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Remote ischemic preconditioning modifies serum apolipoprotein D, met-enkephalin, adenosine, and nitric oxide in healthy young adults

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Key Words:

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Abstract

Remote ischemic preconditioning (RIPC) has been employed as a non-invasive protective intervention against myocardial ischemia-reperfusion injury in animal studies. However, the underlying mechanisms are incompletely defined in humans and its clinical efficacy has been inconclusive. As advanced age, disease, and drugs may confound RIPC mechanisms in patients, our aim was to measure whether RIPC evoked release of adenosine, bradykinin, met-enkephalin, nitric oxide, and apolipoproteins in healthy young adults. Healthy subjects (n=18, 9 males, 23±1.5 years old; 9 females, 23±1.8 years old) participated after informed consent. RIPC was applied using a blood pressure cuff to the dominant arms for 4 cycles of 5-minute cuff inflation (ischemia) and 5-minute cuff deflation (reperfusion). Blood was sampled at Baseline and immediately after the final cuff deflation (Post-RIPC). Baseline and Post-RIPC plasma levels of adenosine, bradykinin, met-enkephalin, apolipoprotein A-1 (ApoA-1), apolipoprotein D (ApoD), and nitric oxide (as nitrite) were measured via ELISA and high-performance liquid chromatography. Mean (±SD) baseline levels of adenosine, bradykinin, met-enkephalin, ApoA-1, ApoD, and nitrite in healthy young adults were 13.8±6.5ng/mL, 2.6±1.9µg/mL, 594.1±197.4pg/mL, 3.0±0.7mg/mL, 22.2±4.0 µg/mL, and 49.8±13.4nmol/L, respectively. Post-RIPC adenosine and nitrite levels increased (59.5±37.9%, p <0.0001; 32.2±19.5%, p <0.0001), whereas met-enkephalin and ApoD levels marginally decreased (5.3 ±14.0%, p =0.04; 10.8±20.5%, p =0.04). Post-RIPC levels were not influenced by sex, age, blood pressure, waist circumference, or BMI. RIPC produces increased levels of adenosine and nitrites, and decreased met-enkephalin and ApoD in the plasma of young healthy adults.

Introduction

Remote ischemic preconditioning (RIPC), by invoking one or more brief and benign cycles of tourniquet-induced ischaemia and reperfusion at a limb, offers a non-invasive, infarct-sparing protective strategy that preserves cardiac function prior to the onset of more extended myocardial ischemia-reperfusion (IR) injury (1, 2). RIPC involves complex interactions with neural, humoral and immune systems, including the release of neurohumoral and other protective factors from the preconditioned site into the blood circulation which activate G-protein coupled receptor (GPCR)-mediated intracellular signalling pathways

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in numerous cell types, producing prosurvival alterations in mitochondrial cellular metabolism, ion homeostasis, and gene transcription (2-6). A large number of classical neurohumoral protective factors, released early after RIPC protocol, including bradykinin, adenosine, and opioid peptides have been shown to activate these prosurvival signalling pathways in animal models of RIPC (3, 7, 8).

The first RIPC clinical trial, performed by Cheung *et al* (9) demonstrated that RIPC significantly reduced troponin I levels, a marker of myocardial injury, and increased release of cardioprotective markers, including TNF α in 37 children with various congenital heart disease. Subsequently Thielmann *et al*, demonstrated that RIPC evoked perioperative cardioprotection and improved outcome following coronary artery bypass graft surgery in 327 adults (10). However, there have been other studies with mixed outcome (11,12), or with no therapeutic benefits of RIPC, including large clinical trials such as the ERICCA (13) and RIPHeart (14) trials with 1,612 and 1,403 respective adult patients with valvular disease or myocardial infarction.

