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Combining remote ischemic preconditioning and aerobic exercise: a novel adaptation of blood flow restriction exercise

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Sprick JD, Rickards CA. Combining remote ischemic preconditioning and aerobic exercise: a novel adaptation of blood flow restriction exercise. Am J Physiol Regul Integr Comp Physiol 313: R497–R506, 2017. First published August 23, 2017; doi:10.1152/ajpregu.00111.2017.—Remote ischemic preconditioning (RIPC) can attenuate tissue damage sustained by ischemia-reperfusion injury. Blood flow restriction exercise (BFRE) restricts blood flow to exercising muscles. We implemented a novel approach to BFRE with cyclical bouts of blood flow restriction-reperfusion, reflecting the RIPC model. A concern about BFRE, however, is potential amplification of the exercise pressor reflex, which could be unsafe in at-risk populations. We hypothesized that cyclical BFRE would elicit greater increases in sympathetic outflow and arterial pressure than conventional exercise (CE) when performed at the same relative intensity. We also assessed the cerebrovascular responses due to potential implementation of BFRE in stroke rehabilitation. Fourteen subjects performed treadmill exercise at 65–70% maximal heart rate with and without intermittent BFR (4 × 5-min intervals of bilateral thigh-cuff pressure followed by 5-min reperfusion periods). Mean arterial pressure (MAP), plasma norepinephrine (NE), and middle and posterior cerebral artery velocities (MCAv and PCAv) were compared between trials. As expected, BFRE elicited higher concentration NE compared with CE (1249 ± 170 vs. 962 ± 114 pg/ml; P = 0.06). Unexpectedly, however, there were no differences in MAP between conditions (overall P = 0.33), and MAP was 4–5 mmHg lower with BFRE versus CE during the reperfusion periods (P = 0.05 for reperfusion periods 3 and 4). There were no differences in MCAv or PCAv between trials (P = 0.22), suggesting equivalent cerebrometabolic demand. The exaggerated sympathoexcitatory response with BFRE was not accompanied by higher MAP, likely because of the cyclical reperfusions. This cyclical BFRE paradigm could be adapted to cardiac or stroke rehabilitation, where exercising patients could benefit from the cardio and cerebroprotection associated with RIPC.

KAATSU; vascular occlusion training; exercise for cardiac rehabilitation; exercise for stroke rehabilitation

REMOTE ISCHEMIC PRECONDITIONING (RIPC) is a therapeutic approach that has been developed to attenuate the damage incurred by ischemia-reperfusion injury (23, 39). Characterized by cyclical occlusions and reperfusions of a remote limb, this therapy has been used in numerous clinical trials in diverse patient populations, including patients undergoing repair of congenital heart defects, coronary artery bypass grafting, and primary percutaneous coronary intervention (6, 16, 44). Because all of these clinical scenarios are associated with an elevated risk of ischemia-reperfusion injury, RIPC is applied preoperatively to reduce the severity of this injury if and when it occurs. Recently, a number of studies have demonstrated the efficacy of RIPC when used prophylactically in patients with significant carotid artery stenosis (30, 31). Five cycles of 5-min bilateral upper arm ischemia and reperfusion applied twice daily over 180–300 days increased cerebral blood flow and decreased the incidence of stroke in these high-risk patients (30, 31). These improvements in cerebrovascular function are consistent with other recent work demonstrating that daily RIPC therapy can also promote improvements in peripheral vascular function (i.e., flow-mediated dilation and cutaneous vascular conduction) (21, 22). These documented benefits in vascular health raise the question of whether RIPC therapy could be used as an adjunct to aerobic exercise, which also facilitates improvements in vascular function (10) and can reduce the severity of ischemic injury (9, 14).

A novel form of exercise that shares some similarities with RIPC is known as blood flow restriction exercise (BFRE) (24, 26, 36, 42). Originally designed to promote muscular hypertrophy, this exercise modality uses restrictive bands or cuffs to reduce blood flow to the active muscles (24). Although most investigations using BFRE have focused on resistance training (26, 27), studies using aerobic exercise have also reported muscular hypertrophy (2, 42) and improved aerobic capacity (1, 36). A key concern that has recently been raised with use of BFRE, however, is the potential for an amplification of the exercise pressor reflex due to greater stimulation of the type III (mechano-sensitive; via cuff compression) and type IV (metabo-sensitive; via cuff restriction) afferent nerves (45). While several studies have reported elevated heart rate (HR) and arterial pressure responses with aerobic BFRE compared with a control condition performed at the same absolute workload (41, 46, 47) (i.e., matching physical work), no studies to date have assessed the sympathetic responses to BFRE when HR is matched between conditions (i.e., matching physiological work). Given that exercise prescriptions for both healthy and clinical populations are typically based on a relative intensity that represents a percentage of maximal HR (HRmax) or HR reserve (4), this is the approach utilized in the present investigation.

