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ORIGINAL ARTICLE

Remote Limb Ischemic Conditioning and Motor Learning: Evaluation of Factors Influencing Response in Older Adults

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Abstract
Remote limb ischemic conditioning (RLIC) is a clinically feasible method of promoting tissue protection against subsequent ischemic insult. Recent findings from our lab demonstrated that RLIC robustly enhances motor learning in young, healthy humans. The next step is to determine which individuals would receive maximum benefit from RLIC before applying these findings to clinical rehabilitation populations such as stroke. Numerous factors, such as age, sex, body mass index (BMI), and cardiovascular comorbidities may influence the response. Sixty-nine participants aged 40–80 were randomized to receive either RLIC ($n = 33$) or sham ($n = 36$) conditioning. Participants underwent seven consecutive sessions consisting of RLIC or sham conditioning with a blood pressure cuff on the upper extremity and motor training on a stability platform balance task, with two follow-up sessions. Balance change (post-test–pre-test) was compared across participants, groups, and the factors of age, sex, BMI, and comorbidities. Participants in both groups improved their performance on the balance task from pre- to post-test. Overall balance change was independently associated with age and BMI. There was no difference in balance change between RLIC and Sham groups. However, RLIC significantly enhanced balance performance in participants with no comorbidities. Compared with our previous study in young adults, middle-aged and older adults demonstrated smaller improvements on the balance task. RLIC enhanced learning in middle-aged and older adults only in the absence of pre-defined comorbidities. RLIC may be a promising tool for enhancing motor recovery, but the accumulation of comorbidity with age may decrease its effectiveness.

Keywords Ischemic preconditioning · Psychomotor performance · Comorbidity · Cardiovascular disease

Introduction

Ischemic conditioning is the phenomenon by which brief, sublethal bouts of ischemia and reperfusion protect a tissue, such as the brain or the heart, from successive, more prolonged ischemic insults [1–3]. Ischemic conditioning can be delivered directly to the target tissue before, during, or after the ischemic insult, or it can be applied to an organ or tissue remote to the tissue to be protected [4, 5]. Remote limb ischemic conditioning (RLIC) is a clinically feasible method of delivering such remote conditioning through repeated inflation and deflation of a blood pressure cuff on an extremity.

Recent work in our lab showed that RLIC robustly enhanced motor learning in young, healthy humans [6, 7]. These studies demonstrated that RLIC may facilitate some of the mechanisms responsible for neural plasticity and learning. RLIC may act on neuroplasticity through humoral and/or neural mediators, similar to the hypothesized mechanism of

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action for other types of tissue protection [2, 8, 9]. Potential applications for enhancement of learning in clinical rehabilitation populations, such as stroke, are numerous. Before we move to clinical populations, however, we need to determine which individuals could receive maximum benefit from RLIC. Numerous factors, such as age, sex, body mass index (BMI), and cardiovascular comorbidities may influence the response to RLIC, based on preclinical and human studies of ischemic conditioning for tissue protection [10–12].

The protective benefits of ischemic conditioning on various tissues are modified with age in a variety of animal and human studies [13–16]; however, other studies suggest no effect of age, including studies performed in humans [17, 18]. Estrogen has cardio- and neuroprotective benefits that may confound the benefit of ischemic conditioning [19, 20]. Studies in animal models currently conflict; some find that females are less responsive to ischemic conditioning [21], while others have found no difference between males and females [22, 23]. Furthermore, the interaction of age, estrogen levels, and menopausal status may play a role in the effectiveness of ischemic conditioning [20, 24]. Other conditions and comorbidities which can accumulate with aging may render tissues less responsive to ischemic conditioning. For example, BMI or physical activity level and age may interact to influence cardioprotection in rodent models and in humans [25–29].

Common cardiovascular comorbidities may also influence the response to ischemic conditioning, including hypertension and hypercholesterolemia. Hypertension has mixed effects on the efficacy of ischemic conditioning in rodent and human studies of cardioprotection, abolishing the effect in some studies but not in others [30–35]. Hypercholesterolemic animal models show variable responses to ischemic conditioning [36–38], as do humans with hypercholesterolemia in clinical trials [31, 35, 39, 40]. Statins, which are commonly prescribed to treat hypercholesterolemia, are capable of conditioning for neuroprotection and cardioprotection by themselves and may modify the response to additional conditioning, though reports are conflicting as to whether statins enhance or blunt the response to ischemic conditioning [35, 41–45].

