SECOND CARDIOLOGY DEPARTMENT

ATTIKON UNIVERSITY HOSPITAL

HEAD: PROFESSOR EK ILIODROMITIS

'THE EFFECTS OF REMOTE ISCHAEMIC CONDITIONING ON ARTERIAL STIFFNESS, ENDOTHELIAL GLYCOCALYX, BIOCHEMICAL FUNCTION, AND VENTRICULAR REMODELLING FOLLOWING PRIMARY PERCUTANEOUS CORONARY INTERVENTION IN PATIENTS WITH STEMI'/

«Η επίδραση της απομακρυσμένης ισχαιμικής προπόνησης στην αρτηριακή σκληρία, τον αρτηριακό γλυκοκάλυκα και τη βιοχημική λειτουργία μετά από πρωτογενή διαδερμική παρέμβαση σε ασθενείς με STEMI»

THREE-MEMBER ADVISORY COMMITTEE

IGNATIOS IKONOMIDIS JOHN LEKAKIS EFSTATHIOS ILIODROMITIS

PHD CANDIDATE

DIMITRIOS VLASTOS

LONDON

2021

PRELUDE

My PhD thesis seeks to elucidate the effects of remote ischaemic conditioning on arterial stiffness, endothelial glycocalyx integrity, and biochemical function in patients undergoing PPCI for STEMI. Expanding on these acute processes, it explores their interaction with ventricular remodelling in an attempt to identify and propose a role for remote conditioning, in the everlasting endeavour to inhibit the progression to ischaemic heart failure.

This research project has taken place in the Laboratory of Preventive Cardiology and Vascular Diseases of the Second Cardiology Department of the Attikon University Hospital, under the expert guidance and support of Professor Ignatios Ikonomidis. It is him who I thank first and foremost for his relentless efforts and invaluable mentoring. His scientific expertise and work ethics set the pillars for the conduction of this demanding and laboursome clinical trial.

I am equally grateful to Professor John Lekakis, who upon examining me in the context of my Cardiology finals, recognised my passion for the cardiovascular system and gave me a ticket to an exciting world of scientific exploration. Without his generosity and disinterest, I would have never been given this amazing opportunity.

For our joint investigation of remote conditioning properties, I am grateful beyond words to Professor Eftasthios Iliodromitis. His world-class expertise in studying these phenomena facilitated our attempts to design our study, comprehend the data obtained, and validly expand on the commonly conflicting evidence pertaining to the cascades underlying ischaemic conditioning.

Words are not enough to express my gratitude to Professor Ioanna Andreadou. By providing us with expert insight into the intriguing biochemical mechanisms of the cardiovascular system, and by overseeing the utilised experimental methods, she played an irreplaceable role

in the completion of our project. I would also like to thank all my colleagues, who contributed to any degree to these efforts, making our Laboratory a formidable centre of medical exploration.

This thesis is dedicated to Katerina, my better half, who has supported me through storms of doubt and fear. It is also dedicated to Nicholas, who I was blessed to have as my brother, and my parents Kostas and Kyriaki, with the hope to give something small in return for dedicating their lives to us.

'If I have seen further, it is by standing on the shoulders of giants.'

Isaac Newton

CONTENTS

DIMITRIOS VLASTOS BRIEF CV

PERSONAL DETAILS

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PLACE OF BIRTH: ATHENS, GREECE

SPECIALTY: CARDIOTHORACIC SURGERY

PRESENT POSITION: THORACIC SURGERY SHO, ROYAL BROMPTON HOSPITAL;

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UNIVERSITY HOSPITAL

EDUCATION AND QUALIFICATIONS

2001-2007: Second High School of Glyfada

2007-2015: National and Kapodistrian University of Athens Medical School, MD

2019: Member of the Royal College of Surgeons (MRCS) PART A-PASS (88%)

2020-2021: Royal College of Surgeons of England, PGCert in Surgery

ELECTIVE PLACEMENTS

- 2013: Western General Hospital (Edinburgh) Colorectal Surgery Department elective placement

SCIENTIFIC MEMBERSHIPS

- Hellenic Society of Cardiology aorta and peripheral arteries Working Group nucleus member
- Member of the Working Group on Aorta & Peripheral Vascular Diseases (European Society of Cardiology)
- Gold Member of the Association for Acute CardioVascular Care (European Society of Cardiology)
- Member of the Society for Cardiothoracic Surgery in Great Britain and Ireland

CAREER HISTORY

2015-2019:

- Research Fellowship in Cardiology (Attikon University Hospital, Athens, Greece)
- Foundation Training in General Surgery (18 months)

2020-2021:

- Clinical Fellowship in Cardiac and Aortic Surgery (Royal Brompton Hospital, London, UK; 6 months)
- Clinical Fellowship in ICU/ECMO (COVID-19 redeployment, Royal Brompton Hospital, London, UK; 6 months)
- Clinical Fellowship in Thoracic Surgery (Royal Brompton Hospital, London, UK; 6 months)
- National Training Number Match: Cardiothoracic-themed Core Surgical training

COURSES ATTENDED

2015: Advanced Life Support Provider (ALS-European Resuscitation Council)

2015: Advanced Life Support Generic Instructor Course (ALS GIC-European Resuscitation Council)

2016: Advanced Trauma Life Support Provider (ATLS-American College of Surgeons)

2017: Basic Surgical Skills (BSS-Royal College of Surgeons of England)

2017: Core Skills in Vascular Surgery (Royal College of Surgeons of Edinburgh)

2018: Surgical Skills in Emergency Surgery and Trauma (SSET-Royal College of Surgeons of England)

2020: Cardiac Surgery Advanced Life Support (CALS) e-course

2020: COVID-19 Critical Care: understanding and application (The University of Edinburgh and Royal College of Physicians of Edinburgh)

2020: Virtual Critical Care Rounds I Adult (Society of Critical Care Medicine)

2020: Fundamental Critical Care Support, 6th Edition (Society of Critical Care Medicine)

2020: Mechanical Ventilation for COVID-19 (Harvard Medical School)

2020: COVID-19: tackling the novel coronavirus (London School of Hygiene and Tropical Medicine and UK Public Health Rapid Support Team)

2020: Introduction to Critical Care Medicine (University of Glascow)

2020: ICU COVID-19 remote course (Brunel University London)

2020: Infection Prevention and Control (IPC) for Novel Coronavirus (COVID-19) (World Health Organization)

2020: Emerging respiratory viruses, including COVID-19: methods for detection, prevention, response and control (World Health Organization)

2020: Clinical Care Severe Acute Respiratory Infection (World Health Organization)

2020: >50 Association for Acute CardioVascular Care and e-Learning for Healthcare mini courses

2020: Essential Skills in Cardiothoracic Surgery (ST2 level; SCTS)

2020: Birmingham Review Course in Cardiothoracic Surgery (SCTS, RCSEn)

2020: My Virtual Anastomosis Coronary Anastomosis Course (Ethicon distant OSATS course)

2020: Core Skills in Laparoscopic Surgery (RCSen; awarded 100% score in laparoscopic suturing OSATS)

2021: Rigid Bronchoscopy (RBHT local course)

2021: PAR Excellence Coronary Anastomosis Course

2021: London Core Review Cardiothoracic Surgery Course (SCTS)

2021: Fundamentals in Cardiac Surgery - Coronary Artery Disease (EACTS)

2021: Introduction to Aortic Surgery (EACTS)

2021: Thoracic Surgery Series 1 (EACTS)

2021: Core Cardiac Surgical Skills course (SCTS)

2021: Introduction to Human Factors (Epsom an St Helier University Hospitals)

2021: Difficult decisions in heart valve surgery: An evidence-based approach (RSM)

COURSES INSTRUCTED

1) eALS (Advanced Life Support) 23 Sep 2020

2) eALS (Advanced Life Support) 24 Sep 2020

3) GIC (Generic Instructor Course) 22 Jun 2019

4) ALS (Advanced Life Support) 10 Mar 2018

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7) ALS (Advanced Life Support) 10 Jun 2017

8) ILS (Immediate Life Support) 04 Mar 2017

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10) ALS (Advanced Life Support) 25 Jun 2016

11) ILS (Immediate Life Support) 11 Jun 2016

12) ALS (Advanced Life Support) 09 Apr 2016

12) ALS (Advanced Life Support) 05 Mar 2016

14) ALS (Advanced Life Support) 30 Jan 2016

COURSES DIRECTED

1) ALS (Advanced Life Support) 05 Oct 2019

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3) ALS (Advanced Life Support) 01 Jun 2019

4) ALS (Advanced Life Support) 06 Apr 2019

5) ALS (Advanced Life Support) 12 May 2018

6) ILS (Immediate Life Support) 24 Feb 2018

RESEARCH EXPERIENCE

- 2008-2013: National and Kapodistrian University of Athens Medical School Department of Anatomy pregraduate academic fellow
- 2015-2021: Attikon University Hospital Second Cardiology Department research fellow

PUBLICATIONS

1) Ikonomidis I*, Vlastos D* (joint first author), Andreadou I, Gazouli M, Efentakis P, Varoudi M, Makavos G, Kapelouzou A, Lekakis J, Parissis J, Katsanos S, Tsilivarakis D, Hausenloy DJ, Alexopoulos D, Cokkinos DV, Bøtker HE, Iliodromitis EK. Vascular conditioning prevents adverse left ventricular remodelling after acute myocardial infarction: a randomised remote conditioning study. Basic Res Cardiol. 2021 Feb 6;116(1):9. doi: 10.1007/s00395-021-00851-1. PMID: 33547969.
2) Dimitrios Vlastos, Franzisco Fernandes, Ishaansinh Chauhan, Saeed Mirsadraee MD, George Asimakopoulos MD. Successful conservative treatment of a type A aortic intramural haematoma. JACC Case Reports 2:1143-1147
3) Ikonomidis I, Vlastos D, Kostelli G, Kourea K, Katogiannis K, Tsoumani M, Parissis J, Andreadou I, Alexopoulos D. Differential effects of heat-not-burn and conventional cigarettes on coronary flow, myocardial and vascular function. Sci Rep. 2021 Jun 3;11(1):11808. doi: 10.1038/s41598-021-91245-9.
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Clinical Practice. Medicina (Kaunas). 2020 Dec 18;56(12):709. doi: 10.3390/medicina56120709. PMID: 33352952

5) Dimitrios C Angouras, Maria Mademli, Dimitrios Vlastos, Dimitrios Dougenis. Successful conservative management of left ventricular pseudoaneurysm caused by a vent catheter. European Journal of Cardio-Thoracic Surgery, ezz243, https://doi.org/10.1093/ejcts/ezz243

6) Tzortzis S, Ikonomidis I, Triantafyllidi H, et al. Optimal Blood Pressure Control Improves Left Ventricular Torsional Deformation and Vascular Function in Newly Diagnosed Hypertensives: a 3-Year Follow-up Study [published online ahead of print, 2020 Jan 2]. J Cardiovasc Transl Res. 2020;10.1007/s12265-019-09951-9. doi:10.1007/s12265-019-09951-9

7) Theodore G. Papaioannou, John Thymis, Dimitrios Benas, Helen Triantafyllidi, Gavriela Kostelli, George Pavlidis, Fotini Kousathana, Kostantinos Katogiannis, Dimitrios Vlastos, Vaia Lambadiari, Evangelia Papadavid, John Parissis, Dimitrios Tousoulis, Ignatios Ikonomidis. Measurement of central augmentation index by three different methods and techniques: Agreement among Arteriograph, Complior, and Mobil-O-Graph devices. J Clin Hypertens. 2019; 21: 1386– 1392. https://doi.org/10.1111/jch.13654

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Undetermined Source. Thromb Haemost. 2019 Aug 17. https://doi.org/10.1055/s-0039-1694752.

9) Ikonomidis, I. , Aboyans, V. , Blacher, J. , Brodmann, M. , Brutsaert, D. L., Chirinos, J. A., De Carlo, M. , Delgado, V. , Lancellotti, P. , Lekakis, J. , Mohty, D. , Nihoyannopoulos, P. , Parissis, J. , Rizzoni, D. , Ruschitzka, F. , Seferovic, P. , Stabile, E. , Tousoulis, D. , Vinereanu, D. , Vlachopoulos, C. , Vlastos, D. , Xaplanteris, P. , Zimlichman, R. and Metra, M. (2019), The role of ventricular–arterial coupling in cardiac disease and heart failure: assessment, clinical implications and therapeutic interventions. A consensus document of the European Society of Cardiology Working Group on Aorta & Peripheral Vascular Diseases, European Association of Cardiovascular Imaging, and Heart Failure Association. Eur J Heart Fail, 21: 402-424. doi:10.1002/ejhf.1436

10) Ignatios Ikonomidis, George Pavlidis, Pelagia Katsimbri, Ioanna Andreadou, Helen Triantafyllidi, Maria Tsoumani, Maria Varoudi, Dimitrios Vlastos, George Makavos, Gavriella Kostelli, Dimitrios Benas, John Lekakis, John Parissis, Dimitrios Boumpas, Dimitrios Alexopoulos, Efstathios Iliodromitis. Differential effects of inhibition of interleukin 1 and 6 on myocardial, coronary and vascular function. Clin Res Cardiol. 2019 Oct;108(10):1093-1101. doi: 10.1007/s00392-019-01443-9. Epub 2019 Mar 11.

11) Dimitrios Benas, Michalis Kornelakis, Helen Triantafyllidi, Gavriela Kostelli, George Pavlidis, Maria Varoudi, Dimitrios Vlastos, Vaia Lambadiari, John Parissis & Ignatios Ikonomidis (2019) Pulse wave analysis using the Mobil-O-Graph, Arteriograph and Complior device: a comparative study, Blood Pressure, 28:2, 107-113, DOI: 10.1080/08037051.2018.1564236

12) Ignatios Ikonomidis, Spyridon Katsanos, Hellen Triantafyllidi, John Parissis, Stavros Tzortzis, George Pavlidis, Paraskevi Trivilou, Georgios Makavos, Maria Varoudi,

Alexandra Frogoudaki, Agathi-Rosa Vrettou, Dimitrios Vlastos, John Lekakis, Efstathios Iliodromitis. Pulse wave velocity to global longitudinal strain ratio in hypertension. Eur J Clin Invest. 2019; 49:e13049. https://doi.org/10.1111/eci.13049

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14) Ignatios Ikonomidis, Astrinos Voumvourakis, George Makavos, Helen Triantafyllidi, George Pavlidis, Konstantinos Katogiannis, Dimitris Benas, Dimitris Vlastos, Paraskevi Trivilou, Maria Varoudi, John Parissis, Efstathios Iliodromitis, John Lekakis. Association of impaired endothelial glycocalyx with arterial stiffness, coronary microcirculatory dysfunction, and abnormal myocardial deformation in untreated hypertensives. J Clin Hypertens. 2018; 20: 672– 679. https://doi.org/10.1111/jch.13236

15) Ignatios Ikonomidis, Dimitrios Vlastos, Kallirrhoe Kourea, Gavriela Kostelli, Maria Varoudi, George Pavlidis, Panagiotis Efentakis, Helen Triantafyllidi, John Parissis, Ioanna Andreadou, Efstathios Iliodromitis, John Lekakis. Electronic Cigarette Smoking Increases Arterial Stiffness and Oxidative Stress to a Lesser Extent Than a Single Conventional Cigarette: An Acute and Chronic Study. Circulation. 2018 Jan 16;137(3):303-306. doi: 10.1161/CIRCULATIONAHA.117.029153.

