeated bouts of IPC may modulate ventilatory efficiency and downstream systemic oxygenation responses associated with hypoxic exercise (9, 10). As greater hypoxic exercise intensities elicit exaggerated adjustments in ventilation and skeletal muscle oxygen demand, it remains unknown how IPC may influence adjustments across hypoxic submaximal intensities. Therefore, this study sought to investigate the ability of a single or 7-day IPC application to improve oxyhemoglobin saturation (Sp_{O_2}) and/or attenuate reduction of muscle oxygenation [tissue saturation index (TSI)] across various hypoxic submaximal exercise intensities. We hypothesized that repeated application (7 days) of IPC would lead to the greatest improvements in oxyhemoglobin saturation and local muscle oxygenation during submaximal exercise performed in moderate hypoxia.

METHODS

Experimental Design

Volunteers reported to the laboratory for six data collection trials (Fig. 1). The first two trials each consisted of a peak graded exercise test, the first completed in conditions of normobaric normoxia (NORM; FI_{O_2} = 0.21) and the second in normobaric hypoxia (HYP; FI_{O_2} = 0.14, ~3,200 m). Hypoxia severity was selected to ensure conditions that would likely elicit hypoxic pulmonary vasoconstriction (22). Identical HYP conditions were used for all hypoxia trials. During the third trial, volunteers performed a normoxic (NORM) submaximal graded exercise test (S-GXT). Subsequently, volunteers completed three experimental trials with identical S-GXT procedures (*trials 4–6*): *1*) hypoxic S-GXT (HYP), *2*) 1-day, single 40-min exposure of ischemic preconditioning before hypoxic S-GXT (IPC1-HYP), and *3*) 7 days of ischemic preconditioning before hypoxic S-GXT (IPC7-HYP). Submaximal graded exercise testing consisted of six discontinuous 6-min efforts [2 sequential 6-min efforts at absolute workloads corresponding to 40%, 60, and 80% HYP peak cycling power output (W_{peak})]. Completion of experimental conditions was performed in the numerical order described, rather than randomized, to avoid the unclearly defined limits of the lasting physiological influence of IPC (23). A minimum of 48-h washout was provided after any session performed in normoxia and 1 wk after any session performed with hypoxia.

The study and informed consent were approved by the Institutional Review Board at the University of Miami. The study conformed to the standards set by the Declaration of Helsinki, except for registration in a clinical trial database. Before completing any study-related activities, volunteers were fully informed of the experimental procedures and possible risks and provided written informed consent.

Volunteers

Fifteen recreationally active (tier 1; Ref. 24) males were recruited for this investigation (Table 1). Volunteers were able to demonstrate a cycle ergometry peak oxygen consumption

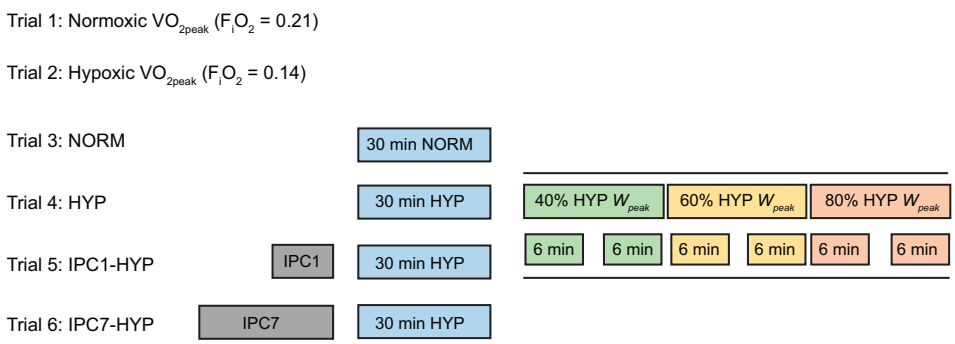

Figure 1. Study design. Absolute power output was identical for all conditions and set as 40%, 60%, and 80% of the hypoxia (HYP) peak power output (W_{peak}) attained in *trial 2*, peak oxygen consumption ($\dot{V}O_{2peak}$) in normobaric hypoxia. FIO_2, oxygen fraction of inspired air; IPC1, single session of ischemic preconditioning (IPC); IPC7, 7 days of IPC; NORM, normoxia.

Table 1. *Volunteer demographics*

Age, yr	24 ± 4
Height, cm	179 ± 5
Body mass, kg	82 ± 11
Body fat, %	15.5 ± 4.4
Normoxia $\dot{V}O_{2peak}$, mL·kg^{-1}·min^{-1}	43.6 ± 4.7
Normoxia W_{peak}	226 ± 28
Hypoxia $\dot{V}O_{2peak}$, mL·kg^{-1}·min^{-1}	42.5 ± 6.8
Hypoxia W_{peak}	199 ± 24*

Values are means ± SE. A paired *t* test was used to evaluate the effect of hypoxia (n = 15). *Significantly different from normoxia ($P < 0.001$). $\dot{V}O_{2peak}$, peak oxygen consumption; W_{peak}, peak cycling power output.

