REMOTE ISCHAEMIC CONDITIONING AFTER STROKE TRIAL (RECAST): a pilot randomised placebo controlled phase II trial in acute ischaemic stroke (ISRCTN 86672015)

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Abstract

Background

Repeated episodes of limb ischaemia and reperfusion (remote ischaemic conditioning, RIC) may improve outcome after acute stroke.

Methods

We performed a pilot blinded placebo-controlled trial in patients with acute ischaemic stroke, randomised 1:1 to receive four cycles of RIC within 24 hours of ictus. The primary outcome was tolerability and feasibility. Secondary outcomes included safety, clinical efficacy (day 90), putative biomarkers (pre- and post-intervention, day 4) and exploratory haemodynamic measures.

Findings

Twenty-six patients (13 RIC, 13 sham) were recruited 15.8 hours (SD 6.2) post onset, age 76·2 years (10.5), blood pressure 159/83mmHg (25/11) and NIHSS 5 [IQR 3.75-9.25]. RIC was well tolerated with 49/52 cycles completed in full. Three patients experienced vascular events in the sham group: two ischaemic strokes and two myocardial infarcts versus none in the RIC group (p=0·076, log-rank test). Compared to sham, there was a significant decrease in day 90 NIHSS in the RIC group, median NIHSS 1 [0.5-5] versus 3 [2-9.5], p=0.04; RIC augmented plasma heat shock protein (HSP) 27 (p<0·05, repeated 2-way ANOVA) and phosphorylated HSP27 (p<0·001) but not plasma S100-beta, matrix metalloprotinase-9, endocannabinoids or arterial compliance.

Conclusions

RIC after acute stroke is well tolerated and appears safe and feasible. RIC may improve neurological outcome and protective mechanisms may be mediated through HSP27. A larger trial is warranted.

Clinical Trial Registration-URL: http://www.isrctn.com. Unique identifier: ISRCTN86672015

BACKGROUND

Applying an ischaemic stimulus distant from the brain (remote ischaemic conditioning, RIC, e.g. transient limb ischaemia) after a stroke can induce neuroprotection.[1] The mechanisms of action underlying this are unclear; the production of a chemical messenger released from the hypoxic limb has been implicated, e.g. nitric oxide, bradykinin, adenosine, heat-shock proteins and endocannabinoids.[2, 3] RIC is an attractive prospect since it bears minimal cost and would be simple to administer. It may decrease stroke risk in patients with intracranial arterial stenosis and applying RIC in pre-hospital stroke patients is feasible.[4, 5] Furthermore, preliminary studies of RIC in patients with acute myocardial infarction are encouraging.[6] In the current study, we aimed to demontrate tolerability and feasibility of RIC in patients presenting to hospital with acute stroke whilst simultaneously investigating potential mechanisms of action.

METHODS

Trial Design

The REmote ischaemic Conditioning After Stroke Trial (RECAST) was a single-centre, randomised, outcome-blinded, placebo-controlled trial (ISRCTN 86672015).

Subjects

Adult patients with an ischaemic stroke in the last 24 hours causing arm and/or leg weakness were eligible. Exclusion criteria included modified Rankin Scale (mRS) >3, thrombolysis for index event and significant co-morbidity. Participants were recruited from Derby Teaching Hospitals NHS Foundation Trust, UK between March 2013 and July 2015.

Randomisation and Intervention

In addition to standard care (single antiplatelets, BP and cholesterol reduction), RIC was performed on the stroke unit immediately after randomisation (web-based, 1:1, minimised on age, sex, NIHSS and systolic BP). Intervention: 4 cycles of intermittent limb ischaemia; alternating 5 minutes inflation (20mmHg above systolic BP) and 5 minutes deflation performed manually using a standard upper arm blood pressure cuff in the non-paretic arm. Patient position was not specified. The control group received a sham procedure (cuff inflation to 30mmHg).

Primary Outcome

The primary outcome was tolerability and feasibility of RIC after acute ischaemic stroke.

Secondary Outcomes

Blood samples were collected (pre-RIC, post-RIC, day 4) for surrogate markers of efficacy (plasma S100-beta, MMP-9, troponin T), inflammation (C-reactive protein [CRP]) and other putative biomarkers (Heat Shock Proteins [HSP], endocannabinoids). Transcranial doppler (TCD) was performed as a continuous beat-to-beat recording during the intervention; central pressures measured with Sphygmocor.[7] Serious adverse events (SAE), mRS, impairment (NIHSS, motricity index), Barthel Index, extended activities of daily living, Zung depression scale, and cognition (MMSE) were measured at day 90. Statistical tests are described in the respective tables/figures.

