The Short-Term Effects of Remote Ischaemic Conditioning on Cerebral Haemodynamics and Cerebral Autoregulation in Healthy Individuals

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Abstract—Remote Ischaemic conditioning (RIC), where brief cycles of ischaemia and reperfusion are applied to the limbs, may improve neurological outcome in acute stroke and reduce recurrent stroke. The short-term effect of RIC on the regulation of cerebral blood flow (CBF), one possible mechanism for these benefits, was investigated. Healthy participants underwent 5-minute recordings in beat-to-beat blood pressure (BP), electrocardiogram (heart rate, HR), end-tidal CO2 and cerebral blood flow velocity (CBFV) with transcranial Doppler insonation of the middle cerebral arteries, before and after four cycles of RIC using 5-min inflation of bilateral thigh cuffs followed by 5-min deflation. Mean values were calculated together with estimates for cerebrovascular resistance, transfer function analysis (TFA) and autoregulation index (ARI). Twenty-five individuals (10 male), aged 28 ± 11 years, had increases in mean and diastolic BP after RIC (113 ± 13 vs. 116 ± 13, p = 0.046; 74 ± 8 vs. 76 ± 9 mmHg, p = 0.03; respectively). Systolic and mean CBFV decreased after RIC (94.5 ± 17.7 vs. 90.0 ± 15.5, p = 0.001; 63.9 ± 11.1 vs. 62.2 ± 10.5 cm s⁻¹, p = 0.02; respectively), as well as pulsatility index (0.77 ± 0.11 vs 0.73 ± 0.11, p = 0.001). No changes occurred in ARI. TFA showed changes in very low frequency range with significantly increased power oscillations (BP and CBFV) and reduction in phase shift (1.04 ± 0.35 vs. 0.76 ± 0.34 radians, p < 0.01). RIC using bilateral thigh cuffs achieved short-term changes to the cerebrovascular and cardiovascular systems. Further evidence is required to establish the effects in a stroke population.

Keywords: remote ischaemic conditioning, cerebral autoregulation, cerebrovascular system, transcranial doppler

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Remote ischaemic conditioning (RIC), where repeated cycles of ischaemia and reperfusion are performed on the limbs, is a physiological phenomenon that could be used to induce endogenous protection to remote organs and tissue such as the heart [1] and brain [2]. There is some debate as to the mechanism and nature of neuroprotection that can be instigated by such a mechanical strategy [3, 4]. It is generally accepted that RIC can trigger humoral, immunological and neuronal pathways that can benefit endogenous and remote tissue, and that there are two windows of protection: acutely and immediately following RIC instigation (early phase), and a delayed phase that can last up to 3 days [3]. There are promising studies in stroke patients reporting that long-term daily RIC exposure may improve neurological outcome in acute stroke and reduce recurrent stroke [5–7], as the confounding factors [8] that have been identified with the use of a single application of RIC as a therapeutic intervention, e.g. diabetes, may be mitigated [9, 10]. Improved vascular and endothelial function have been identified as physiological adaptations that can occur with RIC during the delayed phase [11] or with daily RIC exposure [10, 12]. However, few studies in humans have focused on the short-term effects of RIC and if there are benefits to the cerebrovascular system. Sprick et al [13, 14] showed in healthy individuals that after RIC to the lower limbs there was a steady decrease in cerebral blood flow velocity (CBFV) within the middle cerebral arteries (MCA), that may be due to changes in end-tidal CO₂ (etCO₂). Gonzalez et al. [15] also reported decreases in MCA velocities after RIC to a single lower limb in subarachnoid haemorrhage patients, that were preceded by vasodilation of the cerebral vasculature during the procedure when measured by morphological clustering and analysis of intracranial pulse. Interestingly, one study reported no significant changes to cerebral haemodynamics after performing RIC to a single upper limb of stroke.
patients undergoing endovascular treatment [16]. However, a study in stroke patients receiving pre-hospital RIC in the upper-limb showed that although final infarct size and neurological outcome had not been significantly affected, there was a tendency towards smaller perfusion deficits during admission [5], indicating that there might have been some immediate short-term improvement to cerebral vasculature.