16) Ignatios Ikonomidis, Margarita Marinou, Dimitrios Vlastos, Kallirhoe Kourea, Ioanna Andreadou, Nikolaos Liarakos, Helen Triantafyllidi, George Pavlidis, Elias Tsougos, John Parissis, John Lekakis. Effects of varenicline and nicotine replacement therapy on arterial elasticity, endothelial glycocalyx and oxidative stress during a 3-month

smoking cessation program. Atherosclerosis. 2017 Jul;262:123-130. doi: 10.1016/j.atherosclerosis.2017.05.012. Epub 2017 May 13.

17) I. Ikonomidis, G. Pavlidis, V. Lambadiari, F. Kousathana, H. Triantafyllidi, M. Varoudi, D. Vlastos, S. Vlachos, G. Dimitriadis, J. Lekakis, P673. Improvement of arterial stiffness and myocardial deformation in patients with poorly controlled diabetes mellitus type 2 after optimization of antidiabetic medication, European Heart Journal - Cardiovascular Imaging, Volume 17, Issue suppl_2, December 2016, Pages ii136–ii143, https://doi.org/10.1093/ehjci/jew250.002

18) Ignatios Ikonomidis, Dimitrios Vlastos, John Parissis, Phenotyping heart failure with preserved ejection fraction by echocardiography: a light in the tunnel?, European Heart Journal - Cardiovascular Imaging, Volume 18, Issue 6, June 2017, Pages 636–638, https://doi.org/10.1093/ehjci/jex025

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ORAL PRESENTATIONS

International

1) Contemporary management of thoracic aortic aneurysms
2) Electronic cigarette smoking increases arterial stiffness and oxidative stress to a lesser extent than a single normal cigarette: an acute and chronic study

3) Remote ischemic conditioning confers arterial elasticity improvement, oxidative stress alleviation, and cardioprotective microRNAs upregulation in STEMI patients

National

1) The role of ventriculoarterial coupling in cardiovascular disease
2) Remote ischemic conditioning results in oxidative stress reduction and nitrate-nitrite-nitric oxide pathway activation in acute myocardial infarction patients
3) The effects of smoking cessation on arterial function
4) The effects of ischemic conditioning on arterial function in the context of AMI. Is there a clinical benefit?
5) The role of arterial function in remote ischemic conditioning
6) In-hospital Advanced Life Support resuscitation

ABSTRACTS

72 abstracts in international and national conferences

PRIZES/DISTINCTIONS

Awarding body	Description	Year
WILEY	Top downloaded paper 2018-2019 distinction (The role of ventricular–arterial coupling in cardiac disease and heart failure: assessment, clinical implications and therapeutic interventions. A consensus document of the European Society of Cardiology Working Group on Aorta & Peripheral Vascular Diseases, European Association of	**2020**

	Cardiovascular Imaging, and Heart Failure Association)	
Hellenic Society of Cardiology	As a distinction of my research project: 'Study of the effects of remote ischemic postconditioning on the expression of cardioprotective and vasoactive microRNAs and their correlation with vascular function and cardiac remodelling after STEMI'	**2016**
Hellenic Society of Lipidology of Atherosclerosis and Vascular Disease	As a distinction of the research project I contributed to: 'The effects of electronic cigarette use on arterial stiffness and endothelial glycocalyx of smokers'	**2015**

LETTERS OF RECOMMENDATION

1) Professor of Cardiology Ignatios Ikonomidis, National and Kapodistrian University of Athens, Second Cardiology Department, Attikon University Hospital
2) Professor of Cardiology John Lekakis, Second Cardiology Department, National and Kapodistrian University of Athens, Attikon University Hospital
3) Dr Sarah Trenfield, Consultant Cardiothoracic Anaesthetist, Clinical Lead High Dependency Unit, Clinical Director AICU, Royal Brompton Hospital
4) Miss Sofina Begum, Consultant Thoracic Surgeon, Educational and clinical supervisor; Royal Brompton Hospital, London
5) Mr George Asimakopoulos, Consultant Cardiac Surgeon, Educational and clinical supervisor; Royal Brompton Hospital, London
6) Dr Christine Weaver MBChB MRCP EDIC FFICM, AICU Consultant, Royal Brompton Hospital, London

7) Clinical Senior Lecturer in Colorectal Surgery Hugh Paterson, Western General Hospital, Edinburgh
8) Associate Professor of Descriptive Anatomy Theodore Troupis, National and Kapodistrian University of Athens

ABSTRACT

Aims

Remote ischemic conditioning (RIC) alleviates ischemia-reperfusion injury via several pathways, including micro-RNAs (miRs) expression and oxidative stress modulation. We investigated the effects of RIC on endothelial glycocalyx, arterial stiffness, LV remodelling, and the underlying mediators within the vasculature as a target for protection.

Methods & Results We block-randomised 270 patients within 48h of STEMI post-PCI to either one or two cycles of bilateral brachial cuff inflation, and a control group without RIC. We measured: a) the perfusion boundary region (PBR) of the sublingual arterial microvessels to assess glycocalyx integrity; b) the carotid-femoral pulse wave velocity (PWV); c) miR-144, -150, -21, -208, nitrate-nitrite (NOx) and malondialdehyde (MDA) plasma levels at baseline (T0) and 40 minutes after RIC onset (T3); and d) LV volumes at baseline and after one year. Compared to baseline, there was a greater PBR and PWV decrease, miR-144 and NOx levels increase ($p<0.05$) at T3 following single- than double-cycle inflation (PBR: ΔT0-T3=0.249±0.033 vs 0.126±0.034 μm, p=0.03 and PWV:0.4±0.21 vs -1.02±0.24 m/s, p=0.03). Increased miR-150, -21, -208 ($p<0.05$) and reduced MDA was observed after both protocols. Increased miR-144 was related with PWV reduction ($r=0.763$, $p<0.001$) after the first-cycle inflation in both protocols. After one year, single-cycle RIC was associated with LV end-systolic volume reduction (LVESV) >15% (odds-ratio of 3.75, p=0.029). miR-144 and PWV changes post-RIC were interrelated and associated with LVESV reduction at follow-up ($r=0.40$ and 0.37, $p<0.05$), in the single cycle RIC.

Conclusions

RIC evokes “vascular conditioning” likely by upregulation of cardio-protective microRNAs, NOx production, and oxidative stress reduction, facilitating reverse LV remodelling.

Clinical Trial Registration

http://www.clinicaltrials.gov. Unique identifier: NCT03984123

Funding

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Conflicts of interest/Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Ethics approval; consent to participate

The study was approved by the University General Hospital "Attikon" Institutional Review Board, conforms to the principles outlined in the Declaration of Helsinki, and is registered at the US National Institutes of Health (ClinicalTrials.gov: #NCT03984123). In addition, all participants gave their written informed consent.

INTRODUCTION

Expeditious primary percutaneous coronary intervention (PPCI) constitutes the cornerstone of treating ST-segment elevation myocardial infarction (STEMI) [1,2]. 'Time is muscle', since the duration of ischaemia significantly affects infarct size, and prompt management reduces STEMI morbidity and mortality [3,4]. However, the restoration of blood flow and the abrupt reoxygenation of the ischemic myocardium induces an additional component of ischaemia-reperfusion injury (IRI) which expands infarct size [5]. Importantly, IRI limits the beneficial effects of PPCI on myocardial salvage in patients with STEMI [6]. Remote ischemic conditioning (RIC), with application of brief episodes of ischemia and reperfusion in vascular beds distant to the organ at risk, activates a protective phenotype against IRI [7]. It confers reduced myocardial infarct size and improved myocardial salvage index, decreases the need for pharmacological and mechanical haemodynamic support, and induces superior recovery of left ventricular (LV) systolic function after STEMI [8], with mixed evidence regarding its impact on cardiac mortality and hospitalisation for heart failure [8, 9]. In addition, seven-day RIC improves local and systemic endothelial function and microcirculation in healthy humans [10]. However, the role of RIC on vascular function in STEMI post-PCI patients and the mechanism of its possible protective action have not been evaluated.

Endothelial glycocalyx consists of glycoproteins and proteoglycans that form a surface layer, preventing the direct contact between blood cells and vascular endothelium [11]. It is damaged after exposure to atherogenic risk factors, including hyperglycaemia, dyslipidaemia, hypertension, and smoking [12], and by IRI [13], contributing to coronary microvascular injury (resulting in oedema, vasomotion impairment, coronary microembolization, capillary destruction and haemorrhage). In turn, coronary microvascular injury adversely affects ventricular function and remodelling, and is associated with increased incidence of

cardiovascular complications and mortality [13]. Increased oxidative stress appears to play an important role, since reactive oxygen species (ROS) induce an acute but reversible impairment of glycocalyx structure [14]. In addition to the coronary circulation, endothelial function of the peripheral arteries is also impaired following AMI with the maximal disturbance observed during the first 24 to 72 hours post AMI [15,16], while its assessment within 24 hours of the index event has been shown to predict infarct extension and adverse LV remodelling [15]. Furthermore, endothelial glycocalyx impairment assessed by sublingual microscopy has been associated with microvascular angina, providing additional evidence that the properties of the peripheral arterial system may reflect the state of the coronary microvasculature [17]. In the context of RIC investigation, this method of endothelial glycocalyx integrity measurement might be preferable to assessment of endothelial function using measurement of the flow- mediated dilation (FMD) of the brachial artery. This is because it obviates the need for an additional ischemia-hyperaemia cycle provoked by the extra cuff inflation needed for the FMD study, which would confound the total ischemic burden and stimulus timing of our intervention. Moreover, endothelial glycocalyx integrity is a measure of vascular permeability, while FMD measurement mainly quantifies the capability of NO production by the endothelium. Although RIC has been demonstrated to improve peripheral [7] and coronary endothelial function [18,19] and to reduce the oxidative stress burden associated with IRI [20], its effects on glycocalyx properties have not been defined.

RIC is associated with arterial stiffness alleviation in patients with stable ischemic heart disease [21]. Increased arterial stiffness augments LV afterload and decreases diastolic coronary perfusion [22], reducing oxygen supply to demand ratio. Thus, arterial stiffening may contribute to adverse LV remodelling and thus, to poor prognosis post-AMI [23]. However, the effects of RIC on arterial elastic properties and their interaction with LV remodelling in AMI patients remain unclear.

Oxidative stress enhancement constitutes an important component of IRI [24]. Biomembrane polyunsaturated lipid peroxidation by ROS generated during abrupt reperfusion generates malondialdehyde (MDA) [24]; indeed, increased MDA levels have been reported following PCI and thrombolysis for STEMI [20]. Studies in patients with systemic inflammatory disease have linked increased MDA with impaired LV function and its reduction after treatment with a concomitant LV function improvement [25]. In this respect, RIC reduces lipid peroxidation with attendant decreased MDA levels [20], and thus may induce improvement of myocardial function post-PCI in STEMI.

Nitrate (NO3-) and nitrite (NO2-) have recently been shown to function as recycling substrates in a process of NO regeneration, which is independent of the classic L-arginine-NO-synthase (NOS) pathway [26]. Thus, the nitrate-nitrite (NOx) pool could be perceived as a reservoir of NO bioactivity that complements NOS in states of low-oxygen tension, such as during AMI and could further contribute to vascular protection and myocardial protection post-RIC [27].

In addition, IRI evokes a systemic inflammatory response with significantly increased IL-6 levels [28]. This results in enhanced neutrophil adherence to the cardiovascular endothelium, with deleterious effects [28]. Similarly, IL-6 pathway appears to mediate vascular inflammation in various disease processes [29]. RIC has been reported to decrease IL-6 levels in animal models of systemic inflammation and ischemia [30], but its effects on IL6 production post-STEMI have not been clearly defined.

MicroRNAs (MiRs) are small, single stranded, non-coding RNA molecules that regulate post-transcriptional gene expression in response to cellular or environmental stimuli [31]. MicroRNA-144 (miR-144) has been recognised as an important mediator [32] implicated in RIC signalling both in vascular and myocardial cells. Moreover, miR-150 inhibits apoptosis

and fibrosis in the setting of animal models of myocardial IRI [33]. Additionally, miR-21 has been demonstrated to mediate cardio-protection in coronary artery bypass graft (CABG) patients undergoing remote ischemic conditioning [34]. On the contrary, miR-208 exerts deleterious effects by way of hypertrophy and adverse remodelling induction [35]. However, the effects of RIC on miRs involved in cardiac and vascular function are not fully investigated in the clinical setting.

Following AMI, a non-contractile and expanding infarcted zone of scar tissue is formed. This expansion leads to an increased volume load, which in turn augments the pressure load exerted on non-infarcted regions resulting in adverse LV remodelling. Long-term LV remodelling after AMI may last for up to 2 years after the index event and is associated with cardiovascular mortality [36].

Based on the above observations, the aim of the present study was to determine the "vascular conditioning" potential of RIC by investigating its effects on endothelial glycocalyx, arterial stiffness, and oxidative stress burden after primary PCI, as well as to identify the role of specific miRs, NOx and IL-6 production on vascular function early post MI. With regards to the implemented protocols, there is evidence supporting that a total ischemic period of 5 to 10 minutes may confer the optimum ischemic conditioning stimulus [37,38], while 5 minutes of ischemic inflation induce maximal shear-mediated NO release and vasodilation in FMD studies [39]. Additionally, a single 5-minute inflation-deflation cycle has been demonstrated to confer increased nitrite levels and attendant cyto-protection, both of which progressively weaned following each additional ischemic cycle [40]. Based on the above, we utilised either a single or a double 5-minute cycle ischemic inflation to explore the potential effects of each RIC protocol on "vascular conditioning". Additionally, we intended to shed light on any possible contribution of "vascular conditioning" to long-term reverse LV remodelling.

LITERATURE REVIEW

1. Ischemia-reperfusion injury: the price of coronary reperfusion

IRI encompasses the injurious effects of restoring perfusion and oxygenation of a previously ischaemic organ [5,6,41]. Intriguingly, it further contributes to the existent ischaemic damage and leads to the expansion of the infarcted area [5]. It was first described in a canine model of reperfused myocardium: cellular swelling, myofibrillar contracture, sarcolemmal disruption, and intramitochondrial calcium phosphate precipitation were among the identified histological changes [42]. Myocardial IRI culminates in four types of dysfunction, namely myocardial stunning, no-reflow phenomenon, reperfusion arrhythmias, and lethal reperfusion injury [5]. Myocardial stunning is defined as mechanical dysfunction in spite of coronary flow restoration and the absence of irreversible ischaemic damage [43], while no-reflow phenomenon is an extreme form of microvascular dysfunction [44] that prevents the reperfusion of a previously ischaemic territory [45]. Reperfusion arrhythmias can be malignant [46], while lethal reperfusion injury denotes the reperfusion-induced cardiomyocyte death [5].