The vast majority of studies of aerobic BFRE have utilized short exercise bouts (~2 min) with continuous limb blood flow...
restriction (41, 42, 46, 47), which is the traditional methodological approach for BFRE. One recent study did explore the HR, muscle deoxygenation, and pulmonary responses to intermittent BFRE (upright cycling), but arterial pressures and/or other measures of the exercise pressor reflex were not reported (7). Furthermore, to our knowledge, no studies have assessed the cerebral blood flow and cerebral oxygenation responses to aerobic BFRE; assessment of these responses is necessary for potential implementation within a stroke-rehabilitation setting. Because of our interest in combining RIPC with exercise, the aim of the present investigation was to assess the sympathetic, hemodynamic, and cerebrovascular responses to a novel form of BFRE, which superimposes the RIPC-like paradigm of cyclical blood flow restriction and reperusions with steady-state aerobic exercise via treadmill walking. We hypothesized that there would be an augmentation of the exercise pressor reflex with BFRE compared with conventional exercise (CE) when performed at the same relative HR intensities.

**METHODS**

**Subjects**

Young, healthy volunteers participated in this study conducted at the University of North Texas Health Science Center (UNTHSC) in Fort Worth, TX. All experimental procedures were conducted in accordance with a protocol approved by the UNTHSC Institutional Review Board (IRB no. 2014–149). Before participation, all subjects underwent a medical history evaluation, including seated and standing 12-lead electrocardiogram (ECG) and blood pressure measurements, and were cleared to participate by a physician. Subjects did not routinely use any nicotine products (including tobacco cigarettes, electronic cigarettes, chewing tobacco). Before each experiment, subjects abstained from caffeine, alcohol, dietary supplements, medications, and exercise for 24 h and fasted for at least 8 h (overnight). Female subjects completed a urine pregnancy test to ensure they were not pregnant. All subjects underwent a familiarization session in which they were shown all equipment and experimental procedures that would be performed in the subsequent experimental sessions. Each subject gave written informed consent to participate in this study. All subjects also participated in a resistance BFRE study reported in a companion manuscript (45a). The RIPC protocol that was performed in both studies was identical.

**Maximal Exercise Testing**

All subjects underwent a 1-repetition maximum (1RM) test on a leg press machine before a maximal aerobic exercise test (data presented in 45a). As the maximum load of the leg press machine is 184 kg, individuals who had a 1RM greater than 184 kg were excluded from participation in this study. After a 1-h rest period (15), peak oxygen uptake (VO2peak) was assessed via a treadmill test to volitional fatigue (TMX428CP, TrackMaster, Newton, KS) in accordance with the Bruce Protocol (4). HR (wireless strap; Polar, H1 Series, Polar Electro Oy, Kempele, Finland) that was interfaced with the metabolic cart was also used to verify that subjects were within their target HR range (65–70% HRmax) for the duration of the experiment: ECG data were used for HR analysis. Noninvasive arterial pressure and stroke volume [via the pulse contour method (19)] were measured via finger photoplethysmography (Finometer, Finapres Medical Systems, Amsterdam, The Netherlands). This arm was placed in a sling for stability to ensure accurate detection of the blood pressure waveform throughout the exercise bout. The Finometer was placed on the left arm for most subjects and was on the same arm within each subject for both treadmill experiments. For some subjects, the Finometer was placed on the opposite arm for the RIPC experiment as this condition was included in the current study and in the study reported in a companion paper. Bilateral transcranial Doppler ultrasound (ST3, Spencer Technologies, Seattle, WA) was used to measure middle cerebral artery velocity (MCAv) and posterior cerebral artery velocity (PCAv). The MCAv and PCAv signals were always obtained on opposite sides of the head, which was variable between subjects, but the same within each subject across all experiments. Cerebral oxygen saturation of the frontal cortex (So2) was measured via near-infrared spectroscopy (NIRS; OxiplexTS, ISS, Champain-Urbana, IL) and was calculated as the quotient of oxygenated hemoglobin to total hemoglobin concentrations (THC) multiplied by 100; THC was calculated as the sum of oxygenated hemoglobin and deoxygenated hemoglobin. Cerebral oxygenation measurements were only measured on one side of the forehead and were always selected to be the same side as the TCD-derived MCAv measurement to ensure that regional oxygenation and perfusion were measured on the same side. A venous catheter was inserted into an antecubital vein of the arm contralateral to the blood pressure measurements for collection of venous blood samples. As previously indicated, this arm was the same side for both treadmill experiments, but sometimes the opposite arm for the RIPC experiment. During the blood flow restriction protocols (BFRE and RIPC sessions), 5-cm wide inflatable pressure cuffs (SC5, D.E. Hokanson, Bellevue, WA) were placed around both upper thighs, secured in place with tape, and connected to an inflation system (E20 Rapid Cuff Inflation System, D.E. Hokanson).