Measurements in dynamic cerebral autoregulation (CA) demonstrate the transient response of CBF to sudden changes in BP and it represents the efficiency in the cerebrovasculature to regulate CBF. There is now growing evidence that following stroke there is an impairment of dynamic CA and the ability of the cerebral vessels to respond and regulate blood flow [17, 18]. Guo et al. [19] recently demonstrated in healthy individuals that dynamic CA was improved 6–24 h following RIC to one upper-limb and one lower limb. Carter et al. [20] also recently reported that bilateral arm RIC had no acute effect on cerebrovascular function in healthy individuals. There has yet to be a study in healthy individuals that focuses on the immediate short-term implications of RIC using bilateral thigh cuffs, on the cerebral haemodynamics, with a focus on dynamic CA. It was hypothesised that RIC on the lower limbs would lead to an acute improvement in dynamic CA.

PATIENTS AND METHODS

Healthy volunteers were recruited from the University of Leicester and University Hospitals of Leicester NHS Trust. Volunteers were excluded if they were under the age of 18, were on any medication, had any evidence or history of cerebrovascular disease, peripheral arterial disease, diabetes or haematologic disease, any type of physical disease of the lower limb, hypertension or could not tolerate the ischaemic conditioning session. Informed consent was obtained from all individual participants included in the study.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee, University Ethics Sub-Committee for Medicine and Biological Sciences at the University of Leicester, reference no. 13023-ol32, and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

The study was conducted in a dedicated cardiovascular research laboratory at Leicester Royal Infirmary (Leicester, UK) that had a controlled temperature (20–24°C) and was free from distraction. Each individual abstained from physical activity, caffeine, alcohol and nicotine for at least 4 hours before the assessment. After a minimum of 15 min rest on a bed in an upright position of 70 degrees, a 5 min ‘before RIC’ baseline recording was performed. The following measurements were recorded: brachial BP measurement (Omron Automatic BP 705CP-11, Omron Healthcare, Inc., Lake Forest, IL) on the non-dominant arm; continuous non-invasive arterial BP using a Finometer (FMS, Arnhem, Netherlands) that was placed on the non-dominant hand; CBFV with bilateral insonation of the MCAs (Doppler-Box, DWL, Germany), where two transducers (2 MHz) were attached to a headframe (Compumedics, DWL, Germany) positioned on the temporal bone at an angle and depth (40–60 mm) that yielded the maximum reflected signal; etCO2 using capnography (Capno-check Plus, Kent, UK) and nasal cannulae; and heart rate (HR) with a three-lead electrocardiogram.

The use of bilateral thigh cuffs to perform a thigh cuff manoeuvre and a hypotensive challenge is a common method for assessing CA and the response of the cerebrovascular system to a rapid decrease in CBF [21]. As such, this protocol was emulated within this study, although the period of thigh cuff inflation was extended to 5 minutes rather than the norm of 2 minutes to promote ischaemia in the limbs. Using bilateral thigh cuffs (Hokanson contoured thigh cuffs, model CC22, 24 × 122.5 cm), RIC was performed by using a modified hand-pump to inflate every thigh cuff cycle to 200 mmHg [22]. The thigh cuffs were maintained at this pressure for precisely 5 min. Quick deflation of the thigh cuffs was achieved using the Hokanson quick release mechanism (model RD2), which remained deflated for a minimum of 5 min. After 4 minutes of reperfusion the Finometer was stopped and a brachial BP measurement was taken before the thigh cuff inflation was repeated. Immediately following the fourth RIC cycle and 5 minutes of reperfusion, another brachial BP measurement was taken before a 5 minute ‘after RIC’ physiological recording was performed.

Data editing and analysis have been described in detail previously [23]. In brief, data were simultaneously recorded onto a data acquisition system (PHYSIDAS, Department of Medical Physics, University Hospitals of Leicester NHS Trust) at a sampling rate of 500 samples/s for subsequent off-line analysis. BP was calibrated at the start of each recording using systolic and diastolic values from brachial sphygmomanometry. The R–R interval was automatically marked from the electrocardiogram, and mean BP (MAP) and CBFV (MCFBV) values were calculated for each cardiac cycle. This was further used to calculate the pulsatility index (PI) for each cardiac cycle using the formula PI = (systolic CBFV–diastolic CBFV)/MCFBV. The instantaneous relationship between BP and CBFV was also used to estimate critical closing pressure (CPCP) and resistance area product (RAP) for each cardiac cycle using the first harmonic method [24].