The purpose of the present experiment was to test if RLIC enhanced motor learning in middle-aged and older adults. This population was targeted because it represents a population commonly requiring neurorehabilitation services following stroke. Given the mixed results due to sex and a potential sex by age interaction found in cardio- and neuroprotection literature, recruitment of post-menopausal women was considered a priority. We additionally explored the effects of cardiovascular comorbidities and medication usage on RLIC. The results provide important information in moving this protocol further along the translational pathway towards clinical application. Knowledge gained informs the field about how to target RLIC to a population where it could have the greatest rehabilitative potential.

Methods

Experimental Design

This study used a repeated measures design with nine total sessions to examine the effects of RLIC combined with motor training in neurologically intact, middle-age and older adults. Study and consent procedures were approved by the Washington University Human Research Protection Office. All participants provided written informed consent prior to beginning the study. Participants were compensated for their time. Due to the recent definition change of a clinical trial, this study was retrospectively registered as a clinical trial at http://clinicaltrials.gov (Effects of RLIC on Motor Learning in Middle-aged and Older Adults, ClinicalTrials.gov Identifier: NCT03582943).

Participants

Neurologically intact adults were recruited from the community for participation in this study. Prior to study initiation, a power analysis was done based on our pilot data in young adults. Using the standard deviation of change scores (2.5 s and 1.9 s in RLIC and Sham groups respectively), a power of 0.80 and an alpha of 0.05, 20 total subjects would be sufficient to detect a mean difference in change score of ≥ 3 s on our primary outcome measure. The sample size was increased to a total of 80 (40 per group) in order to handle the increased heterogeneity expected with age and to detect the influence of up to six participant factors in the planned analyses. Participants were included if they (1) were 40–80 years old and (2) had sufficient cognitive skills to provide informed consent and actively participate. Exclusion criteria were determined by self-report and included (a) history of a neurological condition, balance impairment, or vestibular disorder; (b) history of attentional disorders (ADD/ADHD) that could affect learning; (c) history of sleep apnea which could confound the effects of RLIC [46, 47]; (d) presence of lower extremity condition, injury, or surgery that would compromise performance on the balance task; (e) learning disability, sensory, or communication problem that would prevent completion of the study; (f) history of epilepsy, peripheral vascular disease, or blood diathesis which could contraindicate RLIC; (g) current intensive weight lifting or interval training exercise which could confound the effects of RLIC [48, 49]; or (h) current substance abuse or dependence.

Experimental Procedure

The experiment included seven consecutive weekday sessions (D1-D7) and two follow-up sessions (FU1, FU2) as previously described [6, 7]. During the first session (D1), participants provided informed consent and self-reported demographic and health history data, including height and weight for BMI calculations, existing health conditions, and medications. Hypertension, hypercholesterolemia, and their medications were of most interest, but we also recorded the report of diabetes, gastrointestinal disorders (e.g., gastro-esophageal reflux disease), depression, anxiety, and any medications taken for these conditions [10, 50–53]. Participants then completed a pre-test on the balance task. After pre-testing, participants were randomly assigned to the RLIC or the Sham group via a randomization list generated by the study statistician (LC). The randomization was stratified by gender, to achieve an equivalent proportion of females in each group. Participants were blinded to their group assignment. Participants then underwent one set of either RLIC or sham conditioning.

During the second session (D2), participants underwent one set of conditioning with no motor training. Sessions D3-D7 consisted of one set of conditioning, followed by 15 min of training on the balance task. Five training sessions were chosen because five are adequate to assess learning on the balance task, are unlikely to result in performance plateaus, and do not present an excessive time burden for participants [6, 54]. Learning was assessed through a post-test at the end of training on D7. Follow-up sessions took place at 2 and 4 weeks after D7 (FU1 and FU2), and each consisted of an identical balance task post-test to evaluate retention of learning achieved through training.