($\dot{V}O_{2peak}$) ≥ 40 mL·kg^{-1}·min^{-1} and/or 4.0 L·min^{-1} in normobaric normoxia and uninfluenced by having lived at altitude (>2,100 m) for >2 yr during their lifetime or having spent >2 wk at altitude over the last 6 mo. Volunteers were asked to refrain from caffeine consumption for 12 h, be at least 2 h postprandial, and avoid strenuous exercise for 24 h before the start of each session. Volunteers were also asked to match their 24-h food consumption, with emphasis on macronutrient distribution as well as meal timing before laboratory arrival, collected during *trial 1*, before *trials 2–6*. To reduce the influence of variation in hydration status on cardiac hemodynamics, all volunteers were administered a bolus of water proportional to 5 mL/kg of body mass upon arrival at the laboratory for *trials 3–6*. Body fat percentage was measured with bioelectrical impedance analysis (InBody 570; InBody Co., Ltd., Korea).

Exercise Design

$\dot{V}O_{2peak}$ testing (*trials 1* and *2*) was performed with an electromagnetically braked cycle ergometer (Monark Ergometric 829e; Vansbro, Sweden). Before testing, participants were fitted with an oronasal facemask (7450 series; Hans Rudolph, Kansas City, MO) and halo apparatus (2726 Head Support; Hans Rudolph, Kansas City, MO). Participants began cycling at 80 W for 2 min, with workload increased by 30 W every 2 min thereafter until volitional exhaustion. Power at peak oxygen consumption (W_{peak}) was calculated to account for work performed in partially completed stages with the following equation:

$$W_{peak} = \text{power@last completed stage(W)} + \frac{\text{time of stage completed (s)}}{\text{total stage time (s)}} \times 30$$

Expiratory gases were analyzed continuously with an online open-circuit metabolic cart (Sensormedics Vmax Encore 29; Viasys Healthcare, Palm Springs, CA). Before each experimental session, gas analyzers were calibrated with gases within physiological range, and the mass flow sensor was calibrated with a 3.0-L syringe at multiple flow rates. Peripheral oxyhemoglobin saturation (Sp_{O_2}) was noninvasively and continuously monitored with a pulse oximeter (Non 7500; Nonin Medical, Inc., Plymouth, MN) affixed to the left index finger. Heart rate (HR) was continuously monitored with a sternal affixed monitor (Polar Heart Rate Sensor H1; Polar, Finland).

During submaximal graded exercise testing, volunteers were affixed with a noninvasive impedance cardiography device (Physioflow; Manitec Biomedical, Macharen, France) to continuously monitor cardiac output ($\dot{Q}$), stroke volume (SV), and heart rate (HR). Volunteers wore the oronasal mask and halo apparatus further connected to a mass flow sensor and two-way nonrebreathing valve in series to allow for continuous collection of ventilatory and metabolic data. Tissue saturation index (TSI) was measured continuously with a near-infrared spectroscopy (NIRS)-based sensor (Moxy Muscle Oxygen Monitor; Fortiori Design, LLC, Hutchinson, MN) placed over the most distal midline of the vastus lateralis (VL) of the right leg, affixed with self-adherent athletic wrap. Sensor distance from the extended knee was used to guide reapplication of the NIRS device on the same participant. Measurements were reflective of the distance from the midline of the superior border of the patella to each leftmost and rightmost send-receive sensor.

Once outfitted, volunteers rested recumbently while breathing the appropriate test gas for 30 min. Volunteers were made fully supine, and the superficial femoral artery (SFA) of the right leg was imaged with a Doppler ultrasound (Terason USmart 3200T; TeraTech Corporation, Burlington, MA) with a linear array transducer. The volunteer assumed a seated position on the cycle ergometer for a resting (0 W) 6-min measurement block before initiating exercise. For each measurement block, ventilation, cardiac hemodynamic, and TSI data were averaged from *minutes 4–6*, Sp_{O_2} was noted at *minute 6*, and SFA imaging was conducted as immediately as possible after the volunteer discontinued pedaling. Volunteers were instructed to "stop and prop" their right leg to reduce imaging artifact, and imaging was kept to <45 s to minimize decrement of exercise-related increases in blood flow. To further standardize and expedite SFA velocity measurement, an inertial measurement unit (IMU) (BWT61CL IMU; WitMotion ShenZhen, Co., Ltd., Shenzhen City, China) was affixed to the ultrasound linear transducer and synchronized to a custom-written program in LabVIEW (version 18.0; National Instruments, Austin, TX) that provided real-time transducer application angle (°) computed from acceleration data. This allowed for efficient reapplication immediately after each discontinuous exercise bout.

Hypoxic Delivery System

HYP conditions were created with two hypoxic generators (HYP-123; Hypoxico, New York, NY) each capable of an approximate flow rate of 70 L/min in series with two ~180-L 6-mil polyethylene reservoir bags. Volunteers inspired from the reservoir bags and expired into the ambient room using a two-way nonrebreathing valve (2700 series; Hans Rudolph, Inc., Kansas City, MO) in series with a breath-by-breath mass flow sensor. Test-retest reliability indicated excellent reliability (r = 0.9; FI_{O_2} = 0.141 ± 0.001) of the hypoxic environment. In normoxia trials volunteers were affixed with the same flow sensor two-way valve interface without connection to the hypoxic reservoir bags.

Ischemic Preconditioning

Thigh-contoured occlusion cuffs (17 cm in width) were placed on the upper thighs and inflated with an automatic inflation system (E20 Rapid Cuff Inflator; Hokanson, Bellevue, WA) in a supine position. Limb occlusion was verified for each

participant by manual palpation of the dorsal pedal pulse. A single session of IPC (IPC1) consisted of four rounds of bilateral 5-min occlusion/5-min reperfusion at 200 mmHg for a total of 40 min. IPC7 applied single 40-min daily applications at the same time of day for 7 consecutive days for a total of 280 min. Forty-five minutes of recovery was provided between IPC and subsequent exercise to allow muscle oxygenation recovery (25).