Spectral analysis was performed to obtain estimates of BP and CBFV power at each frequency in the range 0.01–0.2 Hz. Estimates of dynamic CA parameters (gain, phase and coherence) were obtained with trans-
fer function analysis, using beat-to-beat values of MAP and MCBFV as input and output waveforms, respectively, in agreement with recommendations from the Cerebral Autoregulation Research Network 2016 White Paper [25] and the averaging of spectral parameters for the very low (VLF, 0.02–0.07 Hz) and low (LF, 0.07–0.2 Hz) frequency ranges. The CBFV response to a hypothetical step change in mean BP was estimated using the inverse fast Fourier transform of the gain and phase frequency responses. Autoregulation index (ARI), which represents dynamic CA, was extracted by using the best least-squares fit between the CBFV step response and one of the 10 model ARI curves proposed by Tiecks et al. [26]. Values of ARI were only accepted for further analyses if strict conditions were met regarding the significance of the transfer function coherence between MABP and CBFV and the normalised mean square error of fit to Tiecks model [27].

Mean values of each variable were calculated from the entire 5 min recordings. Tests for normality were performed using the Shapiro-Wilk normality test. Data are presented as median and interquartile range (25th–75th percentile) and mean (SD) unless stated otherwise. Differences between values derived from the dominant and non-dominant cerebral hemispheres were assessed with paired Student’s t test or the Wilcoxon test to determine whether the hemispheres were comparable. In the absence of significant differences for any of the variables, an average was calculated to represent both hemispheres. Comparison of physiological measurements taken before and after RIC was achieved using paired t-tests or Wilcoxon test. A two-way repeated measures ANOVA was used to assess for the effect between sexes. A p-value of <0.05 was defined as statistical significance.

RESULTS

Of 29 individuals recruited, complete bilateral data sets were collected successfully from 25 subjects (10 male) of mean (SD) age 28 (11) years. All participants except one were Caucasian and all participants except one were right-handed. Of the four individuals that were excluded, one had a lack of temporal acoustic windows, two withdrew from the study after performing one or two cycles of RIC and one individual had poor data quality.

Increases in mean and diastolic BP (mean difference of 2 ± 6 and 3 ± 6 mmHg, respectively) were seen in response to RIC, though no changes were detected in pulse pressure, etCO2 or HR (Table 1). Reductions were seen after RIC in systolic and mean CBFV of 4.5 ± 5.9 and 1.7 ± 3.4 cm s⁻¹, respectively (Table 1). Changes in CBFV waveform were confirmed with a reduction of 6 ± 7% in PI, though there were no changes to other parameters that describe cerebrovascular resistance, including CrCP and RAP (Table 1).

Following RIC, there was no change in ARI, though TFA between BP and CBFV showed increases in VLF power spectral distributions (Table 2, Fig. 1). In addition, with respect to phase shift, there was

| Table 1. Mean values for systemic and cerebral haemodynamic parameters from 5 min recordings taken before and after RIC (n = 25) |
|---------------------------------------------------------------|
| Before RIC | After RIC | p-Value |
|----------------------------------|----------|---------|
| **Systemic Parameters**          |          |         |
| Systolic BP, mmHg                | 113 ± 13 | 116 ± 13 | 0.06   |
| Mean arterial BP, mmHg           | 86 ± 9   | 88 ± 10  | 0.046  |
| Diastolic BP, mmHg               | 74 ± 8   | 76 ± 9   | 0.03   |
| Pulse Pressure, mmHg             | 39 ± 10  | 40 ± 10  | 0.64   |
| HR, beats/min                    | 69 ± 9   | 69 ± 8   | 0.65   |
| etCO2, mmHg                      | 40.0 ± 3.1| 39.6 ± 2.5| 0.26   |
| **Cerebral Haemodynamics**       |          |         |
| Systolic CBFV, cm s⁻¹            | 94.5 ± 17.7| 90.0 ± 15.5| 0.001  |
| Mean CBFV, cm s⁻¹                | 63.9 ± 11.1| 62.2 ± 10.5| 0.02   |
| Diastolic CBFV, cm s⁻¹           | 45.3 ± 7.9| 44.8 ± 7.7| 0.37   |
| Pulsatility Index                | 0.77 ± 0.11| 0.73 ± 0.11| 0.001  |
| CrCP, mmHg                       | 44.0 ± 10.6| 45.9 ± 9.8| 0.41   |
| RAP, mmHg cm s⁻¹                 | 0.68 ± 0.25| 0.70 ± 0.21| 0.73   |
| ARI                              | 5.5 ± 1.1 | 5.2 ± 1.5 | 0.61   |