Oxidative stress serves as a key mediator of IRI [5,47,48], both directly within the realms of the oxygen paradox (whereby the cumulative injury after reoxygenation surpasses the injury caused by the ischemia per se [49]) and indirectly through the reduction of NO bioavailability (which inhibits inflammation, inactivates ROS, and improves coronary flow [50]). The calcium paradox phenomenon further aggravates IRI and is the result of sarcollemal damage and oxidative stress-derived dysfunction of the sarcoplasmic reticulum [51]. It is characterised by calcium overload that overwhelms the myocardial calcium regulation mechanisms and leads to cardiomyocyte hypercontracture, mPTP opening, and a resultant cellular death [51]. The normalisation of pH is an additional trigger of deleterious cascades

(the pH paradox [52]), while sterile inflammation is recognised as an additional component of injury [5,41]. More specifically, neutrophil migration promoted be chemotactic signals and cell-adhesion molecules results in vascular plugging, ROS generation, and release of proteases [53-56].

The deleterious effects of IRI are clinically reflected on the natural history of ischaemic heart disease following PPCI for STEMI [6]. Despite the accumulated experience and the constantly evolving techniques for coronary reperfusion, the morbidity and mortality of heart failure after AMI remain considerable [57,58]. More specifically, 1-year cardiovascular mortality post-PCI varies from 2 to 11% [9,59,60]. In this context, infarct size has been demonstrated as a powerful predictor of outcomes: infarct size at a median time of 4 days after STEMI independently predicted all-cause mortality and rehospitalisation for heart failure within 1 year [61]. Furthermore, IRI is not physiologically isolated in the index reperfused organ and may induce a systemic inflammatory reaction with the potential to progress to multi-organ dysfunction [7,62,63]. The systemic nature of IRI is also underscored by the peripheral endothelial dysfunction that accompanies ACS [64,65]. Interestingly, peripheral endothelial function assessment has prognostic properties, as forearm blood flow response was a significant independent predictor of poor prognosis, while peripheral vasoreactivity response recovery within 8 weeks of the index event predicted further event-free survival after ACS [64] (the endothelial recovery suggests that the ACS caused an additional and potentially reversible insult). Similarly, endothelium-dependent brachial artery dilation independently predicted adverse outcomes within 24.8 months [65].

2. Ischaemic conditioning: the protective properties of brief ischaemia and reperfusion episodes

Ischaemic preconditioning signifies the original cardioprotection paradigm. This phenomenon was first studied in a canine model of myocardial ischaemia, whereby four episodes of 10-minute circumflex occlusion of the circumflex artery, each followed by 20 minutes of reperfusion, resulted in less extensive adenine triphosphate (ATP) loss and necrosis compared with a 40-minute occlusion [66]. In addition, successive occlusions caused diminished lactate production [66]. This study set the stage for the subsequent demonstration of the protective ischaemic preconditioning properties: 4 episodes of 5-minute circumflex occlusion, each followed by 5 minutes of reperfusion, limited the infarction caused by 40 minutes of sustained circumflex ischaemia by 25% in comparison with dogs that had not undergone preconditioning [67]. Ischaemic preconditioning in humans was first applied in patients undergoing elective percutaneous transluminal coronary angioplasty (PTCA): in a landmark study investigating the clinical, haemodynamic, and metabolic response to sequential 90-second left-anterior descending (LAD) artery occlusions it was evident that the second occlusion caused less anginal discomfort, smaller ST-segment deviations, lower mean pulmonary artery pressure, and decreased lactate production [68].

The concept of ischaemic conditioning has been expanded temporally and anatomically. Ischaemic postconditioning entails brief cycles of ischaemia and reperfusion applied after the onset of the index reperfusion: in one of the key experimental studies, it was shown that 3 cycles of 30-second reperfusion and 30-second LAD occlusion at the beginning of reperfusion, i.e., postconditioning, was equally effective with a preconditioning protocol of a 5-minute LAD occlusion and 10 minutes of reperfusion before the index ischaemic event with regards to infarct size reduction, coronary endothelial function preservation, and lipid peroxidation reduction [69]. These findings were later clinically confirmed in patients

undergoing PCI for AMI, where 4 episodes of 1-minute inflation followed by 1-minute deflation of the angioplasty balloon after the culprit lesion stenting leaded to a significant decrease of the creatine kinase area under the curve and a significant increase of blush grade, reflecting a 36% infarct size reduction and improved myocardial reperfusion, respectively [70]. Similarly, postconditioning at PCI for STEMI reduced creatine kinase peak serum levels and reduced myocardial infarct size and oedema, as assessed by magnetic resonance imaging [71]. In the same clinical context, 4 cycles of 1-minute occlusion and 1-minute reperfusion ameliorated coronary microvascular obstruction as signified by the reduced incidence of no-reflow phenomenon [72]. Moreover, in one of the most recent postconditioning trials, 4 cycles of 30-second inflation/deflation at PCI for STEMI inhibited microvascular obstruction and conferred improved LV-remodelling 1 year after the index event as assessed by magnetic resonance imaging [73]. Postconditioning has also been shown to confer improved LV functional recovery: a protocol of 4 1-minute occlusion/reperfusion cycles was associated with a significantly greater EF by 7% and an increased peak systolic strain of the area at risk at 1-year post-PPCI for STEMI [74]. These findings are not indisputable, since among the clinical studies that have investigated the postconditioning phenomenon in patients with STEMI [75-101] evidence can be found of a neutral effect on infarct size, as well as markers of necrosis, myocardial salvage, ejection fraction, and adverse cardiac events during follow-up as presented by the POSTEMI trial [94]. The POST-AMI trial even suggested that there was a possibility of harm, due to a trend for postconditioning patients to develop infarcts of greater size (although the universal applicability of this study is obscured by the utilisation of intravenous abciximab in all patients and by the uneven distribution of diabetics between the intervention and the control arms) [85].

In addition to its temporal variations, ischaemic conditioning has also evolved anatomically [7,102]. In a landmark experimental study of a canine myocardial ischaemia model, four

episodes of 5-minute occlusion/reperfusion of the circumflex artery conferred cardiopotection to the LAD territory from a sustained 1-hour occlusion [103]. Thus, it was inferred that factors mediating conditioning may be transported to or activate protective mechanisms in remote vascular beds [103], marking the dawn of RIC. Subsequent investigations sought to explore the possibility of a circulating humoral factor instigating conditioning. Interestingly, coronary effluents from preconditioned rabbit hearts were infused in virgin recipient hearts and their effects were compared with those of control non-preconditioned effluents. Preconditioned effluent elicited cardioprotection of the virgin hearts against a 40-minute global ischaemia and 60-minute reperfusion episode as evident by the significantly smaller infarct size [104]. The discovery of inter-organ RIC marked a pivotal advance of the ischaemic conditioning concept. More specifically, in an experimental rat model, mesenteric and renal artery occlusions protected from IRI caused by coronary artery occlusion to a degree similar to that of local conditioning [105]; these findings were confirmed by similar studies, where mesenteric or renal artery occlusions elicited RIC [106-108]. Another interesting study explored the effects of limb ischaemia/reperfusion on a swine model of myocardial infarction and on human IRI-induced endothelial dysfunction: in the swine model, four 5-minute lower limb ischaemic stimuli preceded a 40-minute balloon occlusion of the LID, while in the human participants, three 5-minute upper limb ischaemic cycles were applied before a contralateral upper limb 20-minute ischaemic inflation [109]. In the former model, RIC significantly reduced the infarct size, while in the latter RIC preserved the endothelial response to acetylcholine which was otherwise impaired by IRI [109]. Similarly, three 5-minute upper limb ischaemic cycles protected the contralateral upper limb from the IRI-induced impairment of flow-mediated dilation [110]. Importantly, two separate windows of protection were uncovered, one starting immediately after RIC, and another starting within 24 hours and lasting at least up to 48 hours [110]. In a more clinically-oriented investigation,

three cycles of 5-minute forearm ischaemia/reperfusion reduced the troponin release caused by cardioplegic arrest in patients undergoing coronary artery bypass grafting (CABG) [111]. Even in the absence of cardioplegic arrest (off-pump CABG), four 5-minute upper limb ischaemic stimuli resulted in decreased troponin, MDA, and IL-6 levels [112]. In the context of STEMI, one of the most important relevant studies demonstrated that four 5-minute upper limb ischaemic cuff inflation/deflation cycles applied before PPCI conferred improved myocardial salvage index as assessed by myocardial perfusion imaging 30-days after the index reperfusion event [113]. Further expanding on this evidence, the application of three 5-minute lower limb ischaemic cuff inflation/deflation cycles before PPCI reduced infarct size and improved myocardial salvage index, decreased the need for diuretics, pharmacological and mechanical haemodynamic support during the index hospitalisation, improved EF recovery at 12-month follow-up, and reduced cardiac mortality and hospitalisations for HF over a median follow-up period of 2.1 years [8]. The CONDI trial also highlighted a decreased risk for major adverse cardiac and cerebrovascular events, as well as reduced all-cause mortality over a median follow-up period of 3.8 years, in patients undergoing four cycles of 5-minute arm ischaemic cuff inflation/deflation before PCI [114]. Nonetheless, these findings are not unequivocal, as the CONDI-2/ERIC-PPCI trial demonstrated no benefits of utilising this RIC protocol in patients undergoing PPCI for STEMI with regards to cardiac death or hospitalisation for HF at 12 months [9].

3. The role of endothelial glycocalyx in IRI and ischaemic conditioning

Endothelial glycocalyx consists of glycoproteins and proteoglycans that form a surface layer, preventing the direct contact between blood cells and vascular endothelium [11]. It is a major determinant of vascular permeability, leukocyte and platelet adhesion, and endothelial function [115,116]; experimental studies have demonstrated that glycocalyx damage is associated with increased lipoprotein influx [118], enhanced leukocyte and platelet adhesion [118,119], as well as endothelial dysfunction [120,121,122]. IRI exerts deleterious effects on endothelial glycocalyx by inducing shedding of its components [6,123-125]. This contributes to coronary microvascular dysfunction which manifests itself with a multifaceted process that includes myocardial oedema [126], impaired vasomotion [127], platelet-leukocyte aggregation [128], coronary microembolisation [129], and even capillary destruction and intramyocardial haemorrhage with an attendant no-reflow phenomenon [130,131]. Coronary microvascular dysfunction is very frequent with an incidence of up to 70% in the context of reperfusion after acute myocardial infarction (AMI) [132] and is associated with worse long-term outcomes, more extensive LV dysfunction, and enhanced adverse remodelling [133-135]. The systemic nature of IRI is further reflected on the peripheral endothelial dysfunction that accompanies acute coronary syndromes (ACS) [64,65]. Interestingly, impaired peripheral vasomotion has been associated with an increased occurrence of future adverse cardiovascular events [64,65], while restoration of systemic vasodilatory response predicts event-free survival [64]. Ischaemic conditioning alleviates coronary microvascular dysfunction [18]: coronary vasomotion is improved [136,137], myocardial oedema [69,71] and platelet-leukocyte aggregation are reduced [138], as is the risk of no-reflow [139]. Similarly, remote ischaemic conditioning improves peripheral endothelial function in patients undergoing PCI for AMI [140]. Sideview darkfield imaging (SDF) is a modality that provides high-contrast visualisation of the microvasculature in easily accessible vascular beds

[141,142]. It utilises green light illumination which allows imaging of the superficial venules and capillaries based on the principle of green light absorption by haemoglobin [141,142]. Further, the lateral displacement of red blood cells (RBC) within the microvascular lumen can be depicted and calculated as the perfused boundary region (PBR) [143,17]. Hence, PBR calculation is a validated [144-148] method of estimating the depth of RBC penetration which is inversely proportional to glycocalyx thickness. Deeper RBC penetration, i.e., increased PBR has been associated with impaired microvascular perfusion [143] and microvascular angina [17]. Thus, it serves as a marker of systemic glycocalyx-mediated microvascular dysfunction. Despite the existing evidence of the beneficial effects of ischemic conditioning on microvascular function, its direct effects on endothelial glycocalyx integrity have not been explored.

4. The role of arterial stiffness in IRI and ischaemic conditioning

Arterial distensibility is crucial for the determination of the systemic circulation impedance characteristics [22]. High proximal aortic distensibility results in low proximal aortic impedance, which is the hydraulic load imposed on the LV during systole [22]. In addition, adequate distensibility allows the aorta and proximal large arteries to function as an elastic buffer during systole and store about 50% of the stroke volume, which is forwarded during diastole due to their elastic recoil, resulting in a nearly continuous systemic blood flow [91]. This Windkessel effect reduces LV afterload, while augmenting coronary blood flow and facilitating ventricular relaxation [91]. Reversely, increased stiffness and the attendant reduced distensibility result in increased systolic and decreased diastolic blood pressure for any given mean arterial pressure level, increased LV afterload, and decreased diastolic subendocardial perfusion [149,150]. This is further compounded by the acceleration and early return of the reflected pulse waves during systole [151]. Ultimately, an imbalance ensues between increased myocardial oxygen demands and impaired coronary perfusion [150]. Carotid-femoral pulse wave velocity (PWV) is considered the gold-standard for the measurement of arterial stiffness [152]. It is a simple, non-invasive, and reproducible method, whereby the distance between two points in the aorto-femoral pathway is divided by the time of propagation of the pulse wave between them [152]. Aortic stiffness as quantified by carotid-femoral PWV independently predicts adverse cardiovascular events and mortality both in healthy individuals as well as in the presence of cardiovascular risk factors [153-163]. Importantly, it is an independent risk factor for major adverse cardiac and cerebrovascular events, refines risk stratification [23], and is associated with chronic high-sensitivity troponin elevation following STEMI [164]. Moreover, it is known that increased LV afterload, even when transient in nature, can enhance adverse remodelling following MI [165]. In agreement with this notion aortic stiffness was recently demonstrated to independently predict

myocardial healing and infarct size reduction after STEMI [166]. Intriguingly, increased aortic stiffness is also a risk factor for in-stent restenosis following PCI [167]. Chronic remote ischaemic conditioning has been demonstrated to decrease arterial stiffness and central systolic pressure in patients with chronic angina [21]. However, its acute effects in patients following PPCI have not been elucidated.

5. The role of oxidative stress in IRI and ischaemic conditioning

Enhanced oxidative stress has long been established as a key mechanism underlying IRI and exaggerating myocardial injury [5,47,48]. It is mediated by oxygen free radicals and other reactive oxygen species (ROS), which are molecules with unpaired electrons that trigger self-propagating 'chain-reactions' [47]. This contributes to the 'oxygen paradox' phenomenon, whereby the abrupt reoxygenation of the ischemic myocardial tissue results in a cumulative degree of injury which surpasses that caused by ischemia itself [49]. Under normal circumstances, ROS are inactivated by endogenous scavenger systems [168]. However, reperfusion of ischemic tissues leads to generation of increased ROS amounts that overwhelm the cellular antioxidant capacity [169-170]. Beyond a certain threshold membrane lipid peroxidation ensues, causing organelle functional impairment and ultrastructural damage [169,171-174]. Moreover, ROS induce opening of the mitochondrial permeability transition pores (mPTP) culminating in cytosolic calcium overload and cell necrosis [175-177], while mPTP opening is also coupled with mitochondrial cytochrome c release and a resultant triggering of apoptotic cascades [177]. Additional deleterious effects include DNA fragmentation [178], nitric oxide inactivation [179], enhancement of inflammatory cells migration [180,181], and activation of prothrombotic pathways [182]. Myocardial reperfusion enhances oxidative stress in both thrombolysis [183-189] and PCI treatment strategies [189-195]; mitochondria are the main source of excessive ROS production [196,197]. Conversely, several biochemical pathways mediating ischemic conditioning converge on mitochondrial function normalisation [198] and mitigate the oxidative stress response that follows reperfusion [199-200]. Malondialdehyde (MDA) constitutes a stable breakdown product of lipid peroxidation and therefore serves as a widely used marker of oxidative stress in cardiovascular disease [20,201,202]. Against this background, remote ischemic conditioning has been shown to reduce MDA levels in patients with STEMI undergoing PPCI [20].