SFA Blood Flow

Mean superficial femoral artery blood flow was estimated as the product of the mean vessel diameter obtained from two 10-s two-dimensional B-mode video clips with FloWaveUS open-source edge detection source code (26) in MATLAB (MATLAB R2022a; MathWorks, Natick, MA) and the halved time-averaged peak velocity (27) collected over a 10-s clip at a sample frequency of 30 Hz. Mean blood flow was estimated (in mL/min) with the equation

$$\text{estimated mean blood flow}\,(\text{mL}\cdot\text{min}^{-1}) = (V_{\text{peak}}/2)\cdot\pi\cdot\left(\frac{D}{2}\right)^2\cdot 60$$

where V_{peak} is the time-averaged peak velocity of the blood expressed in centimeters per second, π is a mathematical constant, D is the mean diameter of the vessel in centimeters, and 60 is a constant employed to convert units to milliliters per minute (28).

Tissue Saturation Index

MOXY sensors use a continuous-wave NIRS system with 680-, 720-, 760-, and 800-nm light sources. Two send-to-receive spacings of 12.5 and 25 mm are utilized for a measurement penetration of ~12 mm of skin and fat. Adipose tissue thickness (ATT) was measured as the VL skinfold thickness to the nearest millimeter with skinfold calipers (Lange; Beta Technology, Santa Cruz, CA) halved (29). The average values of skin and subcutaneous tissue thickness for the population studied was 6 ± 2 mm (range: 3–11 mm). This thickness, as it was less than half the largest emitter-to-detector distance, is adequate to allow near-infrared light through to muscle tissue (30).

Statistical Analyses

Data were aggregated across four time points for each condition (NORM, HYP, IPC1-HYP, and IPC7-HYP) to include *1*) 0% HYP W_{peak} (seated rest), *2*) 40% HYP W_{peak}, *3*) 60% HYP W_{peak}, and *4*) 80% HYP W_{peak}. Data were extracted and considered for analysis from the second 6-min exercise bout for all exercise intensities as most representative of steady-state submaximal conditions. Because of difficulties with completion of the second 80% intensity bout, data were extracted and analyzed from the first 6-min exercise bout at 80% HYP W_{peak} across all trials for three volunteers.

Fifteen volunteers was determined sufficient to detect a moderate (effect size of 0.3) condition × intensity interaction for Sp_{O_2} and TSI between trials, with an α level of 0.05 and a desired power of 0.95 (31). All analyses were completed with R statistical computing language (R version 4.0.3). Differences between normoxia (NORM), hypoxia (HYP), and hypoxia after 1 and 7 days of IPC treatment (IPC1-HYP and IPC7-HYP) at exercise workloads (W) representative of 40%, 60%, and 80% HYP W_{peak} were compared with linear mixed models, lme4 R package (32), with a random intercept for participant. An analysis of variance (ANOVA) with type 3 sums of squares was utilized to inspect the main effects and interaction between conditions and exercise intensity. Specific comparisons within each model were made using the estimated marginal means through the emmeans R package (33), with a Holm–Bonferroni correction applied to the P values. The results are presented as mean ± the standard error of the estimate or as mean with the 95% confidence interval (e.g., mean = 1.0 [95% CI]; Table 2 and Figs. 2 and 3). In some comparisons, the mean difference (M_{diff}) is reported alongside the standard error.

A supplemental analysis, modeling exercise intensity as a continuous rather than a categorical variable, was also conducted. When responses could be appropriately modeled with a linear mixed model, the lme4 R package (32) with a random intercept for participant and a random slope component for power output (W) was used. In all other analyses, a general additive model was fit with the mgcv R package (34) with restricted estimated maximum likelihood (REML) with an outer optimizer. For the general additive models, a smooth term, using thin plate regression splines, was included for the predictor variable (e.g., power output). Specific comparisons within each model were made with the estimated marginal means through the Emmeans R package (33) and used 80, 120, and 160 W as mean absolute representative workloads.

Main and supplemental figures and analyses can be found within our Open Science Framework repository (https://doi.org/10.17605/OSF.IO/MHTKV). Although results between the two statistical models provide similar conclusions, results reported below reference the outcomes from the statistical model leveraging categorical exercise intensity.

RESULTS

Exercise Stimulus

Mean power outputs corresponding to 40%, 60%, and 80% HYP W_{peak} were 80 ± 10 W, 120 ± 14 W, and 160 ± 19 W, respectively. Exercise HR was not different ($P > 0.05$; Table 2) among the three hypoxia experimental trials, supporting the reliability of the altitude exercise task.