Data are presented as mean (standard deviation). p-Value refers to paired t-test or Wilcoxon signed-rank test. BP, blood pressure; HR, heart rate; etCO2, end-tidal CO2; CBFV, cerebral blood flow velocity; CrCP, critical closing pressure; RAP, resistance area product; ARI, autoregulation index.
decrease in the VLF but not the LF (Table 2, Fig. 1). No other differences were seen in dynamic CA and TFA parameters (Table 2, Fig. 1).

Despite differences between male and females in BP, CBFV, RAP and BP power in VLF range (Table 3), there were no differences between sexes in dynamic CA parameters or in their response to RIC.

### DISCUSSION

This study demonstrated that the use of bilateral thigh cuffs to instigate RIC produced short-term effects on the cardiovascular and cerebrovascular systems that were present up to 10 minutes after the fourth cycle of ischaemia to the lower limbs and release of the thigh cuffs. In summary, RIC had effects on: (i) the cardiovascular system, with increased mean and diastolic BP that maintained a similar pulse pressure; (ii) cerebral haemodynamics, with reduction in mean and systolic CBFV, which prompted a lower PI as diastolic CBFV remained unchanged; (iii) spontaneous fluctuations of BP and CBFV in the VLF range, with increases in spectral power and decreases in phase shift, although no significant changes were detected in ARI.

Using bilateral thigh cuffs to instigate a hypotensive response is an established method to determine CA capacity and occurred within our model of RIC [21]; but the focus of this study was to establish if there were further short-term effects after performing RIC in this way. Our measurements in CrCP and RAP showed that there were no short-term effects in cerebrovascular resistance 10 minutes after the final RIC cycle. This was despite observing short-term reduction in peak systolic CBFV indicating that recovery of cerebral haemodynamics to baseline values had not occurred following the hypotensive challenges, in which thigh cuff inflation and period of ischaemia had caused reactive hyperaemia in the lower limbs, and encourage a transient drop in systemic BP and CBF.

The minor decrease in CBFV that was still present after RIC has been shown in previous studies [14, 15]. Sprick et al [14] suggested that the reduction in CBFV could be due to changes in etCO₂. Despite observing similar changes in CBFV within this study, we did not detect any significant decreases in etCO₂. Gonzalez et al. [15] also observed immediate CBFV decreases within the MCA, but not the internal carotid artery, in a small sample of subarachnoid haemorrhage patients undergoing RIC with a single thigh cuff. Interestingly, diastolic CBFV did recover to before RIC values and thus a consistent reduction in the PI after RIC was observed. Described as a complex marker of arterial compliance and vascular resistance in distal regions [28], this is in contrast to Zhao et al. [16] who did not observe any changes to PI or other cerebral haemodynamic parameters with RIC of a single arm in patients with acute stroke. Further studies are needed to confirm if these differences are due to methodology of instigating RIC or phenotypical characteristics of the population under investigation. Other studies have shown that within minutes (1–3 minutes) of instigating intermittent ischaemia and reperfusion in one limb, vasodilation can occur in other vascular beds such as the coronary arteries [29] and contralateral limbs [30]. However, the main focus of this study was the short-term impact of RIC that occurs 5–10 minutes following the final RIC cycle, and a time point

