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Abstract

Many stroke survivors have reduced cardiorespiratory fitness as a result of their stroke. Ischemic conditioning (IC) is a noninvasive, cost-effective, easy-to-administer intervention that can be performed at home and has been shown to improve both motor function in stroke survivors and vascular endothelial function in healthy individuals. In this study, we examined the effects of 2 wk of remote IC (RIC) on brachial artery flow mediated dilation (FMD) in chronic stroke survivors. We hypothesized that FMD would be improved following RIC compared with a sham RIC control group. This was a prospective, randomized, double-blinded, controlled study. Twenty-four chronic stroke survivors (>6 mo after stroke) were enrolled and randomized to receive either RIC or sham RIC on their affected thigh every other day for 2 wk. For the RIC group, a blood pressure cuff was inflated to 225 mmHg for 5 min, followed by 5 min of recovery, and repeated a total of five times per session. For the sham RIC group, the inflation pressure was 10 mmHg. Brachial artery FMD was assessed on the nonaffected arm at study enrollment and following the 2-wk intervention period. Nine men and fourteen women completed all study procedures. Brachial artery FMD increased from 5.4 ± 4.8 to 7.8 ± 4.4% ($P = 0.030$; $n = 12$) in the RIC group, while no significant change was observed in the sham RIC group (3.5 ± 3.9% pretreatment versus 2.4 ± 3.1% posttreatment; $P = 0.281$, $n = 11$). Two weeks of RIC increases brachial artery FMD in chronic stroke survivors.

NEW & NOTEWORTHY

In this study, we report that 2 wk of remote ischemic conditioning (RIC) improves brachial artery flow-mediated dilation in chronic stroke survivors. Because poor cardiovascular health puts stroke survivors at a heightened risk for recurrent stroke and other cardiovascular events, an intervention that is simple, cost-effective, and easy to perform like RIC holds promise as a means to improve cardiovascular health in this at-risk population.

INTRODUCTION

Stroke is the leading cause of disability of adults in the United States. Approximately 35% of all stroke survivors who present with initial leg weakness do not regain useful function of the leg (14), and 20–25% of chronic stroke survivors are unable to walk without full physical assistance (20). As such, a great deal of focus has been trained on the neural mechanisms of motor recovery after stroke, with the ultimate goal of having stroke survivors regain functional independence. Less studied are the secondary cardiovascular complications and reductions in cardiorespiratory fitness that often occur after stroke. These complications ultimately put stroke survivors at a 1.5– to 6.6-fold increased risk for recurrent stroke (2). Furthermore, a recent study published in the *Journal of the American Medical Association* of more than 15,000 young stroke survivors aged 18–49 yr showed that stroke survivors have approximately a fivefold increase in overall mortality compared with the general population (16). In that study, 17% of the individuals died of cardiovascular disease compared with 10% in the general population (16). In stroke survivors with resultant disability, the deconditioning of the cardiovascular system is likely due to a combination of *1*) premorbid conditions such as obesity, hypertension, and diabetes; *2*) direct effects of the

stroke on cardiovascular centers in the brain; and *3*) reduced physical activity due to disability [reviewed by Billinger et al. (4)].

Brachial artery flow mediated dilation (FMD) is the gold-standard assessment of vascular endothelial health in humans and is predictive of future cardiac events (37), and low FMD is associated with poor outcomes after stroke (38). Stroke survivors have significantly increased cardiovascular risk compared with able-bodied individuals, so it is not surprising that brachial artery FMD is reduced in stroke survivors, even as little as 48 h after stroke (5, 6, 37, 38). Our group has shown that a noninvasive, cost-effective, easy-to-administer intervention called ischemic conditioning (IC) is well tolerated in chronic stroke survivors and that it can improve leg strength, walking speed, and muscle fatigue resistance when it is performed on the affected leg (15, 22). Other groups have shown that local IC of the tested arm and remote IC (RIC) of the contralateral arm or leg can improve brachial artery FMD. Specifically, Moro et al. (32) demonstrated that a single session of either local IC or RIC is sufficient to improve brachial artery FMD in elderly individuals with hypertension, while Jones et al. (23) showed that 7 days of RIC improves brachial artery FMD in young, healthy individuals. Finally, Liang et al. (29) demonstrated that 4 wk of RIC improves brachial artery FMD in patients with coronary heart disease. In this study, we sought to determine whether RIC on the leg is a suitable intervention to improve brachial artery FMD in stroke survivors. We hypothesized that 2 wk of remote RIC on the affected leg would improve brachial artery FMD in the nonaffected arm compared with sham RIC.

