

The Impact of Remote Ischaemic Preconditioning on Cardiac Biomarker and Functional Response to Endurance Exercise

Scott Cocking^{1, 2},
Thijs Landman³,
Mark Benson¹,
Rachel Lord¹,
Helen Jones¹,
David Gaze,⁴
Dick H. J. Thijssen^{1, 3},
Keith George¹

¹Research Institute for Sport and Exercise Science, *Liverpool John Moores University*, UK

²Athlete Health and Performance Research Centre, *Aspetar Orthopaedic and Sports Medicine Hospital*, Doha, Qatar.

³Radboud Institute of Health Sciences, Department of Physiology, *Radboud University Medical Center*, the Netherlands

⁴Department of Chemical Pathology, Clinical Blood Sciences, *St. George's Healthcare National Health Service Trust*, Tooting, London

RUNNING TITLE: Remote IPC lowers exercise-induced cTnT release

WORD COUNT (MANUSCRIPT): 4123

Abstract word count: 253

Number of References: 36

Tables: 3

Author for correspondence:

Professor Keith George, Research Institute of Sports and Exercise Science, Liverpool John Moores University, Tom Reilly Building, Byrom Street, Liverpool, L3 3AF

K.George@ljmu.ac.uk

TEL: +44151 904 6228

DISCLOSURE: No conflicts of interest

Abstract:

Introduction. Remote ischaemic preconditioning (RIPC; repeated short reversible periods of ischaemia) protects the heart against subsequent ischaemic injury. We explored whether RIPC can attenuate post-exercise changes in cardiac troponin T (cTnT) and cardiac function in healthy individuals.

Methods. In a randomised, crossover design, fourteen participants completed 1-hr cycling time-trials (TT) on two separate visits; preceded by RIPC (arms/legs, 4x5-min 220mmHg), or SHAM-RIPC (20mmHg). Venous blood was sampled before and 0, 1 and 3-h post-exercise to assess high sensitivity (hs-)cTnT and brain natriuretic peptide (NT-proBNP). Echocardiograms were performed at the same time points to assess left and right ventricular systolic (ejection fraction; EF & right ventricular fractional area change; RVFAC, respectively) and diastolic (early transmitral flow velocities; E) function.

Results. Baseline hs-cTnT was not different between RIPC and SHAM. Post exercise hs-cTnT levels were consistently lower following RIPC (18 ± 3 vs 21 ± 3 ; 19 ± 3 vs 23 ± 3 ; & 20 ± 2 vs 25 ± 2 ng/L at 0, 1 and 3-h post-exercise, respectively; $P<0.05$). There was no main effect of time, trial or interaction for NT-proBNP and left or right ventricular EF (all $P<0.05$). A main effect of time was evident for E which transiently declined immediately after exercise to a similar level in both trials (0.85 ± 0.04 vs 0.74 ± 0.04 m.s⁻¹, respectively; $P<0.05$).

Conclusion. RIPC was associated with lower hs-cTnT levels after exercise but there was no independent effect of RIPC for NT-proBNP or LV systolic and diastolic function. The lower hs-cTnT levels after RIPC suggests that further research should evaluate the role of ischaemia in exercise-induced elevation in hs-cTnT.

KEY WORDS: cardiovascular function; cardiac fatigue; ischaemic preconditioning

Introduction:

Several previous studies have demonstrated the presence of elevated serum levels of cardiac troponin (cTn), a biomarker of cardiomyocyte damage, during and after an exercise bout (Shave et al., 2010). Furthermore, strenuous exercise can lead to an acute, transient decrease in both systolic and diastolic cardiac function (Oxborough et al., 2011; Scott et al., 2009). Whilst studies reporting cTn-elevation or a decline in cardiac function have typically employed prolonged, strenuous exercise (e.g. marathon, triathlon) (Douglas et al., 1990; Neilan et al., 2006), comparable findings have been reported after short duration exercise (~1 h) (Duttaroy et al., 2012; Tjora et al., 2011). It has been speculated, but never tested, that acute changes in cTn and cardiac function after exercise may be related to the presence of local, sub-clinical ischaemia during strenuous exercise (Ellison et al., 2012). Alternative (non-ischaemia) explanations must also be considered to explain post-exercise elevations in cTn, such as mechanical stress, the atherosclerotic burden and/or the presence of myocardial fibrosis (Möhlenkamp et al., 2008; Breuckmann et al., 2009; Möhlenkamp et al., 2014).

Ischaemic preconditioning (IPC) refers to the exposure of an organ (e.g. the heart) to repeated short bouts of ischaemia and was introduced in 1986 as an effective intervention to attenuate cardiac damage after subsequent prolonged ischaemia in animals (Murry et al., 1986). Interestingly, the protective effects of IPC on the heart are also present when IPC is applied to remote areas, such as the limbs, and is commonly referred to as remote IPC (RIPC). Studies in humans have demonstrated that RIPC reduces cardiac damage in response to acute ischaemic events and cardiac surgery (Cheung et al., 2006; Hausenloy et al., 2007; Hong et al., 2012; Loukogeorgakis et al., 2007; Heusch et al., 2015), although conflicting results have been published (Hausenloy et al., 2015; Meybohm et al., 2015). RIPC, therefore, provides a method capable of attenuating ischaemia-induced damage in the myocardium. Despite this a

single previous study from El Messaoudi et al. found that RIPC did not alter exercise-induced cardiac troponin 1 (cTnI) in healthy volunteers (El Massaoudi et al., 2013). Important limitations in that study were the relatively mild exercise stimulus and RIPC exposure as well as a lack of insight into cardiac function.

Consequently, the present study extended previous work and tested the hypothesis that exercise-related changes in cTn, NT-proBNP and cardiac function can be attenuated when 1-h strenuous cycle exercise is preceded by RIPC and therefore may relate to ischaemia to the heart during strenuous exercise.