Sample Size

Assuming a meaningful delivery of RIC of at least three of the four 5-minute cycles, 26 patients gives 90% power to reject the null hypothesis that intervention and sham are equally tolerated (SD 4 minutes and alpha=0.05). The original sample size was rounded to 30 but with no losses to follow-up or cross-overs, recruitment was stopped at 26.

RESULTS

26 participants were recruited over 27 months (Figure 1). All participants had an average age of 76 years; mean BP 159/83mmHg and median NIHSS 5 (Table 1). More participants in the control group had diabetes (5 vs 0, p=0·04, Fisher's Exact test) and time to randomisation was 15·8 hours.

Tolerability

RIC was well tolerated: 49/52 cycles were completed in full. One participant was intolerant of RIC (due to cuff pressure), with an overall mean difference between groups of 46 seconds (p=0·33). All patients tolerated the sham procedure. Eight (5 RIC, 3 sham) of 26 participants correctly stated at day 90 the intervention they received at randomisation.

Adverse events

There were no procedure related SAEs (Supplementary Table I). Three patients experienced vascular events in the control group: two ischaemic strokes (day 6 and 8) and two myocardial infarcts in the same patient (day 1 and 66), versus none in the RIC group (p=0.076, log-rank test).

Laboratory Measures

In the RIC group, plasma analysis showed a significant increase in total HSP27 (p<0·05, repeated 2-way ANOVA) and phosphorylated HSP27 (pHSP27, p<0·001, Figure 2). HSPs 60, 70 and 90 did not differ between groups or over time. Similarly, plasma CRP, S100-beta, matrix metalloproteinase-9, troponin T and endocannabinoids did not differ between groups (Supplementary Figures I & II).

Haemodynamic measures

RIC did not significantly affect central blood pressure, mean arterial pressure, arterial compliance or Buckberg index (Supplementary Figure III). Most patients were ineligible for TCD assessment (AF n=11; proxy consent n=4, intolerant n=3; refusal n=1, no operator n=3, Supplementary Figure IV).

Clinical outcomes

Day 90 mRS was non-significantly lower in the treatment group (2 versus 3, p=0.8, Supplementary Table II). There was a significant decrease in day 90 NIHSS in the RIC group: median NIHSS 1 (interquartile range 0.5-4), versus 3 (1-4.5) (p=0.04 Mann-Whitney U test).

DISCUSSION

The Remote Ischaemic Conditioning After Stroke Trial has demonstrated that RIC is well tolerated after acute stroke and appears safe and feasible. Furthermore, RIC may improve neurological outcome and reduce vascular event rates evidenced by a significant improvement in NIHSS and a trend to fewer vascular events by day 90. Biomarker studies suggest that protective mechanisms may be mediated through phosphorylated HSP27.

There were no serious adverse events relating to RIC with only one patient reducing treatment due to cuff pressure intolerance. Pre-hospital RIC after acute stroke appears feasible but up to 18% of patients had a transportation time too short for 4 full cycles (the procedure was discontinued on arrival).[4] The primary outcome, penumbral salvage, did not improve with RIC but there were more TIAs and less severe strokes on arrival to hospital in the per-conditioned group.[4] The absence of baseline measurements and an imbalance at randomisation confounds interpretation of their results.

Numerous messengers have been implicated in ischaemic conditioning, and we have demonstrated RIC causes a significant increase in serum total HSP27 and phosphorylated HSP27 (pHSP27) 4 days after intervention, compared to control. Human HSP27 is neuroprotective in experimental stroke and infarct volume reduction is enhanced if HSP is in a phosphorylated form.[8] Pre-clinical models have shown benefit using RIC up to 6 hours post ictus[1] but the role of RIC in the current trial is unlikely to be neuroprotective as the intervention was too late (16 hours). Inflammation post stroke is a process occurring over hours to days, hence, other potential mechanisms include reducing cerebral oedema[1, 3] and inducing ischaemic tolerance to recurrent events. Indeed, mice over-expressing HSP 27 are protected against cerebral infarction.[3]

This trial has a number of limitations. First, the sample size meant the trial was not powered to detect changes in clinical outcomes and the differences seen in neurological improvement and the trend to a reduction in vascular events may be due to chance or a greater cardiovascular risk in the control group. Second, participants receiving stroke thrombolysis were not included; the safety of running RIC in parallel with hyperacute stroke treatments requires further investigation, planned in the RECAST-2 trial, an ongoing pilot study (https://clinicaltrials.gov/ct2/show/NCT02779712). Finally, the longer-term effects of RIC are unknown and should be considered in future trial design; RIC may have both acute and more prolonged effects.[9]

Disclosures

PB is Stroke Association Professor of Stroke Medicine.

