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UNIVERSITY OF TARTU
Press
1632

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Dissertation is accepted for the commencement of the degree of Doctor of Philosophy in Medicine on April 17th, 2024 by the Council of the Faculty of Medicine, University of Tartu, Estonia.

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Commencement: June 20, 2024

Funding: This PhD project was funded by personal research grants from the Estonian Research Council (PRG no. 1437, PUT no. 1169, PRG no. 435 and PRG no. 2078).

ISSN 1024-395X (print)
ISBN 978-9916-27-558-0 (print)
ISSN 2806-240X (pdf)
ISBN 978-9916-27-559-7 (pdf)

University of Tartu Press
www.tyk.ee

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original publications:

I. Kuusik, K.; Kepler, T.; Zilmer, M.; Eha, J.; Vähi, M.; Kals, J. Effects of Remote Ischaemic Preconditioning on Arterial Stiffness in Patients Undergoing Lower Limb Angiographic Procedures: A Randomised Clinical Trial. Eur. J. Vasc. Endovasc. Surg. 2019, 58, 875–882.

II. Kuusik, K.; Kasepalu, T.; Zilmer, M.; Eha, J.; Vähi, M.; Torop, L.A.; Lieberg, J.; Kals, J. The Role of RIPC in Preventing Organ Damage, Inflammation and Oxidative Stress during Lower Limb DSA: A Randomised Controlled Trial. Oxid. Med. Cell. Longev. 2021, 6043550.

III. Kuusik, K.; Kasepalu, T.; Zilmer, M.; Eha, J.; Paapstel, K.; Kilk, K.; Rehema, A.; Kals, J. Effects of RIPC on the Metabolomical Profile during Lower Limb Digital Subtraction Angiography: A Randomised Controlled Trial. Metabolites 2023, 13, 856.

These publications are referred to in the text with their Roman numerals.

Author's contribution:
Papers I-III: Participation in the conceptualisation and planning of the study, gathering of clinical data, analysis of the data and drafting of the paper.

ABBREVIATIONS

a	acyl
aa	diacyl
ACE	angiotensin-converting enzyme
ADMA	asymmetric dimethylarginine
ae	acyl-alkyl
AIx	augmentation index
AIx@75	augmentation index adjusted for a heart rate of 75
AKI	acute kidney injury
ANOVA	analysis of variance
AP-1	activator protein 1
ARB	angiotensin receptor blocker
Arg	L-arginine
ATP	adenosine triphosphate
B2M	beta-$_2$ microglobulin
BCAA	branched-chain amino acid
BH4	tetrahydrobiopterin
C1	large artery elasticity index
C2	small artery elasticity index
Ca^{2+}	calcium ions
CABG	coronary artery bypass graft
CDBP	central diastolic blood pressure
CDP-choline	cytidine 5-diphosphocholine
CI	confidence interval
CK-MB	creatine kinase-myocardial band
COX-2	cyclooxygenase-2
CPP	central pulse pressure
CRP	C-reactive protein
CSBP	central systolic blood pressure
cTnI	cardiac troponin I
DMA	dimethylarginine
DSA	digital subtraction angiography
eGFR	estimated glomerular filtration rate
EPO	erythropoietin
ERK	extracellular signal-regulated kinases
GSK3β	glycogen synthase kinase 3 beta
H^+	hydrogen ions
Hct	haematocrit
HDL	high-density lipoprotein
HGB	haemoglobin
HIF-1α	hypoxia induced factor 1-alpha
hs-CRP	high-sensitivity C-reactive protein
hs-TnT	high-sensitivity troponin T

ICU	intensive care unit
IL	interleukin
INF-γ	interferon gamma
IPC	ischaemic preconditioning
IQR	interquartile range
IRI	ischaemia reperfusion injury
ITT	intention-to-treat
K^+	potassium ion
KIM-1	kidney injury molecule-1
LCAT	lecithin cholesterol acyltransferase
LDL	low-density lipoprotein
LEAD	lower extremity artery disease
L-FABP	liver-type fatty acid-binding protein
LPS	lipopolysaccharide
LVEF	left ventricular ejection fraction
LysoPC	lysophosphatidylcholine
MAP	mean arterial pressure
MAPK	mitogen activated protein kinase
mKATP	mitochondrial ATP-sensitive K^+ channel
MnSOD	manganese superoxide dismutase
MPO	myeloperoxidase
MPTP	mitochondrial permeability transition pore
NGAL	neutrophil gelatinase-associated lipocalin
NFκB	nuclear factor kappa B
NO	nitric oxide
NOS	nitric oxide synthase
NT-proBNP	N-terminal pro b-type natriuretic peptide
Ox-LDL	oxidised low-density lipoprotein
OxS	oxidative stress
PAD	peripheral artery disease
PC	phosphatidylcholine
PCT	procalcitonin
PDBP	peripheral diastolic blood pressure
PEMT	phosphatidylethanolamine methyl transferase
PKC-ε	protein kinase C epsilon type
PKG	protein kinase G
PLA2	phospholipase A2
PLT	platelet
PP	per-protocol
PPP	peripheral pulse pressure
PSBP	peripheral systolic blood pressure
PTA	percutaneous transluminal angioplasty
PTEN	phosphatase and tensin homolog
PWV	pulse wave velocity
RAAS	renin-angiotensin-aldosterone-system