6. The role of nitrate-nitrite-NO pathway in IRI and ischaemic conditioning

In stark contrast to the traditional conception of nitrate and nitrite anions as inert by-products of nitrogen oxide (NO) metabolism, it is now established that they can be endogenously recycled to NO [27,203-205]. While the traditional L-arginine-NO synthase (NOS) pathway constitutes the principal pathway of NO generation, it is dependent on the consumption of molecular oxygen [206,207], imposing an inherent limitation in hypoxic states. Nitrate can be systemically reduced to nitrite [208], which in turn is further reduced to NO through numerous pathways involving haemoglobin [27,209], myoglobin [210,211], xanthine oxidoreductase [212-214], ascorbate [215], polyphenols [216,217], and protons [203,204]. These cascades are accentuated under hypoxic conditions [218], offsetting the attendant deficiency of the traditional L-arginine-NOS mechanism [219,220]. Thus, the nitrate-nitrite-NO pathway may be perceived as a valuable reservoir of NO production in the setting of tissue hypoxia. Experimental data have shown the cytoprotective effects of nitrite in the context of cardiac IRI [221-223]. One of the key underlying mechanisms is the modulation of mitochondrial function and the reduction in mitochondrial-derived ROS [224-226]. In addition, mitochondrial electron transfer is induced with a resultant inhibition of mPTP opening and cytochrome C release [227] (protecting from calcium overload and the triggering of necrotic and apoptotic cascades [26,226,227]). An important aspect of nitrite metabolism is its endocrine nature which allows it to circulate to distant organs and confer remote NO-bioavailability enhancing effects [125]. Indeed, it has been demonstrated to mediate remote ischemic conditioning: experimental femoral ischemia resulted in reduction of mitochondrial respiration, ROS generation, and infarct size reduction in the myocardium [26]. Importantly, the level of nitrite generated by ischemic conditioning depends on the duration of the ischemic stimulus and in this respect, conservative protocols appear to be superior to more

prolonged ischemic stimuli [40]. This is in agreement with findings that suggest that short ischemic stimuli suffice to induce maximal NO vasodilatory responses [228].

7. IL-6 in IRI and ischaemic conditioning

Neutrophils play a key role in the inflammatory processes underlying IRI [5]. In specific, their destructive potential which stems from their oxidants and proteases might be invaluable in the context of bacterial invasion, but is misdirected against endothelial cells and cardiomyocytes during reperfusion [53]. There is substantial evidence of neutrophil activation during IRI [54] and their migration to reperfused myocardial territories coincides with cellular damage [55,230,231]. Their deleterious effects include release of ROS [56], elastase and collagenase [53,54], microvascular embolisation with resultant no-reflow [55,230,232-234], endothelial injury and dysfunction [235-237], as well as platelet activation [238,239]. Cardiomyocyte surface expression of intercellular adhesion molecule-1 (ICAM-1) appears to be a prerequisite for neutrophil-mediated injury [240,241]. To this end, interleukin-6 (IL-6) production is enhanced during acute coronary syndromes (ACS) [242-244] and induces ICAM-1 expression in the reperfused myocardium, facilitating the contribution of neutrophils to IRI [240,241]. Furthermore, IL-6 suppresses myocardial contractility [245-249] and mediates adverse remodelling after AMI [250]. In addition to its cardiac effects, vascular endothelium in general is affected by IL-6 [251]: endothelial activation [252-254], increased endothelial permeability [255,256], and endothelial dysfunction [257,258] have been recognised among the deleterious effects of IL-6 signalling. Remote ischemic conditioning (RIC) has been demonstrated to ameliorate the increase of IL-6 in animal models of IRI [259-261]. Clinical study results have been less consistent in this respect and despite evidence of RIC reducing IL-6 levels [212], data have also been obtained that suggest a neutral relationship [262-264]. Therefore, despite abundant experimental evidence of the prominent role of IL-6 in IRI and the potential of RIC to confer cardiovascular protection by inhibiting its increase, its clinical significance remains to be elucidated.

8. MicroRNAs in IRI and ischaemic conditioning

MicroRNAs (MiRs) are small, single stranded, non-coding RNA molecules that regulate post-transcriptional gene expression in response to cellular or environmental stimuli [31]. Among them, miR-144 appears to play a prominent role in regulating IRI via pleiotropic effects [32, 265-269]. More specifically, it has been demonstrated to reduce infarct size and IRI-induced apoptosis [31,265-267], mitigate the activation of inflammatory signals [268], activate vascular antioxidant mechanisms [269], and decrease DNA fragmentation and the triggering of apoptotic pathways [266]. Crucially, while IRI downregulates miR-144 expression [32,265,268], its levels are increased by ischaemic conditioning [269] and RIC [32,267]. Moreover, systemic administration of miR-144 evokes a cardioprotective response that mimics RIC [32]. Conversely, treatment with anti-miR-144 antibodies inhibits RIC [32] and, similarly, ischaemic conditioning was abrogated in animal models of miR-144/451 cluster knock-out [269]. It has been experimentally shown that miR-144 is upregulated in aortic endothelial and/or smooth muscle cells exposed to stress [28], suggesting a possible vascular source of production during RIC. MiR-150 has also been shown to confer cardio-protection from ischaemic stress: it decreased monocyte migration [270] and reduced cardiomyocyte death [33] in experimental AMI models. MiR-21 is one of the first micro-RNAs discovered and regulates numerous pathways implicated in cardiovascular pathophysiology [271,272]. It is produced in abundancy by endothelial cells [273] and is upregulated upon exposure to ROS [274]. Experimental studies have highlighted its cardioprotective properties that include mitigation of reperfusion-induced apoptosis and myocardial infarct size reduction [275-277]. Its expression is downregulated in infarcted territories, an effect that is reversed by ischaemic preconditioning [275]. In agreement with these notions, ischaemic postconditioning has been similarly found to upregulate miR-21 leading to a reduction of IRI-induced infarct size, inhibition of myocardial apoptosis, and

cardiac function improvement in animal model of reperfusion [278]. Similar results were obtained from a clinical study of RIC in patients undergoing coronary artery bypass surgery (CABG), where miR-21 levels were increased and conferred cardio-protection [34]. Mir-208 is an additional micro-RNA affecting cardiac pathophysiology [35]. Existing evidence thereof are contradictory: on the one hand, miR-21 inhibition leaded to IRI enhancement by way of increased oxidative stress, increased apoptosis, and adversely affected cardiac function [279]; on the other hand, it promotes hypertrophy and adverse remodelling [35]. In this context, despite the rapidly accumulating evidence of the significant role of microRNAs in IRI, their exact effects remain to be explored.

9. Cardiac remodelling: from myocardial infarction to heart failure

Cardiac remodelling encompasses the molecular, cellular, and interstitial changes that lead to alterations in the size, the shape, and the function of the heart in response to excessive loading or injury [280]. The process of ventricular remodelling following AMI has long been established: following AMI, a non-contractile and expanding infarcted zone of scar tissue is formed; this expansion leads to an increased volume load, which in turn augments the pressure load exerted on non-infarcted regions resulting in adverse LV remodelling [281]. Postinfarction remodelling begins within a few hours of the index event [282-284] and has been found to progress for at least two years after STEMI [285]. Eventually heart failure ensues, as LV end-systolic volume index (LVESVi) increases and ejection fraction (EF) decreases, portending a worse prognosis [286,287]. This natural history is affected by the severity of the underlying disease, secondary events (including recurrent ischaemia), neuroendocrine activation, genetic predisposition, and the applied treatment [288-291]. However, the size of the infarct caused by the primary event determines the extent of remodelling: larger infarcts evoke greater dilatation and amplified increases in systolic and diastolic stress [292]. Hence, the significance of IRI is underscored since it may account for up to 50% of the final infarct size [5]. Interestingly, peripheral endothelial dysfunction may also predict the extension of myocardial infarct [15], while increased aortic stiffness was also found to adversely affect myocardial healing post-STEMI and PWV independently predicted infarct size reduction [166]. Moreover, oxidative stress and inflammatory cascades significantly contribute to adverse remodelling after reperfusion [293]. More specifically, ROS and inflammatory cytokines induce matrix metalloproteinase (MMP) activation and decrease the levels of tissue inhibitors of MMPs (TIMPs) [294], both of which are key mediators of LV enlargement [295]. In addition, cardioprotective miRs are conceptually

expected to inhibit LV remodelling; indeed, miR-144 was experimentally found to potently reduce the extent of post-infarction myocardial remodelling [296].

METHODS

Study design and population

The present study was a prospective, randomized trial conducted at the Second Department of Cardiology in Attikon University Hospital, which entailed an acute and a chronic phase. Two hundred seventy patients with STEMI after primary PCI (mean age 53±16 years, 84% male) were recruited and underwent block randomisation (block size 9) to either one (single-cycle, n=90), or two 5-min cycles of bilateral brachial cuff inflations, separated by 5 minutes (double-cycle, n=90), or no cuff inflation (control group, n=90) added to standard care. Randomisation was assigned to a team member who was unmasked to group allocation and was performed via a website generator (Sealed Envelope, London, UK). Data collection and outcome assessment was performed only by members blinded to group allocation. We chose to apply RIC within 48 h after primary PCI (36±12h), in an effort to induce "vascular conditioning" during the period of maximum endothelial dysfunction [15]. Our first protocol utilized two ischemic stimuli by bilateral brachial cuff inflation at 200 mmHg for 5 minutes [38], separated by 5 minutes, after a baseline vascular function assessment (T0). Each ischemic stimulus was followed by a vascular function assessment (T1, T2), with a final assessment 25 minutes after the second cuff deflation (T3). The second protocol was identical to the first, except for the second ischemic stimulus omission; thus, the total ischemic stimulus was either 10 (double-inflation) or 5 minutes (single inflation). Both protocols were preceded by a sham procedure, by way of cuff placement around the ordinary brachial position without inflation. Blood samples were drawn at baseline (T0) and at protocol termination (T3). All patients were in sinus rhythm, while exclusion criteria included age>85 years, cardiogenic shock or Killip class>2 during the index event, administration of nitrates, history of previous known coronary artery or other cardiovascular disease, previous PCI or coronary artery bypass surgery (CABG), as well chronic inflammatory, systemic, or

malignant disease. All patients were scheduled for 2-dimensional echocardiography examination at 12 months after the index hospitalisation to assess the extent of LV remodelling. The study was approved by the University General Hospital "Attikon" Institutional Review Board, conforms to the principles outlined in the Declaration of Helsinki, and is registered at the US National Institutes of Health (ClinicalTrials.gov: #NCT03984123). In addition, all participants gave their written informed consent.

Endothelial glycocalyx assessment

The perfusion boundary region (PBR) is the cell-poor layer that results from the phase separation of flowing red blood cells (RBC) and plasma on the microvessel luminal surface. It includes the glycocalyx component that allows cell penetration. An increased PBR is consistent with deeper penetration of erythrocytes into the glycocalyx, reflecting impairment of glycocalyx barrier properties and reduced glycocalyx thickness [11]; hence, it represents a standardized, reproducible, operator-independent method for assessment of arterial endothelium glycocalyx properties [11]. We measured the PBR of the sublingual arterial microvasculature (diameter span from 5 to 25 μm) using Sidestream Darkfield imaging (Microscan, Glycocheck, Microvascular Health Solutions Inc., Salt Lake City, UT, USA; figure 2).

Arterial stiffness assessment

Arterial stiffness was assessed by carotid-femoral pulse wave velocity (PWV) [297] using arterial tonometry (Complior, Alam Medical, Vincennes, France; normal values <10 m/s [297]). PWV was calculated as the distance between the carotid and femoral arterial pulse palpation site, divided by the respective transit time (m/s). All measurements were performed by the same blinded examiner (intra-observer variability=5%).

Oxidative stress and inflammatory biomarkers

Malondialdehyde (MDA) was determined spectrophotometrically with a commercial kit (Oxford Biomedical Research, Rochester Hills, Mich, colorimetric assay for lipid peroxidation; measurement range 1-20 nmol/L; 3.39% and 4.75% intra-assay and inter-assay variability respectively) [298]. IL-6 was measured by a high-sensitivity immunoassay [human IL-6 Quantikinine (high sensitivity)] that detects values as low as 0.094 (intra-assay variability <5%) [244].

Plasma microRNA levels

Serum miRNAs were obtained from samples using the NucleoSpin miRNA Plasma Kit (MACHEREY-NAGEL GmbH & Co. KG, Duren, Germany**)** according to instructions of the manufacturer.

Nitrate-nitrite-nitric oxide pathway

The concentration of nitrate/nitrite in blood plasma was determined using Griess reaction with a commercially available kit (Cayman's Nitrate/Nitrite Colorimetric Assay Kit 780001) .

LV remodelling

LV remodelling was assessed by way of two-dimensional echocardiography, using a Vivid 7 or E95 (GE Medical Systems, Horten, Norway) phased array ultrasound system. All studies were digitally stored and analysed by two blinded observers, using a computerised station (Echopac 202 GE, Horten, Norway). LV end-diastolic (LVEDV) and end-systolic volumes (LVESV) were calculated from four-and two-chamber views using the modified Simpson biplane method within 48 hours post PCI before RIC and after 1 year. A cut-off of >15% decrease in LVESV was implemented as a criterium of reverse LV remodelling, as this constitutes a validated reverse remodelling marker in the context of ischemic cardiomyopathy [299, 300].

Statistical analysis

Power analysis

In a pilot study of 30 STEMI patients who underwent single-cycle, double-cycle, or no cuff inflation RIC protocol (1:1:1), the response within each subject group for ΔPWV (T0-T3) was normally distributed with a standard deviation of 1 and the calculated effect size was 0.13 with a correlation among repeated measures of 0.2. Thus, we would need 80 patients in each group to reject the null hypothesis that the population means of the single-cycle, double-cycle, and no cuff inflation groups are equal with a probability (power) of 0.8. The Type I error probability associated with the test of this null hypothesis is 0.05 (ANOVA, repeated measures, between factors, G*Power version 3.1.9.6, University of Kiel, Germany). Assuming a 10% loss of patients during follow up and 5% poor echocardiography images, we decided to include 90 patients in each group.