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Table 1. Baseline characteristics

Characteristic	RIC	Sham
	n=13	n=13
Age, years	74.7 (10.8)	77.7 (10.4)
Male	8 (61.5)	9 (69.2)
Blood pressure, mmHg		
Systolic	153.7 (18.9)	164.7 (29.6)
Diastolic	81.5 (7.5)	84.3 (13.7)
Heart rate	78.5 (13.1)	74.1 (17.3)
ECG in AF	4 (30.8)	7 (53.8)
NIHSS	6 [3.5,12]	5 [3.5,9.5]
Premorbid mRS	0 [0,0.5]	0 [0,2]
Time to randomisation, hours	16.3 (5.9)	15.3 (6.6)
Clinical syndrome		
Lacunar	6 (46.2)	6 (46.2)
Partial anterior circulation	3 (23.1)	2 (15.4)
Total anterior circulation	4 (30.8)	4 (30.8)
Posterior circulation	0 (0)	1 (7.7)
Past Medical History		
Hypertension	5 (38.5)	9 (69.2)
Hyperlipidaemia	5 (38.5)	7 (53.8)
Diabetes mellitus	0 (0)	5 (38.5)
Known AF	3 (23.1)	5 (38.5)
Stroke	1 (7.7)	4 (30.8)
TIA	2 (15.4)	1 (7.7)
Ischaemic Heart Disease	2 (15.4)	5 (38.5)
Peripheral Vascular Disease	0 (0)	1 (7.7)

Data presented are mean (standard deviation), median [interquartile range] or number (percentage)