Clinical trials of RIPC have naturally focused on determining whether there are clinical outcomes of therapeutic benefit. As concurrent measurement of the many endogenous factors simultaneously released into the plasma by RIPC have not been widely examined in these trials, it is unclear to what extent the initial baseline presence, release and abundance of these upstream factors may be attenuated, and/or RIPC signalling impeded by disease, senescence, gender, organ immaturity, and drug treatments (i.e., ACE inhibitors, carvedilol, anaesthetics, analgesic agents) (15, 16, 17). These conditions may confound clinical trials by influencing variation in baseline levels of some of these endogenous factors prior to RIPC, potentially limiting the extent that these factors may be further augmented by RIPC. Therefore, as a first step to characterise RIPC in healthy young adults (9 male, 9 female) free of confounding related to age and clinical conditions, the aim of our study was to apply RIPC (4 x 5 minute cycles) in order to measure baseline and post RIPC blood levels of endogenous protective preconditioning factors, including adenosine, met-enkephalin, bradykinin, apolipoprotein A-1 (ApoA-1), apolipoprotein D (ApoD), and nitric oxide (NO).

Results

Table 1 presents the baseline blood pressure and anthropometric measurements of the healthy young adult male and female subjects recruited to undertake the RIPC protocol of 5 minutes of ischaemia followed by 5 minutes of reperfusion applied by blood pressure cuff occlusion and release to their upper arm (4 cycles, see Methods). Baseline and post-RIPC protocol levels of ApoA-1, ApoD, adenosine, met-enkephalin, bradykinin, and nitrite are presented in Table 2. In male study participants, adenosine and nitrite levels were significantly greater than in females at Baseline and Post-RIPC ($p < 0.001$ and $p <$

0.0001). In contrast, Baseline and Post-RIPC ApoA-1 levels were significantly greater in female participants ($p = 0.04$) compared to males. However, there was no influence of sex, age, blood pressure, waist circumference, and BMI on the difference in plasma levels of any metabolite evoked by RIPC. Thus combining male and female results, compared to Baseline, at Post-RIPC, ApoD and met-enkephalin levels significantly reduced by 10.8% and 5.3%, respectively ($p = 0.04$ for each), whereas adenosine and nitrite levels significantly increased by 59.5% and 32.2%, respectively ($p < 0.0001$ for each, Figure 1). There were no significant differences in ApoA-1 and bradykinin levels between Baseline and Post-RIPC.

Discussion

In order to determine the optimal innate response to RIPC in healthy young adults, the aim of the present study was to measure Baseline and Post-RIPC early release levels of ApoA-1, ApoD, adenosine, met-enkephalin, bradykinin, and nitrite in 18 healthy subjects aged 20-25 years old. To date few studies have reported plasma levels of these agents obtained simultaneously, either amongst healthy humans or in settings of clinical pathology. The risk of rapid degradation and clearance of these factors was restricted by collecting blood into a cocktail of enzyme inhibitors, an approach not always fully considered by previous studies of RIPC plasma. Notably, measured metabolites were evident at high levels in our subjects at baseline before any intervention. An important limitation of our study is that as this was an exploratory study, no apriori power calculation was performed due to the multiple concurrent measures of analytes with differing dynamic range and sample size determined by access to study resources.

In response to RIPC shear stress, endothelial nitric oxide synthase produces NO which is rapidly oxidised to nitrite and circulated as a source of NO (17). We therefore measured plasma nitrite, which increased after RIPC relative to baseline levels in males and females. Rassaf, et al. (18), also reported increases in plasma nitrite after RIPC in healthy adult humans ($n=6$, male only, age not detailed). However Nair, et al. (19), reported no change in plasma nitrite after RIPC in 6 healthy males (29.5 ± 7.6 years old). Compared to our study or Rassaf's (18), baseline nitrite was higher (94.1 ± 2.4 nmol/L), which slightly increased after the first cycle and then declined by the fourth cycle. Nair et al.(19), proposed that under some conditions decreases in nitrite were potentially related to episodes of ischemia-reperfusion producing an increase in reactive oxygen species that rapidly scavenge NO to form various nitrogen oxides (and thus less nitrite). As NO synthesis is variable according to vascular health status, age and other factors, plus it is rapidly converted by multiple processes, more detailed studies with highly sensitive NO metabolite detection are required.

RIPC downregulates expressions of bradykinin B₁- and B₂-receptors in neutrophils of healthy humans and cardiac surgery patients (20, 21). Although the decreased kinin receptor expression in neutrophils

may indicate receptor activation and thus internalisation, no change in serum bradykinin levels could be detected immediately following RIPC (21). Similarly, bradykinin levels of our healthy young adult cohort remained unchanged by RIPC, however bradykinin levels were four times greater than the levels reported for older cardiac surgery patients (21).