**Blood flow restriction aerobic exercise session.** This session was used to determine the effects of BFRE on sympathetic, hemodynamic, and cerebrovascular responses. After instrumentation, subjects underwent a 15-min seated baseline, after which they were moved onto the treadmill for the following 40-min exercise bout. At the start of exercise, the thigh cuffs were rapidly inflated to 220 mmHg and the treadmill speed was adjusted to a target of 4 km/h (2.5 mph). While the treadmill speed remained constant (by design), the incline (%) grade was adjusted to achieve the target HR intensity corresponding to 65–70% of HRmax. Thus, the target HR intensity was maintained by adjusting the incline setting only. The cuffs remained inflated to 220 mmHg for 5 min followed by a 5-min deflation and reperfusion sessions (randomized) separated by at least 1 mo each. The three sessions were the following: 1) blood flow restriction aerobic exercise (BFRE), 2) conventional aerobic exercise (CE), and 3) remote ischemic preconditioning (RIPC). Female subjects were tested in the early follicular phase of their menstrual cycle (first 4 days measured by self-report) and completed a urine pregnancy test at the start of each visit to the laboratory to ensure they were not pregnant. All sessions were performed in the morning in a thermo-neutral laboratory (temperature = 23.2 ± 0.1°C, humidity = 53.2 ± 2.2%, barometric pressure = 744.2 ± 0.6 mmHg).

**Instrumentation.** Upon arrival to the laboratory, subjects were encouraged to empty their bladder to ensure optimal comfort and to limit the potential confounding effects of increased sympathetic nervous system activation with bladder distension (11). Subjects were instrumented with a standard II lead ECG (shielded leads, cable, and amplifier, AD Instruments, Bella Vista, NSW, Australia) for measurement of R-R intervals and calculation of HR. A separate wireless HR monitor (wireless strap; Polar, H1 Series, Polar Electro Oy, Kempele, Finland) that was interfaced with the metabolic cart was also used to verify that subjects were within their target HR range (65–70% HRmax) for the duration of the experiment: ECG data were used for HR analysis. Noninvasive arterial pressure and stroke volume [via the pulse contour method (19)] were measured via finger photoplethysmography (Finometer, Finapres Medical Systems, Amsterdam, The Netherlands). This arm was placed in a sling for stability to ensure accurate detection of the blood pressure waveform throughout the exercise bout. The Finometer was placed on the left arm for most subjects and was on the same arm within each subject for both treadmill experiments. For some subjects, the Finometer was placed on the opposite arm for the RIPC experiment as this condition was included in the current study and in the study reported in a companion paper. Bilateral transcranial Doppler ultrasound (ST3, Spencer Technologies, Seattle, WA) was used to measure middle cerebral artery velocity (MCAv) and posterior cerebral artery velocity (PCAv). The MCAv and PCAv signals were always obtained on opposite sides of the head, which was variable between subjects, but the same within each subject across all experiments. Cerebral oxygen saturation of the frontal cortex (So2) was measured via near-infrared spectroscopy (NIRS; OxiplexTS, ISS, Champain-Urbana, IL) and was calculated as the quotient of oxygenated hemoglobin to total hemoglobin concentration (THC) multiplied by 100; THC was calculated as the sum of oxygenated hemoglobin and deoxygenated hemoglobin. Cerebral oxygenation measurements were only measured on one side of the forehead and were always selected to be the same side as the TCD-derived MCAv measurement to ensure that regional oxygenation and perfusion were measured on the same side. A venous catheter was inserted into an antecubital vein of the arm contralateral to the blood pressure measurements for collection of venous blood samples. As previously indicated, this arm was the same side for both treadmill experiments, but sometimes the opposite arm for the RIPC experiment. During the blood flow restriction protocols (BFRE and RIPC sessions), 5-cm wide inflatable pressure cuffs (SC5, D.E. Hokanson, Bellevue, WA) were placed around both upper thighs, secured in place with tape, and connected to an inflation system (E20 Rapid Cuff Inflation System, D.E. Hokanson).
period. This cyclical occlusion and reperfusion protocol was repeated for four cycles throughout the 40 min of exercise. After the 40 min of exercise was completed, the subjects immediately commenced a 5-min active recovery period during which the treadmill speed was reduced to 2.4 km/h (1.5 mph) and the incline was reduced to 0°. Three blood samples (10 ml) were collected throughout the protocol: 1) 5-min into baseline ("pre"); 2) at the end of the second occlusion period ("mid"), and; 3) at the end of the final reperfusion period ("post").