Figure Legends

Figure 1. Trial Flow

Figure 2. Effects of RIC vs sham on plasma levels of (A) Heat Shock Proteins (HSP) 27, (B) phosphorylated HSP27, (C) HSP60, & (D) HSP70 (n=13 per group, ##p<0.01, *p<0.05, ***p<0.001, repeated measures ANOVA with adjustment using Dunnett's multiple comparisons test, where * denotes a difference between groups, #denotes a difference over time). Data presented are mean ± SEM.
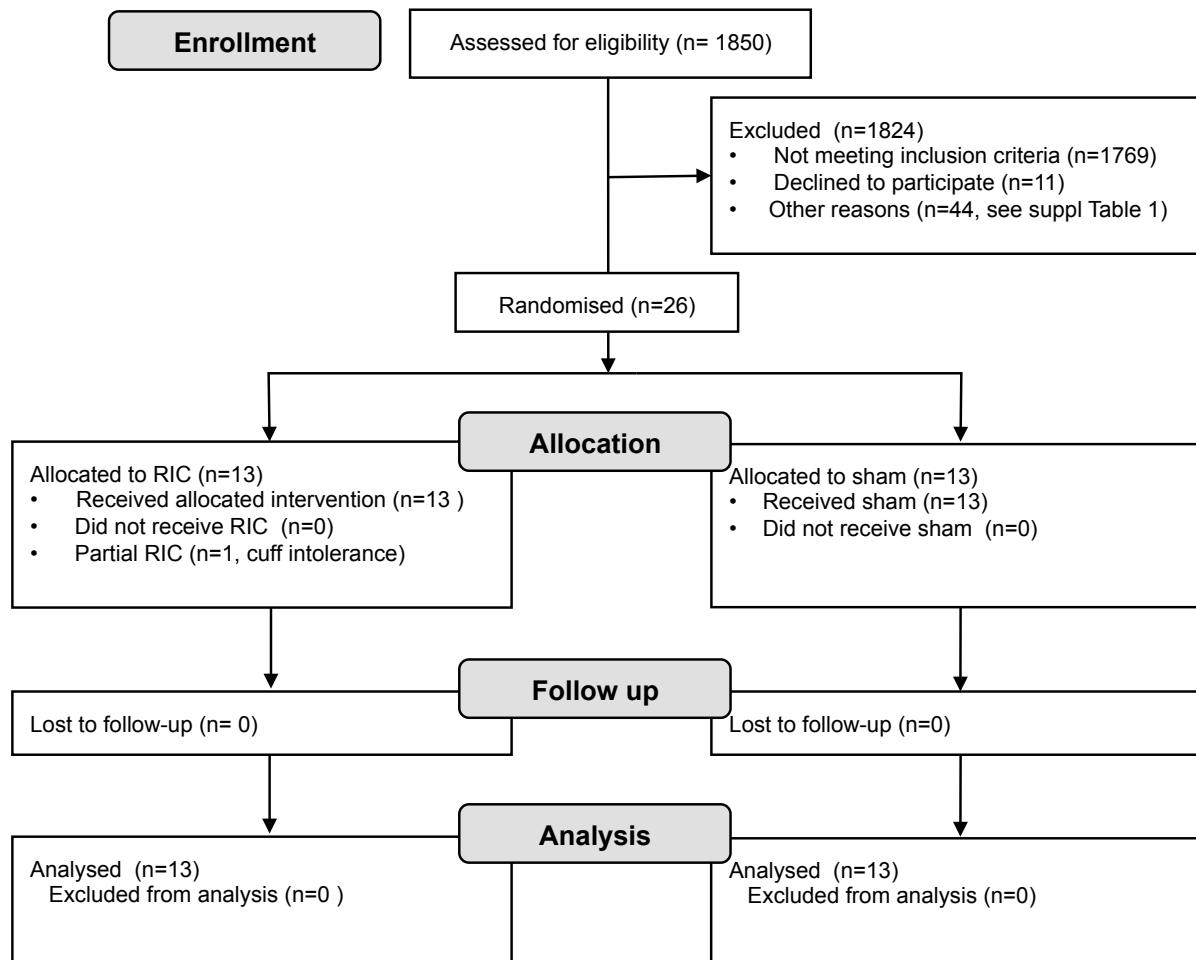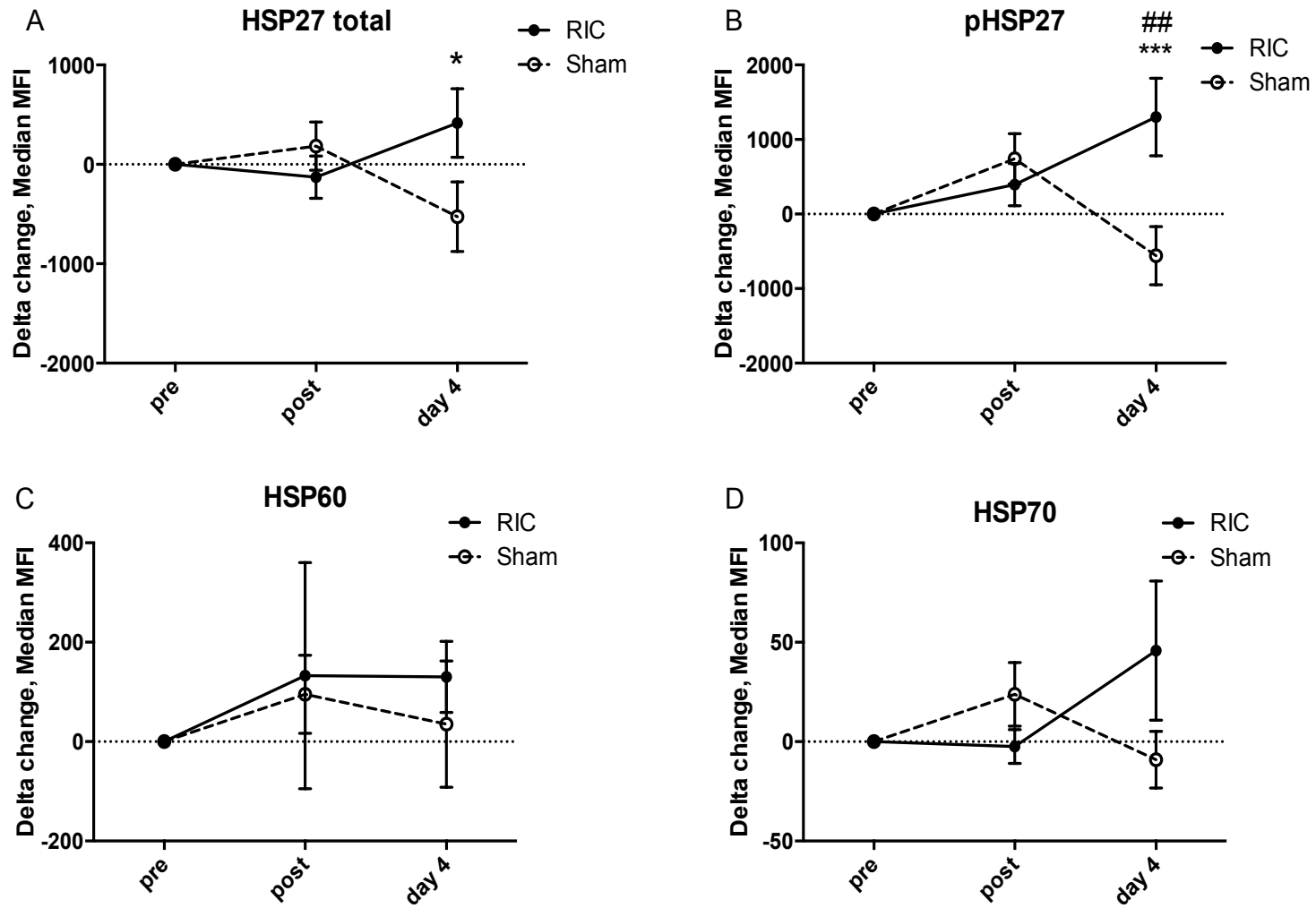
Figure 1