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**Table 2.** Dynamic CA and transfer function analysis parameters in the very low frequency (0.02–0.07 Hz) and low frequency (0.07–0.2 Hz) range from 5 min recordings taken before and after RIC (n = 25)

| Parameter                      | Before RIC | After RIC | p-Value  |
|-------------------------------|------------|-----------|----------|
| Coherence VLF range           | 0.41 ± 0.16| 0.42 ± 0.19| 0.79     |
| Coherence LF range            | 0.72 ± 0.14| 0.69 ± 0.15| 0.44     |
| Gain VLF range, cm s⁻¹ mmHg⁻¹ | 0.87 ± 0.40| 0.86 ± 0.36| 0.92     |
| Gain LF range, cm s⁻¹ mmHg⁻¹  | 1.52 ± 0.58| 1.45 ± 0.45| 0.55     |
| Normalised Gain VLF, % mmHg⁻¹ | 1.08 ± 0.45| 1.10 ± 0.43| 0.83     |
| Normalised Gain LF, % mmHg⁻¹  | 1.92 ± 0.70| 1.87 ± 0.52| 0.76     |
| Phase VLF range, radians      | 1.04 ± 0.35| 0.76 ± 0.34| <0.01    |
| Phase LF range, radians       | 0.55 ± 0.18| 0.59 ± 0.21| 0.43     |
| BP power VLF range, mmHg²     | 11.8 (6.1–21.5)| 19.3 (10.4–35.0)| 0.03     |
| BP power LF range, mmHg²      | 3.2 (1.3–4.5)| 3.8 (1.7–7.3)| 0.51     |
| CBFV power VLF range, (cm s⁻¹)²| 18.5 (12.8–26.6)| 32.9 (19.0–50.9)| <0.0001  |
| CBFV power LF range, (cm s⁻¹)²| 6.3 (4.3–10.9)| 7.7 (4.7–12.9)| 0.40     |

Data are presented as mean ± standard deviation or median (inter-quartile range, 25th to 75th percentile) as appropriate. p-Value refers to paired t-test or Wilcoxon signed-rank test. VLF, very low frequency range; LF, low frequency range; HF, high frequency range; BP, blood pressure; CBFV, cerebral blood flow velocity.
that is known to contain circulating factors that are beneficial to the cardiovascular system [31].

There was significant variability in the ARI responses of individual participants after RIC. Greater than 50% of the participants observed ARI values that changed by a value of 1 or more following the protocol. This is in direct contrast with outcomes from previous experiments investigating hypo- and hypercapnia that had a reproducible response to the intervention [32]. The sex of the participant did not influence the ARI variability after RIC, despite the male and female cerebral haemodynamics being characteristically different [33]. The increase in BP after RIC that have previously been described [14, 15], albeit minor, was confirmed in this study and could be due to increased peripheral resistance during thigh cuff inflation. With sustained increases in MABP after RIC accompanying a decrease in systolic but not diastolic CBFV, it is possible that static CA was intact in these healthy individuals. With regards to dynamic CA, the cerebral vasculature has the ability to buffer oscillatory changes in systolic BP in order to prevent injury and might do this by reducing systolic rather than diastolic fluctuations in CBF [34]. The significant increase in BP power within the VLF range that occurred after RIC (Fig. 1 and Table 2) could also be due to the hyperaemic response without influencing the amplitude (gain) of the fluctuations thus leading to corresponding increases in CBFV power. An increase in BP and CBFV power can occur with moderate exercise (in supine position), but is usually accompanied by an increase in phase shift within the VLF range [35]. Thus, the reduction in phase shift that was detected after RIC within this study, together with no changes to coherence in the VLF range, could indicate other influences on the cerebrovasculature. Nevertheless, a reduction in phase is characteristic of observations where there is hypercapnia [36] that would be in con-
Table 3. Influence of sex (10 males, 15 females) on mean values for systemic, cerebral haemodynamic and selected TFA parameters from 5 min recordings taken before and after RIC