Ventilation

A condition × intensity interaction was present for $\dot{V}_E$ (Fig. 2*A*; $P < 0.001$). As expected, $\dot{V}_E$ was consistently higher during exercise performed at HYP, irrespective of IPC intervention, compared with NORM. IPC7-HYP (117.42 [111.68–123.15] L·min^{-1}, $P = 0.006$) demonstrated heightened $\dot{V}_E$ at 80% HYP W_{peak} compared with HYP (106.95 [101.22–112.68] L·min^{-1}), with IPC1-HYP having a similar direction of effect (114.20 [108.47–119.93] L·min^{-1}, $P = 0.064$). Although all experimental conditions with hypoxia demonstrated lower end-tidal pressure of CO_2 ($P_{ET_{CO_2}}$) at rest ($P = 0.009$–0.030; Fig. 2*D*) and during exercise ($P < 0.001$) compared with NORM, IPC1 and IPC7 did not appear to moderate this effect. A main effect of condition

Table 2. *Hemodynamic responses to normoxic and hypoxic submaximal graded exercise*

	Rest	40% HYP W_{peak}	60% HYP W_{peak}	80% HYP W_{peak}
		Heart rate, beats/min		
NORM	70 [64–76]	107 [101–113]	132 [126–138]	160 [154–166]
HYP	75 [69–80]	120 [114–126]	146 [140–152]	166 [160–172]
IPC1-HYP	73 [67–79]	120 [114–126]	146 [140–151]	167 [161–173]
IPC7-HYP	75 [69–81]	121 [115–127]	149 [143–155]	168 [162–174]
		Cardiac output, L·min^{-1}		
NORM	6.5 [5.3–7.8]	12.4 [11.2–13.6]	15.9 [14.7–17.1]	19.4 [18.2–20.6]
HYP	6.7 [5.5–7.9]	13.3 [12.1–14.5]	16.6 [15.4–17.8]	19.2 [17.9–20.4]
IPC1-HYP	6.1 [4.9–7.3]	13.0 [11.8–14.3]	16.3 [15.1–17.6]	19.5 [18.3–20.7]
IPC7-HYP	6.5 [5.3–7.7]	13.0 [11.8–14.2]	16.5 [15.3–17.7]	19.3 [18.1–20.5]

Values are means [95% confidence interval (CI)]. A two-way analysis of variance was used to evaluate the effect of condition and exercise intensity (n = 15). HYP, hypoxia; IPC1, single session of ischemic preconditioning; IPC7, 7 days of ischemic preconditioning; NORM, normoxia; W_{peak}, peak cycling power output.

indicated that ventilatory equivalent of carbon dioxide [$\dot{V}E/\dot{V}CO_2$ production ($\dot{V}CO_2$)] was greater for all hypoxic conditions compared with NORM ($P < 0.001$; Fig. 2*E*), with neither IPC condition able to mediate this effect. Similarly, ventilatory equivalent of oxygen ($\dot{V}E/\dot{V}O_2$) was greater at rest (P = 0.016–0.039), 60% HYP W_{peak} ($P < 0.001$), and 80% HYP W_{peak} ($P < 0.001$) for all HYP conditions compared with NORM, with no IPC-mediated influence.