Enrollment

Assessed for eligibility (n= 1850)

Excluded (n=1824)
- Not meeting inclusion criteria (n=1769)
- Declined to participate (n=11)
- Other reasons (n=44, see suppl Table 1)

Randomised (n=26)

Allocation

Allocated to RIC (n=13)
- Received allocated intervention (n=13)
- Did not receive RIC (n=0)
- Partial RIC (n=1, cuff intolerance)

Allocated to sham (n=13)
- Received sham (n=13)
- Did not receive sham (n=0)

Follow up

Lost to follow-up (n= 0)

Lost to follow-up (n=0)

Analysis

Analysed (n=13)
  Excluded from analysis (n=0)

Analysed (n=13)
  Excluded from analysis (n=0)

Figure 2

SUPPLEMENTARY MATERIAL

REMOTE ISCHAEMIC CONDITIONING AFTER STROKE TRIAL (RECAST)

Statistical Methods

RIC and control groups were compared using appropriate statistical tests (SPSS Statistics version 22): binary data with chi-squared, Fisher's Exact test or logistic regression with adjustment of baseline prognostic factors; continuous data are compared using t-test and ANCOVA with adjustment for baseline covariates. Repeated and mixed measures ANOVA was used to compare biomarkers, SyphgmoCor and TCD analyses at multiple time-points and between groups, with adjustment using Dunnett's multiple comparisons test (Prism 6 for Mac OS X version 6·0f). Data in the figures are mean values ± SEM unless otherwise stated.

Supplementary Table I

Serious adverse events (SAE)	RIC (n=13)	Sham (n=13)	p*
N^{o} with SAE	4 (30.8)	7 (53.8)	0.23
Non-fatal	4 (30.8)	7 (53.8)	0.23
Fatal	0 (0)	0 (0)	
N^{o} with vascular events	0 (0)	3 (23.1)	0.22
N^{o} with non-fatal stroke	0 (0)	2 (15.4) $^{\partial}$	0.48
N^{o} with non-fatal MI	0 (0)	1 (7.7) †	1
Venous thromboembolism	0 (0)	0 (0)	

∂ Occurred on day's 6 and 8 post randomisation in different patients

† Same patient suffered 2 x NSTEMI on day 1 and day 66 post randomisation

* Analysed using Fisher's Exact test

The data monitoring committee assessed unblinded data (planned) halfway through the trial and, whilst there were no procedure related adverse events, deemed it unnecessary to include patients having received thrombolysis.