Conventional aerobic exercise session. This session was used to compare the sympathetic, hemodynamic, and cerebrovascular responses of BFRE to CE. This protocol was performed in exactly the same manner as the BFRE session with the exception that there were no thigh cuffs applied and hence no occlusive stimulus. Exercise intensity was set at 65–70% of $HR_{max}$ achieved with a treadmill speed of 4 km/h (2.5 mph) and variable incline, as previously described. Blood samples were collected at the same time points as the BFRE condition (pre, mid, and post).

Remote ischemic preconditioning session. This session served as a control condition to isolate the effects of the repeated cuff occlusions and reperusions, independent of exercise. After instrumentation, subjects completed a 15-min seated baseline. The thigh cuffs were then rapidly inflated to 220 mmHg for 5 min, followed by rapid deflation and reperfusion for 5 min. This occlusion/reperfusion protocol was repeated four times over 40 min followed by a 5-min recovery period. Blood samples were collected at the same time points as the exercise conditions (pre, mid, and post).

Data Analysis

All continuous waveform data [ECG, arterial pressure, stroke volume, MCAv, PCAv, eCO2, THC, end-tidal CO2 (etCO2)] were recorded at 1,000 Hz (PowerLab/Labchart, AD Instruments, Bella Vista, NSW, Australia) and analyzed offline via specialized software (WinCPRS, Absolute Aliens, Turku, Finland). R-wave detection was performed on the ECG signal and used to determine the timing of each cardiac cycle. Mean arterial pressure (MAP), mean MCAv, and mean PCav were calculated as the area under the curve from the arterial pressure and cerebral blood velocity waveform. Cardiac output was calculated as HR multiplied by stroke volume, and total peripheral resistance (TPR) was subsequently calculated as MAP divided by cardiac output. For the baseline period, minutes 5–10 were averaged. For the 40 min of exercise, all hemodynamic variables were averaged for each 5-min period; these time intervals corresponded to each occlusion and reperfusion period, and recovery. The data were evaluated in this way so that each occlusion and reperfusion period could be analyzed independently and compared with the matching time point during the CE condition with no occlusive stimulus and with the RIPC condition without the exercise stimulus.

Whole blood was collected in EDTA tubes treated with glutathione as a preservative (1.23 mg glutathione/1 ml whole blood) and centrifuged at 1,500 RPM for 15 min at 4°C. Plasma was separated and snap frozen in liquid nitrogen and then stored at −80°C until analyzed. Norepinephrine (NE) was measured in duplicate via enzyme-linked immunosorbent assay (BA E-6200, Rocky Mountain Diagnostics, Colorado Springs, CO). Only duplicate samples with a coefficient of variation <15% were included in the final analysis. As a result, $N = 9$ for NE data. Hematocrit was also assessed from the “pre” blood sample to ensure equivalent hydration status between conditions.

Statistics

Two-way repeated measures ANOVAs (factor 1: time, factor 2: condition, BFRE, CE, RIPC) were used to compare the effects within and between each condition over time. A one-way (condition only) repeated measures ANOVA was used to compare baseline hematocrit between conditions. Tukey post hoc tests were performed when a significant interaction was indicated by the ANOVA values. For comparison of treadmill incline, a paired t-test was performed between the BFRE and CE conditions. Exact $P$ values are reported for all comparisons. Unless otherwise stated, all data are presented as means ± SE.

RESULTS

Twenty-three subjects were recruited to participate in this study. Of these 23 subjects, 2 were excluded due to VO2peak <30 ml·kg⁻¹·min⁻¹, 3 were excluded due to a 1RM >184 kg, 2 were excluded due to medication use, and 2 withdrew due to scheduling or personal reasons. As a result, 14 subjects completed all three experimental conditions (8M/6F, age 28 ± 2 yr, height 170 ± 3 cm, weight 71 ± 3 kg, BMI 24.5 ± 0.7 kg/m²).

The average $HR_{max}$ was 189 ± 2 beats/min, and the average VO2peak was 35.8 ± 1.8 ml·kg⁻¹·min⁻¹. There were no differences in baseline hematocrit between the three conditions ($P = 0.68$). During the two exercise trials (CE and BFRE), all subjects achieved the target HR intensity of 65–70% $HR_{max}$ and were able to maintain this intensity throughout the entire 40 min of exercise (Fig. 1A). In one case, the treadmill speed was adjusted below 4 km/h during the occlusion periods to maintain the subject’s HR within the target range. For one other subject, the treadmill incline was not recorded due to a technical failure. As a result, $N = 13$ for treadmill incline. While the treadmill speed remained constant at 4 km/h (by design), subjects were able to reach this target HR intensity at a lower treadmill incline during BFRE compared with CE (7.3 ± 0.3° for CE vs. 6.0 ± 0.3° for BFRE, $P < 0.001$).