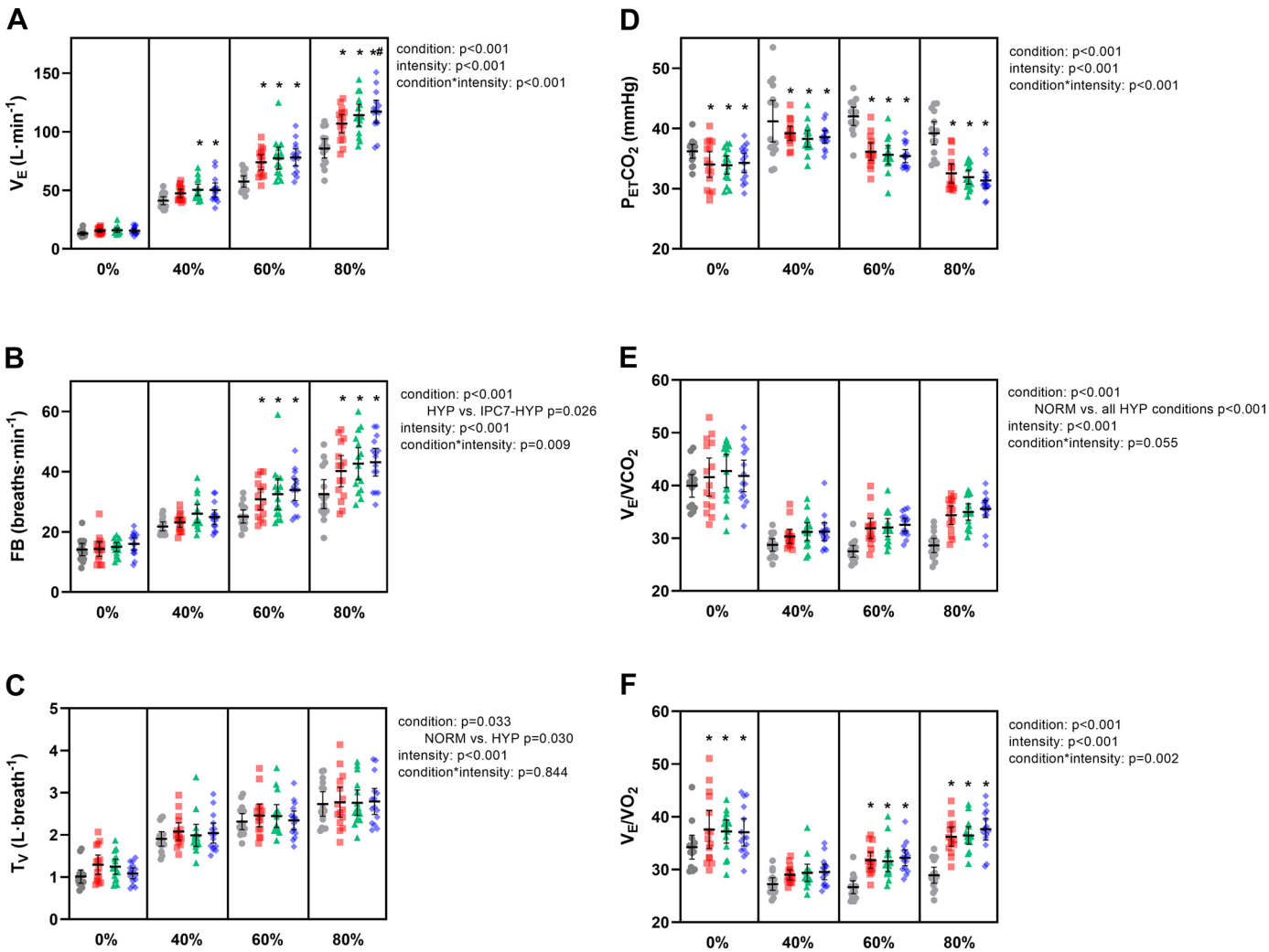

Figure 2. Ventilatory responses to normoxic and hypoxic submaximal graded exercise. Data presented as mean [95% confidence interval (CI)]. A two-way analysis of variance was used to evaluate the effect of condition and exercise intensity (n = 15). *A*: minute ventilation ($\dot{V}E$). *B*: breathing frequency (FB). *C*: tidal volume (T_V). *D*: end-tidal pressure of carbon dioxide ($P_{ET}CO_2$). *E*: ventilatory equivalent of carbon dioxide [$\dot{V}E/\dot{V}CO_2$]. VCO_2, CO_2 production. *F*: ventilatory equivalent of oxygen [$\dot{V}E/\dot{V}O_2$]. VO_2, O_2 production HYP (red squares), hypoxia; IPC1-HYP (green triangles), single session of ischemic preconditioning (IPC) followed by hypoxic exercise; IPC7-HYP (blue diamonds), 7 sessions of daily IPC followed by hypoxic exercise; NORM (gray circles), normoxia. *Significant ($P < 0.05$) difference compared with NORM; #significant ($P < 0.05$) difference compared with HYP.