Supplementary Table II
Comparison of the RIC and sham treated groups with respect to clinical outcome measures at day 90

| Functional measures (day 90)	RIC (n=13)	Sham (n=13)	Unadjusted values		Adjusted values*	
			Mean difference (95% CI)	p	Mean difference 95% CI	p
Modified Rankin Scale (/6)						
Mean	2.46 (1.39)	2.69 (1.79)	-0.23 (-1.5, 1.1)	0.72	-0.12 (-0.87, 1.1)	0.82
Median	2 [1, 4]	3 [1, 4.5]		0.8		
Barthel Index (/100)						
Mean	69.6 (35.6)	62.3 (41.0)	7.3 (-23.7, 38.4)	0.63	4.9 (-28.7, 18.8)	0.67
Median	85 [25, 100]	85 [17.5, 100]		0.84		
NIHSS						
Mean	2.7 (3.0)	6.1 (5.6)	-3.4 (-7.0, 0.26)	0.067	-3.5 (-6.3, -0.7)	**0.016**
Median	1 [0.5, 5]	3 [2, 9.5]		**0.044**		
Motricity Index (/100)						
Mean	74.9 (30.7)	64.5 (39.2)	10.4 (-18.6, 39.4)	0.47	8.9 (-12.5, 30.3)	0.4
Median	89 [61, 100]	74 [32.5, 100]		0.85		
NEADL (/66)						
Mean	36.6 (23.7)	24.5 (23.1)	12.1 (-6.9, 31)	0.2	10.8 (-1.8, 23.4)	0.09
Median	41 [6.5, 58]	28 [3.5, 47]		0.08		
Zung depression score						
Mean	49.0 (20.1)	55.4 (12.7)	-6.4 (-20.4, 7.6)	0.36	-4.1 (-9.6, 1.6)	0.15
Median	52.5 [30, 65]	55 [48.1, 64.4]		0.41		
Mini-Mental State Examination†						
Mean	26.5 (3.3)	23.2 (5.7)	3.3 (-0.6, 7.1)	0.09	3.1 (-0.7, 7)	0.11
Median	27 [25.5, 28.5]	25 [19, 27]		0.12		

Data are mean (SD) or median [IQR].

* Adjusted for age & stroke severity (NIHSS), analysis by ANCOVA (analysis of covariance). Median values are compared using a Mann-Whitney U test.

† One patient in the sham group unable to complete MMSE due to aphasia; Data presented are mean values (standard deviation)

NIHSS, National Institutes of Health Stroke Scale; NEADL, Nottingham Extended Activities of Daily Living