Previous studies of cardiac opioid peptides have indicated that proenkephalin is high during early development, decreases after maturation and then progressively increases with advanced age (22). Proteolytic cleavage of cardiac proenkephalin to met-enkephalin occurs continuously during normoxia, but is further augmented by IPC and chronic cyanosis (23-25). Two randomised double-blind trials of RIPC in infants with hypoxic congenital heart disease undergoing heart surgery found no significant benefit of RIPC (26, 27), potentially due to intrinsic hypoxic signalling being already adapted before RIPC (27). In our present study, picogram amounts of met-enkephalin were already in circulation at baseline, but after RIPC were decreased. We hypothesise that in healthy humans, decreases in met-enkephalin following RIPC may represent uptake and degradation following activation of *in vivo* cellular signalling. In the clinical setting, the early RIPC signalling response interaction with anaesthesia is unclear due to mixed results following the simultaneous use of propofol with RIPC (15-17, 28), likely due to drug interactions with endogenously activated GPCR-signalling pathways (29).

As an important regulatory metabolite of adenosine triphosphate (30), adenosine, via the interaction of adenosine A₁ receptors and δ -opioid receptors, triggers signalling protection against myocardial IR injury (31, 32). A distinct rise in adenosine was evident for males and females after RIPC. Although adenosine levels have not been previously reported for healthy adult humans undergoing RIPC protocol, baseline venous adenosine in healthy volunteers (n=10 male and female adults, combined group with unstated n or age) has been recently reported to be 13 ± 7 nmol/L (33), which is comparable to an adenosine level presenting between the female and male baseline values in our current study.

Baseline ApoA-1 levels measured in our present study reflect previous findings reported by Pang *et al.*, of mg amounts of ApoA-1 in healthy volunteers (34). Our present study showed no change in ApoA-1 levels after RIPC, whereas Pang *et al.* found ApoA-1 to be significantly decreased after RIPC at 3-8 hours, but not at one hour or 24-48 hours. Gedik *et al.* (5), reported that elderly patients treated with RIPC before undergoing coronary artery bypass graft surgery had no change in ApoA-1 or 24 other humoral factors including inflammatory cytokines. In contrast, Hepponstall *et al.* (35) found in healthy (older) adult males that serum ApoA-1 increased at 15 minutes but decreased at 24 hours. In a recent proteomic analysis of serum from 6 healthy volunteers subjected to RIPC protocol, 394 plasma proteins were quantified, but only 4 of these proteins were detected to change in response to RIPC, though the changes were small (<10%) and below the threshold of technical confidence due to sample handling and assay

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methodological limitations that prevent quantitative evaluation of protein isoforms, posttranslational modifications and degraded proteins (36).

Our study recruited a very narrow age range of young adult males and females. Females undertook RIPC at a similar time in their oestrus cycle. Whilst the differences in Baseline and Post-RIPC levels were not significantly influenced by gender, we noted that compared to males, females had an overall greater level of Baseline and Post-RIPC ApoA-1 and lower adenosine and nitrite levels. This may, in part, reflect the natural differences in anthropometric measurements between male and female (i.e. muscle/lipid mass, body size). Although it has been reported that age and gender may impact the *release* of factors triggered by RIPC, and the targets' response capacity for cardioprotection may also be affected by age and sex *per se* (8, 37), detailed examination in mechanistic studies of healthy humans has been limited. Examination of the intracellular signalling molecules involved downstream of the GPCR complexes or the end effectors cardioprotective protein targets of RIPC was beyond the scope of the current study, however this will be important in future studies of age- and gender-dependent signalling potency and response efficacy.

Conclusions

The aim of this study has been to identify RIPC-induced early release of factors in healthy adult human that have previously been reported to be cardioprotective in animal models of RIPC. These factors are short lived in plasma due to short half-life and rapid enzymatic degradation or conversion and thus have had limited study during RIPC protocols, particularly in healthy humans. Baseline levels of all measured metabolites are robustly expressed in the healthy young male and female adults in our study. RIPC further increases plasma levels of adenosine and nitrites, and decreases met-enkephalin and ApoD.