RBC	red blood cells
RIPC	remote ischaemic preconditioning
RISK	reperfusion injury salvage kinase
ROS	reactive oxygen species
SAFE	survivor activating factor enhancement
SD	standard deviation
STAT	signal transducer and activator of transcription
STEMI	ST-segment elevation myocardial infarction
SVR	systemic vascular resistance
TG	triglycerides
TNFα	tumour necrosis factor alpha
TRPV	transient receptor potential cation channels of the vanilloid subfamily
WBC	white blood cells

1. INTRODUCTION

Ischaemia-induced injuries are among the predominant concerns in modern medicine due to their close association with a variety of vascular diseases. To mitigate ischaemia and prevent irreversible damage, timely restoration of blood flow is essential. However, this reperfusion can paradoxically cause further tissue damage and significantly expand the risk area for cell death, a process known as ischaemia-reperfusion injury (IRI) (Becker, 2004). Such injuries are particularly prevalent during medical interventions that involve reperfusion as a treatment component, notably in interventional radiological procedures of the lower limbs.

Remote ischaemic preconditioning (RIPC) represents a non-invasive and cost-effective strategy that employs the body's innate defence mechanisms to shield distant tissues and organs from the effects of IRI. By initiating repeated brief ischaemic episodes at a remote site, such as an upper limb, RIPC induces a state of resilience against subsequent episodes of IRI. This prophylactic process is typically implemented through the inflation and deflation of a blood pressure cuff, creating cycles of short ischaemia followed by reperfusion. The principal idea behind RIPC is to use these intermittent, controlled episodes of ischaemia as a preparatory measure to strengthen organs and tissues against potential prolonged ischaemic insults, thereby helping to mitigate ischaemic damage in critical areas distant from the site of preconditioning.

Despite its theoretical promise, the clinical application of RIPC has yielded inconsistent outcomes, particularly in patients undergoing vascular interventions. The results vary widely, with some studies highlighting significant benefits, while others note minimal or no effect. This inconsistency can be attributed to the complex nature of IRI and the diverse mechanisms through which RIPC operates, potentially involving modifications in endothelial function, inflammatory responses and metabolic pathways. This divergence in findings underscores the necessity for a comprehensive evaluation of RIPC, one that encompasses a broader spectrum of physiological responses.

The prevalence of peripheral artery disease (PAD), characterised by steno-occlusive lesions affecting arteries in the upper and lower extremities, further complicates this landscape. As the global population ages, the incidence of PAD and its complications continue to increase, heightening the risks of morbidity and mortality from associated coronary and cerebrovascular diseases. This growing prevalence has correspondingly led to an increase in the utilisation of diagnostic and therapeutic procedures such as digital subtraction angiography (DSA) and percutaneous transluminal angioplasty (PTA). These necessary interventions for managing symptomatic lower extremity artery disease (LEAD) can themselves trigger IRI, thus exacerbating tissue damage and broadening the area at risk of cell death.

In light of these complexities, this research was initiated to systematically evaluate the effectiveness of RIPC against IRI in patients with LEAD. By focusing on the impact of RIPC on arterial stiffness, organ damage, oxidative stress,

inflammatory biomarkers and systemic serum metabolic alterations in patients undergoing DSA and endovascular treatment, the study aims not only to advance our understanding of RIPC's potential benefits but also to explore new therapeutic strategies within the vascular system. This endeavour seeks to provide a clearer delineation of the conditions under which RIPC may be most beneficial, thereby offering a solid foundation for future clinical applications and improving outcomes for patients with vascular diseases.