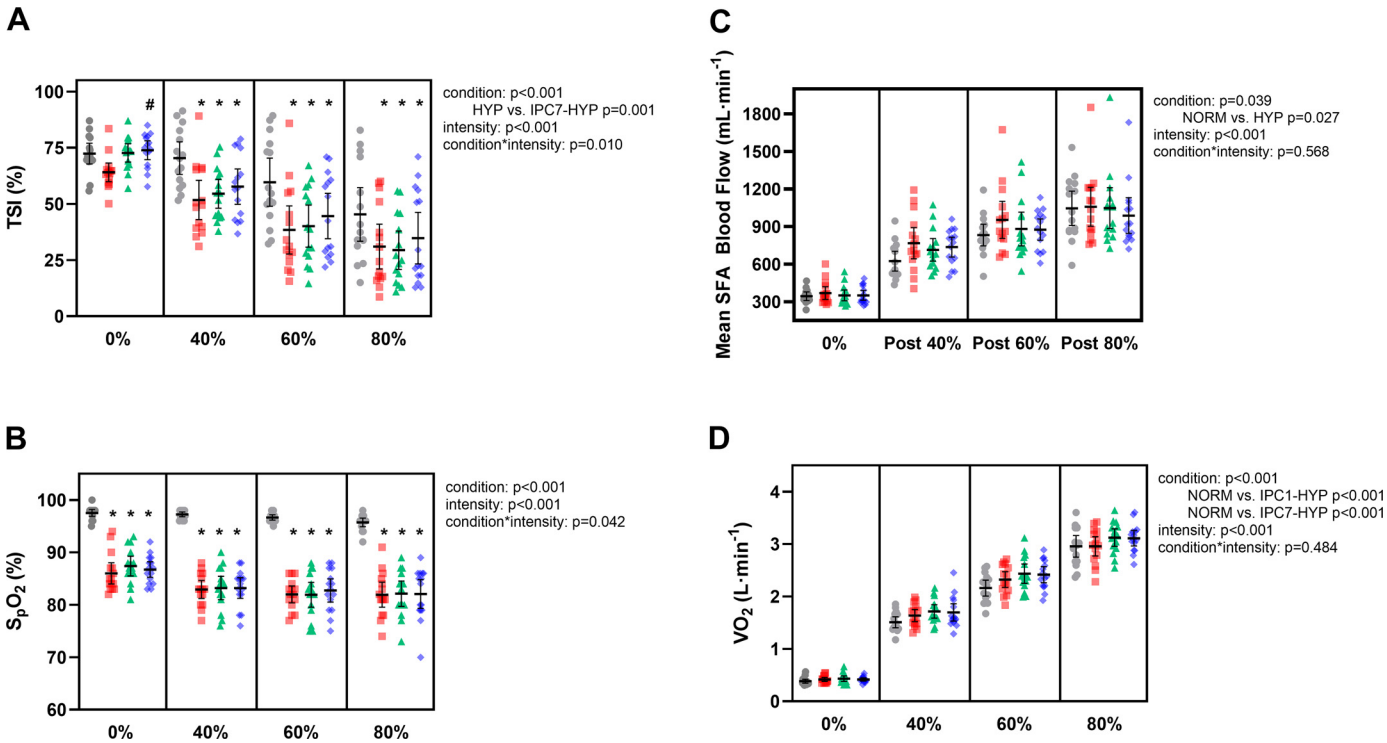

Figure 3. Local and systemic oxygenation, leg blood flow, and oxygen consumption during normoxic and hypoxic submaximal graded exercise. Data presented as mean [95% confidence interval (CI)]. A two-way analysis of variance was used to evaluate the effect of condition and exercise intensity (n = 15). *A*: tissue saturation index (TSI). *B*: oxyhemoglobin saturation (Sp_{O_2}). *C*: superficial femoral artery (SFA) blood flow. *D*: volume of oxygen consumed ($\dot{V}O_2$). HYP (red squares), hypoxia; IPC1-HYP (green triangles), single session of ischemic preconditioning (IPC) followed by hypoxic exercise; IPC7-HYP (blue diamonds), 7 sessions of daily IPC followed by hypoxic exercise; NORM (gray circles), normoxia. *Significant ($P < 0.05$) difference compared with NORM; #significant ($P < 0.05$) difference compared with HYP.

Breathing frequency (FB) was the primary driver of higher minute ventilation with all HYP conditions compared with NORM ($P < 0.001$; Fig. 2*B*). IPC7-HYP tended to elicit higher FB compared with HYP, irrespective of exercise intensity (P = 0.026). Tidal volume (V_T) (Fig. 2*C*) was greater, irrespective of exercise intensity, with HYP compared with NORM (M_{diff} = 0.160 ± 0.056 L, P = 0.030), whereas IPC1-HYP (M_{diff} = 0.119 ± 0.056 L, P = 0.177) and IPC7-HYP (M_{diff} = 0.075 ± 0.056 L, P = 0.554) tidal volume were indistinguishable from NORM across all exercise intensities. Collectively, it appears that IPC7-HYP encourages a greater reliance on breathing frequency as opposed to tidal volume to support elevation of $\dot{V}_E$ during progressively more intense hypoxic exercise.

Oxyhemoglobin Saturation

A condition × exercise intensity interaction was present for Sp_{O_2} (P = 0.042). Sp_{O_2} was reduced in hypoxia across rest and all exercise intensities ($P < 0.001$); however, neither IPC1 (P = 0.891) nor IPC7 (P = 0.891) was able to modify HYP Sp_{O_2} (Fig. 3*B*).

Leg Oxygenation and Blood Flow

IPC7-HYP demonstrated greater resting TSI values compared with HYP (M_{diff} = +9.9 ± 3.6%, P = 0.040; Fig. 3*A*). During exercise, each HYP condition, irrespective of IPC involvement, elicited lower TSI values compared with NORM ($P < 0.001$). Estimated mean SFA blood flow (Fig. 3*C*), irrespective of oxygen environment or IPC, tended to increase with increasing exercise intensity ($P < 0.001$), as expected. Interpreting a significant main effect of condition for mean SFA blood flow, HYP tended to elicit greater SFA mean blood flow compared with NORM (P = 0.027), irrespective of exercise intensity. Although the direct comparisons of hypoxic conditions indicated no significant differences (P = 0.309–0.550), an absence of condition-driven difference between NORM and IPC1-HYP (P = 0.550) and IPC7-HYP (P = 0.648) indicates that mean SFA blood flow after IPC1 and IPC7 were indistinguishable from NORM.

Increasing exercise intensity surfaced as the main driver of mean SFA velocity elevation ($P < 0.001$). Mean diameter also tended to increase as exercise intensity increased ($P <$ 0.001), with any differences between conditions becoming smaller with increasing exercise intensity. Irrespective of exercise intensity, mean SFA diameter tended to be greater with IPC1-HYP (0.013 ± 0.004 cm, P = 0.018) and IPC7-HYP (0.016 ± 0.004 cm, P = 0.002) compared with NORM. The condition main effect of HYP mean SFA diameter was not significantly different from NORM (P = 0.124).

Cycling $\dot{V}O_2$

A main effect of condition ($P < 0.001$) was detected for $\dot{V}O_2$. $\dot{V}O_2$ was not different between HYP and NORM (Fig. 3*D*; M_{diff} = 0.08 ± 0.04 L·min^{-1}, P = 0.093), with $\dot{V}O_2$ predictably increasing with greater exercise intensity ($P < 0.001$). However, both IPC1-HYP (M_{diff} = 0.17 ± 0.04 L·min^{-1}, $P <$ 0.001) and IPC7-HYP (M_{diff} = 0.16 ± 0.04 L·min^{-1}, $P < 0.001$)

elicited greater $\dot{V}O_2$ across all exercise intensities compared with NORM, suggesting greater metabolic demand during hypoxia following both IPC conditions.

Hemodynamics

Differences in HR between conditions was dependent upon exercise intensity ($P = 0.039$). All HYP conditions demonstrated an elevated HR compared with NORM during submaximal exercise except for HYP at 80% HYP W_{peak} ($P = 0.075$). As would be expected, $\dot{Q}$ tended to increase with increasing exercise intensity across all conditions ($P < 0.001$; Table 2).

DISCUSSION

Our repeated-measures study design sought to investigate the ability of a single or 7-day IPC application to improve oxyhemoglobin saturation (Sp_{O_2}) and/or attenuate reduction of muscle oxygenation (TSI) during hypoxic submaximal exercise. Sp_{O_2} was unchanged by either IPC condition at any hypoxic exercise intensity (Fig. 3*B*). Reduction of vastus lateralis TSI during resting hypoxic exposure was attenuated by IPC7-HYP compared with HYP alone. Considering all exercise intensities, IPC7-HYP also tended to attenuate reductions in TSI resulting from HYP. Interestingly, 7 days of repeated IPC (IPC7) increased minute ventilation at 80% HYP W_{peak}, likely as a function of increased breathing frequency.