STATA v.11 and SPSS v.22 were used to analyse the data. The Shapiro-Wilk test was used to examine whether the data were normally distributed, whereas the Levene test was used to examine the homoscedasticity of the data. All non-parametric variables were compared using the Wilcoxon test for comparisons between baseline and post-intervention values and were transformed into ranks for multivariate analysis. In all analyses, we used two tailed tests with $p<0.05$. We used parametric (Pearson r) and non-parametric (Spearman rho) correlation coefficients to examine cross-sectional associations. Analysis of variance (ANOVA) for clinical and biological data was performed to test the differences among groups and all non-parametric variables were transformed into ranks before entering the analysis using a previously published methodology [298]. Two-way ANOVA (general linear model, SPSS 22, SPSS Inc, Chicago, Ill) for repeated measurements was applied on the examined vascular function and biochemical markers (at T0, T1, T2, and T3 for the vascular markers and T0

and T3 for biomarkers) with the parameter of time used as a within-subject factor and the applied protocol, age, sex, BMI, dyslipidaemia, diabetes, hypertension, concomitant medical treatments, MI location, and number of diseased coronary vessels (>70% stenosis) used as between-subject factors; ANCOVA (analysis of covariance) was applied to investigate the effect of the baseline values of the investigated marker and myocardial enzyme elevation. The Greenhouse-Geisser correction was used when the sphericity assumption, as assessed by Mauchly's test, was not met. Post hoc comparisons were performed with Bonferroni correction. A p-value of <0.05 was considered as statistically significant. Inter- and intra-observer variabilities (%) of vascular and biochemical markers were calculated as the SD of the differences between the first and second measurements, and expressed as a percentage of the average value in 30 healthy volunteers. Logistic regression analysis using the presence of LV remodelling at 1-year follow-up as the dependent variable and the application or not of RIC as the independent variable was performed. Similar to the acute phase measurements, ANOVA and ANCOVA were used to examine the effects of age, sex, BMI, dyslipidaemia, diabetes, hypertension, concomitant medical treatments, MI location, myocardial enzymes, number of diseased coronary vessels (>70% stenosis), and baseline values.

RESULTS

Study population characteristics

Out of the 270 patients, 126 (47%) suffered from an anterior STEMI, 119 (44%) had single-vessel, 123 (45%) two-vessel, and 28 (11%) three-vessel CAD. Median high-sensitivity (hs)-troponin T was 3886 [807-9779] ng/mL. Patients undergoing RIC had similar clinical characteristics to the control patients (p>0.05, Table1). All except three single-cycle, two double-cycle inflation, and 3 control patients had achieved TIMI 3 flow by PCI at angiographic reperfusion assessment. All were free from angina, arrhythmias, and any

significant ECG change during PCI. There were no differences in the angiographic or biochemical characteristic of STEMI, time from onset of symptoms to hospital admission, and time from admission to reperfusion (total ischemic time) between the groups assigned to single-, double-cycle inflation, or no RIC (Table 1).

Medical treatment included antiplatelet therapy (aspirin and clopidogrel) and anticoagulation (enoxaparin). Additional bolus infusions of unfractionated heparin (UFH) were given during PCI. β-blockers, ACE inhibitors, and statins were given to all patients. No difference was noted between the drug therapy given to the study groups (Table 1).

Glycocalyx barrier properties

Compared to baseline, all patients had decreased PBR at T2 and T3 ($p<0.05$, Figure 3, Table 2). By ANOVA there was a statistically significant interaction between the changes of PBR and the RIC protocol ($p=0.03$), suggesting that the magnitude of PBR changes along time was different between the 2 protocols and controls. The single-cycle inflation group achieved a greater improvement of PBR than the double-cycle inflation group at termination of the protocol (T3) ($\Delta T1=-0.259\pm0.031$ vs -0.3 ± 0.029 μm, $p=0.7$; $\Delta T2=-0.245\pm0.025$ vs -0.149 ± 0.02 μm, $p=0.04$; $\Delta T3=-0.249\pm0.033$ vs -0.126 ± 0.034 μm, $p=0.03$, for the single versus the double-cycle inflation protocol, respectively). A greater improvement of glycocalyx properties was observed after the first inflation cycle at T1 in patients with baseline PBR> 2.1 μm ($n=50$; mean difference in PBR improvement=0.5 ± 0.03 μm at T1, $p<0.001$). No changes in PBR were induced by sham inflation ($p=0.7$, data not shown). No changes in PBR were observed in the control group of no inflation ($p=0.9$, Table 2).

Biochemical markers

There was no statistically significant difference in the baseline MDA, NOx, or IL-6 levels among the studied groups ($p>0.05$). Compared to baseline, MDA was significantly reduced at

T3 ($p<0.001$) in both protocols (ΔMDA=-0.49±0.29 vs -0.48±0.21 nmol/L, $p=0.9$, for the single- versus the double-inflation protocol, respectively) (Figure 4). Additionally, the single-inflation protocol promoted an increase in NOx levels, in contrast to the double-inflation protocol, which resulted in reduced NOx levels at protocol termination (T3) (ΔNOx= 2.85±0.81 μmol/l vs -1.88±0.62 μmol/l, $p= 0.01$, for the single- versus the double-inflation protocol, respectively). IL-6 levels were not affected by any intervention ($p>0.05$). No changes in the examined biomarkers were observed following sham ($p>0.05$, data not shown) or no inflation ($p>0.05$, Table 3).

MiRs

Compared to baseline, all patients had increased Micro-RNA plasma concentration post-RIC ($p<0.05$, Table 3). There was a significantly greater increase in miR-144 concentration following the single- compared to the double-inflation protocol (Δmir144= 48.5±15.3 vs 32.3±12.1 /U6sn, $p=0.02$, for the single- versus the double-cycle protocol, respectively). The increase in miR-144 levels correlated with PWV reduction measured 5 minutes following the first cuff deflation in both protocols (T1; $r=0.763$, $p<0.001$). Both RIC protocols induced a similar increase in miR -150, -21, and -208 levels compared to baseline ($p<0.05$, Table 3) (Δmir-150=1.6±0.4 vs 1.5±0.5 /U6sn, $p=0.9$; Δmir-21=0.9±0.3 vs 0.9±0.4 /U6sn, $p=0.99$; and Δmir-208=0.5±0.2 vs 0.37±0.19 /U6sn, $p=0.6$, for the single- versus the double-cycle protocol, respectively) (Table 3, Figure 5). No changes in miRs were observed in the control group ($p=0.9$, Table 3).

Arterial stiffness

PWV was significantly affected by our intervention ($p<0.05$; table 2, figure 4). By ANOVA, there was a statistically significant interaction between PWV changes and the RIC protocol exploited ($p=0.03$). Compared to baseline, ΔT1=-0.55±0.19 vs -0.49±0.17 m/s, $p=0.7$; ΔT2=-

0.7±0.2 vs -0.69±0.21 m/s, p=0.9; ΔT3: -0.4±0.21 vs +1.02±0.24 m/s, p=0.03, for the single- versus the double-inflation protocol, respectively (Figure 6). Thus, there was a decrease of PWV at T1 and T2 in both protocols (P<0.05), but at protocol termination (T3, 25 min after the second inflation) there was as a net PWV decrease in the single- compared to an increase in the double-inflation group. Regardless of the protocol used, patients with baseline PWV >11 m/s benefited from a larger aortic elasticity improvement than patients with lower baseline PWV after the first inflation cycle (T1: mean difference in PWV improvement= 3.5±0.6 m/s, p< 0.002). No changes were observed following sham (p=0.7, data not shown) or no inflation (p=0.8, Table 3).

LV remodelling

We assessed every patient at 1-year follow-up be echocardiography and compared the LV volumes changes in the single- and double-cycle group with the respective changes in the control group. Out of 180 patients who underwent RIC, 85 of the single-cycle group and 87 of the double-cycle group were found for follow–up echocardiography and were compared with 85 patients without RIC. Single-cycle was associated with a significantly greater decrease in LVEDV and LVESV within 12 months of the index event compared with double- and no inflation (ΔLVEDV= -23±3 vs -7±2 vs -6±2 ml, p<0.001; ΔLVESV: -10±2 vs -3±1 vs -2±1, p<0.001, respectively; table 4, figure 7). By binary logistic regression, single-inflation RIC was related to reverse LV remodelling (LVESV change>15%) with an odds ratio of 3.75 (95% CI: 1.120-8.675, p=0.03), after adjusting for patient age, sex, BMI, dyslipidaemia, diabetes, hypertension, concomitant medical treatments, MI location, myocardial enzymes, number of diseased coronary vessels (>70% stenosis), and baseline values of the LVEDV and LVESV. Interestingly, within this group, the increase in miR-144 post-RIC was significantly correlated with the respective decrease in LVESV (r=-0.40, p=0.001). Additionally, compared to baseline, the reduction of PWV at protocol termination (PWV T0-PWV T3) in

the single-inflation group was related with the respective LVESV reduction at follow-up (r=0.37, p=0.002), after adjustment for age, sex, BMI, dyslipidaemia, diabetes, hypertension, concomitant medical treatments, MI location, myocardial enzymes, number of diseased coronary vessels (>70% stenosis), and baseline PWV.

DISCUSSION

In this study, we have shown that RIC with a single 5 min cycle of bilateral brachial cuff inflation conferred improvement of endothelial glycocalyx properties and reduction of aortic stiffness, at 5 and 35 minutes post-inflation in STEMI patients. This improvement in vascular function was in parallel with upregulation of protective miRs, namely miR-144, -150, and -21, oxidative stress burden reduction, and increase in NOx levels. More specifically, increased miR-144 concentration was closely associated with PWV improvement after RIC. These changes were not evident in the control group without cuff inflation. Single-cycle protocol was demonstrated to be superior regarding improvement of endothelial glycocalyx properties, miR-144 levels, aortic stiffness reduction, and increased NO bioavailability. Furthermore, the RIC-induced increase of miR-144 levels and improvement of PWV in the early phase of AMI were interrelated and both associated with a greater decrease of LVESV at 1 year of follow-up. Moreover, RIC by a single cuff inflation cycle was associated with a 3-fold higher probability of reverse LV remodelling within 12 months of the index event compared to the double-cycle or no RIC.

IRI induces coronary microcirculation injury with endothelial glycocalyx shedding, resulting in myocardial oedema, resistant vasoconstriction, platelet-leukocyte aggregation, coronary microembolization, and capillary destruction [7,13,18]; peripheral endothelial function is similarly affected [7]. RIC has been demonstrated to ameliorate coronary endothelial dysfunction, reducing myocardial oedema and infarct size [13,18,19], as well as

peripheral endothelial dysfunction, preserving flow-mediated dilation [7]. Interestingly, coronary microvascular dysfunction is reflected on the peripheral endothelial glycocalyx impairment as assessed by sublingual microscopy [17]. Further expanding this notion, our study is the first in our knowledge to provide direct evidence of improved endothelial glycocalyx properties in patients undergoing RIC after primary PCI. This could be at least partially mediated by the attendant oxidative stress alleviation, which has been shown to cause a rapid glycocalyx cadherin externalisation and gap junction restoration [14], normalising glycocalyx permeability.

Nitrate-nitrite-NO pathway has been demonstrated to mediate myocardial protection from IRI by modulating mitochondrial membrane electron transfer and inhibiting apoptosis [26]; RIC increases circulating nitrite levels in both human and animal models of IRI [26]. Interestingly, one 5-minute inflation-deflation cycle has been found to induce superior levels of plasma nitrite and associated cyto-protection compared with multiple cycles, with progressively diminished effects after every successive ischemic stimulus [40]. In agreement with the above-mentioned findings, our single-cycle intervention conferred increased nitrate and nitrite levels; on the contrary, the double-cycle protocol caused a net decrease in the measured concentrations, suggesting a possible consumption of the NOx pool by the ischemic insult of the second inflation-deflation (T2) cycle.

Enhanced oxidative stress with attendant membrane phospholipid peroxidation plays a major role in IRI and MDA constitutes a breakdown product of lipid peroxide β-cleavage [4]. Indeed, increased MDA levels have been reported following thrombolysis and PCI for STEMI [20]. In this respect, RIC has been demonstrated to ameliorate oxidative stress and reduce MDA concentration following PCI [20]. In agreement with this, our intervention resulted in MDA levels reduction, irrespective of the protocol applied. Moreover, IRI evokes a systemic inflammatory response with significantly increased IL-6 levels. Interestingly,

myocytes produce increased IL-6 levels in response to hypoxia, resulting in enhanced neutrophil adherence to the cardiovascular endothelium with deleterious effects [301]. Similarly, activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB)-/IL-6 pathway appears to mediate vascular inflammation in various disease processes [29]. On the one hand, RIC has been reported to activate heme oxygenase -1 (HO-1), thereby inhibiting NFκB and decreasing IL-6 levels in animal models of systemic inflammation and ischemia [30]. Remote ischemic conditioning (RIC) has been demonstrated to ameliorate the increase of IL-6 in animal models of IRI [259-261]. Clinical study results have been less consistent in this respect and despite evidence of RIC reducing IL-6 levels [212], data have also been obtained that suggest a neutral relationship [262-264]. It could therefore be hypothesised that IL-6 could be a circulating factor of RIC instead of a solely deleterious mediator, but evidence has been inconclusive. In our study, IL-6 levels were not affected by any of the applied protocols; this remains an interesting area for future investigation.

MiR-144 is a key effector of RIC [32], whose non-coding nature, small size, and direct effects on ribosomal function [31] allow it to rapidly modulate multiple cascades that abrogate IRI. It serves as a pivotal mediator of cellular adaptation to hypoxia [302] and experimental studies have demonstrated that its expression is upregulated in aortic endothelial and/or smooth muscle cells in response to stress [28]. Our finding suggests a possible vascular source of miR-144 production during RIC. One of its main mechanisms of action is the rapid -within 60 minutes [32] - activation of the reperfusion injury salvage kinase (RISK) pathway which constitutes a common pro-survival signalling pathway of remote pre- and post-conditioning [303]. Moreover, miR-144 evokes a crucial vascular antioxidative mechanism, in the form of Rac-1 downregulation [269]. To this end, modulation of ROS endothelial signalling appears to be one of its main mechanisms of action [304]. Indeed, we demonstrated that RIC results in increased miR-144 concentration, in

parallel with oxidative stress reduction as assessed by MDA reduction, and in correlation with arterial elasticity improvement as assessed by PWV reduction.

Increased NO bioavailability [22] and oxidative stress alleviation [10] are promoted by miR-144 expression and have been found to reduce arterial stiffness. Indeed, in our study increased miR-144 levels were correlated with PWV reduction. This finding suggests that our single-cycle intervention through increased miR-144 expression, increased NOx levels, reduced oxidative stress and improved glycocalyx properties likely resulted in PWV reduction [32,269,303]; on the contrary, double-cycle inflation caused a net decrease in the measured NOx concentrations, suggesting a possible consumption of the NOx pool by the ischemic insult caused by the second inflation-deflation cycle, possibly contributing to the increased PWV values at protocol termination. RIC has been previously demonstrated to improve arterial elasticity in patients with CAD [21], but our study is the first to describe PWV improvement within 48 hours of primary PCI.