2. REVIEW OF THE LITERATURE

2.1. Overview of Remote Ischaemic Preconditioning

Ischaemic preconditioning first emerged in scientific literature in 1986, with a landmark study that demonstrated its ability to limit infarct size (Murry et al., 1986). This reduction was achieved through four cycles of 5-minute circumflex occlusions in a canine model, each separated by 5 minutes of reperfusion, followed by 40 minutes of ischaemia (Murry et al., 1986). Researchers postulated that the underlying mechanism behind these protective findings could either be a curtailment in ATP exhaustion or a minimised accumulation of metabolites during prolonged occlusion (Murry et al., 1986). In 1993, it was discovered that ischaemic events, even in remote tissues, could enhance resistance to subsequent ischaemia-reperfusion injuries, thereby reducing the size of myocardial infarctions (Przyklenk et al., 1993). This discovery laid the foundation for what is now termed RIPC. Over the years, numerous animal studies have shown that effects of RIPC against IRI seem to be consistent across various organs and tissues (Addison et al., 2003; Hausenloy & Yellon, 2008; Kharbanda et al., 2002; Liauw et al., 1996; Pell et al., 1998; Przyklenk et al., 1993; Tokuno et al., 2002). A 2015 meta-analysis of 503 studies showed that ischaemic preconditioning reduced myocardial infarction by 24.6% [95% CI: 23.5–25.6] in animals, with no significant difference in efficacy between local and remote preconditioning (Wever et al., 2015). Beyond the early protective effect lasting up to two hours following ischaemic preconditioning, a later phase of heightened resistance to ischaemic injury surfaces after 24 hours and can persist for as long as 72 hours (Baxter et al., 1997; Kuzuya et al., 1993; Loukogeorgakis et al., 2005; Murry et al., 1986).

2.1.1. Implementing Remote Ischaemic Preconditioning in Research

Unlike local ischaemic preconditioning, RIPC presents unique advantages, especially when direct intervention is impractical (Lang & Kim, 2022). Among the organs and tissues suitable for performing RIPC (Ateş et al., 2002; D. Singh & Chopra, 2004; Varga et al., 2019), limbs are considered the safest for clinical use due to its non-invasive nature and ease of application (Vasdekis et al., 2013). The use of a blood pressure cuff for performing RIPC has been validated through numerous clinical trials making it highly accessible for both clinical and experimental settings (Hoole et al., 2009; Meybohm et al., 2015; Zarbock et al., 2015).

There is no universally established protocol for performing RIPC (Heusch & Rassaf, 2016). Major RIPC trials typically involve three to five cycles of five minutes of ischaemia followed by five minutes of reperfusion (Liu et al., 2021; Pei et al., 2014). Research indicates that four to six cycles yield significant cardioprotection, with no additional benefit observed from eight cycles (Johnsen et al., 2016). Additionally, the cycle length that provides optimal protection has been suggested to last 2 to 5 minutes, while 10-minute cycles negate the protective

effect (Johnsen et al., 2016). The cuff pressure is generally set to 200 mmHg or up to 50 mmHg above the systolic blood pressure to safely block arterial blood flow, thereby ensuring effective occlusion while minimising discomfort and the risk of tissue damage (Pei et al., 2014). The implementation of sham controls, which involve inflating the cuff to a lower pressure that does not stop blood flow and thus does not induce ischaemia, is common in RIPC studies (Zarbock et al., 2015). This method is vital to verify that the observed beneficial effects are genuinely due to ischaemic preconditioning and not influenced by other confounding factors (Hohenschurz-Schmidt et al., 2023).

2.1.2. Contemporary Human Studies of Remote Ischaemic Preconditioning

In neurology and neurosurgery, remote ischaemic conditioning has been investigated as a potential neuroprotective strategy (Purroy et al., 2020; Sales et al., 2017). Although RIPC cannot be performed prior to an acute stroke event, its application in chronic conditions like intracranial arterial stenosis has been demonstrated to provide significant protection against subsequent strokes (Meng et al., 2012).

In cardiac surgery, a meta-analysis revealed that RIPC significantly reduces troponin release in patients not taking sulfonylureas (X. Wang et al., 2023). This aligns with further evidence suggesting that RIPC significantly reduces troponin release and myocardial injury, although it does not significantly affect the incidence of acute myocardial infarction, acute kidney injury (AKI) or overall mortality (Xie et al., 2018). Subgroup analysis indicated that RIPC might reduce mortality and AKI rates specifically in patients receiving volatile anaesthetic agents (Xie et al., 2018). These findings are supported by a systematic review that involved a cohort of 5999 adult patients across thirty-three randomized clinical trials, which found that in subgroups exposed to volatile anaesthetics during cardiac surgery with cardiopulmonary bypass, RIPC significantly reduced the incidence of AKI and marginally reduced intensive care unit (ICU) stay durations (Deferrari et al., 2018). This suggests that the benefits of RIPC, particularly in reducing myocardial injury and AKI, are influenced by the choice of treatment regimen.

In non-cardiac surgery, a meta-analysis of 3660 patients, RIPC was shown to reduce cardiovascular events during non-cardiac surgery without significantly affecting the rates of AKI or all-cause mortality (Wahlstrøm et al., 2021). In another meta-analysis that focused on non-cardiac, non-vascular surgeries, RIPC was shown to mitigate the ischaemic consequences of surgical interventions, potentially decreasing postoperative morbidity and reducing the length of hospital stays (Papadopoulou et al., 2023). However, the considerable variability among individual studies requires cautious interpretation due to significant heterogeneity and the limited scope of some research, underscoring the need for nuanced application of RIPC and highlighting the importance of targeted studies to fully determine its benefits and limitations (Papadopoulou et al., 2023).