MATERIALS AND METHODS

General methods.

This was a prospective, double-blinded, parallel-group, randomized controlled study, and the primary study outcome was the change in brachial artery FMD. The trial was registered on clinicaltrials.gov with the unique identifier NCT03095755. Written informed consent was obtained from all individuals before any study activities, and all study protocols were approved by the Institutional Review Boards at the Medical College of Wisconsin and Marquette University (protocol PRO00019103). All study participants served as their own controls and were studied twice: once at study enrollment and once 24–48 h after completing their final session of either RIC or sham RIC (see below). Inclusion criteria for the study were as follows: *1*) between the ages of 18 and 85 yr, *2*) able to give informed consent, *3*) ≥6 mo postdiagnosis of unilateral cortical or subcortical stroke, and *4*) residual leg paresis. Exclusion criteria were as follows: *1*) substance abuse, *2*) head trauma in last 6 mo, *3*) neurodegenerative disorder, *4*) any uncontrolled serious medical condition, *5*) inability to follow multistep commands, *6*) inability to walk ≥10 ft without physical assistance, *7*) history of major psychiatric disorder, *8*) myocardial infarction in the last year, and *9*) uncontrolled hypertension.

Participant recruitment.

All study participants were recruited from either a secure, online REDCap database of stroke survivors who previously received care at Froedtert Memorial Lutheran Hospital and consented to be contacted for research studies, or by word of mouth. Potentially eligible study participants were contacted by phone by a member of the study team and were asked health-related questions to confirm eligibility. Study participants then reported to the laboratory at Marquette University. and written informed consent was obtained. After obtaining informed consent, the Fugl-Meyer test and 10-m walk test were performed by the same licensed physical therapist who was blinded to group assignment. These measures were included in this study to provide detail related to the degree of motor impairment of the study participants. Baseline assessment of brachial artery FMD was then performed, and study participants were randomized to receive either RIC or sham RIC using an online randomizer.

Ischemic conditioning procedure.

All study participants performed either RIC or sham RIC at home every other day for 2 wk as we and others have previously described (1, 3, 12, 15). Briefly, after demonstrating they could perform the RIC or sham RIC task independently or with the help of their caregiver, study participants were issued a DS400 hand-held aneroid sphygmomanometer (D. E. Hokanson, Bellevue, WA) and a SC12 thigh occlusion cuff (D. E. Hokanson). They were instructed to perform five sets of intermittent cuff inflations while they were lying supine every other day for 2 wk. Participants in the RIC group were instructed to inflate the cuff on their affected thigh to a pressure of 225 mmHg for 5 min, followed by 5 min of recovery, a total of five times per session. Similarly, participants in the sham RIC group were instructed to inflate the cuff to 10 mmHg. This inflation pressure is sufficient to perceive cuff tightness, but not high enough to occlude blood flow to the limb. Study participants were blinded to the purpose of the different cuff inflation pressures, which is consistent with our previous work and other studies that use IC as an intervention and have a sham control group (10, 11, 15, 22, 35).