| Table 3. Influence of sex (10 males, 15 females) on mean values for systemic, cerebral haemodynamic and selected TFA parameters from 5 min recordings taken before and after RIC |
|------------------------------|---------|---------|---------|---------|---------|
| **Systemic Parameters**      | Before RIC | After RIC | Sex | RIC | Int. |
| Systolic BP, mmHg            | M 119 ± 16 | 122 ± 13 | 0.04 | 0.08 | 0.67 |
|                               | F 109 ± 10 | 113 ± 12 |       |      |      |
| Mean arterial BP, mmHg       | M 90 ± 9  | 92 ± 8   | <0.05 | 0.07 | 0.56 |
|                               | F 82 ± 8  | 85 ± 11  |       |      |      |
| Diastolic BP, mmHg           | M 78 ± 8  | 80 ± 6   | 0.07  | 0.04 | 0.62 |
|                               | F 71 ± 7  | 74 ± 10  |       |      |      |
| Pulse Pressure, mmHg         | M 42 ± 14 | 42 ± 12  | 0.30  | 0.67 | 0.92 |
|                               | F 37 ± 8  | 38 ± 8   |       |      |      |
| HR, beats/min                | M 69 ± 14 | 68 ± 10  | 0.62  | 0.60 | 0.67 |
|                               | F 70 ± 4  | 70 ± 6   |       |      |      |
| etCO₂, mmHg                  | M 40.1 ± 4.5 | 39.5 ± 3.3 | 0.99 | 0.22 | 0.45 |
|                               | F 39.9 ± 1.8 | 39.7 ± 2.0 |      |      |      |
| **Cerebral Haemodynamics**   |         |         |       |      |      |
| Systolic CBFV, cm s⁻¹        | M 85.9 ± 14.4 | 81.4 ± 12.6 | 0.03 | <0.01 | 0.99 |
|                               | F 100.3 ± 17.7 | 95.7 ± 14.8* |      |      |      |
| Mean CBFV, cm s⁻¹            | M 58.1 ± 11.0 | 56.1 ± 9.6  | 0.02 | 0.02 | 0.71 |
|                               | F 67.7 ± 9.6* | 66.2 ± 9.2* |      |      |      |
| Diastolic CBFV, cm s⁻¹       | M 41.9 ± 8.5 | 41.2 ± 7.7  | 0.06 | 0.34 | 0.63 |
|                               | F 47.5 ± 6.9 | 47.3 ± 7.0  |      |      |      |
| Pulsatility Index            | M 0.76 ± 0.09 | 0.72 ± 0.10* | 0.80 | <0.01 | 0.99 |
|                               | F 0.77 ± 0.12 | 0.73 ± 0.12* |      |      |      |
| CrCP, mmHg                   | M 44.9 ± 13.3 | 45.3 ± 12.1 | 0.93 | 0.49 | 0.61 |
|                               | F 43.3 ± 8.9 | 46.3 ± 8.5  |      |      |      |
| RAP, mmHg cm s⁻¹             | M 0.82 ± 0.30 | 0.84 ± 0.25 | <0.01 | 0.71 | 0.87 |
|                               | F 0.59 ± 0.17* | 0.60 ± 0.12* |      |      |      |
| ARI                          | M 5.4 ± 0.97 | 5.2 ± 1.6  | 0.74  | 0.63 | 0.98 |
|                               | F 5.5 ± 1.3 | 5.3 ± 1.5  |      |      |      |
| **Transfer Function Analysis**|        |         |       |      |      |
| Phase VLF range, radians     | M 1.05 ± 0.35 | 0.75 ± 0.32 | 0.99 | 0.01 | 0.90 |
|                               | F 1.03 ± 0.36 | 0.76 ± 0.37 |      |      |      |
| BP power VLF range, mmHg²    | M 23.1 ± 14.4 | 29.8 ± 16.4 | <0.01 | 0.03 | 0.76 |
|                               | F 9.6 ± 5.0* | 18.4 ± 13.9 |      |      |      |
| CBFV power VLF range (cm s⁻¹)²| M 16.9 ± 7.0 | 30.8 ± 17.8 | 0.15 | <0.001 | 0.34 |
|                               | F 24.2 ± 13.9 | 46.7 ± 34.3* |      |      |      |