Methods

Participants

Fourteen healthy, recreationally-trained to well-trained cyclists (Age 29 ± 8 ; Body Mass 71.6 ± 11.5 kg; Height 179 ± 9 cm; BMI 22.4 ± 1.6) were recruited. Participants were undertaking regular endurance training (>3 aerobic exercise sessions per week). We excluded subjects with any illness or injury that had the potential to impact the 1-hour cycling time trial (TT) performance. Participants who had any personal or early family history of cardiovascular disease were excluded from participating in the study. This study was approved by the local Ethics Committee and all participants provided informed consent after reading participant information sheets and were fully familiarized to all testing procedures.

Experimental design

In a single blind, randomised crossover-design study, participants undertook three exercise sessions within the physiology laboratories at Liverpool John Moores University involving a maximal cycling test and two time trials (TT). On the first laboratory visit, a maximal incremental cycling test to volitional exhaustion was completed in order to assess peak

oxygen uptake and classify participant training status. The graded cycling test was completed at least 7 days before the first experimental TT. Visits 2 & 3 involved the completion a 1 hour cycling TT (maximum distance achieved in 1 hour) protocol preceded by RIPC or SHAM. TTs were separated by a minimum of 7 days. Data collection of blood samples and echocardiography was performed before and after (0, 1 and 3 h) the TT. All tests were completed at the same time of day to minimize the impact of circadian variation on exercise performance and outcome parameters (Atkinson and Reilly, 1996). Participants were asked to attend the lab in a euhydrated state and to have abstained from intense exercise, caffeine and alcohol intake for a minimum of 24-hr before the exercise.

Experimental protocols

Visit 1 (maximal cycle test).

The graded maximal cycle test was completed on a cycle ergometer (Lode, Excalibur sport VS, The Netherlands). The exercise test was preceded by a warm-up of 5 min at 50 W. The incremental test began at a workload of 50 W and increased by 25 W per min until volitional exhaustion was achieved. Oxygen consumption was continually assessed using an automated online gas analysis system (Oxycon, COSMED, Italy). Heart rate was measured continuously using short-range telemetry (Polar Electro Oy, Finland). Achievement of $\text{VO}_{2\text{max}}$ was deemed when 2 of the 3 following criteria was met: 1). A plateau in VO_2 despite a continual increase in cycling power output. 2). A heart rate (HR) 10 beats of age predicted maximum. 3). A respiratory exchange ratio of above 1.10 (Howley et al., 1995)

Remote ischaemic preconditioning (RIPC) and SHAM.

Before TT commencement, RIPC or SHAM was performed in the supine position using bilateral arterial occlusion on both the arms and legs (alternating between left and right).

Automated pressure cuffs (Hokanson, Washington, USA) were placed proximally around the (left or right) upper arms and thighs. Pressure cuffs were unilaterally inflated to 220 mmHg (RIPC or 20 mmHg (SHAM) on ipsilateral upper and lower limbs. Limb occlusion lasted for 5 minutes to block arterial inflow and was interspersed with 5 minutes of reperfusion. As the pressure cuffs were deflated, the blood pressure cuffs on the opposite side of the body and were inflated on both limbs for 5 minutes. This process was repeated 4 times (40 minutes total IPC time).

Visits 2&3 Exercise Protocol:

Immediately after the RIPC or SHAM procedure, participants completed a maximal 1-hr cycling TT using a computerised cycling program (Racer Mate CompuTrainer, UK). Participants used their own bikes where possible to improve consistency between both road- and lab-cycling positions during experimental trials. Rate of perceived exertion (RPE) was monitored every 10 minutes using the Borg scale (Borg, 1982). Heart rate (HR) was measured throughout exercise using short-range telemetry and average power output (W) was recorded from the CompuTrainer software. Participants were made aware of time and HR throughout the TT, but were blinded to all other physiological feedback until the completion of the final TT. Pre- and immediately post-exercise, body mass was recorded using an electronic weighing scale (SECA, Germany) along with heart rate, systolic and diastolic blood pressure using an automated sphygmomanometer (Dinamap, GE Medical, Wisconsin, USA). Biomarkers and cardiac function were assessed over four time points: Pre- (PRE); immediately post (POST-OH); 1-hr post (POST-1H) and 3-hr post (POST-3H) TT exercise.

Measurements

Biomarkers.

Serum cardiac troponin T (cTnT) and NT-proBNP were assessed from whole blood sampled by repetitive venipuncture. In total, 4 blood samples of 5 mL were taken per trial. Whole blood was spun down, pipetted and serum was stored at -80°C. All serum samples were analysed in a single session.

Cardiac Troponin

The Roche diagnostics high sensitivity cardiac troponin T (hs-cTnT) assay was used. The hs-cTnT measurements were performed using an Elecsys Cobas E-unit (Roche diagnostics, Haywards Heath, UK). The detection limit of the assay is 3ng/L with an upper limit of 10000ng/L. The claimed 10% coefficient of variation (CV) is 13ng/L with a 99th centile of 14ng/L of a healthy reference population.

N terminal pro B-type natriuretic peptide.

NT-proBNP was measured by a solid phase two site chemiluminescent sandwich immunoassay using an Immulite 2500 (Siemens Healthcare Diagnostics, Frimley, UK). The detection limit of the assay was 20ng/L with a measuring range up to 35000ng/L. The interassay % CV was 5.0 to 4.0% in the range 40.9 to 32096ng/L.

Cardiac function.