All study participants were given a calendar to indicate when they should perform the interventions, and they completed worksheets documenting the time of day that the interventions were performed and the peak cuff inflation pressure of each inflation period. We chose to perform RIC on the affected leg based on our previous work and the work of others, showing that RIC of the affected leg of stroke survivors is well tolerated and can result in motor benefits (15, 18, 22, 27). Practically, it is also easier for an individual with upper extremity dysfunction to independently place and operate the cuff on the leg versus the arm. We also chose to have participants perform the procedures every other day, as opposed to every day, to reduce the burden on participants. RIC has two well-described windows of cardioprotection, one that occurs 0–12 h post-RIC (first window) and one that occurs 24–72 h post-RIC (second window) (26). By performing RIC every other day, participants were constantly in the second window of protection. Finally, we chose a two-week intervention period to be consistent with our previous work in stroke survivors (15) and as a compromise between the different intervention periods of longitudinal studies by Jones et al. (23) and Liang et al. (29) that examined the effects of repeated bouts of RIC on brachial artery FMD.

Brachial artery FMD assessment.

Participants reported to the laboratory for assessment of brachial artery FMD 24–48 h after performing their last remote RIC or sham RIC session. Brachial artery FMD assessments were performed in accordance with established guidelines (41), with necessary modifications, as follows. All study participants reported to the laboratory between the hours of 0700 and 1000 and were instructed to fast and abstain from alcohol or caffeine for 12 h, as well as to refrain from exercise for 24 h. Participants reported at the same time of the morning for all pretreatment/posttreatment measurements. Participants were not asked to discontinue their medications for safety concerns.

After the participants had rested in the supine position for 15 min, their nonaffected arm was abducted ~80°, and an SC10 pneumatic cuff (D. E. Hokanson) was placed around their forearm. Baseline brachial artery diameter and blood flow velocity through the artery were measured 3–5 cm proximal to the antecubital fossa using a GE Vivid i ultrasound with a 12L-RS linear array probe (6.0–13.0 MHz; GE Healthcare, Milwaukee, WI) positioned parallel to the vessel to visualize lumen-intimal interfaces. An insonation angle of 60° was used for all measures. Thirty seconds of baseline images were captured on a laptop using Vascular Imager (Medical Imaging Applications, Coralville, IA) and the established ECG-gated methodologies. Next, the cuff was inflated around the forearm to 225 mmHg for 5 min to occlude blood flow to forearm, wrist, and hand using an E20 Rapid Cuff Inflation System (D. E. Hokanson). Peak blood flow velocity during the first five cardiac cycles was measured immediately after cuff release. Continuous duplex imaging of the arterial wall and pulse-wave velocity could not be performed with the resolution necessary to measure brachial artery diameter with the precision required for proper assessment of FMD due to the limitations of the ultrasound system. After the first five cardiac cycles

postcuff release, the artery diameter was continuously captured in B-mode for 3 min and measured off-line using automated edge-detection software (Brachial Analyzer; Medical Imaging Applications) to assess vasodilation. After completion of the first test session, the distance of the measurement site to the antecubital fossa was measured to ensure the same arterial segment would be measured during the second test session.

Peak brachial artery diameter was determined using a moving average of five cardiac cycles. Percent FMD was defined as 100 × (maximum diameter during reactive hyperemia − resting diameter)/resting diameter. Peak and mean shear rate (s^{-1}) were calculated from the pulse wave using the following equation: 8 × $V/BA_{Diameter}$ where V is either peak or mean flow velocity (in cm/s) and $BA_{Diameter}$ is the diameter of the brachial artery (in cm) at cuff release. The same blinded assessor performed the FMD procedures and data analysis.

Statistical analysis.