In vascular surgery, a meta-analysis found that RIPC does not significantly impact mortality, renal dysfunction, myocardial infarction or the length of hospital stays (Stather et al., 2019). Additionally, a comprehensive Cochrane review, encompassing 14 trials with 1295 participants, revealed that RIPC offers no significant benefits in reducing perioperative mortality, myocardial infarction or renal impairment in both vascular and endovascular surgery settings when compared to not using RIPC (Liang et al., 2023). This analysis also assessed the certainty of evidence for these outcomes as moderate, implying that future, high-quality research might revise these findings (Liang et al., 2023). In contrast, research from the University of Tartu suggests that RIPC is effective in reducing kidney injury biomarkers in patients undergoing vascular surgery (Kasepalu et al., 2020b). These conflicting results underscore the uncertain clinical efficacy of RIPC in vascular surgery, emphasising the need for further investigation.

2.2. Biological Mechanisms of Remote Ischaemic Preconditioning

Much of our current understanding regarding the mechanisms of protective effects after RIPC stems from studies that investigated heart damage following cardiac ischaemic preconditioning in animal models (Lang & Kim, 2022). These studies often focus on the preconditioning's ability to mitigate IRI, a complex cascade of events that leads to additional cellular damage upon the restoration of blood flow to ischaemic tissues after prolonged ischaemia. While IRI remains an active research area, the interplay between OxS, calcium overload and mitochondrial dysfunction is crucial.

In short, during ischaemia, oxygen supply is rapidly depleted, causing cells to shift to anaerobic metabolism. This metabolic shift cannot generate sufficient adenosine triphosphate (ATP) to maintain the ion gradients and mitochondrial membrane potential. As ATP becomes depleted, ion pumps fail, causing cells to swell in a process termed oncosis, indicative of an imminent, pre-lethal cellular injury (Davidson et al., 2020). This state is a precursor to cell necrosis characterised by the chaotic disruption of the cell membrane (Davidson et al., 2020). The reintroduction of oxygen supply reactivates mitochondrial respiration, but simultaneously induces OxS through the production of reactive oxygen species (ROS), a phenomenon that can also occur due to the presence of residual oxygen during low-flow ischaemia (Cadenas, 2018). Rapid replenishment of ATP leads to a sudden increase of intracellular calcium (Ca^{2+}) and to hypercontraction in cardiomyocytes, further contributing to cellular damage (Rodríguez-Sinovas et al., 2007). Elevated Ca^{2+} uptake into mitochondria triggers Ca^{2+} overload, which, alongside increased production of ROS and normalisation of pH during reperfusion, stimulates the opening of mitochondrial permeability transition pore (MPTP) (Davidson et al., 2006; Rodríguez-Sinovas et al., 2007). This results in mitochondrial depolarisation, uncoupling of oxidative phosphorylation and ATP depletion (Davidson et al., 2006; Rodríguez-Sinovas et al., 2007). The opening

of the MPTP in the initial minutes of reperfusion amplifies both apoptosis and necrosis, underscoring the need for administering therapeutic interventions either before or immediately at the onset of reperfusion (Davidson et al., 2020; Eefting et al., 2004; Ong et al., 2015).

2.2.1. Mechanisms of Protection Against Ischaemia Reperfusion Injury

In biopsies taken from patients undergoing coronary artery bypass surgery, there is evidence that those who were preconditioned ATP levels were maintained post-ischaemia in comparison to control subjects, highlighting the role of ischaemic preconditioning in preserving mitochondrial function (Yellon et al., 1993). Preconditioning has also been shown to increase resistance to IRI by delaying the opening of MPTP when exposed to elevated Ca^{2+} and ROS levels (Argaud et al., 2004).

The transduction of RIPC signal to intracellular response can be mediated through various receptors, including G-protein-coupled receptors, cytokine receptors and receptor tyrosine kinases or can occur in a receptor-independent manner (Kleinbongard, 2023; Miura et al., 2010). As a result, cytosolic signalling pathways involving protein kinases and phosphatases are activated (Hausenloy & Yellon, 2016). The intracellular molecular pathways, however, are complex, intertwined and only partially understood to this day (Hausenloy et al., 2016). Although variations in pathway activation between species have been observed, the nitric oxide (NO)/protein kinase G (PKG), reperfusion injury salvage kinase (RISK) and survivor activating factor enhancement (SAFE) pathways are identified as the predominant pathways activated in response to ischaemic preconditioning signals (Burley et al., 2007; Hausenloy et al., 2016; Skyschally et al., 2015). The mentioned pathways exhibit biphasic activation with the initial response occurring during reperfusion of preconditioning cycling and the second transpiring in the first minutes of reperfusion (Hausenloy et al., 2016; Hausenloy & Yellon, 2007; Rossello et al., 2018). RISK and SAFE pathways ultimately activate cytosolic Akt (protein kinase B) and extracellular signal-regulated kinase (ERK) 1/2, which in turn stimulates mitochondrial protein kinases (Miura et al., 2010). The indirect activation of mitochondrial protein kinase C epsilon type (PKC-ε) by cytosolic PKG, Akt and ERK has been shown to cause the mitochondrial ATP-sensitive K^+ channels (mKATP) to open (Costa et al., 2005; Miura & Tanno, 2012). With the influx of K^+ and efflux of H^+ due to the opening of mKATP channels, the electron transport chain is inhibited. This increases ROS generation and limits the ischaemia-induced overload of mitochondrial Ca^{2+} levels (Andrukhiv et al., 2006; L. Wang et al., 2001). Limited flow of ROS, generated during ischaemia, serves as a redox signalling that is vital for activating subsequent kinases, as evidenced by the observed abolition of the preconditioning effect when either mKATP channel inhibitors or free radical scavengers were administered (Costa et al., 2006; Hausenloy et al., 2016; Miura et al., 2010; Pain et al., 2000). Furthermore, activation of the mKATP channel prior to an ischaemic