Remote Limb Ischemic and Sham Conditioning

Each set of ischemic or sham conditioning consisted of five cycles of 5 min of blood pressure cuff inflation followed by 5 min of deflation on the non-dominant upper extremity, chosen for convenience. This number and duration of RLIC cycles produced effects in human cardio- and neuroprotective trials [55, 56]. RLIC was achieved via blood pressure cuff inflation to at least 20 mmHg above the participant's resting systolic blood pressure. This RLIC pressure was shown in our previous work to be as effective as the standard 200 mmHg at enhancing learning on the balance task, with fewer side effects [7]. Sham conditioning was achieved via blood pressure cuff inflation to 10 mmHg below the individual's diastolic blood pressure. The sham pressure was chosen because it gives participants the sensation of cuff inflation but does not cause tissue ischemia, and our previous work has found this method of sham conditioning to be an adequate active control [6].

To confirm the presence or absence of ischemia, a pulse oximeter was periodically placed on the index finger of the conditioned arm, the radial pulse was checked, and the color of the conditioned limb was visually inspected. An oxygen saturation reading of 0 or "error," an absent radial pulse, and a pale or dusky distal limb indicated ischemia was occurring. An oxygen saturation reading similar to the preconditioning measure, the presence of the radial pulse, and unchanged color of the limb indicated that ischemia was not occurring in the Sham group. If ischemia was not confirmed for a participant in the RLIC group at any point during conditioning, the blood pressure cuff was inflated an additional 10 mmHg or until ischemia was confirmed.

Oxygen saturation, heart rate, and blood pressure were measured before, during, and after each set of conditioning on the arm not undergoing conditioning to monitor safety. Participants were asked each day to rate their average discomfort associated with conditioning on an 11-point Likert-type pain scale ranging from 0 (none) to 10 (worst pain imaginable). Pain rating data in this cohort were similar to our previous experiments [6, 7] and are not reported further.

Motor Task

The primary motor task used was a stability platform balance task (Lafayette Instrument model 16030L) [6, 57, 58]. This motor task was chosen because it is ecologically valid [59] and engages a broad range of brain systems, serving as a probe to determine the global response of the motor learning system to RLIC. The stability platform includes electronic tilt angle measurements (resolution 1.0°), selectable balance thresholds, digital angle readouts, and built-in timing functions (resolution 0.001 s). The platform was placed at a setting lower to the floor compared to our previous work in young adults [7] to restrict the platform tilt angle and decrease the difficulty for the older age group. Participants were instructed to stand on the platform with feet facing forward and to keep the platform level for as long as possible during a trial. Performance of the task was demonstrated to participants prior to pre-testing. All trials were 30 s long. Performance was quantified by measuring the cumulative amount of time that a participant kept the platform within $\pm 3°$ of horizontal during each trial. Participants completed 5 pre-test trials during D1, 15 training trials during each day D3-D7, and 5 post-test trials on each follow-up day (FU1 and FU2), for a total of 90 trials over the duration of the study. The final five trials during D7 (trials 76–80) were averaged for the post-test measure of performance on the stability platform. Trials were separated by 30 s of rest, with additional rest time (1–2 min) after each 5-trial block during which participants stepped away from the stability platform and were permitted to sit or otherwise rest.

A handrail was positioned in front of the stability platform for safety. Participants were allowed to use the handrail for initial positioning and during rest breaks, but were not allowed to use it for support during a trial. Trials during which a participant held onto the handrail to maintain balance were excluded from analyses. Participants wore a gait belt for safety, but experimenters did not touch the gait belt except in the case of a loss of balance requiring external support to regain balance. After each trial on the stability platform, participants were given feedback on how many seconds they maintained the platform in balance (± 3°). No feedback was given regarding balance strategies, allowing participants to try out and select their own techniques and strategies for task performance based on trial and error (i.e., discovery learning).