An a priori power analysis was not performed for this study. We chose to enroll 12 study participants per group based on three previous studies that enrolled 10–20 study participants per group and showed significant IC- or RIC-induced improvement in brachial artery FMD (23, 29, 32). All continuous variables are summarized by means ± SD, and all statistical analyses were performed using IBM SPSS Statistics 26 (International Business Machines Corporation, Armonk, NY). Differences in characteristics between the study participants in the RIC and sham RIC groups were compared using parametric unpaired Student's *t* tests or nonparametric Mann-Whitney *U* tests where appropriate, after testing the data for normality using the Shapiro-Wilk test. Within-group pretreatment versus posttreatment differences in all parameters related to brachial artery FMD were also tested for normality using the Shapiro-Wilk test and then compared using paired *t* tests when normality was not violated. Between-group baseline differences for the same measures were compared using unpaired *t* tests. For our primary outcome, the change in percent brachial artery FMD, the robustness of *t* test-based conclusions was evaluated using the analysis of covariance (ANCOVA) model. After confirming normality (Shapiro-Wilk test), equal variance (Levene test), and equal slopes, the pretreatment/posttreament changes between the groups were compared controlling for the effect of baseline (pretreatment values). A P value <0.05 was used for statistical significance.

RESULTS

A total of 37 chronic stroke survivors were assessed for eligibility for this study, and 24 stroke survivors who met the inclusion criteria were enrolled (see Fig. 1 for study design). One study participant in the sham RIC group withdrew from the study after falling at home during nonstudy-related activities. The average age of all study participants who completed the study was 60 ± 16 yr. Fourteen of the study participants were female, and nine were male. The average time since stroke for all study participants was 8 ± 9 yr, and 16 participants previously had experienced an ischemic stroke, while 7 had a hemorrhagic stroke. The average time between pretreatment and posttreatment tests was 19 ± 7 days for the RIC group and 17 ± 8 days for sham RIC group (P = 0.57). Scheduling conflicts or transportation issues often did not permit "post" testing exactly 14 days after "pre" testing; therefore, participants were instructed to wait until 14 days before the scheduled posttest to begin the interventions. The total number of cuff inflation sessions performed by the study participants was 7 ± 2 for RIC and 7 ± 2 for sham RIC groups (P = 0.99). Four participants (two in RIC group and two in sham RIC group) were forced to reschedule their posttest sessions due to transportation issues. We chose to have these participants continue the RIC or sham RIC interventions every other day, in accordance with our protocol, until the day before their posttest session rather than withdrawing them from the study. A maximum of 10 sessions was allowed (versus the planned seven sessions).

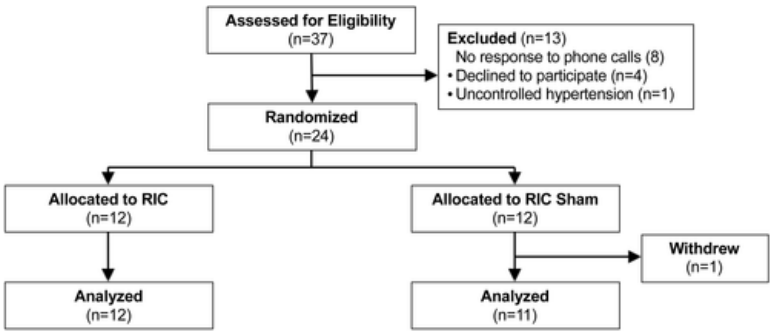

Fig. 1. Diagram of the clinical trial.

As shown in Table 1, there were no differences in the study participants' characteristics between the RIC or sham RIC groups. The data for the participants' ages, weights, and years since stroke were not normally distributed. No differences in age, weight, and time since stroke were detected between the groups using the Mann-Whitney *U* test. The medians and ranges of those biological variables are as follows: Age, years (RIC: median, 64; range, 25–80; sham RIC: median, 59; range, 26–80), weight, kg (RIC: median, 76; range, 54–102; sham RIC: median, 74; range, 58–102); time since stroke, years (RIC: median, 8; range, 1–32; sham RIC: median, 4; range, 1–10). A list of medications taken by the study participants is shown in Table 2.