Stroke volume and cardiac output increased with exercise for both CE and BFRE ($P < 0.001$) and remained elevated above baseline throughout the exercise periods (Fig. 1, B and C). While cardiac output remained elevated above baseline throughout the exercise bouts ($P < 0.001$), stroke volume progressively fell throughout the exercise bout for both CE and BFRE ($P ≤ 0.03$ from 25-min into exercise vs. onset of exercise). While there was no change in cardiac output throughout the RIPC session ($P ≥ 0.99$), stroke volume fell below baseline values during the final three occlusion periods ($P ≤ 0.07$), suggesting a decreased venous return with limb occlusion. TPR decreased with exercise for both CE and BFRE ($P < 0.001$), and while there were no differences between CE and BFRE ($P = 0.73$), TPR was lower for both exercise conditions compared with RIPC throughout the intervention ($P ≤ 0.001$) (Fig. 1D).

In support of our hypothesis, plasma NE concentration increased with exercise ($P < 0.001$ for both BFRE and CE) and was higher with BFRE compared with CE at the “post” time point ($P = 0.06$; Fig. 2A). NE concentration did not increase from baseline with RIPC ($P ≥ 0.91$). MAP increased with exercise for both CE and BFRE ($P < 0.001$) and was higher with BFRE during the first occlusion period compared with CE ($P = 0.08$) (Fig. 2B). MAP remained elevated with CE with no differences between minutes 5 and 40 of exercise ($P ≤ 0.18$ vs. 5-min time point). In contrast, MAP progressively fell with BFRE throughout the exercise period ($P ≤ 0.02$ vs. 5-min time point) and was lower than CE during the third and fourth reperfusion periods ($P ≤ 0.05$). Interestingly, MAP also increased progressively with RIPC ($P = 0.04$ vs. baseline by the final reperfusion period), reaching values equivalent to the BFRE condition by the third reperfusion period ($P ≥ 0.46$). Based on the observation that BFRE elicited a lower MAP...
response than CE during a number of the reperfusion periods when all three conditions were included in the ANOVA (BFRE, CE, RIPC), a separate two-way ANOVA was conducted between CE and BFRE only to isolate the MAP responses to the two exercise conditions independent of RIPC. This analysis revealed that during BFRE, MAP was still initially higher during the first occlusion period compared with CE ($P = 0.03$), but then was lower during all four reperfusion periods ($P = 0.05$) and during recovery ($P = 0.07$).

Mean MCAv increased from baseline with the onset of exercise ($P < 0.001$), then progressively fell throughout exercise for both CE and BFRE ($P < 0.001$ from 15 min into exercise vs. onset of exercise), with no differences between these two conditions ($P = 0.99$) (Fig. 3A). For mean PCAv, $N = 7$ due to the inherent difficulties of acquiring and maintaining the PCA signal throughout each of the three experimental sessions. Mean PCAv also increased from baseline with the onset of exercise ($P < 0.001$) and remained elevated throughout the exercise bout for CE ($P \geq 0.42$ vs. onset of exercise) but decreased progressively toward baseline with BFRE ($P \geq 0.09$ vs. baseline by occlusion 3). Overall, there were no differences in mean PCAv between the two exercise conditions ($P = 0.22$). Unexpectedly, mean PCAv was slightly higher at baseline with BFRE compared with RIPC ($P = 0.02$), but not between CE and RIPC, or between BFRE and CE (both $P \geq 0.25$). etCO2 increased with the onset exercise for both CE and BFRE ($P \leq 0.001$) and remained higher during both exercise conditions compared with RIPC throughout the intervention ($P \leq 0.03$) (Fig. 3C). There was an overall time by condition effect ($P = 0.05$) for ScO2 and this appears to be primarily driven by the exercise conditions ($P = 0.07$ for BFRE vs. RIPC; $P = 0.10$ for CE vs. RIPC), with no difference between BFRE and CE ($P = 0.98$) (Fig. 3D).

**DISCUSSION**

In the current investigation we assessed the sympathetic, hemodynamic, and cerebrovascular responses to an acute bout of steady-state aerobic BFRE with cyclical occlusions and reperusions, reflecting the RIPC paradigm. The major findings were the following: 1) in support of our hypothesis, we observed an increase in sympathetic activity with BFRE, evi-
During the exercise bouts in these studies, it is certainly possible that the attenuated arterial pressure response observed with BFRE in the present investigation was due to the superimposition of the RIPC model with exercise, resulting in an enhanced local vasodilation with each reperfusion period. Unfortunately, our only measure of vascular resistance in the current investigation is TPR, so we are unable to determine if local vasodilation of the active muscle beds was enhanced during each reperfusion period; this phenomenon certainly requires further investigation.