Influence of IPC on Hypoxic Exercise Ventilation

There are limited data on the ability of IPC, in any application scheme, to modulate ventilation during hypoxic submaximal exercise. However, the application of a single bout of IPC among recreational cyclists and runners was unable to modulate $\dot{V}E$ during submaximal exercise performed in normoxia (35, 36). Similarly, Rieger et al. (12) demonstrated no effect of acute normobaric hypoxia [end-tidal pressure of O_2 (PET_{O_2}) = 50 mmHg] on resting ventilation after a single exposure of IPC but did elicit an increase in resting hypoxic ventilatory responsiveness (HVR) with chronic hypobaric hypoxia 24 h after a single IPC session (12). Our resting $\dot{V}E$ data in acute normobaric hypoxia were also similar between the IPC conditions (IPC1 16.02 [10.29–21.75], IPC7 15.55 [9.82–21.28] L·min^{-1}) and HYP (15.56 [9.83–21.30] L·min^{-1}), yet our results indicate that IPC7 (+ 9.8%) and, to a lesser extent IPC1 (+ 6.8%), appear capable of augmenting ventilation during high-intensity submaximal hypoxic exercise. To the best of our knowledge, this is the first investigation to demonstrate that IPC may alter ventilation during steady-state hypoxic exercise. Our data contrast with those of Kim et al. (9), who indicated that $\dot{V}E$ is likely lower with very low-intensity exercise (30 W) in normobaric hypoxia (FI_{O_2} = 0.125) as indicated by reductions in $\dot{V}E/\dot{V}CO_2$ and an increase in PET_{CO_2} from pre to post after a single bout of IPC. PET_{CO_2} and $\dot{V}E/\dot{V}CO_2$ were not different between conditions at hypoxia at any exercise intensity in the present study (Fig. 2, *D* and *E*). Exercise intensities greater than very low, and the commensurate increase in oxygen demand, likely induce exaggerated and differently elicited ventilatory responses to maintain Sp_{O_2}, at least in those who are "reasonably chemosensitive" (37).

Despite an increase in ventilation at 80% HYP W_{peak} for IPC7, exercise Sp_{O_2} was not significantly elevated. This result contrasts with previously reported IPC-induced augmentation of Sp_{O_2} during exercise performance efforts (10, 13). Still, Chopra et al. (18) demonstrated no difference in Sp_{O_2} or submaximal cycling ventilation (absolute 100 W) in severe hypoxia (FI_{O_2} = 0.103) after 7 days of IPC. The present study was also unable to demonstrate an influence of IPC7, or IPC1 for that matter, on ventilation or Sp_{O_2} at 40% or 60% HYP W_{peak} (equivalent to mean power outputs of ~80 and 120 W). Chopra et al. (18) demonstrated a condition effect of IPC on minute ventilation during an incremental ramp test, where ventilation rates were more comparable to those at our 80% HYP W_{peak} intensity (Chopra: ~99.1–113.4 vs. ~107.0–117.4 L·min^{-1}). Exercise ventilation rates that contribute significantly to mechanical and metabolic stress at hypoxia may be necessary to demonstrate modulation by repeated IPC. Interestingly, $\dot{V}E$ during exercise after IPC7, in the present study, is most probably elevated via increases in FB rather than VT. Twelve of fifteen volunteers demonstrated elevations in FB at 80% HYP W_{peak} during IPC7-HYP compared with HYP. Considering the maintenance of PET_{CO_2} across all hypoxic conditions, it is unlikely that the augmented breathing frequency was able to impact alveolar ventilation, rendering the lack of Sp_{O_2} change plausible. Furthermore, the work of breathing and resultant oxygen demand by the respiratory muscles tends to be greater per unit of alveolar ventilation if $\dot{V}E$ is increased via FB rather than VT (38). In line with this, both IPC1 and IPC7 demonstrated greater exercise oxygen consumption, across all exercise intensities, compared with normoxia, whereas the hypoxic control condition remained indistinguishable. We suggest that repeated IPC exposure (IPC7) may decrease hypoxic ventilatory efficiency by increasing respiratory frequency during submaximal exercise of high intensity. The contributing physiological mechanisms are currently unknown, and the ventilation adjustments appear unique to hypoxic submaximal exercise. Although IPC1 appears able to modulate ventilation to some degree, our limited population data do not appear sufficient to speak to a meaningful influence.