Table 1. Characteristics of study participants

	RIC (n=12)	Sham RIC (*n*=11)	P Value
Age, yr	60±16	60±18	0.917 [a]
Sex			
Male, *n*	6	3	NA
Female, *n*	6	8	NA
Height, cm	171±11	167±10	0.358 [b]
Weight, kg	77±15	79±18	0.559 [a]
Body mass index, kg/m^2	26±5	28±5	0.498 [b]
Systolic blood pressure, mmHg	132±16	128±13	0.604 [b]
Diastolic blood pressure, mmHg	80±8	81±7	0.708 [b]
Heart rate, bpm	66±9	69±10	0.554 [b]
Time since stroke, yr	12±11	4±3	0.052 [a]
Type of stroke			
Ischemic, *n*	7	9	NA
Hemorrhagic, *n*	5	2	NA
Affected side			
Left, *n*	6	6	NA
Right, *n*	6	5	NA
Assistive device			
Yes, *n*	5	5	NA
No, *n*	7	6	NA
Self-selected walking speed, m/s	0.87±0.28	0.93±0.41	0.752 [b]
Lower extremity Fugl-Meyer score, 0-34	26±6	26±5	0.934 [b]
[a]Mann-Whitney rank sum test;			
[b]Two-sample Student's t test;			

Table 2. Medications taken by study participants

Medication	RIC (*n*=12)	Sham RIC (*n*=11)
Angiotensin-converting enzyme inhibitor	2	4
Angiotensin receptor blocker	0	1
Beta blocker	4	3
Calcium channel blocker	1	4
Antiarrythmic	2	1
Diurectic	3	1
Antihistamine	0	3
Statin	7	7
Bronchodilator	2	1
Corticosteroid	0	2
Chloride channel activator	0	1
Proton pump inhibitor	2	2
Histamine H2 antagonist	1	0
5HT3 receptor antagonist	1	0
Analgesic	2	7
Anticonvulsant	2	0
Barbiturate	1	0
Benzodiazepine	1	3
Cholinesterase inhibitor	0	1
GABA abalog	2	0
Muscle relaxant	3	5
Nonsteroidal anti-inflammatory drug	0	3
Pyrrolidine anticonvulsant	0	1
Salicylate	3	8
Anticoagulant	3	3
Bisphosphonate	1	0
Glucose-elevating agent	0	1
Insulin	0	1
Selective serotonin reuptake inhibitor	5	4
Serotonin-norepinephrine reuptake inhibitor	1	2
Antidepressant	1	2
Antirheumatic	0	1
Estrogen	1	1
n, number of participants.		

The pooled mean brachial artery FMD of all study participants during the pretest session was 4.5 ± 4.4%, which agrees with other studies that have shown that FMD is reduced in stroke survivors compared with age-matched controls (5, 6, 37, 38). Characteristics of the brachial artery are shown in Table 3. At study enrollment, there were no baseline differences between the RIC and sham RIC groups in resting brachial artery diameter (P = 0.31), maximal brachial artery diameter (P = 0.39), time to peak dilation (P = 0.36), cuff release peak flow velocity (P = 0.37), cuff release peak shear rate (P = 0.13), cuff release mean flow velocity (P = 0.62), and cuff release mean shear rate (P = 0.42). Percent FMD during pretesting was also not significantly different between the RIC and sham RIC groups (P = 0.31). The analysis of covariance model (ANCOVA) confirmed t test findings of significantly different FMD changes between the RIC and sham RIC groups. In the ANCOVA model, there was an effect of baseline FMD on the response to RIC or sham RIC (P = 0.009), but even when adjusting for the baseline,

the change in percent FMD between the groups was highly significant ($P = 0.002$) with a difference of means of 4.33%. There was no interaction between baseline FMD and group ($P = 0.55$). The covariate effects of age and time since stroke were not statistically significant ($P = 0.10$ and $P = 0.09$, respectively). Controlling for the effects of age and time since stroke in the ANCOVA model also did not change the statistical significance of the RIC-induced improvement in percent FMD ($P = 0.015$ and $P = 0.001$, respectively). As shown in Fig. 2*A*, brachial artery FMD increased significantly following 2 wk of RIC (5.4 ± 4.7% pre to 7.8 ± 4.4% post; $P = 0.030$), while there was no change in FMD following 2 wk of sham RIC (3.5 ± 3.9% pre to 2.4 ± 3.1% post; $P = 0.281$). Individual pretreatment and posttreatment FMD values for the RIC and sham RIC groups are shown in Fig. 2, *B* and *C*, respectively. As shown in Fig. 3, RIC caused a relative increase in FMD of 2.5 ± 3.4%, while there was a negligible difference of −1.1 ± 3.2% in the sham RIC group.