Methods

This study was approved by The Royal Children's Hospital Human Research Ethics Committee. All subjects (n = 18) received RIPC to assess its effect on serum ApoA-1, ApoD, adenosine, bradykinin, met-enkephalin, and NO (as total nitrite) in healthy 20-25 year old adults. Each subject's respective baseline levels served as their own control.

Subject recruitment

Healthy 20 to 25-year-old males (n=9) and females (n=9) were recruited by signed informed consent. All participants had no history of smoking and no pre-existing or past medical conditions, such as vascular disorders, diabetes mellitus, or chronic drug treatments. Females were assessed at a similar time in their oestrus cycle. The participants fasted for a minimum of 8 hours and abstained from alcohol and high caloric meals for a minimum of 48 hours prior to the study. Body-mass-index (BMI), waist circumference, and baseline blood pressure were measured 30 minutes prior to commencement of the study.

Study protocol

RIPC was applied to the upper arm using an adult blood pressure cuff. Each participant received 4 cycles of 5-minute arm ischemia followed by 5-minute arm reperfusion. This was achieved through 5-minute blood pressure cuff inflation to 30mmHg exceeding the participant's systolic blood pressure to mimic ischemia, and 5-minute cuff deflation to mimic reperfusion. Blood flow interruption and restoration were established using manual detection of the radial artery pulse on the RIPC arm. From each participant, 45ml of venous blood was collected from the antecubital fossa of the RIPC arm prior to the application of RIPC (Baseline) and a further 45ml immediately after the final cuff deflation, at commencement of reperfusion (Post-RIPC). Blood was collected via a 20-gauge butterfly needle into prepared ethylenediamine tetraacetic acid (EDTA) tubes (Sarstedt, Germany). All RIPC procedures and sampling were performed from 7AM to normalise circadian effects.

Measurements of plasma metabolites levels

A cocktail of inhibitors was added to each EDTA tube prior to sample collection to prevent degradation of plasma metabolites. Inhibitors included (all Sigma-Aldrich): 1 μ M dilazep-dihydrochloride to inhibit adenosine uptake into and release from red blood cells; 10 μ M erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA) to block adenosine deaminase activity; 2 μ g/ml indomethacin to inhibit nucleotide release from platelets; 40 μ M EDTA and 40 μ M O,O'-bis(2-aminoethyl)ethyleneglycol-N,N,N'-N'-tetraacetic acid (G-EDTA) to inhibit platelet aggregation and release of adenosine from platelets, and 2 μ M dipyridamole to inhibit adenosine transport. A cocktail of amastatin, apstatin, bestatin, captopril, thiorphan, phosphoramidon, (0.1 μ mol/ml each) was used to preserve enkephalins and bradykinin by preventing proteolytic activity of angiotensin-converting enzyme, aminopeptidases, neutral endopeptidases, and carboxypeptidases. Whole blood samples were held on ice, then centrifuged for plasma separation (2,000 x g, 10 minutes, 4°C), and plasma was frozen and stored at -80°C until assayed.

Quantitative detection of the levels of bradykinin (Enzo Life Sciences), met-enkephalin (LifeSpan BioSciences Inc), ApoA-1 (Abcam), and ApoD (LifeSpan BioSciences Inc) in the collected plasma at

Baseline and Post-RIPC were measured using commercially available enzyme-linked immunoassay (ELISA) kits as described. Protein quantification was conducted following the manufacturer's specifications and each sample was tested in duplicate. A 4-parameter-logistic (4PL) curve was used to determine the concentration of proteins on GraphPad Software.

Adenosine and nitrite levels were measured using high performance liquid chromatography (HPLC). Adenosine was separated from neutralised potassium perchlorate extracts of each plasma sample using HPLC with a Waters Alliance e2695 with a C₁₈ column (3 µm particle size), mobile phase elution (isocratic) in 10 mM sodium phosphate, 5 mM tetrabutyl ammonium phosphate, 2% acetonitrile in water, and sample absorbance was measured at 254nm (Waters 2998 photodiode array) and levels were determined against known adenosine standards. Total nitrite levels were measured using high sensitivity ozone chemiluminescence using a 208i NOA (Sievers Instruments, GE Analytical Instruments), against a calibration curve of sodium nitrite standards. Nitrite free HPLC water was used in the preparation of stock sodium nitrite (100nM) and serial dilution for each standard. Injected samples (50 µL) were assayed in duplicate.