event has been shown to trigger intracellular signalling that inhibits the glycogen synthase kinase 3 beta (GSK3β) by phosphorylation and consequently prevents the opening of MPTP during the reperfusion phase (Miura & Tanno, 2012).

The second window of protection is mainly carried out by the transcription and synthesis of de novo proteins, which act as protective agents against ischaemic damage during the late phase (Hausenloy & Yellon, 2010). Triggered by initial ischaemic preconditioning stimuli, early mediators (such as PKC, ERK 1/2 and phosphatidylinositol 3-kinase/Akt) are activated, which in turn mobilise transcription factors such as NFκB, AP-1, HIF-1α and signal transducer and activator of transcription (STAT) 1/3/5 of the SAFE pathway (Hausenloy & Yellon, 2010). In fact, the phosphorylation of STAT-5, which is typically activated by members of the cytokine family and growth hormones, has been associated with cardioprotection conferred by RIPC (Heusch, 2015; Heusch et al., 2012). The pathways activated following RIPC have been shown to influence gene expression, resulting in the suppression of genes associated with chemotaxis, adhesion, migration, exocytosis, apoptosis and innate immunity, while simultaneously enhancing genes that protect against OxS and promote cytoprotection (Konstantinov et al., 2004, 2005; G. Li et al., 2004; Onody et al., 2003). These changes in gene expression are evident as early as 15 minutes after RIPC but become more pronounced 24 hours later (Konstantinov et al., 2004). The subsequent synthesis of proteins, including cyclooxygenase-2 (COX-2), heat stress proteins, inducible nitric oxide synthase (NOS), manganese superoxide dismutase (MnSOD) and aldose reductase, is believed to facilitate the protection in late phase of RIPC (Hausenloy & Yellon, 2010).

2.2.2. Humoral, Neuronal and Systemic Pathways of Remote Ischaemic Preconditioning

During brief episodes of ischaemia and reperfusion, a remote ischaemic tissue or organ releases a range of substances. RIPC stimulates the release of humoral factors, activates neural pathways and initiates systemic mechanisms, which include both anti-inflammatory responses and changes in gene expression (Donato et al., 2021). Depending on the anatomical location one or several of these pathways and substances may be engaged.

2.2.2.1. Humoral pathways of RIPC

The humoral pathway conveys RIPC-induced protective agents through the bloodstream to target organs and tissues, where they activate intracellular signalling pathways. Studies have shown that rabbits receiving transfusions or plasma dialysate from donors subjected to direct or remote ischaemic preconditioning exhibited a reduced myocardial infarct size compared to controls, highlighting the role of the humoral preconditioning signal delivery method (Dickson et al., 2001; Shimizu et al., 2009). Notably, cardiomyocytes treated with RIPC plasma dialysate exhibited similar protection, as seen in cells that underwent direct

ischaemic preconditioning (Breivik et al., 2011; Shimizu et al., 2009). Based on this study, it can be inferred that the initiators of ischaemic tolerance are molecules weighing less than 15 kilodaltons and possessing hydrophobic characteristics (Shimizu et al., 2009).