Table 3. Characteristics of the brachial artery

	RIC (n=*12)*			Sham RIC (*n*=11)		
	Pre	**Post**	**P Value**	**Pre**	**Post**	**P Value**
Baseline brachial artery diameter, mm	3.66±0.76	3.69±0.86	0.702	4.02±0.91	4.18±1.07	0.128
Maximum brachial artery diameter, mm	3.85±0.77	3.97±0.90	0.157	4.15±0.92	4.21±1.10	0.170
Change in brachial artery diameter, mm	0.19±0.17	0.28±0.16	0.014	0.13±0.15	0.09±0.12	0.347
Time to peak dilation, s	67±47	77±46	0.097	44±22	53±34	0.109
Peak flow velocity at cuff release, cm/s	145±43	143±39	0.630	123±26	128±35	0.631
Peak shear rate at cuff release, s^{-1}	33±12	34±16	0.895	26±10	26±11	0.461
Mean flow velocity at cuff release, cm/s	39±17	43±18	0.052	36±10	40±20	0.352
n, number of particpants						

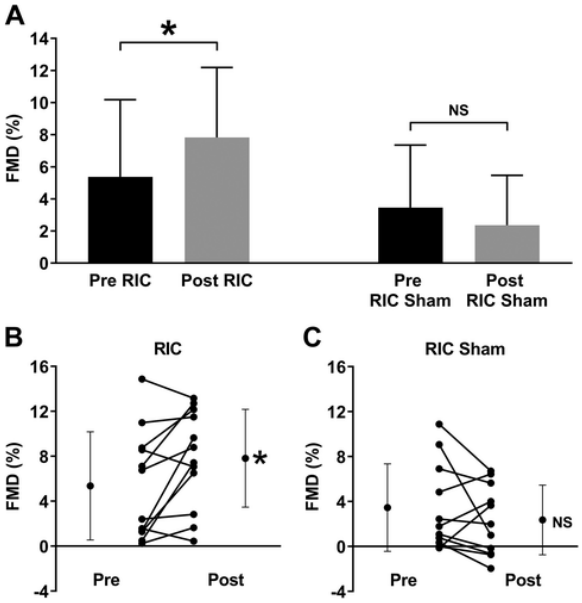

Fig. 2. Brachial artery flow mediated dilation (FMD) before and after 2 wk of either remote ischemic conditioning (RIC) or sham RIC. *A*: percent brachial artery FMD increased in the RIC group (*n* = 12 participants), while no change was observed in the sham RIC group (*n* = 11 participants). Individual responses within the RIC (*B*) and sham RIC(*C*) groups are shown. *$P < 0.05$ vs. Pre RIC. NS, no significance. All data shown are expressed as means ± SD.

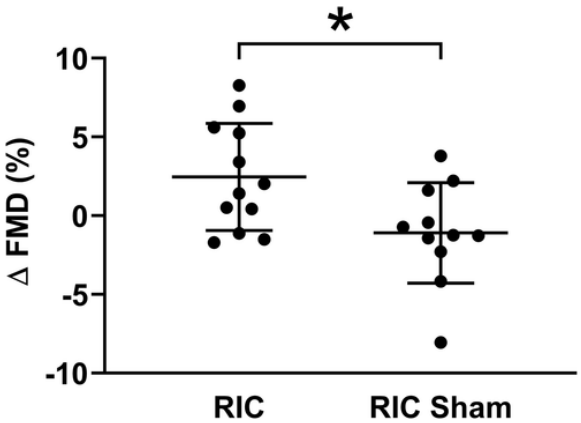

Fig. 3. Change in brachial artery flow mediated dilation (FMD) in the remote ischemic conditioning (RIC) and sham RIC groups. The absolute change in FMD was greater in the RIC group (*n* = 12 participants) compared with the sham RIC (*n* = 11 participants) group. *$P < 0.05$ ischemic conditioning (IC) vs. Sham RIC. All data are expressed as means ± SD.