Data Analysis

Data were stored in a secure REDCap database (Vanderbilt University, Nashville, TN) [60]. All statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC) with two-sided tests at a significance level of 0.05. For the balance task, the balance change (in seconds) for an individual from pre-test (D1) to post-test (D7) was computed (post-test–pre-test average time in balance, in seconds). Descriptive statistics for continuous variables (mean, standard deviation, median, and range) and for categorical variables (frequency tables) were obtained for the sample. Linear regression analysis was performed to compare means of balance change between RLIC and SHAM and to assess participant factors as independent predictors of the outcomes. Analysis of covariance (ANCOVA) was used to compare balance change between RLIC and SHAM adjusting for participant factors as potential confounders. The possible interactions between group and factors were considered in ANCOVA. Factors of interest were age, gender, post-menopausal status, BMI, hypertension, hypercholesterolemia, and medications. In considering comorbidities, we were constrained by the human clinical reality that multiple comorbidities frequently occur in the same individual. Because of co-occurrence and small numbers of some reported comorbidities, we created an additional factor categorizing those with one or more comorbid conditions vs. those that had none of the pre-defined comorbid conditions. A one-sample t test was performed to examine retention of balance change between follow-up (D8, D9) and D7, and two sample t tests to test for group differences in retention. Careful attention was paid to ensuring that data satisfied assumptions required of a particular analytic strategy. Similarly, we evaluated regression residuals before we reached conclusions based on linear regression analysis.

Results

Figure 1 shows the CONSORT diagram, describing the flow of participants through the study. Eighty-two adults were randomized and 69 were included in the analyses ($n = 33$ in the RLIC group; $n = 36$ in the Sham group). Table 1 shows the demographic and comorbidity characteristics of the final sample included in the analyses. No significant differences were found between RLIC and Sham groups for the demographic characteristics and frequency of comorbidities.

Overall, middle-aged and older adults in both RLIC and Sham groups responded similarly, showing modest improvement in the balance task across the 5-day training protocol with improvements largely retained at follow-up (Fig. 2a). Individuals in the RLIC and Sham groups both exhibited an increase in their ability to balance on the platform at the post-test time point relative to the pre-test, but the extent of this increase did not differ between the two groups (Fig. 2b, $p = 0.983$). Likewise, there were no group differences at follow-up (FU1, $p = 0.489$, FU2, $p = 0.657$).

Age and BMI were found to have independent effects on balance change (post-test–pre-test time in balance, seconds), regardless of group. Balance change decreased with increase in age ($p = 0.004$), and with increase in BMI ($p = 0.002$). Scatterplots for balance change with age and BMI are shown in Fig. 3. We next examined the interaction of each of the participant factors with group. Interactions between group and factors of age, BMI, sex, post-menopausal, hypertension, hypertension medications, hypercholesterolemia, and statins were not significant. Statistical results for these interactions are shown in Table 2. Only the interaction between group and presence of any comorbidity was significant between RLIC and Sham, with the RLIC group showing a significantly greater balance change in participants without any comorbidities. Figure 4 displays a 77% increase in balance change in RLIC group with no comorbidities vs. Sham group with no comorbidities. Characteristics (mean ± standard deviation, range) of the 13 participants with no comorbidities in the RLIC group were the following: age, 55.8 ± 8.9, 43–74; BMI, 25.2 ± 4.4, 20.8–37.4; 8 females (8/8 post-menopausal). Characteristics of the 16 participants with no comorbidities in the Sham group were the following: age, 59.3 ± 9.9, 41–74; BMI, 25.3 ± 4.5, 18.8–34.5; 10 females (10/10 post-menopausal).

Discussion

This study tested if RLIC would enhance learning in middle-aged and older adults with various co-morbidities and medications. Irrespective of conditioning, age and BMI were independent predictors of the extent of motor learning, as measured by balance change scores. RLIC did not enhance motor learning of the balance task overall, as indicated by no between-group differences in balance change. In the absence of any pre-defined comorbidities, however, RLIC enhanced learning by more than 70%, as indicated by balance change scores that were greater in the RLIC group without comorbidities compared to the Sham group without comorbidities.

Previous studies in our lab demonstrated robust enhancement of motor learning on the stability platform test in young, healthy adults without comorbid conditions or medications. Young adults following an identical conditioning and motor training protocol demonstrated average balance