Cardiac function was examined using trans-thoracic echocardiography. Scans were performed by a trained sonographer using standardised protocols (American Society of Echocardiography [ASE]) (Mor-Avi et al., 2011). All images were collected with the participant lying in the left lateral decubitus position. Acquisition and analysis were performed using VividQ and EchoPac (GE Medical, Norway). During scans all settings were optimized to obtain maximum signal-to-noise ratio. Key images and outcome measures were

as follows. From a parasternal long axis view, M-mode scans characterized left ventricle (LV) dimension at end-diastole (LVED) and at end-systole (LVES). Apical 2- and 4-chamber views allowed the examination of LV end-diastolic (LVEDV) and end-systolic (LVESV) volumes using the Simpson Biplane method. This facilitated the estimation of ejection fraction (EF). Right ventricular (RV) size and dimension were measured at end diastole from the outflow tract using a parasternal long-axis view and at the inflow from an apical four-chamber orientation. RV end-diastolic area and end-systolic area were calculated by tracing around the endocardium from a modified apical four-chamber orientation and RV fractional area change (RVFAC) was calculated. LV diastolic filling was assessed using pulsed-wave Doppler echocardiographic recordings from the apical 4-chamber view. Specifically, spectral Doppler envelopes characterized from a 4 mm sample volume allowed measurement of peak flow velocity of the early rapid filling wave (E-wave) and peak flow velocity of the late filling wave due to atrial contraction (A-wave). The ratio E/A was calculated. In the same apical view, and using a 2 mm sample volume, tissue Doppler assessment of basal septal, LV lateral wall as well as RV lateral wall peak early diastolic (E') and late atrial (A') myocardial velocities were completed. From apical 4 chamber images we completed post-hoc analysis of longitudinal cardiac deformation analysis in the RV and LV. Myocardial speckle tracking analysis of regional strain (ϵ) and strain rates (SR: systole, early diastole and atrial diastole) were determined in basal, mid and apical wall segments for the septum, LV lateral wall and RV lateral wall. In all cases semi-automated wall tracking was applied with frame rates as high as possible (but below 90 fps) and all images were optimized with gain, compression, and dynamic range to enhance myocardial definition.

Statistics

Statistical analysis was performed using SPSS 21.0 (SPSS, Chicago, Illinois) software. All data was reported as mean \pm SD and statistical significance was assumed at $P \leq 0.05$. Two

way repeated measures ANOVA (time [4 levels] - pre vs. 0 vs. 1 vs. 3 hr post-exercise, intervention [2 levels] - RIPC vs. control) was used to assess changes across the TT in our primary and secondary outcome parameters, and whether these changes differ when exercise was preceded by RIPC. When a significant main or interaction-effect was observed, post-hoc analysis using Bonferroni testing to correct for multiple comparisons was performed.

Results:

Maximal cycling test: The mean $\text{VO}_{2\text{peak}}$ was $50.1 \pm 7.6 \text{ mL.kg.min}^{-1}$. Peak HR was $182 \pm 9 \text{ beats.min}^{-1}$ and the average peak power attained was $346 \pm 63 \text{ W}$. All individuals reported that lower limb local fatigue was responsible for cessation of exercise.

1-hr time trial performance: Both average power output and cycle distance were not different between RIPC and SHAM groups (W: 179 ± 47 vs. 183 ± 53 , $P > 0.05$; & Distance: $31.96 \pm 3.08 \text{ km}$ vs. $32.10 \pm 2.47 \text{ km}$, $P > 0.05$, respectively). No significant changes in body mass or blood pressure were found between or within trials ($P > 0.05$, respectively). Average HR was not different between RIPC and SHAM ($154 \pm 16 \text{ beats.min}^{-1}$ vs. $151 \pm 16 \text{ beats.min}^{-1}$, $P > 0.05$). Similarly, there was no difference in average RPE between both RIPC and SHAM conditions (15 ± 2 vs. 15 ± 1 , respectively, $P > 0.05$).

Cardiac biomarkers: At rest, hs-cTnT was not different between RIPC and SHAM trials ($P > 0.05$). Absolute hs-cTnT levels were lower after RIPC than SHAM across all time points post-exercise (main effect for trial: $P < 0.05$), but there was no main effect of time or time-by-trial interaction (both $P > 0.05$, Figure 1a). Maximal values for hs-cTnT occurred POST-3H (RIPC: $20 \pm 9 \text{ ng/L}$ vs. SHAM: $25 \pm 8 \text{ ng/L}$, $P = 0.08$). No main effect of time, trial or time-by-trial interaction was observed for NT-proBNP (all $P > 0.05$, Figure 1b).

Cardiac function: There was an effect of time for HR after exercise ($P < 0.001$) but no time-by-trial interaction (Table 1). There was no main effect of time, trial or time-by-trial interaction for LVEDV, a surrogate of preload, LVEF or RVFAC ($P > 0.05$, Table 1). A main effect of time was evident for E peak velocity ($P < 0.05$) with E dropping immediately after exercise in both trials with a recovery by POST-3H. Changes in E contributed to a drop in E/A immediately post-exercise, but this did not reach statistical significance ($P = 0.06$). Additionally, there was no change in E/e' ratio between conditions ($P > 0.05$). A main effect of time was also noted for E' in the LV (Table 2), although these changes were small and did not differ between SHAM and RIPC trials. A main effect of time was also observed for LV longitudinal peak SSR with values increasing at POST-1H and POST-3H ($P = 0.003$ and 0.007 , respectively, Table 2). A main effect of time was noted for LV longitudinal ASR with increased values at POST-3H ($P = 0.006$) as well as a main effect of trial with lower values reported throughout the RIPC trial ($P = 0.011$). No effect of time or trial was observed for peak ϵ , ESR or SSR ($P > 0.05$).

Discussion

The primary aim of this study was to investigate whether RIPC influenced exercise-induced changes in cardiac biomarker release and cardiac function. The key findings from the current study are; (1) there is some evidence that hs-cTnT release following 1-hour TT cycling is attenuated when exercise is preceded by RIPC, (2) post-exercise NT-proBNP were not altered by RIPC, and (3) RIPC prior to a 1-hour cycling time trial did not influence post-exercise cardiac function. The association of lower hs-cTnT levels after RIPC suggests that ischaemia may contribute (in part) to exercise-induced elevation in hs-cTnT.