While stroke volume was not different between BFRE and CE, the decreased stroke volume observed during occlusion only with the RIPC condition suggests that the restrictive stimulus of the cuffs was sufficient to decrease venous return. This decrease in stroke volume with cuff inflation was offset by the addition of exercise during the BFRE condition, however, most likely due to engagement of the skeletal muscle pump. Although partial restriction of venous return with cuff inflation might still be occurring with BFRE, it is possible that the increased sympathetic activity we observed with BFRE (reflected in the NE response) could increase cardiac contractility (12), subsequently increasing stroke volume and compensating for any decreases in venous return induced by cuff inflation. Several investigations have reported lower stroke volume with BFRE compared with a control condition where subjects exercised at the same absolute workload (41, 46, 47). In these studies, however, continuous vascular occlusion was used, which may elicit a greater restriction of venous return, compared with the cyclical occlusion-reperfusion paradigm used in our investigation.

To our knowledge, this is the first investigation to superimpose an RIPC-like paradigm of 4 × 5-min cycles of limb blood flow restriction/reperfusion with steady-state aerobic exercise. This is in contrast to most BFRE protocols, which use a continuous occlusive stimulus throughout the exercise bout.
ScO$_2$ was higher for CE and BFRE than RIPC at several time points (factor 1) and between conditions (factor 2). Tukey post hoc tests were performed when an interaction effect was present. *P < 0.05 for CE and BFRE vs. RIPC; ‡P < 0.01 for BFRE vs. RIPC.

Fig. 3. Regional cerebral blood velocity, cerebral oxygenation, and end-tidal CO$_2$ (etCO$_2$) responses to conventional exercise (CE; ○), blood flow restriction exercise (BFRE; □), and remote ischemic preconditioning (RIPC; ▲). Occlusion periods denoted by vertical gray bars. Mean middle cerebral artery velocity (MCAv; A), mean posterior cerebral artery velocity (PCAv; B, N = 7), and etCO$_2$ (C) increased over time with exercise (P < 0.001) and were higher with CE and BFRE compared with RIPC (P < 0.02). There was an overall condition effect (P = 0.05) for frontal lobe cerebral oxygen saturation (ScO$_2$; D, N = 12); ScO$_2$ was higher for CE and BFRE than RIPC at several time points (P < 0.09). A two-way repeated measures ANOVA was performed to compare differences across time (factor 1) and between conditions (factor 2). Tukey post hoc tests were performed when an interaction effect was present. *P < 0.05 for CE and BFRE vs. RIPC; ‡P < 0.01 for CE vs. RIPC; ‡‡P < 0.05 for BFRE vs. RIPC.

One recent study did utilize an intermittent BFRE protocol (7), but the duration and frequency of the occlusive stimulus was quite different to traditional RIPC protocols (10 × 2-min bouts of exercise with occlusion, each separated by 1-min of active recovery without occlusion). Furthermore, the increased muscle deoxygenation and metabolic strain reported with intermittent BFRE in that study are not directly related to the sympathoexcitatory responses explored in the current investigation. In regards to aerobic BFRE with continuous occlusion, several studies have demonstrated that treadmill walking with blood flow restriction resulted in a greater increase in MAP compared with a nonocclusive control condition performed at the same treadmill speed (3.2–4 km/h); i.e., the same absolute workload (41, 46, 47). Not surprisingly, HR was consistently higher with BFRE compared with the control condition; i.e., subjects were exercising at a higher relative intensity with BFRE, due to constant stimulation of the exercise pressor reflex with a continuous occlusive stimulus. In the present investigation, HR was matched between trials to ensure that the same relative intensity was achieved in both conditions. As exercise prescriptions are typically based on a percentage of HR$_{max}$ or HR reserve, we opted to match HR between conditions to be consistent with current recommendations (4).