Statistical analysis

Statistical analysis was performed on GraphPad Prism 7 (GraphPad Software, Inc.). Data for all Baseline and Post-RIPC measurements were normally distributed (Kolmogorov–Smirnov test) and presented as mean ± SD. The influence of gender, age, and baseline blood pressures were measured using 2-way ANOVAs with Bonferroni corrected post-hoc tests. Comparisons of mean Baseline and Post-RIPC values were performed using paired *t*-tests. A *p*-value of <0.05 was considered statistically significant.

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References

1. Heusch G. 25 years of remote ischemic conditioning: from laboratory curiosity to clinical outcome. *Basic Res Cardiol*. 2018;113(3):15.
2. Hausenloy DJ, Yellon DM. Ischaemic conditioning and reperfusion injury. *Nat Rev Cardiol*. 2016;13(4):193-209.
3. Basalay MV, Davidson SM, Gourine AV, Yellon DM. Neural mechanisms in remote ischaemic conditioning in the heart and brain: mechanistic and translational aspects. *Basic Res Cardiol*. 2018;113(4):25.
4. Lieder HR, Kleinbongard P, Skyschally A, Hagelschuer H, Chilian WM, Heusch G. Vago-Splenic Axis in Signal Transduction of Remote Ischemic Preconditioning in Pigs and Rats. *Circ Res*. 2018;123(10):1152-63.
5. Gedik N, Kottenberg E, Thielmann M, Frey UH, Jakob H, Peters J, et al. Potential humoral mediators of remote ischemic preconditioning in patients undergoing surgical coronary revascularization. *Sci Rep*. 2017;7(1):12660.
6. 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(1-2):3-13.
7. Galagudza MM, Sonin DL, Vlasov TD, Kurapeev DI, Shlyakhto EV. Remote vs. local ischaemic preconditioning in the rat heart: infarct limitation, suppression of ischaemic arrhythmia and the role of reactive oxygen species. *Int J Exp Pathol*. 2016;97(1):66-74.
8. Kleinbongard P, Skyschally A, Heusch G. Cardioprotection by remote ischemic conditioning and its signal transduction. *Pflügers Archiv - Eur J Physiol*. 2017;469(2):159–181.
9. Cheung MM, Kharbanda RK, Konstantinov IE, Shimizu M, Frndova H, Li J, et al. Randomized controlled trial of the effects of remote ischemic preconditioning on children undergoing cardiac surgery: first clinical application in humans. *J Am Coll Cardiol*. 2006;47(11):2277-82.

10. Thielmann M, Kottenberg E, Kleinbongard P, Wendt D, Gedik N, Pasa S, et al. Cardioprotective and prognostic effects of remote ischaemic preconditioning in patients undergoing coronary artery bypass surgery: a single-centre randomised, double-blind, controlled trial. *Lancet*. 2013;382(9892):597-604.
11. Man C, Gong D, Zhou Y, Fan Y. Meta-analysis of remote ischemic conditioning in patients with acute myocardial infarction. *Sci*. 2017;7:43529.
12. Le Page S, Bejan-Angoulvant T, Angoulvant D, Prunier F. Remote ischemic conditioning and cardioprotection: a systematic review and meta-analysis of randomized clinical trials. *Basic Res Cardiol*. 2015;110 (2):11.
13. Hausenloy DJ, Candilio L, Evans R, Ariti C, Jenkins DP, Kolvekar S, et al. Remote ischemic preconditioning and outcomes of cardiac surgery. *N Engl J Med*. 2015;373(15):1408-17.
14. Meybohm P, Bein B, Brosteanu O, Cremer J, Gruenewald M, Stoppe C, et al. A multicenter trial of remote ischemic preconditioning for heart surgery. *N Engl J Med*. 2015;373(15):1397-407.
15. Ferdinandy P, Hausenloy DJ, Heusch G, Baxter GF, Schulz R. Interaction of risk factors, comorbidities, and comedications with ischemia/reperfusion injury and cardioprotection by preconditioning, postconditioning, and remote conditioning. *Pharmacol Rev*. 2014;66(4):1142-74.
16. Heusch G, Rassaf T. Time to Give Up on Cardioprotection? A Critical Appraisal of Clinical Studies on Ischemic Pre-, Post-, and Remote Conditioning. *Circ Res*. 2016;119(5):676-95.
17. Cho YJ, Nam K, Kim TK, Choi SW, Kim SJ, Hausenloy DJ, Jeon Y. Sevoflurane, propofol and carvedilol block myocardial protection by limb remote ischemic preconditioning. *Int J Mol Sci*. 2019; 20(2). pii: E269. doi: 10.3390/ijms20020269.
18. Rassaf T, Totzeck M, Hendgen-Cotta UB, Shiva S, Heusch G, Kelm M. Circulating nitrite contributes to cardioprotection by remote ischemic preconditioning. *Circ Res*. 2014;114(10):1601-10.
19. Nair A, Khan S, Omar S, Pei XQ, McNeill K, Chowienczyk P, et al. Remote ischaemic preconditioning suppresses endogenous plasma nitrite during ischaemia-reperfusion: a randomized controlled crossover pilot study. *Br J Clin Pharmacol*. 2017;83(7):1416-23.