Cardiac Biomarkers

Baseline hs-cTnT was found to be slightly higher (> 14 ng/L) in the current study. A previous large sample study ($n = 545$) reported a 99th percentile of 30 ng/L (Collinson et al., 2012) which suggests the current data are likely within the 'normal' spectrum. A post-exercise elevation in hs-cTnT, a highly specific marker of cardiomyocyte insult, has been observed previously (Duttaroy et al., 2012; La Gerche et al., 2008). The increase in hs-cTnT values post-exercise in the current study was smaller than those observed in past research (Duttaroy et al., 2012), which may be partially explained by between-study differences in exercise intensity and/or exercise duration (Fu et al., 2009) or the higher baseline levels observed compared to previous work.

A systematically lower set of hs-cTnT data was observed after exercise in the RIPC compared to the SHAM trial (significant main effect of trial). This provides the first, tentative, suggestion that RIPC can attenuate hs-cTnT release that occurs as a consequence of acute endurance exercise. This contradicts the only previous study that examined the effects of RIPC on exercise-related changes in cTnI, which did not find an effect of RIPC (El Massaoudi et al., 2013). The difference in outcomes may be explained by a combination of factors, related to the exercise- and RIPC-stimulus, but also variability in participant training status. El Massaoudi et al. (2013) included relatively untrained subjects, whilst the intensity of exercise was lower than in the present study. Furthermore, El Massaoudi et al. employed a 3x5 minute bilateral forearm RIPC protocol occluding a smaller muscle mass for a shorter duration compared to the current study, which may have limited the cardio-protective stimulus.

It is tempting to suggest that the mechanism(s) underpinning the lower hs-cTnT data after exercise in the RIPC trial are consistent with the ischaemia-reperfusion induced cardio-protective adaptation observed in previous animal studies using IPC (Jean-St-Michel et al., 2011). While direct evidence between RIPC and inhibition of mitochondrial permeability transition pore (mPTP) opening is yet to be established (Sharma et al., 2015), it is apparent that mitochondrial K_{ATP} channel opening does occur. RIPC inhibits mPTP opening upon reperfusion and offers cardio-protection in similar fashion to both the reperfusion injury salvage kinase (RISK) and survivor activating factor enhancement (SAFE) pathways, which are also activated following RIPC (Heusch et al., 2015; Sharma et al., 2015). Opening of K_{ATP} channels additionally activates downstream signalling of protein kinase C- ϵ , shown to also offer cardio-protective effects (Wolfrum et al., 2002). It is suggested both humoral and neural pathways could be responsible for the transmission of cardio-protective signals to the heart following RIPC (Sharma et al., 2015), however the exact contribution of each pathway remains unclear. Therefore any resultant change from RIPC does not necessarily provide direct evidence for an ischaemic mediated mechanism or event.

Sphingosine-1-phosphate (S1P) is a molecule recently demonstrated to reduce ischaemia/reperfusion (I/R) induced cardiac damage (Santos-Gallego et al., 2016) and to mediate the mechanism of RIPC-induced cardio-protection (Jin et al., 2004). Interestingly, S1P can also be produced in skeletal muscle (Baranowski et al., 2015), thus intramuscular S1P release in response to RIPC could influence the cardio-protective response following exercise. Additionally, the observation that plasma S1P levels are only elevated by high-intensity exercise (Baranowski et al., 2015) could explain why in previous work (Neilan et al., 2006), athletes with less training experience (i.e. less S1P release) showed more severe exercise-induced cardiac damage.

Whilst our study may provide the first indirect evidence of the role of ischaemia in exercise-induced hs-cTnT elevation, there are some important caveats. Firstly, the elevation in hs-cTnT after exercise in the SHAM trial was small compared to previous studies in different exercise circumstances (Shave et al., 2010). Secondly there was no significant statistical interaction term (time by trial) for the hs-cTnT data. Finally, there was no other corroborative evidence of ischaemia, such as ECG changes. At this preliminary stage in the evaluation of mechanism(s) underpinning hs-cTnT changes after acute exercise (and the ability of RIPC to prevent such changes) we cannot, categorically, rule out other potential mechanisms.

We did not observe any effect of time, trial or an interaction of these factors on NT-proBNP. An elevation in NT-proBNP has been positively correlated with exercise duration, with significantly higher serum levels occurring post-ultramarathon (100km run) when compared to marathon running (Scharhag et al., 2005). The time (or exercise volume) dependent increase in NT-proBNP reflects an increased BNP expression in over-stretched myocytes in vitro. On the basis that no changes in NT-proBNP were reported after both short-endurance and high-intensity exercise (Benda et al., 2015; El Massaoudi et al., 2013), it is suggested the exercise volume in the current study may have been insufficient to stimulate a significant NT-proBNP response.

Cardiac Function

No post-exercise changes in LV systolic function were observed in either trial. This is in line with previous studies employing a relatively short (1 hr) endurance exercise exposure (Oxborough et al., 2012). For example, Palatini et al. reported no change in LV systolic function following 50-80 minutes of cycling to exhaustion at anaerobic threshold (Palatini et

al., 1994). It would seem that systolic function is well maintained in the face of this type of acute exercise exposure. Nonetheless, small changes in diastolic function were observed during recovery from exercise in both trials in our study, and as such, changes were not mediated by RIPC. A transient decline in peak E diastolic flow velocity after exercise, with a return to baseline by the POST-3H, has been confirmed in a range of previous studies and this effect is summarised in a meta-analysis (Middleton et al., 2006). There is evidence to suggest transient declines in diastolic, but not systolic function can occur following shorter duration exercise (<6 hours) (Middleton et al., 2006). A post-exercise, transient decline in diastolic function has been postulated to be due to a decrease in preload, ischaemia and damage, and/or other intrinsic processes (Oxborough et al., 2010). The current study would tend to suggest that ischaemia is not a key candidate, due to the consistency of post-exercise changes in these measures of diastolic function in both the RIPC and SHAM trials. Further research is needed, preferably in situations where the heart is exposed to a longer period of exercise.