It is noteworthy that although HR was matched between conditions, the treadmill incline required to elicit the target HR response was lower in the BFRE condition compared with CE (lower absolute workload). We speculate that this response stems from augmented activation of the exercise pressor reflex with the occlusive stimulus, eliciting an elevation in HR. Consequently, to maintain HR within the predetermined target range (i.e., 65–70% HR$_{max}$), the treadmill incline had to be reduced in the BFRE condition. The lower treadmill incline we observed with aerobic BFRE is analogous to the lower training intensities traditionally utilized in blood flow restriction resistance exercise (20% of 1RM rather than 65% of 1RM) (24). This finding could have implications for application of BFRE to the rehabilitation setting, where individuals may benefit from the lower mechanical stress associated with a decreased treadmill incline, particularly elderly individuals and/or patients with musculoskeletal injuries. If the same benefits known to be associated with conventional aerobic exercise are also
VO2peak, the level of exercise previously reported to cause a significant increase in cerebral blood flow. Exercise intensities utilized in this study are above 60% of VO2peak in parallel with increases in metabolically derived arterial CO2. As exercise intensity increases above 60% of VO2peak, hyperventilation-induced reductions in arterial CO2 cause cerebral vasoconstriction and a subsequent reduction in cerebral blood flow. In the present investigation, we observed cerebral blood flow responses in the anterior (indexed by mean MCAv) and posterior (indexed by mean PCAv) circulations that are consistent with this model, as these responses also tracked changes in etCO2 (used as a surrogate for arterial CO2). Metabolic production of CO2 combined with increases in MAP resulted in an initial increase in cerebral blood flow with exercise which was then followed by a progressive decrease due to hyperventilation-induced hypocapnia. This response was expected as the exercise intensities utilized in this study are above 60% of VO2peak, the level of exercise previously reported to cause a hypocapnia-induced cerebral vasoconstriction (35). While MAP was lower at multiple time points during the BFRE condition compared with CE, this pattern of response was not reflected in cerebral blood flow. Since we recruited only young, healthy subjects in the present study, the relative stability of cerebral blood flow despite variations in arterial pressure is likely due to intact cerebral autoregulation (49). Future studies are required, however, to determine if this stability of cerebral blood flow is still maintained in clinical populations such as stroke patients, who often exhibit impairments in cerebral autoregulation (5). We also observed similar frontal lobe oxygen saturation (ScO2) responses between the two exercise conditions. As the cerebral NIRS signal is understood to primarily be indicative of oxygen saturation of the venous blood rather than tissue (i.e., prefrontal cortex) oxygen saturation (29), alterations in ScO2 can be suggestive of changes in cerebral oxygen extraction. That is, a decrease in ScO2 would indicate either a reduction in O2 supply (i.e., cerebral blood flow) and/or an increase in O2 demand (i.e., cerebral oxygen consumption). By coupling this metric with simultaneous measurements of cerebral blood flow (via velocity), the balance of cerebral oxygen supply and demand can be assessed. Because there were no differences in cerebral blood flow and ScO2 responses between exercise conditions, we interpret these outcomes to suggest an equivalent cerebro-metabolic demand. As it has been suggested that the exercise-induced increases in cerebral blood flow are a key feature underlying the enhancements in cerebrovascular function associated with exercise (28), these findings have promising implications for application of BFRE to stroke rehabilitation, where patients may benefit from the lower arterial pressures and equivalent cerebrovascular responses compared with conventional exercise paradigms.

There are several methodological considerations that need to be mentioned as they relate to interpretation of these findings. First, assessment of cerebral blood flow via transcranial Doppler ultrasound relies on the assumption that the diameter of the insonated artery remains constant. Recent studies using high resolution magnetic resonance imaging, however, have demonstrated that periods of pronounced hypercapnia (+9 mmHg) or hypocapnia (−13 mmHg) can induce diameter changes in the MCA (8, 48). Since the etCO2 responses observed in the present study are much lower in magnitude (approx. +5 mmHg) than those reported in these aforementioned studies, changes in diameter are not likely. Furthermore, even if vessel diameters were increasing in response to elevations in arterial CO2, the reported MCAv measurements would actually be underestimated rather than overestimating changes in cerebral blood flow. While the effects of changes in arterial CO2 on PCA diameter are still unknown, the observation that there were no differences in cerebral blood velocity between the two exercise conditions, diminishes the potential confounds of this limitation.

Second, our NIRS-derived cerebral oxygenation data are limited as we are only able to assess oxygen saturation of the frontal lobe. While more invasive techniques could provide an evaluation of overall cerebral oxygen consumption (i.e., performing arterial-venous blood sampling across the brain), we have minimized the potential confounds of this limitation by simultaneously measuring cerebral blood velocity within the same brain region being assessed by NIRS (via MCAv). Another possible confounding factor related to the cerebral NIRS measurement is the potential for contamination from increases in skin blood flow, which would be expected during exercise (32). However, by using a spatially resolved NIRS sensor consisting of four emitters placed at varying distances from the detector, extraneous measurements from extracranial sample volume (i.e., skin, muscle, fat) can be removed from the NIRS signal.