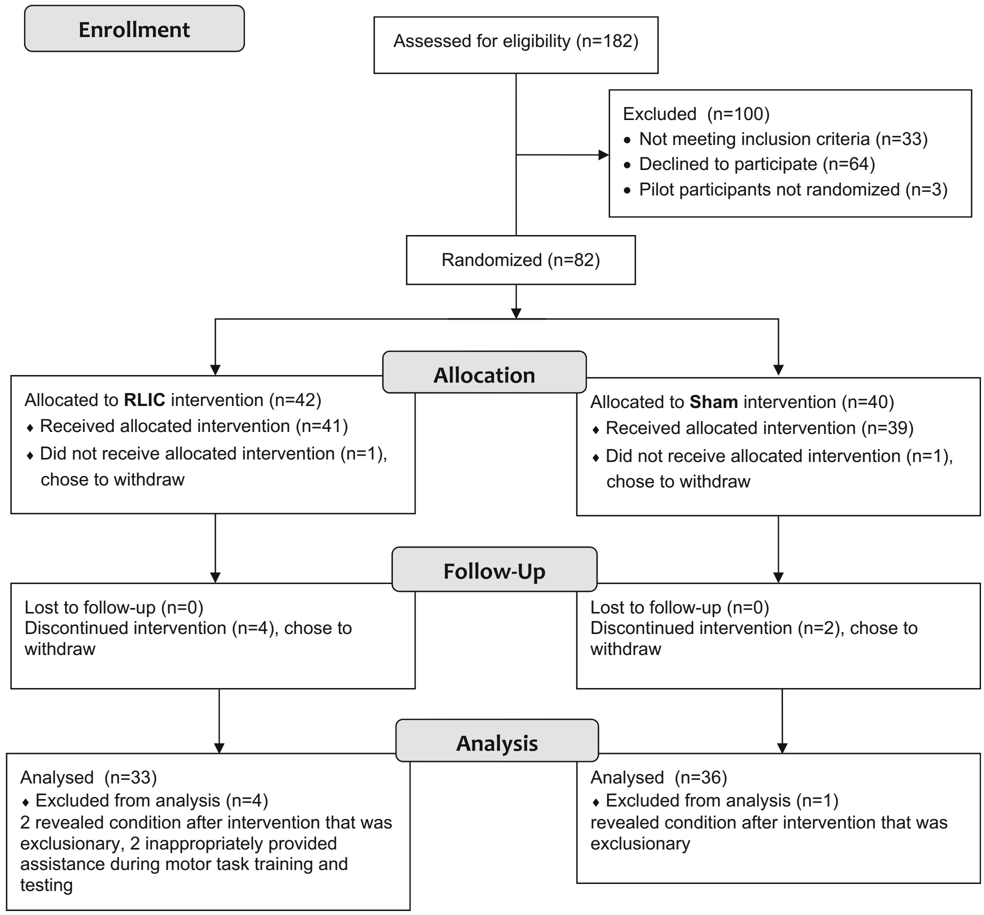

Fig. 1 CONSORT diagram showing flow of participants through the study

improvements from pre-test to post-test of 17.6 s in the RLIC group and 10.8 s in the Sham group [6]. Motor learning in middle-aged and older adults in the present study was diminished (4.79 s in the RLIC group and 4.81 s in the Sham group) compared with younger adults in our previous studies [6, 7]. Our collective results are consistent with the idea that efficiency of motor learning declines with age for many motor tasks, and that learning of novel motor tasks may occur more slowly in older adults than in younger adults [61]. Specific to balance learning, in a study of dynamic balance training of older vs. younger adults on a shifting platform, older and younger adults both demonstrated improvement, but older adults used more rigid balance strategies and improved to a lesser extent [62]. In our study, age was an independent predictor of balance change, potentially reflecting the adoption of less efficient balance strategies and decline in motor learning

Table 1 Participant demographics and presence of comorbidities in RLIC and Sham groups

	RLIC ($n = 33$)	Sham ($n = 36$)	p value
Age, years	60.4 ± 10.5	61.8 ± 9.0	0.553
Sex, # females	22	25	0.805
Post-menopausal females, #	18	25	0.127
Race (% non-Caucasian)	24%	28%	0.322
Body mass index	26.5 ± 4.4	26.8 ± 5.1	0.786
Hypertension, # (# on meds)	11 (11)	7 (6)	0.189
Hypercholesterolemia, # (# on meds)	6 (4)	10 (6)	0.345
Diabetes, # (# on meds)	2 (2)	3 (3)	0.716
Gastrointestinal disorders, # (# on meds)	3 (0)	6 (2)	0.350
Depression, # (# on meds)	5 (3)	4 (3)	0.618
Anxiety, # (# on meds)	3 (1)	3 (2)	0.911
1+ comorbidities, #	20	20	0.671