Supplementary Figure I

Effects of RIC vs sham on plasma levels of protein S100-beta, matrix metalloproteinase 9 (MMP-9), Troponin-T and C-reactive protein (CRP). Analysis by repeated measures ANOVA did not reveal a difference over time or between groups (n= 13 per group, data are mean ± SEM). Assays were performed blinded by Luminex technology using commercially available assays (Merck Millipore Ltd, UK).
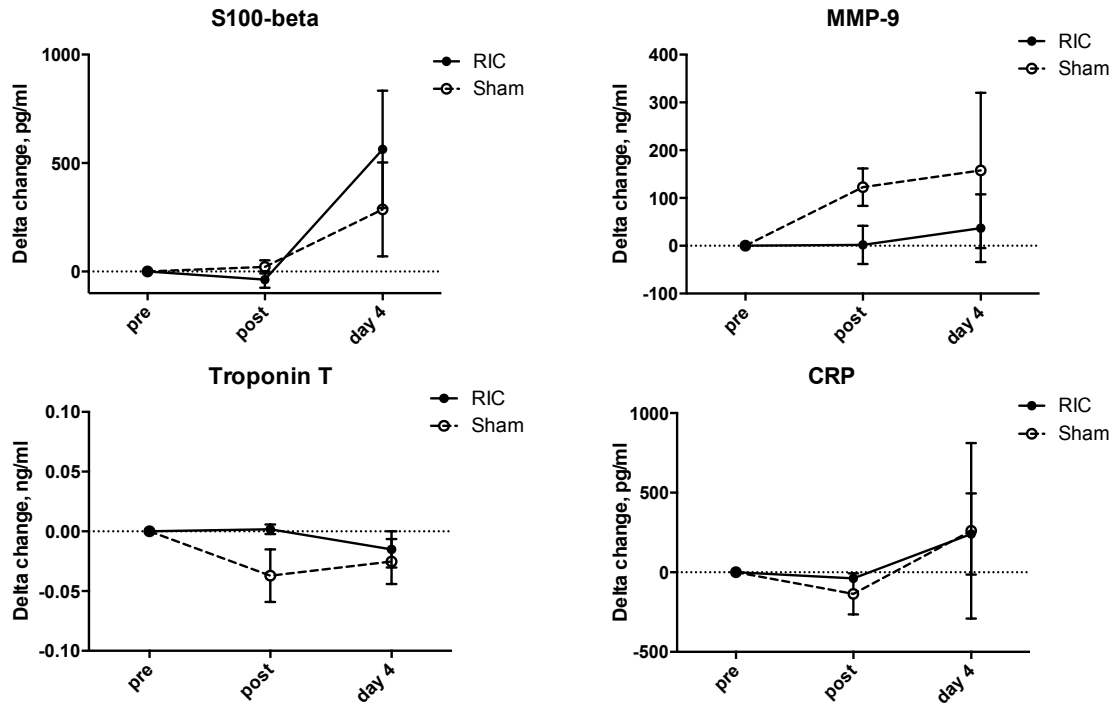
Supplementary Figure II: Effects of RIC vs sham on plasma levels of endocannabinoids (A) anandamide (AEA), (B) oleoylethanolamide (OEA), (C) palmitoylethanolamide (PEA) and (D) 2-arachidonoylglycerol (2-AG) (n=11 RIC, 13 Sham, [####] $p<0.0001$, [###] $p<0.001$, repeated measures ANOVA with adjustment using Dunnett's multiple comparisons test, where [#] denotes a difference over time). Data presented are mean ± SEM. eCBs were measured by liquid chromatography- tandem mass spectrometry based on a validated method.[1]
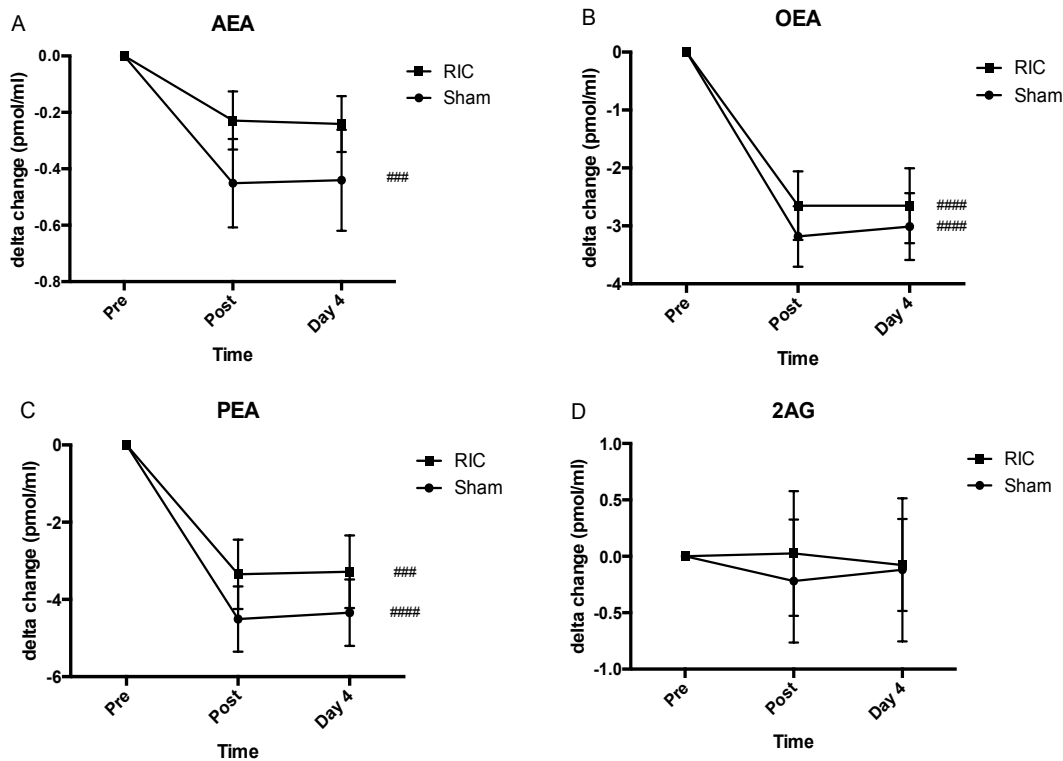
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Supplementary Figure III