Moreover, Giricz et al. noted an enhanced release of extracellular vesicles, typically rich in RNAs and proteins, following an ischaemic preconditioning, implying their role as intercellular signalling conveyors in mediating RIPC's organoprotective effect (Giricz et al., 2014). When this dialysate was administered to isolated mouse hearts, it reduced the infarct size in a manner comparable to the cardioprotection achieved through ischaemic preconditioning (Giricz et al., 2014). In another study, the transfer of post-RIPC dialysate from pigs to mice led to a significant reduction in infarct size and indicated that activation of glycine receptors contributes to the cardioprotective effects (José Alburquerque-Béjar et al., 2017). Exosomes derived from cardiomyocytes have been shown to modulate cell proliferation, migration, differentiation, survival and angiogenesis in response to ischaemic incidences (Røsand & Høydal, 2021). Furthermore, cardiac fibroblast-derived exosomes have demonstrated significant potential in reducing fibrosis and enhancing cardiomyocyte viability under hypoxic conditions (Røsand & Høydal, 2021). Additionally, microRNA-144 has emerged as a potential mediator of RIPC protection; it was found to be upregulated by RIPC and downregulated in myocardial IRI (J. Li et al., 2014). The administration of microRNA-144 rapidly intensified a pro-survival kinase signature with increased phospho-Akt, phospho-GSK3β and phospho-ERK 1/2 MAPK and promoted autophagy signalling, thus improving functional recovery and diminished the infarct size (J. Li et al., 2014).

Similarly, the observed increase in lightweight cytokines in right atrial tissue, namely interleukin (IL)-1β, IL-8 and tumour necrosis factor alpha (TNFα), after performing RIPC in humans undergoing cardiopulmonary bypass, suggests that cytokines can also be involved in the RIPC humoral pathway signalling (Albrecht et al., 2012). In a related study, upon measuring cytokines from arterial plasma in patients undergoing coronary artery bypass surgery following the implementation of RIPC, only the levels of IL-1α exhibited a significant increase compared to the control group (Gedik et al., 2017).

Also, erythropoietin (EPO), largely produced in the kidneys and occasionally labelled as a cytokine for its signalling properties in non-hematopoietic tissues, showed a roughly 40% increase in serum levels one hour post-RIPC in mice (Calvillo et al., 2003; Oba et al., 2015). The cardioprotective effect of RIPC in mice was negated by anti-EPO antibodies and renal denervation, hinting that EPO acts as a humoral factor of RIPC, influenced by the renal nerve during limb ischaemic preconditioning (Oba et al., 2015).

Furthermore, RIPC appears to trigger a protective cascade by deactivating phosphatase and tensin homolog (PTEN) and stimulating STAT3, leading to enhanced expression of IL-10 in skeletal muscle and its increased presence in circulation after 24 hours (Cai et al., 2012). In the same study, this elevation in IL-10 was linked to the activation of Akt and endothelial NOS, suggesting that

IL-10 is a humoral mediator in the delayed phase of RIPC (Cai et al., 2012). Such a response aligns with the critical role of endothelial NOS in generating nitrite that accumulates in the heart, significantly contributing to the reduction of myocardial infarct size (Rassaf et al., 2014). Additionally, in human subjects, RIPC induces an increase in plasma nitrite concentrations via the activation of endothelial NOS due to endothelial shear stress, a mechanism that appears to operate independently of the phosphorylation states of Akt and ERK (Rassaf et al., 2014).

Adenosine, primarily recognised as a neural signal conductor, has also been proposed as a humoral mediator because of its observed elevated levels in the blood after RIPC (Tsibulnikov et al., 2019). However, given its short half-life of ten seconds, it likely acts locally within tissues, casting doubt on its function as a sustained humoral agent (Tsibulnikov et al., 2019).

Many other agents as potential humoral mediators of the RIPC effect have been proposed. Glycine has been shown to be significantly elevated in plasma-dialysates after performing RIPC on pigs (José Alburquerque-Béjar et al., 2017), and leukotrienes have been proposed as mediators of cardioprotection in rats (B. Singh et al., 2016).

2.2.2.2. Neural communication pathways of RIPC

Organ-protective effects of RIPC are mediated through a neurogenic pathway, as evidenced by the loss of this protective effect following the administration of neurogenic pathway blockers or nerve transection (Aulakh et al., 2017; Donato et al., 2013; Lim et al., 2010) and is important both in the early as well as in the delayed phase of preconditioning (Loukogeorgakis et al., 2005). The crucial role of sensory (afferent) nerve integrity in transmitting the RIPC signal was underscored in studies with diabetic patients, where dialysate only from those without peripheral neuropathy significantly reduced infarct size and improved haemodynamic recovery in rabbit models (Jensen et al., 2012). Furthermore, prior research has demonstrated that RIPC signalling leads to the activation of the vagal nerve and muscarinic receptors associated with the parasympathetic nervous system, resulting in a shift in the sympathovagal balance towards increased parasympathetic activity (Donato et al., 2013; Gardner et al., 2020; Pickard et al., 2016). It is hypothesised that vagal nerve activation acts as a bridge that links the activation of sensory nerves by RIPC in the limb to the release of humoral agents (Pickard et al., 2016). This is supported by studies with efferent vagal nerve stimulation, which preserved the connexin 43 gap junctions when applied before or during coronary occlusion (Ando et al., 2005). Preservation of the connexin 43 gap junctions is crucial for cardiomyocyte communication and may display antiarrhythmic properties, regardless of alterations in heart rate (Ando et al., 2005; Katare et al., 2009).