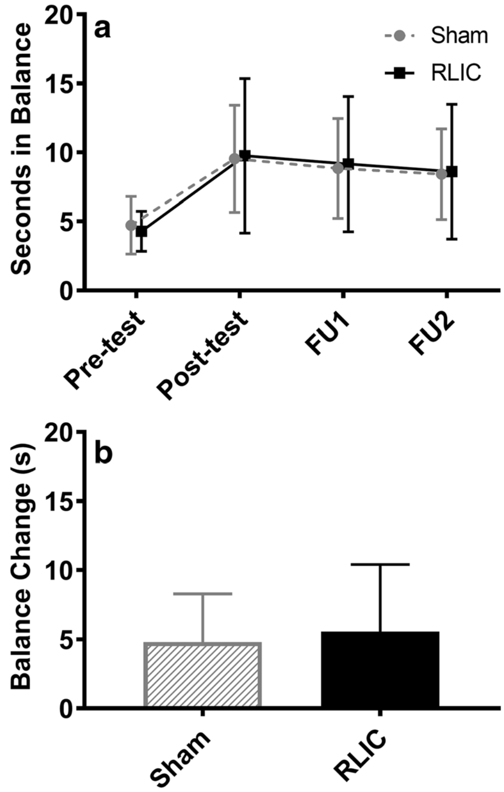

Fig. 2 Balance changes for RLIC and Sham groups. **a** Average number of seconds in balance out of 30 s, averaged across 5 trials at each time point. There was no significant difference between groups (RLIC and Sham) at any time point. Black solid line represents RLIC, gray dashed line represents Sham group. Error bars represent standard deviations. FU, follow-up. **b** Average balance change (post-test–pre-test) in seconds for all participants in each group. Error bars represent standard deviations

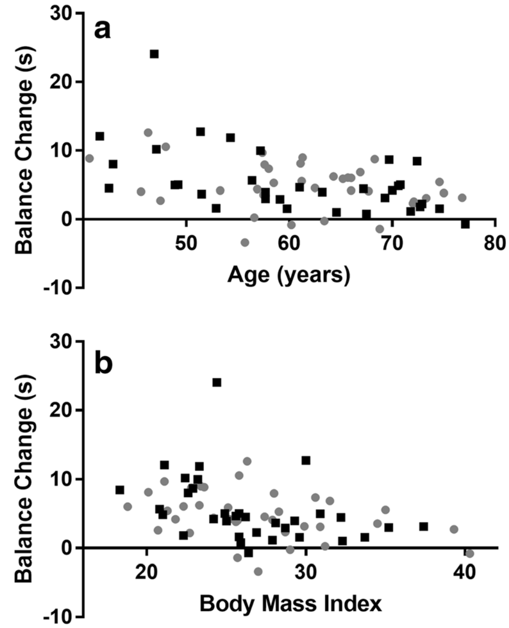

Fig. 3 Age and BMI are independent predictors of learning on the balance task. Scatter plots illustrate the independent effects of age (**a**) and BMI (**b**) on balance change, regardless of group. Black squares indicate RLIC and gray circles indicate Sham participants. Linear regression analysis revealed independent effects of age ($R^2 = 0.118$, $p = 0.004$) and BMI ($R^2 = 0.137$, $p = 0.002$)

efficiency as age increases. Other work, however, indicates motor learning potential (the capability to achieve a level of performance with extended practice) may be preserved in aging, given extended motor practice and an appropriate pace [61]. Increased task difficulty may reduce motor learning in older adults due to the high variability of motor outputs [63]. We attempted to decrease the difficulty of the task for the middle-aged and older adults by placing the stability platform on a lower setting to the floor than had been previously used in younger adults. The lower setting restricts the tilt angle of the platform. Despite this adjustment, the possibility exists that the motor learning task was relatively more difficult for the middle-aged and older adults in this sample, and that greater learning could have been observed with more practice.