Implications

Based on our observations and the post-exercise kinetics of cTnT release observed in other work (Middleton et al., 2008), it is likely the small post-exercise changes in hs-cTnT do not represent long term irreversible myocardial damage (La Gerche et al., 2008; Shave et al., 2010). The observation that RIPC may at least partially mediate changes in hs-cTnT appearance after prolonged exercise warrants on-going study. It is also apparent that post-exercise changes in LV diastolic function are small, transient and likely to have no performance and/or clinical relevance (Middleton et al., 2006). In the current study, there was no association between hs-cTnT and changes in LV diastolic function. The lack of association between changes in cTn and cardiac function after endurance exercise is common

(Shave et al., 2010) and supports the hypothesis that increases in cTn and decreases in LV function represent two different phenomenon that are not causally linked.

Limitations

Individual variability in hs-cTnT response in the current study likely restricted statistical significance on post-hoc analysis. The relatively small sample size used (N=14) may have also contributed to absence of statistical significance for the post-hoc comparisons. The limited exercise volume and blood sampling protocol employed in the current study may have led to “blunted” hs-cTnT data. Whilst there is little data assessing cTn kinetics during and after endurance cycling, Middleton et al. (2008) noted that cTnT peaked between 60-120 min during treadmill running with a secondary peak between 3-12 hours post exercise. A more frequent blood sampling protocol (between 3 & 12 hours post-exercise) may have further clarified any between-trial differences observed in cTnT based on the trend for increasing post-exercise hs-cTnT levels in SHAM at POST-3H, and subsequently, a progressive increase in the difference between SHAM vs RIPC. It is therefore advisable that future RIPC studies should employ more frequent blood draws and employ a larger exercise stimulus. The use of a 7 day washout between trials ensured participants were in similar physical condition. A longer washout period may be advisable in future studies following new observations of circulating cardio-protective factors 6 days after RIPC treatment in animals (Hildebrandt et al., 2016). The use of counterbalanced and randomized trial order in the present study should have accounted for any delayed humoral alterations during SHAM condition exercise. All participants were instructed to undertake no exercise 24 hours prior to laboratory visits but we cannot confirm if any (intense) exercise performed 24-48 hour prior to laboratory testing, influenced baseline hs-cTnT data.

Conclusion

Overall, serum hs-cTnT concentrations were lower in the RIPC vs SHAM condition. Whilst there was a trend for an increasing difference in hs-cTnT between conditions as time progressed, this was not met with a significant main effect for time, or time by trial interaction. This provides the first, indirect, evidence that ischaemia may be implicated as a mechanism in changes in hs-cTnT post-exercise. RIPC did not mediate post-exercise changes in NT-proBNP, LV systolic function or LV diastolic function, providing further support to the hypothesis that exercise-induced changes in cTn may not be directly related to changes in cardiac function.

NEW FINDINGS / BRIEF PERSPECTIVE:

RIPC is associated with reduced myocardial infarct following ischaemic exposures. The effect RIPC can have on both cTnT response and cardiac function following an intense exercise TT bout has not previously been explored.

We show that RIPC is associated with lower cTnT elevation following cycling TT exercise, providing the first, indirect evidence that ischaemia may be implicated as a mechanism in changes in cTnT post-exercise.

References:

- Atkinson G, Reilly T. Circadian variation in sports performance. *Sports Med* 1996; 21: 292–312.
- Benda NM, Eijssvogels TM, Van Dijk AP, Hopman MT, Thijssen DH. Changes in BNP and cardiac troponin I after high-intensity interval and endurance exercise in heart failure patients and healthy controls. *Int. J. Cardiol* 2015; 184: 426–427.
- Baranowski M, Błachnio-Zabielska AU, Charmas M, Helge JW, Dela F, Ksiazek M, Długołęcka B, Klusiewicz A, Chabowski A, Górski J. Exercise increases sphingoid base-1-phosphate levels in human blood and skeletal muscle in a time- and intensity-dependent manner. *Eur J Appl Physiol* 2015; 115: 993–1003
- Breuckmann F¹, Möhlenkamp S, Nassenstein K, Lehmann N, Ladd S, Schmermund A, Sievers B, Schlosser T, Jöckel KH, Heusch G, Erbel R, Barkhausen J. Myocardial late gadolinium enhancement: prevalence, pattern, and prognostic relevance in marathon runners. *Radiology*. 2009 Apr;251(1):50-7.