RIPC leads to an elevation in adenosine levels (Ng et al., 2019), which activates the local afferent nerves and myocardial adenosine receptors during early phase of reperfusion, leading to a reduction in heart infarct size after coronary artery occlusion (Liem et al., 2002). These protective effects are successfully

mimicked by adenosine infusion, yet are abolished by the administration of both ganglion blockers and adenosine receptor antagonists (Liem et al., 2002). Furthermore, an effluent taken from preconditioned isolated perfused rabbit hearts also have elevated adenosine levels, the presence of which has been associated with the preservation of mitochondrial integrity and function (Leung et al., 2014). In human studies, intra-arterial administration of adenosine activated adenosine receptors, initiating the cardioprotection associated with RIPC (Contractor et al., 2016). However, when caffeine was used to block these receptors, it effectively nullified this protective effect, suggesting that adenosine receptor activation in the triggering organ is crucial for the initial mechanisms of RIPC (Contractor et al., 2016). Bradykinin, a physiological mediator of pain, exhibits a dual role: at high doses, it can contribute to IRI, while at low doses, it may offer protection against myocardial IRI (Saxena et al., 2013). It has been proposed to convey RIPC stimulus from the periphery, as both bradykinin receptor blockers and local anaesthetics have shown to abrogate cardioprotection (Sharma et al., 2015). This highlights a function of nociception in initiating RIPC signalling.

The role of peripheral sensory signalling in RIPC is further underscored by the involvement of transient receptor potential cation channels of vanilloid subfamily (TRPV) channels, particularly TRPV1 and TRPV4, that are located both in cardiac and peripheral sensory nerves, as well as in dorsal root ganglion neurons, and are activated by ischaemic preconditioning (Randhawa & Jaggi, 2015). Activation of TRPV1 channels, triggered by the increased production of arachidonic acid metabolites, leads to the release of calcitonin gene-related peptide and substance P, both recognised for their cardioprotective effects (Randhawa & Jaggi, 2015). TRPV4 channels may contribute to preservation of vascular function during preconditioning by promoting endothelial NOS and endothelium-derived hyperpolarising factor activation, enhancing smooth muscle hyperpolarisation and vasodilation (Randhawa & Jaggi, 2015). The LEAD patients may benefit from this, as these mechanisms can potentially improve vascular function and reduce ischaemic injury.

2.2.2.3. Systemic and anti-inflammatory effects of RIPC

There is some evidence that RIPC modifies inflammatory response by changes in gene transcription in humans (Konstantinov et al., 2004) and may upregulate systemic anti-inflammatory cytokine concentrations (Cai et al., 2012). In rat stroke models, RIPC was shown to decrease levels of INF-γ and elevate IL-4 and IL-10 in the peripheral blood, indicating a shift in the inflammatory response (J. Yang et al., 2018). In another animal study, RIPC conferred a late phase of protection against myocardial IRI by upregulating interleukin expression in remote muscle that also led to increased levels of circulating IL-10 (Cai et al., 2012). In patients who underwent cardiac surgery, RIPC significantly raised pre-surgical serum levels of IL-8, IL-1β and TNFα, while post-surgical levels of cardiac tissues showed increased myeloperoxidase (MPO) and IL-1β levels (Albrecht et al., 2012). In another study on elective coronary artery bypass graft

(CABG) patients, only IL-1α levels were significantly increased following RIPC (Gedik et al., 2017). However, the anti-inflammatory impact of RIPC in CABG patients has been observed at the cellular level, where it markedly reduced the expression of bradykinin receptors (kinin B1 and B2) in neutrophils, which are important for bradykinin signalling (Saxena et al., 2013). This change persisted up to 24 hours post-surgery (Saxena et al., 2013).

The extent to which changes in the inflammatory response following RIPC are directly attributable to reductions in tissue damage remains unclear. RIPC has been shown to decrease the number of pro-inflammatory monocytes and increase the number of Tie-2 positive monocytes, which are involved in angiogenesis and tissue repair (Hummitzsch et al., 2021). Additionally, RIPC modulates plasma cytokine levels, increasing the concentrations of certain factors such as growth hormone and IL-1α, which have roles in reducing inflammation and promoting cardiovascular protection (Hummitzsch et al., 2021). In animal studies, ischaemic conditioning has demonstrated an ability to modify the inflammatory response following lipopolysaccharide (LPS) administration, enhancing survival in models of sepsis (Honda et al., 2019; Joseph et al., 2017). However, these results contrast with studies in non-surgical settings. For instance, in healthy volunteers who underwent RIPC prior to LPS administration there was no significant impact on systemic cytokine release, but RIPC alone prior to LPS induced renal markers of cell-cycle arrest (Zwaag et al., 2019). While results vary across contexts, a study on living donor liver transplant recipients revealed that RIPC significantly lowered plasma levels of pro-inflammatory markers and markers of glycocalyx damage, suggesting a reduction in systemic inflammation (Tosun et al., 2021). Conversely, in patients undergoing transcatheter aortic valve replacement, remote ischaemic preconditioning did not significantly alter plasma levels of inflammatory markers, such as leucocyte count, CRP, PCT, or IL-6, nor did it improve inflammation-associated survival at six months post-procedure (Zhang et al., 2022).