Hypoxic Exercise Local Oxygenation Responses after IPC

Literature suggests that 7 days of IPC is sufficient to prompt local and remote improvements in vascular reactivity, resting skin microcirculation (23), and reductions in exercise deoxygenated hemoglobin attributable to modulation of mitochondrial and/or vascular function (16). In the present study, IPC7 elevated vastus lateralis TSI during resting hypoxic exposure and considering all exercise intensities, indicating improvements in local oxygen delivery and/or reductions in local skeletal muscle oxygen consumption. Estimated mean blood flow at the superficial femoral artery was consistently greater, considering rest and all exercise intensities, during hypoxia alone, yet IPC1-HYP and IPC7-HYP displayed mean blood flow values indistinguishable from normoxia. This agrees with data from Cocking et al. (39) suggesting no tendency toward an increase in mean blood flow during exercise despite an enlarged arterial diameter during exercise after local IPC. Our mean diameter

measures support this notion with visualized differences at rest and after early low-intensity exercise with IPC7. It is postulated that improvements in TSI during hypoxic rest and submaximal exercise are primarily attributable to reductions in local skeletal muscle oxygen extraction. Jeffries et al. (16) saw reductions in normoxic resting muscle oxygen consumption after 7 days of IPC, extending support for plausibility. Further investigation is warranted to substantiate this speculation. Limited data exist describing the influence of IPC on constant-load hypoxic exercise, with studies that indicate improvements in hypoxic cycling time trial performance following IPC demonstrating greater levels of deoxygenation across the more intense efforts (13, 14). Recently, Chopra et al. (18) demonstrated an ~19% reduction in gastrocnemius oxygen extraction during steady-state 100-W cycling in severe ($F_{I_{O_2}}$ = 0.103) hypoxia. In the present investigation, TSI during exercise was reduced in all hypoxic conditions in agreement with existent literature (40, 41), with IPC able to significantly modulate the reduction when all exercise intensities were considered. Notably, trends were visualized indicating 11–16% increases in TSI during exercise of intensity similar to that used by Chopra et al. (40 and 60% HYP W_{peak}) after IPC7.

Limitations of this investigation should be transparently acknowledged. Because of an incomplete understanding of repeated IPC's duration of influence (23), completion of experimental conditions was not randomized. The authors acknowledge this as a limitation; however, because of the submaximal nonperformance nature of the exercise and the washout durations used, it is suggested that the influence of a learning or carryover effect is likely minimal. Because of a logistical inability to measure SFA blood flow by Doppler ultrasound during exercise, immediate postexercise measures are utilized as reflective of exercise SFA blood flow. Notably, SFA blood flow values are therefore lower than those expected during exercise. The moderate fitness level ($\dot{V}O_{2peak}$ 43.6 ± 4.7 mL.kg^{-1}.min^{-1}) and exercise modality unfamiliarity of our volunteers also undoubtedly influenced the complex oxygenation adjustments induced by the coexistence of hypoxia, exercise, and IPC (15, 42). It is suggested that fitness level and exercise modality familiarity are key modulators of physiological response to exercise after IPC. The relationship between and magnitude of ventilatory responses and local metabolic efficiency during hypoxic exercise would be expected to significantly differ in well-trained athletes because of the coexistence of significant training adaptations, including improvements in respiratory muscle strength and efficiency (43). Finally, it is worth noting that evidence suggests that blood flow and muscle oxygenation responses to IPC may differ between biological males and females, attributable to differences in hormonal milieu and muscle metabolism (44). Further research should aim to emulate hypoxic submaximal exercise investigations with female volunteers, especially given their generally smaller pulmonary structures, to verify the most appropriate populations for IPC.

Perspectives and Significance

IPC7 augments $\dot{V}_E$ increasingly throughout hypoxic exercise to an ~10% increase at 80% HYP W_{peak}, primarily driven by increased breathing frequency. Control of respiration consists of a complex interplay between central and peripheral chemoreceptors (45), lung/airway mechanoreceptors, as well as group III/IV skeletal muscle mechano- and metaboreceptors (46, 47). It is possible that changes in ventilation with IPC7 are modulated by activation of skeletal muscle hypoxia-hypoxia-inducible factor (HIF)-1 pathways with systemic influence (19) and/or an "overpriming" of group III/IV skeletal muscle afferents. IPC is linked to the release of a variety of local factors including adenosine and bradykinin (48), both of which have been linked to augmentation of group III and IV afferent discharge rates and/or sensitivity (49, 50) capable of enhancing communication of local muscular stress to the central nervous system (CNS) and consequently influencing medullary ventilatory control. Regardless, greater ventilation without a commiserate Sp_{O_2} defense, especially at high exercise intensities, provides no ergogenic benefit. Furthermore, as indicated by increases in hypoxic oxygen consumption for IPC7, elevations in ventilation at submaximal intensities are likely to negatively influence exercise efficiency. A single session of IPC may also modulate hypoxic ventilation; however, our present population was unable to demonstrate this fact with any certainty. Locally, it does appear probable and replicable that IPC7 improves muscle oxygen saturation (reduces muscle oxygen extraction) at hypoxic rest and exercise. Seven days of IPC appears a superior ergogenic intervention, compared with a single session, to modify muscle oxygenation responses to hypoxic rest and exercise. Furthermore, caution should be exercised when using ischemic preconditioning as a preexercise interventional strategy with both healthy and clinical populations until its influence on hypoxic ventilation is more fully understood.

SUPPLEMENTAL DATA

Supplemental materials: https://doi.org/10.17605/OSF.IO/MHTKV.

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DISCLAIMERS

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

A.D.S., L.P.C., S.A., A.C.P., B.A., and K.A.J. conceived and designed research; A.D.S. performed experiments; A.D.S. and A.R.C. analyzed data; A.D.S. and A.R.C. interpreted results of experiments; A.D.S. and A.R.C. prepared figures; A.D.S. drafted manuscript; A.D.S., A.R.C., L.P.C., S.A., A.C.P., B.A., and K.A.J. edited and revised manuscript; A.D.S., A.R.C., L.P.C., S.A., A.C.P., B.A., and K.A.J. approved final version of manuscript.

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