20. Saxena P, Shaw OM, Misso NL, Naran A, Shehatha J, Newman MA, et al. Remote ischemic preconditioning stimulus decreases the expression of kinin receptors in human neutrophils. *J Surg Res*. 2011;171(1):311-6.
21. Saxena P, Aggarwal S, Misso NL, Passage J, Newman MA, Thompson PJ, et al. Remote ischaemic preconditioning down-regulates kinin receptor expression in neutrophils of patients undergoing heart surgery. *Interactive Cardiovascular & Thoracic Surgery*. 2013;17(4):653-8.
22. Boluyt M, Caffrey J, Younes A, Lakatta E. Age-associated increase in rat cardiac opioid production. *The American Journal of Physiology*. 1993;265(1):H212-9.
23. Younes A, Pepe S, Barron BA, Spurgeon HA, Lakatta EG, Caffrey JL. Cardiac synthesis, processing, and coronary release of enkephalin-related peptides. *American Journal of Physiology - Heart & Circulatory Physiology*. 2000;279(4):H1989-98.
24. Younes A, Pepe S, Yoshishige D, Caffrey JL, Lakatta EG. Ischemic preconditioning increases the bioavailability of cardiac enkephalins. *American Journal of Physiology - Heart & Circulatory Physiology*. 2005;289(4):H1652-61.
25. van den Brink OW, Cochrane AD, Rosenfeldt FL, Penny DJ, Pepe S. Increased myocardial methionine-enkephalin with reduced arterial oxygenation in congenital heart disease. *J Paediatr Child Health*. 2014;50(10):E63-7.
26. Jones BO, Pepe S, Sheeran FL, Donath S, Hardy P, Shekerdemian L, et al. Remote ischemic preconditioning in cyanosed neonates undergoing cardiopulmonary bypass: a randomized controlled trial. *J Thorac Cardiovasc Surg*. 2013;146(6):1334-40.
27. Pepe S, Liaw N, Hepponstall M, Sheera F, Yong M, d'Udekem Y, et al. Effect of Remote Ischemic Preconditioning on Phosphorylated Protein Signaling in Children Undergoing Tetralogy of Fallot Repair- A Randomized Controlled Trial. *Journal of American Heart Association*. 2013;2(3).
28. Hausenloy DJ, Candilio L, Yellon DM. Remote Ischemic Preconditioning and Cardiac Surgery. *N Engl J Med*. 2016;374(5):491-2.