- Cheung MMH, Kharbanda RK, Konstantinov IE, Shimizu M, Frndova H, Li J, Holtby HM, Cox PN, Smallhorn JF, Van Arsdel GS, Redington AN. Randomized Controlled Trial of the Effects of Remote Ischemic Preconditioning on Children Undergoing Cardiac Surgery. *J. Am. Coll. Cardiol* 2006; 47: 2277–2282.
- Collinson PO, Heung YM, Gaze D, Boa F, Senior R, Christenson R, Apple FS. Influence of population selection on the 99th percentile reference value for cardiac troponin assays. *Clinical Chemistry* 2012; 58 (1): 000-000
- Douglas PS, O'Toole ML, Woolard J. Regional wall motion abnormalities after prolonged exercise in the normal left ventricle. *Circulation* 1990; 82: 2108–2114.
- Duttaroy S, Thorell D, Karlsson L, Börjesson M. A single-bout of one-hour spinning exercise increases troponin T in healthy subjects. *Scand. Cardiovasc. J* 2012; 46: 2–6.
- Ellison GM, Waring CD, Vicinanza C, Torella D. Physiological cardiac remodelling in response to endurance exercise training: cellular and molecular mechanisms. *Heart* 2012; 98: 5–10.
- El Messaoudi S, Vissers A, Thijssen D, Riksen N P, Rongen G A. The effect of remote ischemic preconditioning on exercise-induced plasma troponin I appearance in healthy volunteers. *Int J Cardiol* 2013; 168 (2): 1612-3
- Fu F, Nie J, Tong T. Serum Cardiac Troponin T in Adolescent Runners: Effects of Exercise Intensity and Duration. *Int. J. Sports Med* 2009; 30: 168–172.
- Hausenloy DJ, Candilio L, Evans R, Ariti C, Jenkins DP, Kolvekar S, Knight R, Kunst G, Laing C, Nicholas J, et al. Remote Ischemic Preconditioning and Outcomes of Cardiac Surgery. *N. Engl. J. Med.* 2015; 373, 1408–1417.
- Hausenloy DJ, Mwamure PK, Venugopal V, Harris J, Barnard M, Grundy E, Ashley E, Vichare S, Di Salvo C, Kolvekar S, et al. Effect of remote ischaemic preconditioning on myocardial injury in patients undergoing coronary artery bypass graft surgery: a randomised controlled trial. *The Lancet* 2007; 370: 575–579.
- Heusch G, Bøtker HE, Przyklenk K, Redington A, Yellon D. Remote Ischemic Conditioning. *J. Am. Coll. Cardiol* 2007; 65: 177–195.
- Hildebrandt HA, Kreienkamp V, Gent S, Kahlert P, Heusch G, Kleinbongard P. Kinetics and Signal Activation Properties of Circulating Factor(s) From Healthy Volunteers Undergoing Remote Ischemic Pre-Conditioning. *JACC Basic Transl. Sci.* 2016; 1: 3–13.
- Hong DM, Jeon Y, Lee CS, Kim HJ, Lee JM, Bahk JH, Kim KB, Hwang HY. Effects of remote ischemic preconditioning with postconditioning in patients undergoing off-pump coronary artery bypass surgery--randomized controlled trial. *Circ J* 2012; 76 (4): 884-90.
- Howley ET, Bassett DR, Welch HG (1995). Criteria for maximal oxygen uptake: review and commentary. *Med. Sci. Sports Exerc* 1995; 27 (9): 1292-1301
- Jean-St-Michel E, Manlhiot C, Li J, Tropak M, Michelsen MM, Schmidt MR, McCrindle BW, Wells GD, Redington AN. Remote Preconditioning Improves Maximal Performance in Highly Trained Athletes. *Med. Sci. Sports Exerc* 2011; 43: 1280–1286.
- Jin ZQ, Goetzl EJ, & Karliner JS. Sphingosine Kinase Activation Mediates Ischemic Preconditioning in Murine Heart. *Circulation* 2004; 110: 1980-1989
- La Gerche A, Connelly KA, Mooney DJ, MacIsaac AI, Prior DL. Biochemical and functional abnormalities of left and right ventricular function after ultra-endurance exercise. *Heart* 2008; 94: 860–866.
- Loukogeorgakis SP, Williams R, Panagiotidou AT, Kolvekar SK, Donald A, Cole TJ, Yellon DM, Deanfield JE, MacAllister RJ. Transient Limb Ischemia Induces Remote Preconditioning and Remote Postconditioning in Humans by a KATP Channel Dependent Mechanism. *Circulation* 2007; 116: 1386–1395.
- Meybohm P, Bein B, Brosteanu O, Cremer J, Gruenewald M, Stoppe C, Coburn M, Schaele G, Böning A, Niemann B, et al. A Multicenter Trial of Remote Ischemic Preconditioning for Heart Surgery. *N. Engl. J. Med.* 2015; 373: 1397–1407
- Middleton N, George K, Whyte G, Gaze D, Collinson P, Shave R. Cardiac Troponin T Release Is Stimulated by Endurance Exercise in Healthy Humans. *J. Am. Coll. Cardiol* 2008; 52: 1813–1814.