The effects of RIC vs sham on central blood pressure ((A) systolic, (B) diastolic, (C) mean arterial (MAP)), (D) arterial compliance (Augmentation index) and (E) Buckberg index. Data are mean ± SEM. (SyphgmoCor analyses at multiple time-points and between groups, with adjustment using Dunnett's multiple comparisons test)
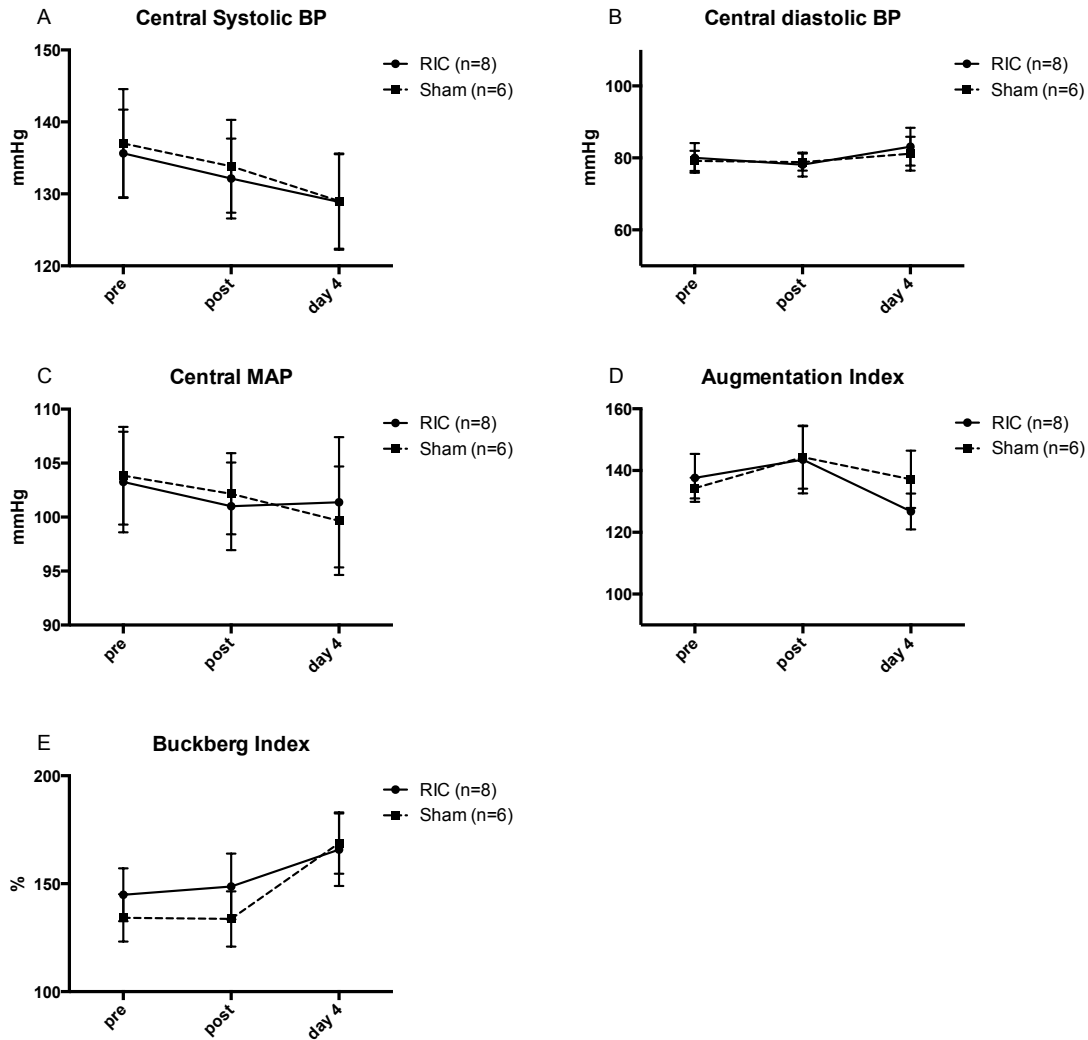
Supplementary Figure IV
The effects of upper limb RIC or sham on ipsilateral middle cerebral artery blood flow. The values represent the mean value (±standard deviation) taken from continuous beat-to-beat recording over each inflation/deflation cycle (sham n=2, RIC n=2)