Data are presented as mean ± standard deviation. *p < 0.05 vs. Pre RIC of same sex (Sidak’s multiple comparison analysis). **p < 0.05 vs. Pre RIC during same time period (Sidak’s multiple comparison analysis). P-value refers to two-way Repeated Measures ANOVA between Sex and RIC and their interaction (Int.). #p < 0.05 vs. Males during same time period (Sidak’s multiple comparison analysis). BP, blood pressure; HR, heart rate; etCO₂, end-tidal CO₂; CBFV, cerebral blood flow velocity; CrCP, critical closing pressure; RAP, resistance area product; ARI, autoregulation index; VLF, very low frequency.
contrast to the reduced CBFV measured in this study. With absence of any clear changes to etCO₂, it could be that other vasoactive substances such as nitrite [37] that are released during RIC could still be influencing the cerebral vessels. Although impairment in CA can also be associated with a reduction in phase, it can be associated with increases in transfer gain, which was not apparent in this study [38].

This study has shown that RIC with bilateral thigh cuffs is feasible, safe and acceptable in a healthy population. Although some of the short-term changes were statistically significant, whether these reflect physiological changes that are clinically significant remain to be seen. An intervention that can reduce the PI within the MCA is a promising result as it has been studied extensively over the years where it is known to increase with age [39] and can be associated with vascular diseases including stroke [40] and recurring vascular events [41]. However, an overall reduction in mean CBFV would not be favourable in patients with acute stroke, who are known to have a deterioration in CBF and CA during the first 2 weeks of injury [42]. Further studies are required to determine if repeated application using RIC would benefit this population or those with cardiac problems, who also have impaired CA amongst other comorbidities [43, 44]. Nevertheless, bilateral thigh cuffs to determine CA capacity has been used extensively over the last thirty years and there have been no complications except for the moderate pain associated with cuff inflation and the compliance of participants to undertake such procedure [45, 46]. Further studies are needed to determine if instigating RIC with bilateral thigh cuffs will show the same promising results to dynamic CA as Guo et al [19], 6–24 hours following its application. One of the key limitations to this study is the absence of a sham control as a comparison. This would have allowed determination of the inter-subject variability on the results which may have been particularly useful considering the variability noticed in some measurements such as ARI [47]. Another limitation to this protocol was the absence of confirming whether blood flow had been completely restricted during each RIC cycle with the thigh cuffs. The absence of peripheral pulses to palpation was used in some cases to suggest some degree of ischaemia in the lower limb had occurred, however due to the confinements of using a hand pump to inflate the thigh cuff, this was not possible for each subject at each RIC cycle. The ischaemic period was consistently kept to 5 min duration, but due to the nature of the thigh cuff inflation and re-setting of recording instruments, the reperfusion period did vary by up to one-minute in-between each cycle. Although moderate pain to the thigh cuff inflation was predominantly during the first cycle due to an unfamiliar sensation to the protocol, it was a significant factor for two participants in our study who had to withdraw from the study following one to two RIC cycles.

CONCLUSIONS

This study demonstrated that there were short-term changes to cardiovascular and cerebral haemodynamics following four 5-minute cycles of RIC using bilateral thigh cuffs. When dynamic CA was assessed using ARI there was variability in the response of the participants to RIC. TFA and phase measurements indicated that CA could be compromised in the short-term after RIC, with minor reduction in mean CBFV compensated for by increases in BP and CBFV power. Further studies are warranted to determine how long it takes for the cerebrovascular system to return to baseline values following RIC and whether there are any benefits to daily RIC exposure using bilateral thigh cuffs.

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COMPLIANCE WITH ETHICAL STANDARDS

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

All participants had provided informed consent in compliance with local ethics committee approvals.

AUTHOR CONTRIBUTIONS

Study design: O. Llwyd, T. G. Robinson, R. B. Panerai; Data collection and data preparation: O. Llwyd, A. Badrick; Data analysis: O. Llwyd, A. Badrick; Interpretation of data: O. Llwyd, T. G. Robinson, R. B. Panerai, A. Badrick; Drafted manuscript: O. Llwyd, T. G. Robinson, R. B. Panerai, A. Badrick; Edited and revised manuscript: O. Llwyd, T. G. Robinson, R. B. Panerai, A. Badrick; Approved final version of manuscript: O. Llwyd, T. G. Robinson, R. B. Panerai, A. Badrick.

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