As with age, BMI was an independent predictor of motor learning, irrespective of group. It is unclear what specific physiological properties represented by BMI (e.g., adiposity, physical activity level, relationship to cardiovascular health) affected the balance scores. Overweight or obese individuals exhibit decreased motor performance on postural stability and dynamic balance tasks [64, 65], which may have made the stability platform more difficult. Similarly, if one considers BMI an indicator of physical activity, then it is logical that individuals with lower BMIs would perform better on a task with physical demands. Note, however, that we did not distinguish between lean muscle mass and fat mass contributions to high BMI in our sample. While persons with high BMIs frequently present with other cardiovascular comorbidities [66], the cohort with no comorbidities for whom RLIC enhanced motor learning contained individuals with a wide range of BMI values. Therefore, while BMI had an overall impact on performance on the balance task, our data suggest it is not directly linked to a diminished response to RLIC.

Our most impactful finding is that RLIC only enhanced learning in the absence of the selected comorbidities in our sample. Lack of enhancement from RLIC observed in the presence of comorbidities is supported by some tissue protection literature, where comorbidities are hypothesized to underlie the decreased responsiveness of clinical populations to ischemic conditioning when compared with the response in healthy animals [10–12]. Though findings are mixed, in many animal models and human trials, hypertension and some medications for hypertension inhibited or abolished the protective response to ischemic conditioning [30–32, 67]. High cholesterol and statins also limit the effects of ischemic conditioning in some animal models and human trials [37–40, 43, 45]. Diabetes has been found to limit cardioprotection by ischemic conditioning [50, 68], though RLIC may particularly be inhibited in the presence of peripheral neuropathy [69]. There have been limited reports of

Table 2 Summarized interactions between group and participant factors

	Sham		RLIC		
	N	Balance change (s)	*N*	Balance change (s)	*p* value
All participants	36	4.81 ± 3.47	33	5.55 ± 4.85	0.984
Age[a]					
< 55	6	7.16 ± 4.07	11	9.18 ± 6.18	0.455
55–64.99	15	4.91 ± 3.72	9	4.06 ± 2.65	
≥ 65	15	3.78 ± 2.63	13	3.51 ± 2.83	
Sex					
Female	25	5.10 ± 3.43	22	5.68 ± 3.73	0.803
Male	11	4. 16 ± 3.63	11	5.30 ± 6.78	
BMI[a]					
< 25	13	6.26 ± 2.49	14	8.48 ± 5.47	0.233
25–29.99	15	4.22 ± 4.17	12	2.62 ± 1.79	
≥ 30	8	3.56 ± 2.94	7	4.72 ± 4.09	
Post-menopausal					
Absent	11	4.16 ± 3.63	15	6.23 ± 5.92	0.776
Present	25	5.10 ± 3.43	18	4.99 ± 3.83	
Hypertension					
Absent	29	5.08 ± 3.12	22	6.98 ± 5.16	0.341
Present	7	3.73 ± 4.81	11	2.69 ± 2.44	
HTN medication					
Absent	30	4.86 ± 3.31	22	6.98 ± 5.16	0.203
Present	6	4.64 ± 4.40	11	2.69 ± 2.44	
Hypercholesterolemia					
Absent	26	4.82 ± 3.38	27	5.80 ± 5.14	0.876
Present	10	4.79 ± 3.90	6	4.42 ± 3.40	
Statins					
Absent	30	5.30 ± 3.39	29	5.75 ± 5.04	0.879
Present	6	2.37 ± 3.03	4	4.13 ± 3.31	
Comorbidities					
Absent	16	4.55 ± 3.29	13	8.05 ± 5.97	0.029*
Present	20	5.02 ± 3.69	20	3.93 ± 3.17	

N, number of participants in each category; balance change is presented as seconds ± standard deviation; and *p* values are presented for the interactions between group and participant factor

*Indicates significance at $p < 0.05$. [a] For continuous variables of age and BMI, scores are grouped for display purposes, but analysis and *p* values presented were obtained from the continuous variable. *BMI*, body mass index; *HTN*, hypertension

possible negative effects of depression [52] and proton pump inhibitors [51] for GERD on ischemic conditioning. Several recent large-scale clinical trials of RLIC in cardiac surgery, with samples containing comorbidities and medications similar to those described here, have shown no clinical efficacy [70, 71]. In contrast to our results, however, several clinical trials report overall effectiveness of ischemic conditioning protocols (including RLIC) for tissue protection, including neuro- and cardioprotection, in populations similar to the one studied here with a broad distribution of cardiovascular and other comorbidities [35, 55]. Considerable variability exists across studies in sample sizes, sample characteristics, and conditioning protocols (e.g., remote vs. local conditioning, pre- vs. post-conditioning delivery), which complicates direct comparisons. It is unknown what factors allow successful neuro- or cardioprotection in some samples but not others, and unfortunately the present study was designed to only investigate human behavior, not mechanisms. Future large-scale clinical trials of ischemic conditioning for tissue protection may clarify which characteristics are required.