DISCUSSION

The significant finding of this study is that 2 wk of remote RIC can improve brachial artery FMD in chronic stroke survivors. This finding indicates that RIC is a suitable and efficacious intervention that has potential to improve cardiovascular health in the chronic phase of stroke recovery. Thus, our study adds to the growing body of literature indicating that RIC can potentially improve outcomes and overall physical fitness for stroke survivors (15, 17, 22, 28).

Ischemic conditioning was first described three decades ago as a technique to protect vital organs from ischemic injury by transiently making the tissue of interest ischemic before a longer ischemic insult (33). Work has since shown that RIC of a limb exerts similarly protective effects on vital organs, both in animals (7) and in humans (9, 24, 30). Most of the work related to IC and RIC has been focused on cardioprotection, but in the last decade, increased attention has been given to how RIC can confer neuroprotection and improve outcomes after a stroke (28). The first stroke-related RIC studies in humans demonstrated that RIC on the leg was safe and well tolerated in patients with acute subarachnoid hemorrhage (18, 27). Very recently, pilot studies have shown that RIC has the potential to reduce National Institutes of Health stroke score when performed within 24 h of cerebrovascular accident (17). Ongoing trials are currently examining the efficacy of RIC to reduce infarct size during acute cerebral infarction (NCT02169739) or within 6 mo of stroke (NCT02189928). In addition, our group was the first to demonstrate that local IC on the affected leg can improve muscle performance and walking ability in chronic stroke survivors (15, 22).

To our knowledge, only aerobic exercise has been shown to improve brachial artery FMD after a stroke (5). Unfortunately, a disproportionate number of stroke survivors are not able to exercise independently at home or exercise to an intensity sufficient to receive cardiovascular benefit. Furthermore, long-term compliance to exercise regimens is low, just as it is even in able bodied individuals (8, 34, 40). In our study, all participants completed the expected number of RIC or sham RIC sessions, which underscores the ideal nature of RIC as a potential alternative intervention to improve cardiovascular health after a stroke.

In this study, RIC was performed on the affected leg and FMD was assessed on the nonaffected arm. We chose to measure endothelial function at a site distant from the cuff inflation site because we sought to determine whether RIC could improve systemic endothelial function. Thus, the observed improvement in brachial artery FMD was due to remote effects of IC, not local effects. The exact pathways and mechanisms by which RIC exerts protective effects on remote organs and tissues have been the focus of decades of research. The current body of literature indicates dozens of discrete and interconnected signaling pathways may be necessary for remote cardioprotection [reviewed by Heusch (21)]. While evaluating the mechanisms responsible for the observed improvement in FMD is beyond the scope of this study, there are several likely pathways involved.

Kimura et al. (25) showed that daily IC on the upper limb for one month in healthy young men increased forearm blood flow in response to acetylcholine infusion in the limb exposed to the repeated bouts of IC. Furthermore, the magnitude of the improvement was positively correlated with an increased concentration of endothelial progenitor cells. Improvement in acetylcholine-mediated vasodilation was also abolished in that study with the nitric oxide synthase inhibitor N^G-monomethyl-l-arginine. Together, these findings suggest that improvement of endothelial function following IC is likely due to increased nitric oxide bioavailability in the conditioned limb (25). Of note, and in contrast to our study, improvement of endothelial function was not observed in the contralateral limb of that study.