- Middleton N, Shave R, George K, Whyte G, Hart E, Atkinson G. Left Ventricular Function Immediately following Prolonged Exercise: A Meta-Analysis. *Med. Sci. Sports Exerc* 2006; 38: 681–687.
- Mor-Avi V, Lang RM, Badano LP, Belohlavek M, Cardim NM, Derumeaux G, Galderisi M, Marwick T, Nagueh SF, Sengupta PP, et al. Current and Evolving Echocardiographic Techniques for the Quantitative Evaluation of Cardiac Mechanics: ASE/EAE Consensus Statement on Methodology and Indications Endorsed by the Japanese Society of Echocardiography. *Eur. J. Echocardiogr* 2011; 12: 167–205.
- Möhlenkamp S, Lehmann N, Breuckmann F, Bröcker-Preuss M, Nassenstein K, Halle M, Budde T, Mann K, Barkhausen J, Heusch G, Jöckel KH, Erbel R; Marathon Study Investigators; Heinz Nixdorf Recall Study Investigators. Running: the risk of coronary events : Prevalence and prognostic relevance of coronary atherosclerosis in marathon runners. *Eur Heart J*. 2008 Aug;29(15):1903-10.
- Möhlenkamp S, Leineweber K, Lehmann N, Braun S, Roggenbuck U, Perrey M, Broecker-Preuss M, Budde T, Halle M, Mann K, Jöckel KH, Erbel R, Heusch G. Coronary atherosclerosis burden, but not transient troponin elevation, predicts long-term outcome in recreational marathon runners. *Basic Res Cardiol* 2014; 109 (1): 391
- Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 1986; 74: 1124–1136.
- Neilan TG, Januzzi JL, Lee-Lewandrowski E, Ton-Nu T-T, Yoerger DM, Jassal DS, Lewandrowski KB, Siegel AJ, Marshall JE, Douglas PS, et al. Myocardial Injury and Ventricular Dysfunction Related to Training Levels Among Nonelite Participants in the Boston Marathon. *Circulation* 2006; 114: 2325–2333
- Oxborough D, Birch K, Shave R, George K. “Exercise-Induced Cardiac Fatigue”-A Review of the Echocardiographic Literature: Exercise-Induced Cardiac Fatigue. *Echocardiography* 2010; 27: 1130–1140.
- Oxborough D, Sharma S, Shave R, Whyte G, Birch K, Artis N, Batterham AM, George K. The Right Ventricle of the Endurance Athlete: The Relationship between Morphology and Deformation. *J. Am. Soc. Echocardiogr* 2012; 25: 263–271.
- Oxborough D, Shave R, Warburton D, Williams K, Oxborough A, Charlesworth S, Foulds H, Hoffman MD, Birch K, George K. Dilatation and Dysfunction of the Right Ventricle Immediately After Ultraendurance Exercise: Exploratory Insights From Conventional Two-Dimensional and Speckle Tracking Echocardiography. *Circ. Cardiovasc. Imaging* 2011; 4: 253–263.
- Palatini P, Bongiovi S, Marcor F, Michieletto M, Mario L, Schiradi C, Pessina AC. Left ventricular performance during prolonged exercise and early recovery in healthy subjects. *Eur J Appl Physiol* 1994; 69: 396-401
- Santos-Gallego CG, Vahl TP, Goliasch G, Picatoste B, Arias T, Ishikawa K, Njerve IU, Sanz J, Narula J, Sengupta PP, et al. Sphingosine-1-Phosphate Receptor Agonist Fingolimod Increases Myocardial Salvage and Decreases Adverse Postinfarction Left Ventricular Remodeling in a Porcine Model of Ischemia/Reperfusion. *Circulation* 2016; 133: 954-966
- Scharhag J, Herrmann M, Urhausen A, Haschke M, Herrmann W, Kindermann W. Independent elevations of N-terminal pro-brain natriuretic peptide and cardiac troponins in endurance athletes after prolonged strenuous exercise. *Am. Heart J* 2005; 150: 1128–1134.
- Sharma V, Marsh R, Cunniffe B, Cardinale M, Yellon DM, Davidson SM. From Protecting the Heart to Improving Athletic Performance – the Benefits of Local and Remote Ischaemic Preconditioning. *Cardiovasc. Drugs Ther* 2015; DOI:10.1007/s10557-015-6621-6
- Shave R, Baggish A, George K, Wood M, Scharhag J, Whyte G, Gaze D, Thompson PD. Exercise-Induced Cardiac Troponin Elevation. *J. Am. Coll. Cardiol* 2010; 56: 169–176.
- Wolfrum S, Schneider K, Heidbreder M, Nienstedt J, Dominiak P, Dendorfer A. Remote preconditioning protects the heart by activating myocardial PKC ϵ -isoform. *Cardiovasc. Res* 2002; 55: 583–589.

Additional information:

Competing interests:

No conflicts of interest were declared by the authors.

Funding:

No funding was received for this project.

Figures Legend:

Figure 1: hs-cTnT (A) and NT-proBNP (B) release at pre, POST-0H, POST-1H, & POST-3H exercise time points during both IPC (open squares) and SHAM (solid circles) conditions in healthy volunteers (n = 14). Error bars represent SD.

Table 1: Cardiac loading and rate changes, POST-0H, POST-1H, & POST-3H exercise time points during both RIPC and SHAM conditions in healthy volunteers (n = 14)

	Intervention				<i>P</i> values	
	Pre	POST-0H	POST-1H	POST-3H		
HR (beats.min ⁻¹)						
RIPC	55 ± 9	72 ± 12	64 ± 13.3	65 ± 10	Condition	0.40
SHAM	56 ± 8	70 ± 14	70 ± 13	63 ± 13	time	< 0.001
					Con*time	0.60
LVEDV (ml)						
RIPC	126 ± 22	123 ± 21	127 ± 24	129 ± 24	Condition	0.42
SHAM	130 ± 25	124 ± 21	124 ± 27	124 ± 26	time	0.30
					Con*time	0.37
SBP (mmHg)						
RIPC	119 ± 9	122 ± 18	118 ± 9	119 ± 7	Condition	0.24
SHAM	122 ± 12	119 ± 11	119 ± 11	125 ± 10	time	0.74
					Con*time	0.59
DBP (mmHg)						
RIPC	63 ± 6	64 ± 13	61 ± 5	62 ± 6	Condition	0.28
SHAM	66 ± 11	64 ± 10	63 ± 8	65 ± 9	time	0.76
					Con*time	0.92

* Significantly different between IPC and SHAM conditions

Abbreviations: HR, Heart Rate; LVEDV, Left Ventricular End Diastolic Volume; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure

Table 2: Measures of left ventricular functional response at pre, POST-0H, POST-1H, & POST-3H exercise time points during both RIPC and SHAM conditions in healthy volunteers (n = 14).