Increased arterial stiffness increases LV afterload while reducing diastolic coronary perfusion with resultant subendocardial ischemia [22,149,150] and ventricular-arterial decoupling [150]. These effects within 48 hours of AMI contribute to adverse LV remodelling and prognosis [305,306]. Moreover, it has been experimentally demonstrated that even transient increases in LV afterload may detrimentally affect remodelling [165]. In support of these findings, our single inflation protocol was associated with a greater reduction of LVESV and increased prevalence of reverse remodelling (LVESV decrease >15%) at 12 months post-AMI compared to double or no inflation likely through reduction of PWV at a critical for myocardial salvage time. Importantly, the reduction of PWV at protocol termination as well as the miR-144 concentration were associated with the LVESV decrease at follow-up. Previous studies regarding the effects of ischemic conditioning on LV remodelling have produced mixed results. On the one hand, repeated RIC has failed to improve LV remodelling

in the context of chronic ischaemic heart failure, with the exception of longitudinal deformation improvement in a subgroup of patient with higher NT-proBNP plasma levels [307]. On the other hand, repeated RIC inhibited adverse LV remodelling and prolonged survival in an animal model of IRI [308]. Similarly, per-conditioning by staccato reperfusion has been associated with reduced LV volumes within 12 months of PCI [305], while post-conditioning has been found to confer improved LV remodelling, as assessed by LVESV, within 1 year of AMI [73].

The discrepancy between our single- and double-cycle protocol effects highlights the association between the number of ischemic cycles, the total ischemic time, and the underlying ischemic damage with RIC effectiveness [37]. In more detail, the second cycle may have crossed the ischemic burden threshold above which the beneficial effects of RIC on arterial stiffness are lost. This is concordant with previous findings of superior nitrite levels and cyto-protection with a single, compared to multiple inflation-deflation cycles [40]. Given the prominent role of NO in muscular arteries stiffness modulation [309], it can be postulated that the second ischemic stimulus consumed a component of the NOx pool, thereby reducing NO bioavailability with an attendant increase in aortic stiffness and failure to promote reverse LV remodelling. Similarly, the single-cycle protocol conferred superior restoration of the endothelial glycocalyx integrity. This could reflect coronary microvascular impairment amelioration to a greater extent compared to the double cycle RIC [17] and is in agreement with existing evidence of coronary microvascular injury adversely affecting LV remodelling [13]. The above mechanisms may explain the similar changes in LV volumes between the double- and no inflation group. These observations are also in accordance with the notion that one 5 min ischemic cycle may induce a favourable ischemic conditioning response [12], compared with multiple ischemic cycles [9,310-313].

There are some limitations to the interpretation of the results of our study, which are pertinent to the effects of PWV improvement on LV remodelling. On the one hand, it is known that increased LV afterload can adversely affect LV remodelling after AMI, even when it is transient in nature [165] and thus an early reduction of afterload may prohibit adverse LV remodelling. On the other hand, staccato reperfusion [305] and ischemic postconditioning [73] have also been demonstrated to have direct beneficial effects on the myocardium, leading to reverse LV remodelling. Our design does not permit to investigate the causality between LV remodelling and changes of vascular function caused after RIC versus the direct effects of RIC on the myocardium given the fact that aortic stiffness alleviation was an inherent result of our conditioning protocols. In addition, NOx levels represent the cumulative measurement of nitrates and nitrites, the levels of which (especially of nitrates) may be affected by food intake. The relatively high percentage of male subjects in our study should be also acknowledged as a limitation as female subjects may be underrepresented.

CONCLUSIONS

In conclusion, our findings suggest that RIC within 48 hours of STEMI acutely modulates the cardiovascular biochemical environment, evoking “vascular conditioning”. More specifically, miR-144 is upregulated, nitrate-nitrite-NO pathway is activated, and oxidative stress burden is reduced. Consequently, endothelial glycocalyx properties are improved, resulting in arterial stiffness alleviation. Ultimately, reverse LV remodelling is encouraged. The above protective effects of “vascular conditioning” occur at a time window after the first critical 90 min needed to diagnose STEMI and rush the patient to primary PCI, facilitating the application of RIC in clinical practice at a more convenient time for both the patient and the medical team, to encourage positive LV remodelling. Another future implication of our study would be to investigate the potential of interventions to restore NO

bioavailability early post STEMI, aiming to reduce aortic stiffness and inhibit adverse LV remodelling.

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FIGURE LEGENDS

Figure 1: Double-cycle, single-cycle, and no bilateral cuff inflation protocols

Figure 2: Sideview darkfield imaging assessment of endothelial glycocalyx Dper: diameter of perfusion; PBR: perfusion boundary region; RBC: red blood cell; RBCW: red blood cell width a) Sideview darkfield (SDF) imaging utilises the light emitting diode (LED) light reflected from haemoglobin to visualise the red blood cells (RBC) flowing in sublingual microvessels. The lateral distribution of the observed RBC columns demarks the boundaries of the perfused area of the vascular lumen, quantified by the Diameter of perfusion (Dper). Thus, an increased Dper infers deeper RBC penetration and a reduced non-permeable endothelial component, which signifies glycocalyx structural damage. b) The measurement process begins with image capturing, where the perfused luminal area is depicted as dark contrast flow. c) Thereafter, vascular segments are automatically identified. d) This is followed by RBC column width (RBCW) calculation, the distribution of which is used to calculate the perfused area diameter (Dperf) and the perfusion boundary region (PBR) according to the formula: PBR= (Dperf – RBCW) / 2.

Figure 3: Effects of remote conditioning on perfusion boundary region PBR: perfusion boundary region (μM); RIC: remote ischemic conditioning. The vertical lines represent the standard deviation of the mean. Two-way ANOVA for repeated measurements was applied with the parameter of time (T0,T1,T2,T3) used as a within-subject factor, and single- versus double-inflation, age, sex, BMI, dyslipidaemia, diabetes, hypertension, concomitant medical treatments, MI location, myocardial enzymes and the number of diseased coronary vessels (>70% stenosis) as between subject factors; ANCOVA was applied to investigate the effect of the baseline values of the respective markers. Post hoc comparisons were performed with Bonferroni correction and the adjusted p values for the comparison between T0 versus T1, T2,

and T3 are shown. The interaction between RIC protocol [single- vs double-cycle vs no cuff inflation (controls)] and changes of PBR over time were also examined, and the p values of the interaction are shown.

Figure 4: Effects of remote conditioning on pulse wave velocity PWV (m/sec): pulse wave velocity; RIC: remote ischemic conditioning. The vertical lines represent the standard deviation of the mean. Two-way ANOVA for repeated measurements was applied with the parameter of time (T0,T1,T2,T3) used as a within-subject factor, and single- versus double-inflation, age, sex, BMI, dyslipidaemia, diabetes, hypertension, concomitant medical treatments, MI location, myocardial enzymes and the number of diseased coronary vessels (>70% stenosis) as between subject factors; ANCOVA was applied to investigate the effect of the baseline values of the respective markers. Post hoc comparisons were performed with Bonferroni correction and the adjusted p values for the comparison between T0 versus T1, T2 and T3 are shown. adjusted p values are shown. The interaction between RIC protocol [single- vs double-cycle vs no cuff inflation (controls)] and changes of PBR over time were also examined, and the p values of the interaction are shown.

Figure 5: Effects of remote conditioning on serum malondialdehyde levels MDA: malondialdehyde (nmol/L); RIC: remote ischemic conditioning. The vertical lines represent the standard deviation of the mean. Two-way ANOVA for repeated measurements was applied with the parameter of time (T0,T1,T2,T3) used as a within-subject factor, and single- versus double-inflation, age, sex, BMI, dyslipidaemia, diabetes, hypertension, concomitant medical treatments, MI location, myocardial enzymes and the number of diseased coronary vessels (>70% stenosis) as between subject factors; ANCOVA was applied to investigate the effect of the baseline values of the respective markers. The interaction between RIC protocol [single- vs double-cycle vs no cuff inflation (controls)] and changes of PBR over time were also examined, and the p values of the interaction are shown.

Figure 6: Effects of remote post-conditioning in miR-144 expression RIC: remote ischemic conditioning. The fold change in expression level compared to the housekeeping gene, U6sn, was calculated using the $2^{-\Delta\Delta Ct}$ method. The vertical lines represent the standard deviation of the mean. Two-way ANOVA for repeated measurements was applied with the parameter of time (T0, T3) used as a within-subject factor, and single- versus double-inflation, age, sex, BMI, dyslipidaemia, diabetes, hypertension, concomitant medical treatments, MI location, myocardial enzymes and the number of diseased coronary vessels (>70% stenosis) as between subject factors; ANCOVA was applied to investigate the effect of the baseline values of the respective markers. The adjusted p values for the comparison between T0 and T3 are shown. The interaction between RIC protocol [single- vs double-cycle vs no cuff inflation (controls)] and changes of PBR over time were also examined, and the p values of the interaction are shown.

Figure 7: Effects of remote conditioning on LV remodelling LV: left ventricular, LVEDV: left ventricular end-diastolic volume, LVESV: left ventricular end-systolic volume, RIC: remote ischemic conditioning. The vertical lines represent the standard deviation of the mean. Two-way ANOVA for repeated measurements was applied with the parameter of time (baseline, 1 year follow-up) used as a within-subject factor, and single- versus double-inflation, age, sex, BMI, dyslipidaemia, diabetes, hypertension, concomitant medical treatments, MI location, myocardial enzymes and the number of diseased coronary vessels (>70% stenosis) as between subject factors; ANCOVA was applied to investigate the effect of the baseline values of the respective markers. The interaction between RIC protocol [single- vs double-cycle vs no cuff inflation (controls)] and changes of PBR over time were also examined, and the p values of the interaction are shown.

TABLES

Table 1: Baseline patient characteristics

Patient characteristics	**Single-cycle RIC (n=90)**	**Double-cycle RIC (n=90)**	**Control (n=90)**	**p-value**
Age (years)	53±16	54±16	52±16	0.70
Sex (Male %)	72 (80%)	73(82%)	75 (83%)	0.69
BMI	27±4	27±5	27±5	0.72
Hypertension	24 (27%)	26 (29%)	27 (30%)	0.70
Diabetes mellitus	15 (17%)	15 (17%)	16 (18%)	0.74
Dyslipidemia	21 (24%)	23 (26%)	22 (25%)	0.69
Smoking	47 (53%)	49 (55%)	51 (56%)	0.60
1 vessel disease	38 (42%)	41 (46%)	40 (44%)	0.50
2 vessel disease	41 (46%)	40 (44%)	42 (47%)	0.31
3 vessel disease	11 (12%)	9 (10%)	8 (9%)	0.22
Infarct related artery				
LAD	42 (47%)	43 (48%)	41 (46%)	0.40
Cx	23 (25%)	22 (24%)	21 (23%)	0.41
RCA	25 (28%)	25 (28%)	28 (31%)	0.21
hs-Troponin (ng/mL)	3843 [991-9338]	3926 [600-10000]	3890 [832-10000]	0.31
Symptom to balloon time (min)	179 [133-280]	181 [131-279]	180 [135-278]	0.12
First medical contact to balloon time (min)	104 [80-130]	102 [79-131]	105 [82-129]	0.18

WBC (/mcL)	8.790±2.577	8.540±2.588	8.680±2.592	0.20
CRP (mg/L)	191±3	170±4	185±4	0.30
EF (%)	44±13	46±14	46±10	0.50
Systolic BP (mmHg)	121±18	120±17	122±19	0.70
Diastolic BP (mmHg)	78±15	79±14	80±14	0.77

control: no inflation; BMI: body mass index; BP: blood pressure; CRP: C-reactive protein; Cx: circumflex artery; EF: ejection fraction; hs-Troponin: high sensitivity Troponin; LAD: left anterior descending artery; RCA: right coronary artery; RIC: remote ischemic conditioning; WBC: white blood cells count

Table 2: Effects of RIC on endothelial glycocalyx integrity and aortic stiffness

Vascular assessment	Group	T0	T1	T2	T3
PBR (μm)	Single	2.31±0.05	2.05±0.04*	2.06±0.06*	2.06±0.05*
	Double	2.34±0.04	2.04±0.06*	2.19±0.05*	2.21±0.06*
	Control	2.32±0.07	2.32±0.08	2.31±0.1	2.32±0.09
PWV (m/s)	Single	12.09±0.6	11.54±0.7*	11.39±0.7*	11.71±0.65*¶
	Double	12.06±0.5	11.57±0.6	11.37±0.7*	13.8±0.7*¶
	Control	11.7±0.8	11.6±1	11.6±1	11.7±1.5

PBR: perfusion boundary region; PWV: pulse wave velocity; T0: baseline; T1: after first cuff inflation; T2: after second inflation (or omission of 2nd inflation); T3: 20 min after second (or omission) inflation; controls=no inflation; *: $p<0.05$ for comparison with T0; ¶: $p<0.05$ for single- vs double-cycle protocol

Table 3: Effects of RIC on oxidative stress, cumulative nitrate-nitrite levels, and miRs expression

Biochemical assessment	**Group**	**T0**	**T3**
MDA (nmol/L)	Single	2.57±0.16	2.08±0.14*
	Double	2.61±0.15	2.13±0.15*
	Control	2.5±0.29	2.5±0.18
NOx (µmol/L)	Single	8.25±1.18	11.1±2*¶
	Double	10.79±1.18	8.91±2*¶
	Control	9.5±1	9.4±0.8
IL-6 (pg/ml)	Single	6.55±4.02	6.78±4.21
	Double	6.61±4.18	6.58±4.5
	Control	6.54±4,26	6.63±4.19
miR-144(/U6sn)	Single	7.4±0.7	55.9±0.8*¶
	Double	7.65±0.5	39.87±0.7*¶
	Control	5±0.6	4.8±0.5
miR-150(/U6sn)	Single	1.8±0.8	3.4±0.9*
	Double	2.05±0.5	3.53±0.6*
	Control	1.7±0.6	3.1±1.8
miR-499(/U6sn)	Single	1.6±0.4	3.5±0.4*
	Double	1.72±0.5	2.96±0.4*
	Control	1.4±0.3	1.8±0.3
miR-21(/U6sn)	Single	1.2±0.3	2.1±0.3*
	Double	1.28±0.4	2.18±0.5*

	Control	1.1±0.3	1.3±0.3
miR-208(/U6sn)	Single	1.9±0.5	2.4±0.4*
	Double	1.99±0.4	2.36±0.4*
	Control	1.8±0.4	2±0.3

T0: baseline; T3: 20 min after second (or omission of 2nd) inflation IL-6: interleukin-6; MDA: malondialdehyde; NOx: nitrate-nitrite; *: $p<0.05$ for baseline vs T3; ¶: $p<0.05$ for single- vs double-cycle protocol

Table 4: Progression of LV remodelling within 12 months of the index event

	group	**baseline**	**12 months**	**p-value**
LVEDV (mL)	single	105±34	83±31*¶	0.03
	double	107±29	100±32*¶	0.04
	control	110±29	104±32*¶	0.04
LVESV (mL)	single	59±31	49±25*¶	0.02
	double	60±25	57±25¶	0.06
	control	63±25	61±25¶	0.06
LVEF (%)	single	44±13	44±12	0.16
	double	45±10	43±12	0.1
	control	46±10	44±12	0.1