The hypothesis that nitric oxide is central to the vasoactive response following RIC is supported by work showing that plasma nitrite increases following RIC (13, 36). Remote IC can also exert cardioprotective effects by increasing circulating bradykinin levels (19, 39). Those protective effects of RIC can also be abolished with HOE-140, a selective B_2 bradykinin receptor antagonist (19, 39). Finally, K_{ATP} channels have also been shown to be involved in the vasoprotective RIC response. Loukogeorgakis et al. (31) demonstrated that remote RIC prevented a reduction in FMD induced by ischemia-reperfusion injury in healthy human volunteers. Those protective effects could be abolished with oral administration of glibenclamide, an inhibitor of K_{ATP} channels (31). How these signaling pathways potentially converge or work in concert on the vascular endothelium to enhance brachial artery FMD following RIC is still unknown.

Study limitations and future directions.

We recognize several study limitations and propose multiple future study directions. First, our study is relatively small, and enrollment was not based on an a priori power analysis; therefore, because our study is underpowered, the results should be interpreted with caution and would best be used for further hypothesis generation. On the basis of the body of literature that has examined the mechanisms of RIC-induced cardioprotection, we now know there are two distinct “windows” of protection. The first window of protection occurs immediately following RIC, lasts up to 12 h, and is largely mediated by changes in ion channel permeability, posttranslational modification of proteins, and autocoid secretion (26). The second window of protection begins ~24 h post-RIC and lasts up to 72 h. This window is largely mediated by changes in inflammatory signaling and gene expression (26). Because we measured FMD 24–48 h after the final RIC session, we cannot say specifically which window of protection is mediating the response, although it is most likely the second window based on the timing of the postintervention FMD assessment. Also, because our participants performed seven sessions of RIC over 2 wk, we cannot say whether the changes in FMD would be present after one session of whether they are additive/cumulative, whereby more RIC sessions produce a larger improvement in FMD until a ceiling effect occurs. Thus, future studies should be performed that examine the effects of the timing and dosing of RIC on brachial artery FMD after stroke.

Second, because many stroke survivors rely on caregivers or medical transport services for transportation, we chose to have study participants perform the RIC or sham RIC interventions at home to minimize the number of trips to the laboratory that were required to participate in the study. All participants were given strict

instructions to fill out the daily log sheets truthfully, and study compensation was in no way tied to the number of successfully completed RIC or sham RIC sessions. All study participants completed 100% of their assigned sessions based on their self-reported logs, which underscores the ideal nature of RIC as an intervention in that it is cost-effective, easy to perform, and home-based.

Third, because of limitations of our ultrasound system, we could not perform duplex imaging of the brachial artery in B-mode and flow velocity in pulse-wave mode with acceptable resolution; thus, area under the curve analysis could not be performed. However, all study participants served as their own controls, and no changes in peak shear rate were observed following either RIC or sham RIC. We also chose not to perform nitroglycerin-mediated dilation of the brachial artery to assess endothelium-independent vasodilation. Because of the propensity of nitroglycerin to cause headaches and because many stroke survivors can experience headaches regularly, we chose not to administer nitroglycerin for this study, which is consistent with other studies that have evaluated FMD after stroke (5, 6, 37, 38).

Finally, we recognize that while there was not a statistical difference in time since stroke between the RIC and sham RIC groups, the difference is relatively large (12 ± 11 vs. 4 ± 3 yr). Because most motor recovery occurs within the first 90 days after stroke, we would not expect there to be significant differences in motor function and cardiovascular deconditioning in individuals >1 yr after stroke. Furthermore, none of our study participants were actively participating in physical therapy or structured exercise routines.

Summary and conclusions.

This study is the first to show that RIC can improve systemic endothelial function in chronic stroke survivors. Future, larger studies should be conducted to determine the mechanisms responsible for RIC-induced improvement in brachial artery FMD after stroke. Additional studies are warranted to examine whether RIC-induced improvement in FMD is similar to what has been reported with aerobic exercise or whether RIC combined with aerobic exercise causes larger improvements in FMD after stroke.

GRANTS

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

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

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