	Intervention				<i>P</i> values	
	Pre	POST-0H	POST-1H	POST-3H		
LV EF (%)						
RIPC	53 ± 7	53 ± 6	54 ± 5	56 ± 4	Condition	0.26
SHAM	55 ± 5	54 ± 6	55 ± 6	54 ± 6	time	0.50
					Con*time	0.44
LV S' (m.s ⁻¹)						
RIPC	0.11 ± 0.02	0.11 ± 0.02	0.12 ± 0.03	0.12 ± 0.02	Condition	0.93
SHAM	0.11 ± 0.02	0.12 ± 0.03	0.11 ± 0.03	0.12 ± 0.02	time	< 0.001
					Con*time	0.62
LV SSR (.s ⁻¹)						
RIPC	(-)1.1 ± 0.2	(-)1.1 ± 0.1	(-)1.3 ± 0.2	(-)1.2 ± 0.1	Condition	0.24
SHAM	(-)1.2 ± 0.2	(-)1.2 ± 0.2	(-)1.3 ± 0.2	(-)1.5 ± 0.3	time	0.001
					Con*time	0.90
LV E (m.s ⁻¹)						
RIPC	0.86 ± 0.14	0.75 ± 0.16	0.77 ± 0.16	0.84 ± 0.18	Condition	0.83
SHAM	0.81 ± 0.15	0.73 ± 0.14	0.81 ± 0.13	0.86 ± 0.17	time	0.035
					Con*time	0.71
LV A (m.s ⁻¹)						
RIPC	0.44 ± 0.12	0.49 ± 0.14	0.45 ± 0.12	0.50 ± 0.12	Condition	0.75
SHAM	0.46 ± 0.11	0.47 ± 0.15	0.50 ± 0.1	0.49 ± 0.13	time	0.58
					Con*time	0.80
LV E/A						
RIPC	2.04 ± 0.46	1.59 ± 0.38	1.78 ± 0.57	1.76 ± 0.56	Condition	0.60
SHAM	1.82 ± 0.4	1.64 ± 0.44	1.7 ± 0.53	1.82 ± 0.46	time	0.06
					Con*time	0.60
LV E/e'						
RIPC	4.69 ± 1.05	4.25 ± 1.16	4.26 ± 1.24	4.30 ± 1.12	Condition	0.35
SHAM	4.34 ± 1.11	4.05 ± 1.14	4.25 ± 0.92	4.39 ± 1.07	time	0.12
					Con*time	0.67
LV E' (m.s ⁻¹)						
RIPC	0.19 ± 0.04	0.18 ± 0.03	0.19 ± 0.02	0.20 ± 0.03	Condition	0.69
SHAM	0.19 ± 0.04	0.19 ± 0.03	0.19 ± 0.04	0.20 ± 0.04	time	0.018
					Con*time	0.90
LV A' (m.s ⁻¹)						
RIPC	0.07 ± 0.02	0.08 ± 0.02	0.09 ± 0.02	0.08 ± 0.02	Condition	0.14
SHAM	0.07 ± 0.01	0.07 ± 0.02	0.09 ± 0.02	0.08 ± 0.02	time	< 0.001
					Con*time	0.48
LV ESR (.s ⁻¹)						
RIPC	1.9 ± 0.3	1.9 ± 0.5	2.0 ± 0.4	1.9 ± 0.3	Condition	0.27
SHAM	2.0 ± 0.4	1.8 ± 0.4	2.1 ± 0.5	2.3 ± 0.8	time	0.16
					Con*time	0.20

* Significantly different between IPC and SHAM conditions

Abbreviations: LV, Left Ventricle; RV, Right Ventricle; EF, Ejection Fraction; S', Systolic myocardial tissue velocity; SSR, Systolic strain rate; E, Early diastolic filling; A, Late diastolic filling; E/A, Early to late diastolic ratio; E', Early diastolic myocardial velocity; A', Late diastolic myocardial velocity; ESR, Early diastolic strain rate

Table 3: Measures of right ventricular response at pre, POST-0H, POST-1H, & POST-3H exercise time points during both RIPC and SHAM conditions in healthy volunteers (n = 14).

	Intervention				<i>P</i> values	
	Pre	POST-0H	POST-1H	POST-3H		
RVFAC (%)						
RIPC	43.2 ± 6.4	43.6 ± 5.2	45.5 ± 8.2	44.4 ± 4.7	Condition	0.71
SHAM	42.6 ± 7.6	45 ± 7.6	44.1 ± 7.3	43.6 ± 6.9	time	0.49
					Con*time	0.77
RV S' (m.s ⁻¹)						
RIPC	0.14 ± 0.02	0.13 ± 0.02	0.15 ± 0.02	0.16 ± 0.02	Condition	0.10
SHAM	0.13 ± 0.02	0.13 ± 0.02	0.15 ± 0.02	0.15 ± 0.03	time	< 0.001
					Con*time	0.79
RV SSR (.s ⁻¹)						
RIPC	(-)1.5 ± 0.2	(-)1.4 ± 0.3	(-)1.5 ± 0.4	(-)1.2 ± 0.2	Condition	0.27
SHAM	(-)1.4 ± 0.2	(-)1.6 ± 0.3	(-)1.5 ± 0.3	(-)1.6 ± 0.2	time	0.59
					Con*time	0.68
RV E' (m.s ⁻¹)						
RIPC	0.16 ± 0.02	0.16 ± 0.04	0.16 ± 0.02	0.16 ± 0.03	Condition	0.94
SHAM	0.16 ± 0.03	0.16 ± 0.03	0.17 ± 0.02	0.16 ± 0.04	time	0.73
					Con*time	0.93
RV A' (m.s ⁻¹)						
RIPC	0.11 ± 0.03	0.09 ± 0.03	0.12 ± 0.03	0.13 ± 0.03	Condition	0.18
SHAM	0.10 ± 0.04	0.09 ± 0.04	0.12 ± 0.03	0.11 ± 0.03	time	< 0.001
					Con*time	0.53
RV ESR (.s ⁻¹)						
RIPC	2.3 ± 0.3	2.3 ± 0.7	2.1 ± 0.5	2.0 ± 0.4	Condition	0.06
SHAM	2.0 ± 0.4	2.1 ± 0.3	1.9 ± 0.4	2.2 ± 0.4	time	0.11
					Con*time	0.97

* Significantly different between IPC and SHAM conditions

Abbreviations: RV, Right Ventricle; RVFAC, Right ventricular fractional area change; S', Systolic myocardial tissue velocity; SSR, Systolic strain rate; E, Early diastolic filling; A, Late diastolic filling; E/A, Early to late diastolic ratio; E', Early diastolic myocardial velocity; A', Late diastolic myocardial velocity; ESR, Early diastolic strain rate