LVESV left ventricular end systolic volume LVEDV, left ventricular end diastolic volume LVEF left ventricular ejection fraction; *: $p<0.05$ for baseline vs 1 year follow up; ¶: $p<0.05$ for single-cycle vs double-cycle or control (no inflation)

FIGURES

Figure 1

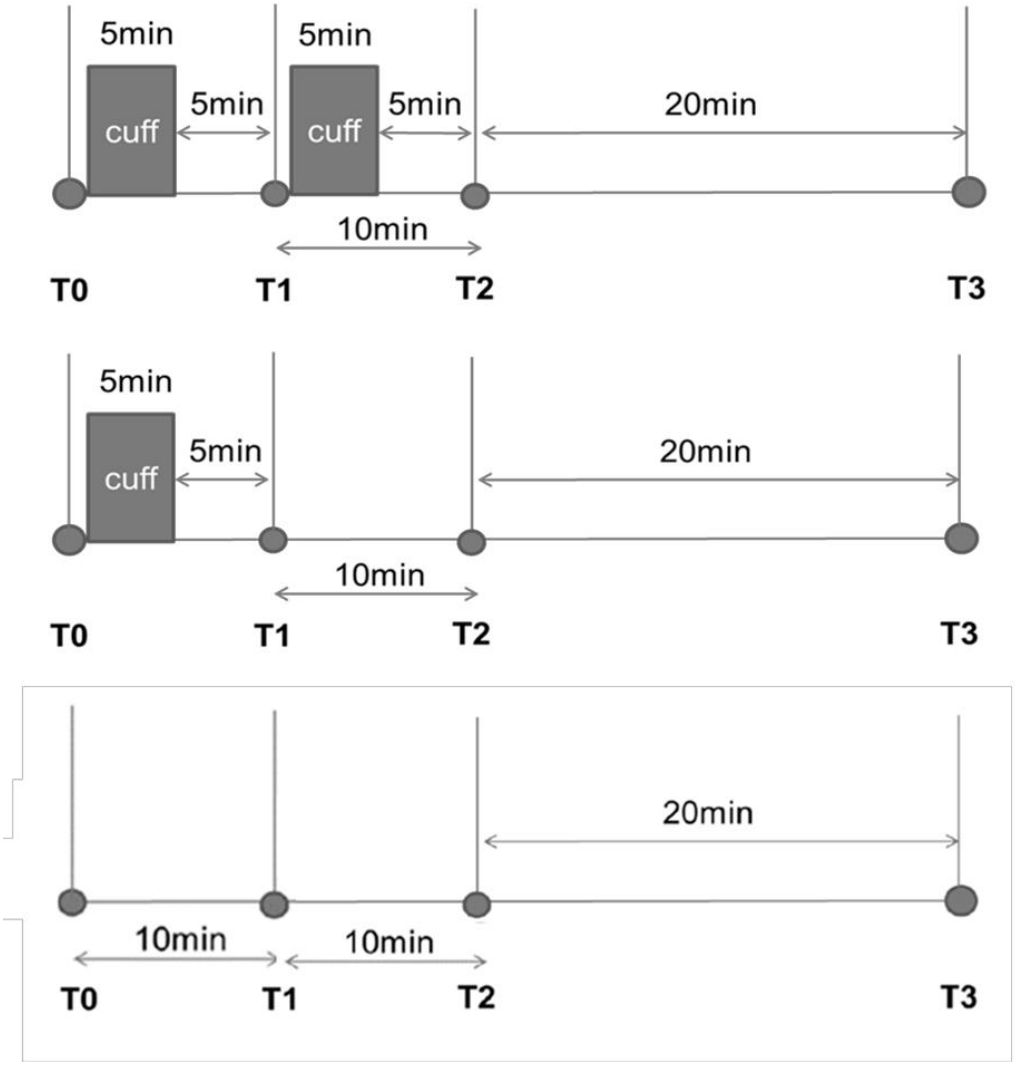


Figure 2

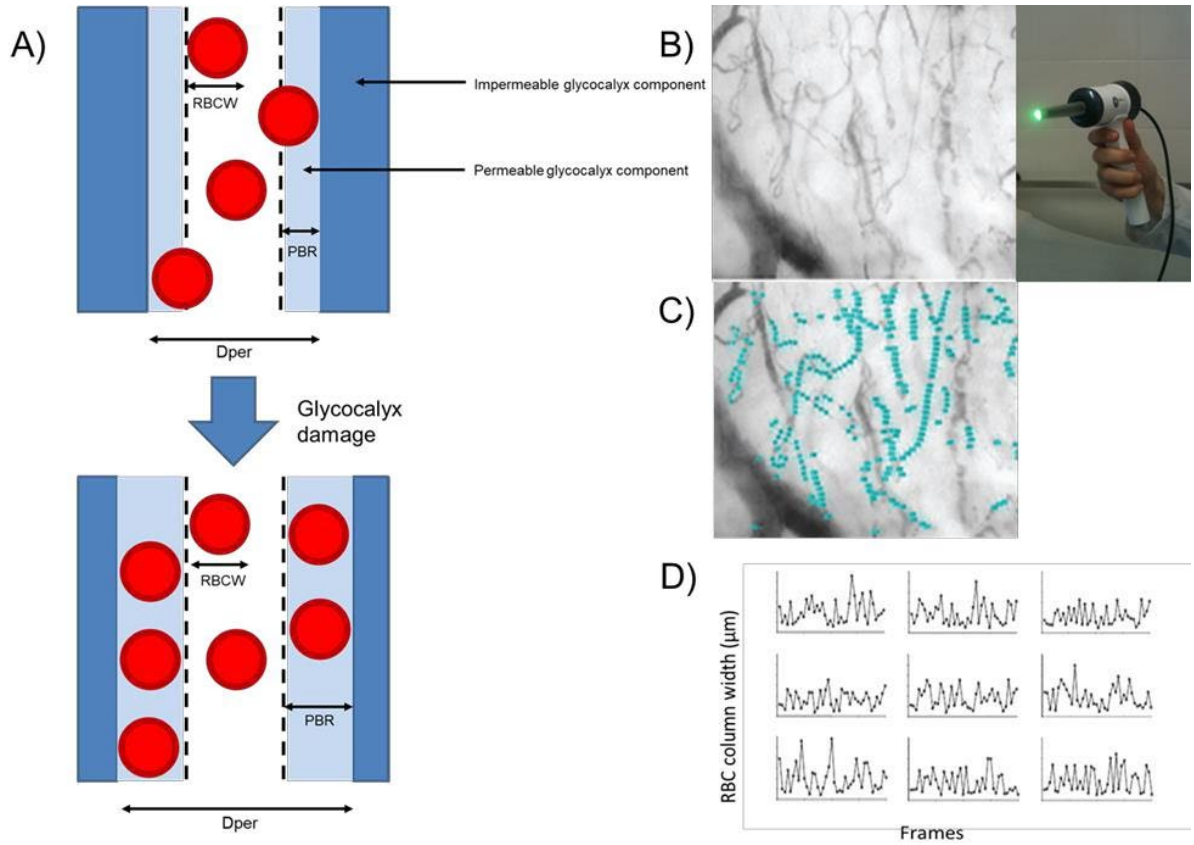

A)
RBCW
Impermeable glycocalyx component
Permeable glycocalyx component
PBR
Dper
Glycocalyx damage
RBCW
PBR
Dper
B)
C)
D)
RBC column width (μm)
Frames

Figure 3

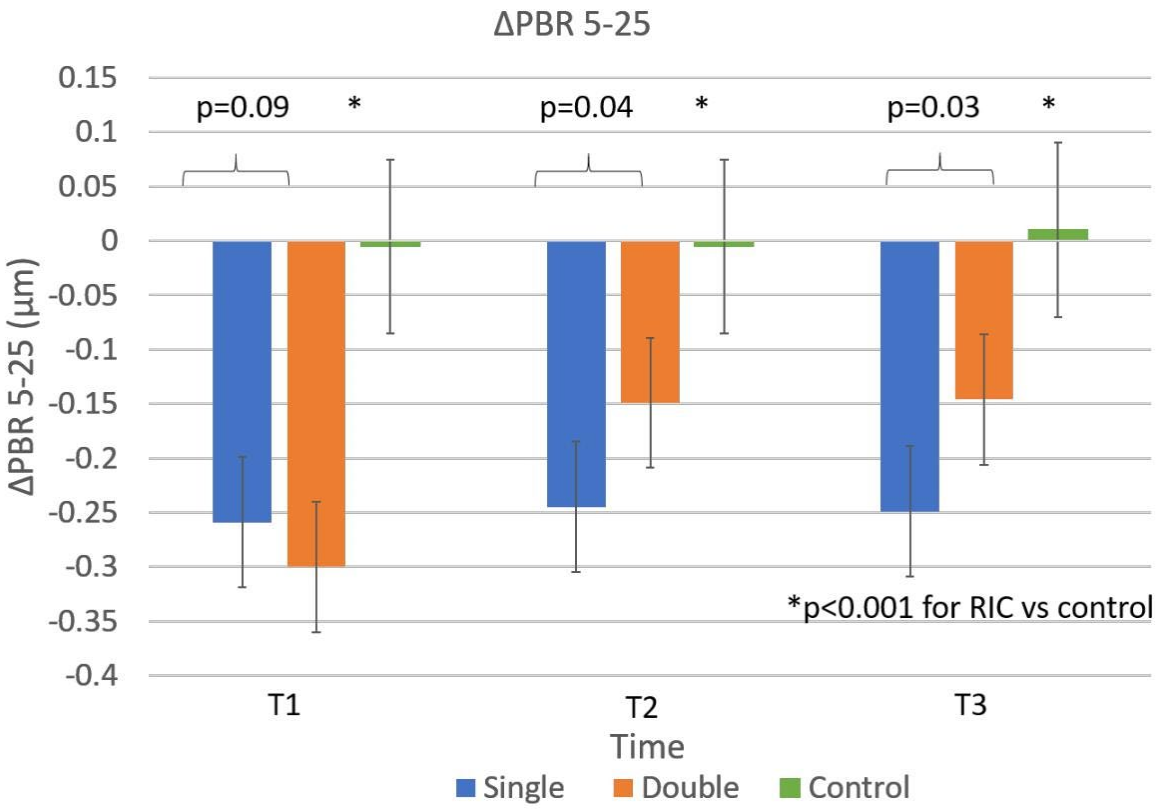

ΔPBR 5-25
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
-0.25
-0.3
-0.35
-0.4
ΔPBR 5-25 (µm)
p=0.09
*
p=0.04
*
p=0.03
*
*p<0.001 for RIC vs control
T1
T2
T3
Time
Single
Double
Control

Figure 4

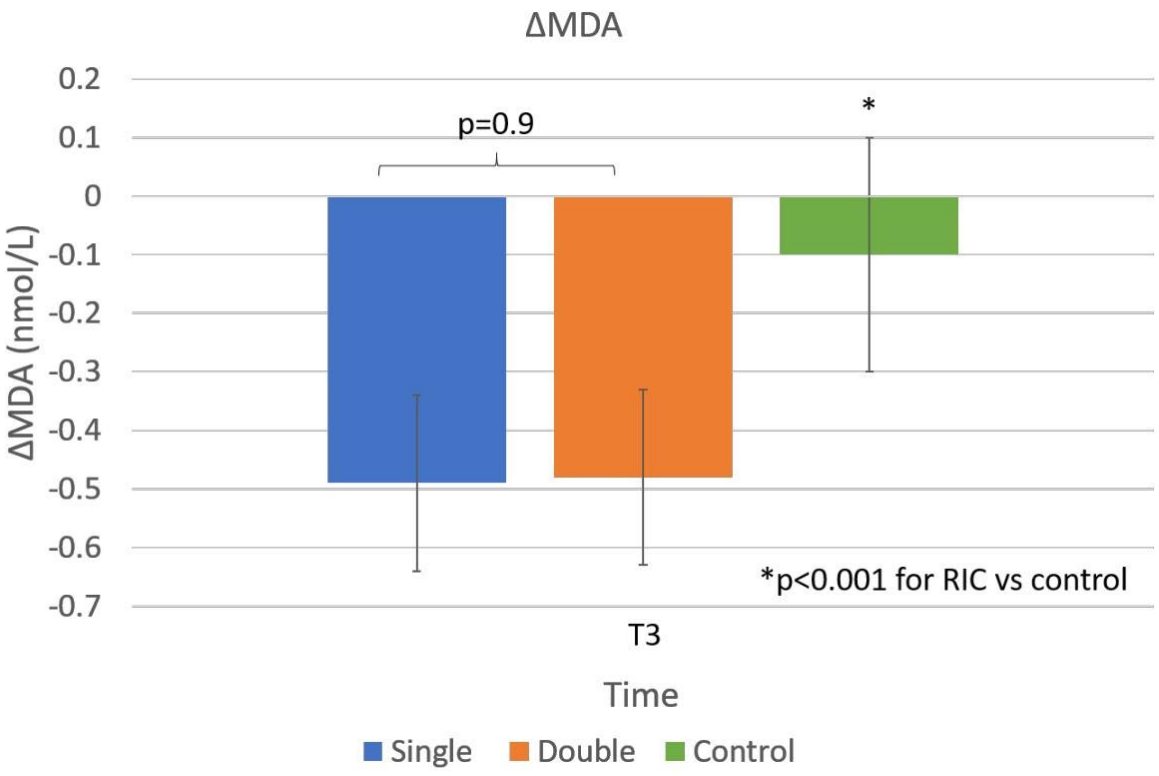

ΔMDA
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
ΔMDA (nmol/L)
p=0.9
*
*p<0.001 for RIC vs control
T3
Time
Single
Double
Control

Figure 5

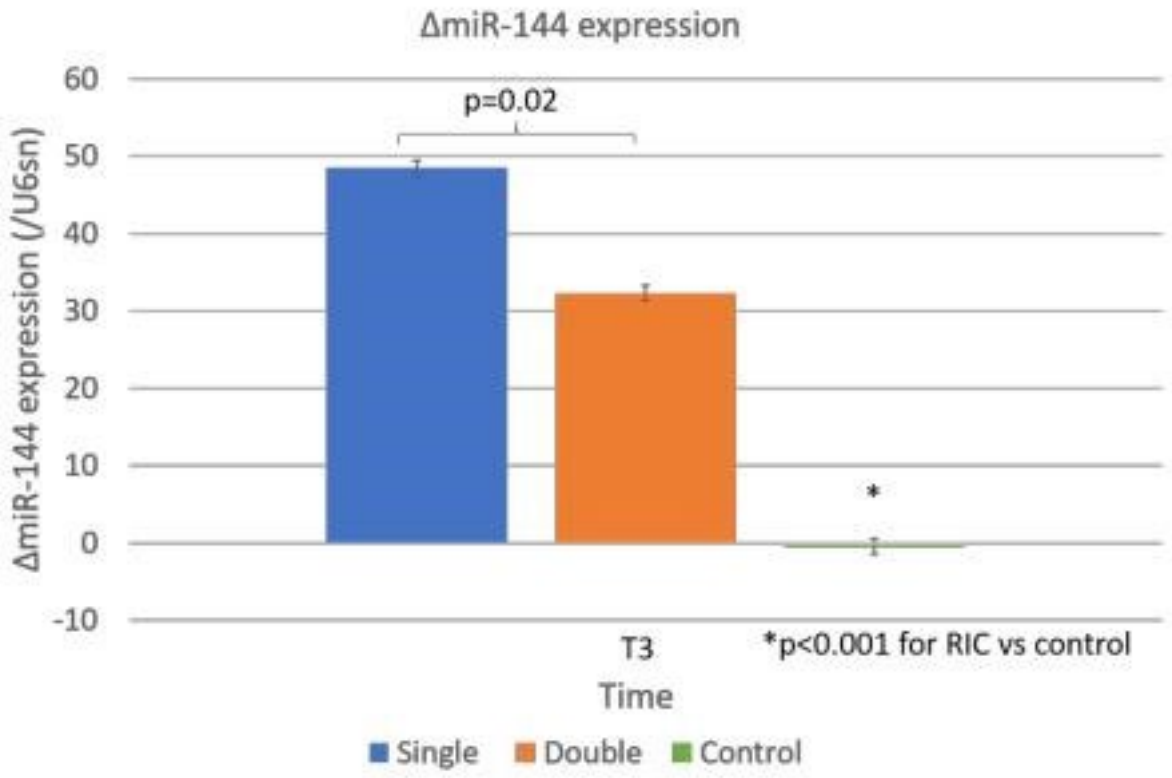

ΔmiR-144 expression
60
50
40
30
20
10
0
-10
ΔmiR-144 expression (/U6sn)
p=0.02
*
T3
*p<0.001 for RIC vs control
Time
Single
Double
Control