Collectively, our findings seem to suggest that it may not be aging, BMI, or post-menopausal status specifically which attenuate the response to RLIC, but rather an accumulation of various comorbidities that tend to occur with greater frequency in older populations with higher BMIs. Among our cohort with no comorbidities, a wide range of ages and BMIs is represented, as well as post-menopausal females. Therefore, we do not

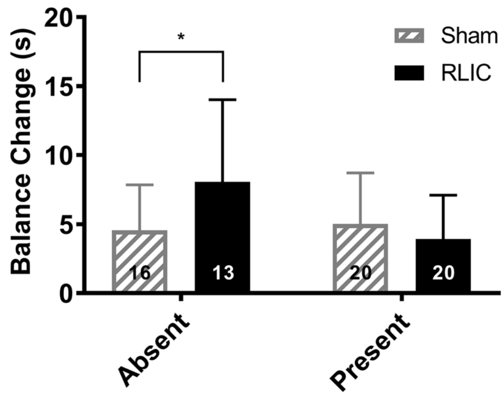

Fig. 4 Effect of 1+ comorbidities on balance change. Effect of 1+ comorbidities on balance change (post-test–pre-test) averaged for RLIC and Sham groups. Only in the absence of any pre-defined comorbidities did RLIC significantly enhance motor learning in our sample. Gray bars indicate Sham participant average balance change; black bars indicate RLIC participant average balance change. Error bars represent standard deviation. Small numbers inside bar graph indicate number of participants in each subgroup. An asterisk indicates significance at $p < 0.05$

observe that status as a post-menopausal female affected enhancement of motor learning by RLIC in our sample. Protective effects from ischemic conditioning generally decline with aging, which has particularly been shown in cardioprotection literature [13, 14]. It is unknown, however, if the decreased efficacy is a result of decreased humoral response to RLIC, or decreased ability of a particular tissue to be protected. Since protection of a tissue was not the goal of the current experiment to enhance motor learning, our data might be interpreted to suggest that hypertension, hypercholesterolemia, and other pre-defined comorbidities reduced the general humoral response to RLIC in our sample. At present, there are no established serum markers of RLIC to confirm the effect of these comorbidities on the humoral response. One or more markers are sorely needed to determine responders and advance ischemic conditioning further down the translational pathway.

This study was limited by a small overall sample size and small sample sizes for some of the comorbidities tested, particularly diabetes. Diabetes, however, often co-occurs with other cardiovascular comorbidities. Given the human presentation of multiple co-occurring conditions in the same individual, it was not possible to explore all factors individually for their effects on RLIC. Therefore, we were unable to determine specifically which comorbidities may most strongly influence the effectiveness of RLIC. Due to the small sample size, it is also possible that the randomization failed to equally distribute unknown confounders related to comorbidities and medication use not evaluated in our study. Because this is an early phase, learning study, we did not adjust for multiple comparisons nor did we state the secondary endpoint of comparing RLIC with and without comorbidities a priori. This is a further limitation. Given the pilot nature of this study, exploratory analysis was necessary to generate this hypothesis. These findings will need to be replicated in a larger sample size.

In conclusion, RLIC only enhanced motor learning in a small sample of middle-aged and older adults in the absence of pre-defined comorbidities. These results need to be replicated in larger samples. Future translation of RLIC to neurorehabilitation studies will need to carefully consider which populations might be most appropriate. Our original intent was to pair RLIC with stroke rehabilitation but we will now need to reconsider, since that population will be likely to have these comorbid conditions. Different clinical populations, particularly younger individuals without cardiovascular comorbidity, might be the best target for exploring potential benefits of using RLIC as an adjunct to rehabilitation therapies.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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