29. Wagner NM, Lu Y, Gross ER. Remote Ischemic Preconditioning and Cardiac Surgery. *N Engl J Med*. 2016;374(5):490.
30. Randhawa PK, Jaggi AS. Unraveling the role of adenosine in remote ischemic preconditioning-induced cardioprotection. *Life Sci*. 2016 Jun 15;155:140-6.
31. Peart JN, Gross GJ. Adenosine and opioid receptor-mediated cardioprotection in the rat: evidence for cross-talk between receptors. *American Journal of Physiology - Heart & Circulatory Physiology*. 2003;285(1):H81-9.
32. Surendra H, Diaz RJ, Harvey K, Tropak M, Callahan J, Hinek A, et al. Interaction of delta and kappa opioid receptors with adenosine A1 receptors mediates cardioprotection by remote ischemic preconditioning. *J Mol Cell Cardiol*. 2013;60:142-50.
33. Löfgren L, Pehrsson S, Hägglund G, Tjellström H, Nylander S. Accurate measurement of endogenous adenosine in human blood. *PLoS One*. 2018 Oct 25;13(10):e0205707. doi: 10.1371/journal.pone.0205707.
34. Pang T, Zhao Y, Zhang NR, Jin SQ, Pan SQ. Transient limb ischemia alters serum protein expression in healthy volunteers: complement C3 and vitronectin may be involved in organ protection induced by remote ischemic preconditioning. *Oxid Med Cell Longev*. 2013; 2013:859056.
35. Hepponstall M, Ignjatovic V, Binos S, Monagle P, Jones B, Cheung MH, et al. Remote ischemic preconditioning (RIPC) modifies plasma proteome in humans. *PLoS ONE*. 2012; 7(11): e48284.
36. Helgeland E, Breivik LE, Vaudel M, Svendsen OS, Garberg H, Nordrehaug JE, et al. Exploring the human plasma proteome for humoral mediators of remote ischemic preconditioning--a word of caution. *PLoS ONE*. 2014;9(10):e109279.
37. Heinen A, Behmenburg F, Aytulun A, Dierkes M, Zerbin L, Kaisers W, et al. The release of cardioprotective humoral factors after remote ischemic preconditioning in humans is age- and sex-dependent. *Journal of Translational Medicine*. 2018;16(1):112.

Tables

Table 1. Male and female subject baseline blood pressure and anthropometric measurements (mean±SD).

	Female	Male	<i>p</i> -value
Number	9	9	-
Age	23 ± 1.8	23 ± 1.5	0.78
Systolic blood pressure (mmHg)	97.2 ± 10.8	121.7 ± 10.8	<0.001
Diastolic blood pressure (mmHg)	60.1 ± 9.9	79.4 ± 7.2	<0.001
Waist circumference (cm)	74.7 ± 4.5	85.6 ± 5.1	<0.001
Body mass index (cm/kg ²)	21.8 ± 2.4	25.6 ± 2.7	0.01
Height (cm)	167.7 ± 10.2	178.6 ± 5.1	0.01
Weight (kg)	61.7 ± 10.2	81.5 ± 7.5	<0.001

Table 2. Plasma metabolites and proteins sampled from the arm cuffed for RIPC protocol at Baseline and Post-RIPC levels (mean ± SD, n=9; * *p* = 0.02, ** *p* = 0.001, *** *p* < 0.0001 vs female).

	Female			Male		
	Baseline	Post-RIPC	<i>p</i> -value	Baseline	Post-RIPC	<i>p</i> -value
Apolipoprotein A-I (mg/mL)	3.2 ± 0.7	3.2 ± 0.4	0.9	2.8 ± 0.6	2.7 ± 0.8	0.8
Apolipoprotein D (μg/mL)	22.8 ± 3.5	19.3 ± 5.3	0.1	21.1 ± 4.5	19.9 ± 6.2	0.6
Adenosine (ng/mL)	9.9 ± 1.2	16.0 ± 2.7	0.0001	17.6 ± 7.5 *	26.8 ± 10.4 *	0.04
Met-enkephalin (pg/mL)	635 ± 220	610 ± 180	0.8	608 ± 115	520 ± 115	0.3
Bradykinin (μg/mL)	3.7 ± 2.4	5.8 ± 7.9	0.5	9.9 ± 21	3.9 ± 4	0.4

Nitric Oxide (as nitrite, nmol/L)	39.0 ± 2.3	52.9 ± 6.5	0.0001	60.5 ± 11 **	76.1 ± 7 ***	0.003
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Figure Legend

Figure 1. The effect of RIPC on plasma metabolite levels relative to respective Baseline levels expressed as percentage difference (mean ± SD, n=18).

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