Figure 6

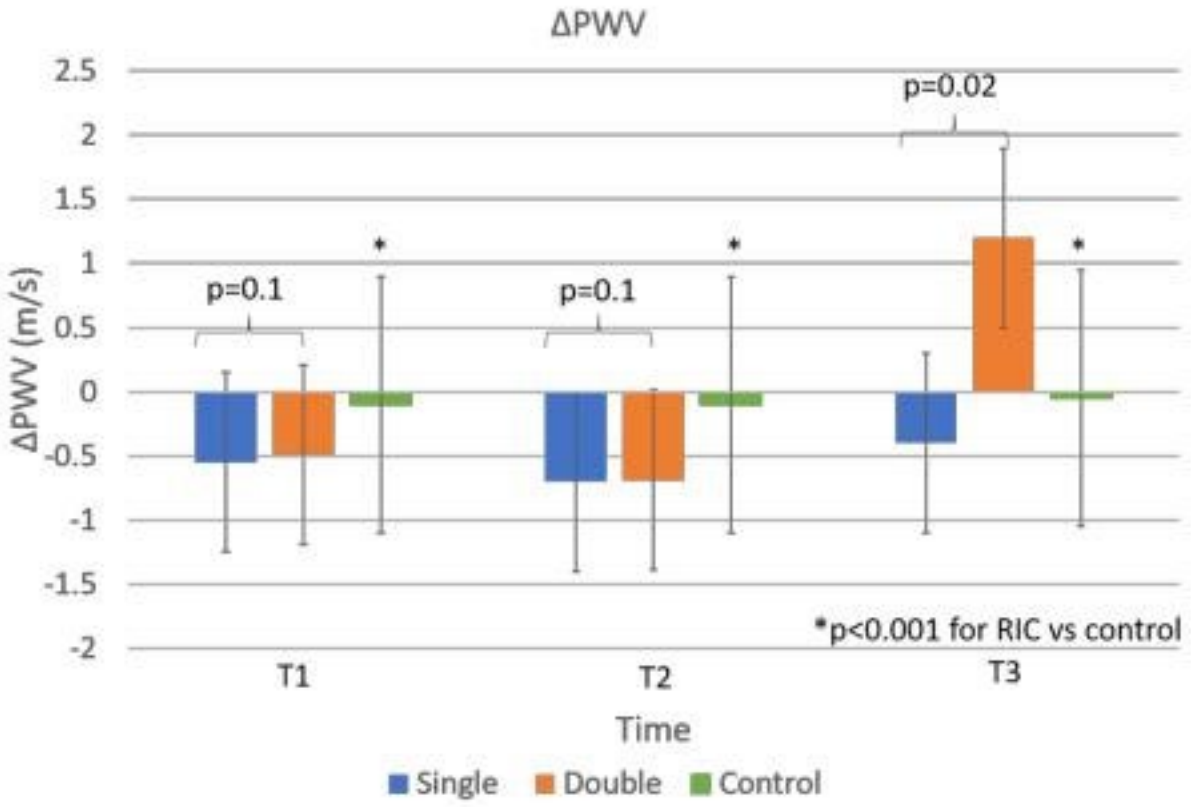
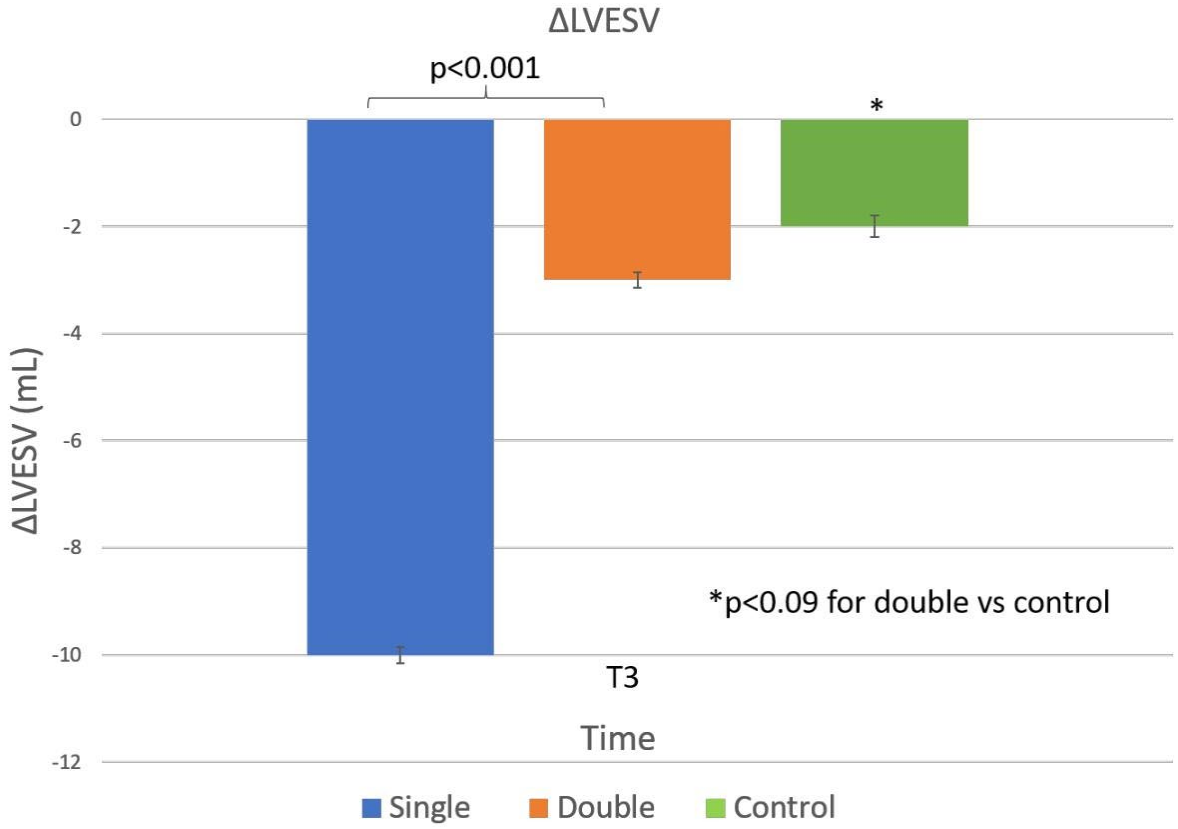

ΔPWV
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
ΔPWV (m/s)
p=0.1
p=0.1
p=0.02
*
*
*
*p<0.001 for RIC vs control
T1
T2
T3
Time
Single
Double
Control

Figure 7


ΔLVESV
p<0.001
*
0
-2
-4
-6
-8
-10
-12
ΔLVESV (mL)
*p<0.09 for double vs control
T3
Time
Single
Double
Control

Original Contribution | Published: 06 February 2021

Vascular conditioning prevents adverse left ventricular remodelling after acute myocardial infarction: a randomised remote conditioning study

Ignatios Ikonomidis, Dimitrios Vlastos, Ioanna Andreadou, Maria Gazouli, Panagiotis Efentakis, Maria Varoudi, George Makavos, Alkistis Kapelouzou, John Lekakis, John Parissis, Spiridon Katsanos, Damianos Tsilivarakis, Derek J. Hausenloy, Dimitrios Alexopoulos, Dennis V. Cokkinos, Hans-Eric Bøtker & Efstathios K. Iliodromitis

Basic Research in Cardiology **116**, Article number: 9 (2021) | Cite this article

Abstract

Aims

Remote ischemic conditioning (RIC) alleviates ischemia–reperfusion injury via several pathways, including micro-RNAs (miRs) expression and oxidative stress modulation. We investigated the effects of RIC on endothelial glycocalyx, arterial stiffness, LV remodelling, and the underlying mediators within the vasculature as a target for protection.

Methods and results

We block-randomised 270 patients within 48 h of STEMI post-PCI to either one or two cycles of bilateral brachial cuff inflation, and a control group without RIC. We measured: (a) the perfusion boundary region (PBR) of the sublingual arterial microvessels to assess glycocalyx integrity; (b) the carotid-femoral pulse wave velocity (PWV); (c) miR-144,-150,-21,-208, nitrate-nitrite (NOx) and malondialdehyde (MDA) plasma levels at baseline (T0) and 40 min after RIC onset (T3); and (d) LV volumes at baseline and after one year. Compared to baseline, there was a greater PBR and PWV decrease, miR-144 and NOx levels increase ($p < 0.05$) at T3 following single- than double-cycle inflation (PBR:ΔT0–T3 = 0.249 ± 0.033 vs 0.126 ± 0.034 μm, $p = 0.03$ and PWV:0.4 ± 0.21 vs −1.02 ± 0.24 m/s, $p = 0.03$). Increased miR-150,-21,-208 ($p < 0.05$) and reduced MDA was observed after both protocols. Increased miR-144 was related to PWV reduction ($r = 0.763$, $p < 0.001$) after the first-cycle inflation in both protocols. After one year, single-cycle RIC was associated with LV end-systolic volume reduction (LVESV) > 15% (odds-ratio of 3.75, $p = 0.029$). MiR-144 and PWV changes post-RIC were interrelated and associated with LVESV reduction at follow-up ($r = 0.40$ and 0.37, $p < 0.05$), in the single-cycle RIC.

Conclusion

RIC evokes “vascular conditioning” likely by upregulation of cardio-protective microRNAs, NOx production, and oxidative stress reduction, facilitating reverse LV remodelling.

Clinical Trial Registration

http://www.clinicaltrials.gov. Unique identifier: NCT03984123.

The role of microRNA expression in remote ischemic conditioning improvement of aortic elastic properties and endothelial glycocalyx integrity in acute myocardial infarction

Session Role of non coding RNAs in cardiovascular diseases

Speaker Doctor Ignatios Ikonomidis

Event : ESC Congress 2018

- Topic : basic science
- Sub-topic : Vascular Tone, Permeability, Microcirculation
- Session type : Moderated Posters

Authors : I Ikonomidis (Athens,GR), D Vlastos (Athens,GR), M Gazouli (Athens,GR), D Benas (Athens,GR), M Varoudi (Athens,GR), I Andreadou (Athens,GR), H Triantafyllidi (Athens,GR), P Efentakis (Athens,GR), G Makavos (Athens,GR), C Kontogiannis (Athens,GR), A Kapelouzou (Athens,GR), J Lekakis (Athens,GR), D Cokkinos (Athens,GR), EK Iliodromitis (Athens,GR)

The role of microRNA expression in remote ischemic conditioning improvement of aortic elastic properties and endothelial glycocalyx integrity in acute myocardial infarction

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Remote ischemic post-conditioning may prevent cardiac remodeling within two years of STEMI by cardioprotective microRNA upregulation

Session Poster session 1

Speaker Doctor Ignatios Ikonomidis

Event : EuroEcho 2019

- Topic : imaging
- Sub-topic : Imaging of Coronary Artery Disease
- Session type : Poster Session

Authors : I Ikonomidis (Athens,GR), D Vlastos (Athens,GR), S Katsanos (Athens,GR), M Gazouli (Athens,GR), J Thymis (Athens,GR), C Triantafyllou (Athens,GR), M Varoudi (Athens,GR), I Andreadou (Athens,GR), H Triantafyllidi (Athens,GR), G Makavos (Athens,GR), A Kapelouzou (Athens,GR), AR Vrettou (Athens,GR), A Frogoudaki (Athens,GR), D Cokkinos (Athens,GR), EK Iliodromitis (Athens,GR)

Remote ischemic conditioning by single cuff inflation improves aortic elastic properties and endothelial glycocalyx thickness in acute myocardial infarction patients

Session Endothelial dysfunction

Speaker Doctor Ignatios Ikonomidis

Event : ESC Congress 2017

- Topic : basic science
- Sub-topic : Vascular Biology and Physiology
- Session type : Rapid Fire Abstracts

Authors : I Ikonomidis (Athens,GR), D Vlastos (Athens,GR), S Vlachos (Athens,GR), D Benas (Athens,GR), M Varoudi (Athens,GR), I Andreadou (Athens,GR), H Triantafyllidi (Athens,GR), P Efentakis (Athens,GR), G Makavos (Athens,GR), J Lekakis (Athens,GR), E Iliodromitis (Athens,GR)

Remote ischemic conditioning results in oxidative stress reduction and nitrate- nitrite-nitric oxide pathway activation in acute myocardial infarction patients

Session Cardioprotection

Speaker Doctor Ignatios Ikonomidis

Event : ESC Congress 2016

- Topic : basic science
- Sub-topic : Cardiac Diseases
- Session type : Advances in Science

Authors : I Ikonomidis (Athens,GR), D Vlastos (Athens,GR), I Andreadou (Athens,GR), P Efentakis (Athens,GR), M Varoudi (Athens,GR), G Pavlidis (Athens,GR), G Makavos (Athens,GR), S Vlachos (Athens,GR), D Mpenas (Athens,GR), H Triantafyllidi (Athens,GR), E Seitanidi (Athens,GR), EK Iliodromitis (Athens,GR), J Lekakis (Athens,GR)

Remote ischemic conditioning results in oxidative stress reduction and nitrate- nitrite-nitric oxide pathway activation in acute myocardial infarction patients

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- Topic : basic science
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Authors : I Ikonomidis (Athens,GR), D Vlastos (Athens,GR), I Andreadou (Athens,GR), P Efentakis (Athens,GR), M Varoudi (Athens,GR), G Pavlidis (Athens,GR), G Makavos (Athens,GR), S Vlachos (Athens,GR), D Mpenas (Athens,GR), H Triantafyllidi (Athens,GR), E Seitanidi (Athens,GR), EK Iliodromitis (Athens,GR), J Lekakis (Athens,GR)