Third, the measurement of plasma NE as an index of overall sympathetic drive does not represent the total NE that is released from postsynaptic neurons. While performing direct measurements of muscle sympathetic nerve activity would be ideal, the repeated measures design of this study combined with the inherent difficulty of maintaining muscle sympathetic nerve activity signals throughout 45-min of upright treadmill exercise necessitated assessment of sympathetic activity via plasma NE. Additionally, it is generally accepted that venous NE is an appropriate marker of overall sympathetic activity (13), particularly during the steady-state exercise protocol employed in this investigation. Furthermore, we cannot rule out the potential role of pain and/or discomfort from the occlusive cuffs on sympathetic outflow, independent of the exercise protocols. Previous work has demonstrated that RIPC can induce pain with different cuff pressures (43), which could be manifest in an increase in circulating catecholamines. While we did not directly quantify pain in our investigation, subjects did not indicate that inflation of the cuffs was painful per se (although some indicated tolerable discomfort), and no experiments were terminated due to pain.

Fourth, while the 1-mo intervening period between experiments was necessary to control for menstrual cycle phase in our female subjects, we acknowledge that this prolonged length of time could have increased the variability of responses. However,
as the order of experiments was randomized and the key findings were statistically robust, this extended duration of participation did not appear to influence the outcomes of this study.

Fifth, the RIPC session was conducted with the subjects in the upright, seated position, while the two exercise conditions were performed with the subjects in the upright standing position. These postural differences could have influenced hemodynamic and sympathetic responses, independent of the interventions. As the RIPC session was 1 h in duration, however, we opted to keep the subjects in the seated position to ensure that they were as relaxed and comfortable as possible. This posture was a compromise between completely supine and upright. Having subjects in the upright posture for this length of time would very likely have increased sympathetic nervous system activity and engagement of the muscle pump, both potential confounding factors that we were aiming to avoid.

Finally, the variability in cuff sizes and occlusive pressures used between BFRE investigations makes it difficult to compare outcomes across studies. Other investigations have used cuffs ranging from 3 to 18 cm in width (7, 37, 46, 50), whereas others do not report the width of the cuff at all (41, 47). Several studies, including the present investigation use a standardized predetermined occlusive pressure across all participants (4, 47), whereas others use very individualized approaches to determine the target pressures for each subject, including consideration of limb circumference, limb adiposity, and arterial pressure (7, 46). As differences in cuff width (20), cuff pressure (25), as well as the individual physical and physiological characteristics of each subject (18) can all have a profound influence on the achieved degree of arterial occlusion, these variables should be considered when comparing findings across studies. As this was the first investigation to superimpose the cyclical RIPC-like paradigm with steady-state aerobic exercise, we opted to use the same absolute cuff pressure for each subject, which was selected based on pressures commonly used in RIPC studies (16, 21, 22) and those reported within the BFRE literature (2–4). It is important to note, however, that most RIPC studies use a cuff pressure and limb (usually the upper arm) that would result in complete arterial occlusion, which is in contrast to the partial blood flow restriction used in our study (with cuffs around the upper thighs). As highlighted by Spranger et al. (45), differences in cuff width would likely have a dramatic effect on the activation of the exercise pressor reflex, with wider cuffs resulting in a greater activation. These considerations highlight the need for future studies to identify the optimal cuff widths and pressures and limb(s) that should be utilized in this RIPC-like cyclical BFRE paradigm.

Perspectives and Significance

In this investigation we utilized a novel BFRE paradigm that superimposes the cyclical RIPC model of four cycles of 5-min occlusion and 5-min reperfusion with steady-state aerobic exercise at a relative intensity of 65–70% of HR_{max}. While this model resulted in increased sympathetic activity compared with conventional exercise, this response was not accompanied by higher arterial pressure, potentially due to the cyclical nature of the occlusions and reperusions that is unique to this model. Furthermore, although HR was matched between conditions, this target was reached with a lower treadmill incline during the BFRE condition and with an equivalent cerebro-metabolic demand. A key question that remains to be answered is whether the benefits associated with RIPC can also be achieved through the use of this novel exercise paradigm. A recent review by Quindry is promising in this regard, highlighting numerous similarities in the signal transduction pathways underlying cardioprotection facilitated by both exercise and RIPC, including K\textsubscript{ATP} channels, endogenous opioids, and circulating cytokines (40). Furthermore, as repeated RIPC and exercise training can both independently promote improvements in vascular health (21, 22) and reduce the damage incurred by ischemia-reperfusion injury (38), future studies are necessary to explore the possibility that these benefits could be additive within the context of this novel BFRE paradigm. Moreover, as the majority of RIPC studies utilize a restrictive stimulus that most likely results in complete restriction of blood flow, future work should also explore whether complete cessation of blood flow is required to elicit the protective effect or if only partial restriction (as was used in this investigation) can still result in cardio- and cerebro-protective effects, in combination with, or independently of exercise.

This model of exercise could potentially be explored in clinical settings such as cardiac- and stroke rehabilitation, where patients are already exercising and could also benefit from the positive responses associated with RIPC, including reduced tissue damage from ischemia-reperfusion injury. Future longitudinal studies are required, however, before this application could be made.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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

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