While we understand that RIPC involves complex signaling cascades, the exact molecular intermediates and the cross-talk between different pathways remain poorly defined. RIPC's impact on the inflammatory response varies considerably between animal models and human clinical settings, indicating the need for context-specific evaluation. This suggests that RIPC could potentially be beneficial for patients with LEAD, but further research is needed to confirm these effects.

2.3. Clinical Applications and Challenges of Remote Ischaemic Preconditioning

Clinical implementation of RIPC necessitates an intervention that must be administered before the onset of the ischaemic event. This preconditioning strategy is most viable in scenarios where the onset of ischaemia is predictable, such as in surgical or procedural settings.

2.3.1. Remote Ischaemic Preconditioning in Cardiovascular Diseases

RIPC has consistently exhibited protective cardiovascular effects in various animal models, leading to numerous human studies that explore its potential benefits. The first human study that showed cardiovascular benefits of RIPC was conducted on children undergoing repair of congenital heart defects (Cheung et al., 2006). It revealed significantly lower cardiac troponin I (cTnI) levels and reduced inotropic requirements in the RIPC group (Cheung et al., 2006). However, in large clinical trials, RIPC has not shown clinically significant effects in CABG patients. For example, the ERICCA trial involving 1612 patients undergoing elective on-pump CABG surgery did not find significant improvement in clinical outcomes with RIPC, including cardiovascular events, myocardial injury, inotropic support requirements, kidney injury, hospital stay duration, physical function and quality of life (Hausenloy et al., 2015). Similarly, the RIPHeart trial, which included 1403 patients undergoing elective cardiac surgery with cardiopulmonary bypass under total anaesthesia using intravenous propofol, also found no beneficial effects of RIPC in reducing the composite outcome of death, myocardial infarction, stroke, or acute renal failure up to the time of hospital discharge (Meybohm et al., 2015). Furthermore, a one-year follow-up of the RIPHeart trial also indicated no significant impact of RIPC on intraoperative myocardial dysfunction, neurocognitive function or long-term outcomes (Meybohm et al., 2018).

Despite the lack of significant differences in mortality or morbidity rates in clinical trials, a reduction in cTnI leakage due to RIPC was noted in a meta-analysis of cardiac surgery patients (L. Yang et al., 2014). In contrast, another meta-analysis focusing on patients undergoing both surgical and transcatheter valvular interventions demonstrated that RIPC significantly reduced periprocedural troponin release, suggesting cardioprotection, though without improving early post-operative clinical outcomes (Moscarelli et al., 2021). Moreover, a pooled analysis of randomised controlled trials on on-pump CABG patients revealed that RIPC did not reduce postoperative mortality or morbidity, nor ICU or hospital stays, despite reduction of the duration of mechanical ventilation (Yi et al., 2017). Importantly, a notable reduction in mechanical ventilation duration, ICU stays and AKI incidence (particularly AKI stage I), with more pronounced benefits in younger patients undergoing less complex surgeries, was observed in a meta-analysis of 21 randomised controlled trials involving 6302 adult cardiac surgery patients, underscoring RIPC's potential benefits (Zhou et al., 2017). These findings suggest that while RIPC may have limited impact on major clinical outcomes, it can still confer procedural risk mitigation benefits.

Previously, our research group has demonstrated that in patients undergoing non-cardiac vascular surgery, RIPC effectively mitigates the increase in high-sensitivity troponin T (hs-TnT) and N-terminal pro b-type natriuretic peptide (NT-proBNP) (Kepler et al., 2020). The implementation of RIPC in non-cardiac surgeries has thus the potential to reduce cardiovascular events, including myocardial infarctions, cardiac arrests, new arrhythmias and low cardiac output

syndromes. Support for this comes also from a comprehensive meta-analysis of 43 randomised controlled trials including 3660 patients (Wahlstrøm et al., 2021). However, this meta-analysis reported no significant effect of RIPC on acute kidney injury or overall mortality rates (Wahlstrøm et al., 2021). Conversely, a focused analysis on vascular surgery revealed that RIPC did not significantly affect mortality, renal dysfunction, myocardial infarction, myocardial injury or length of stay in a meta-analysis of 13 randomised controlled trials involving 1097 patients (Stather et al., 2019), highlighting the need for further large multi-centre trials to explore the benefits of RIPC in major surgeries.

In the study by Bøtker *et al.*, 333 patients suspected of acute myocardial infarction were assessed, revealing that RIPC applied before hospital admission, in conjunction with angioplasty, significantly increased myocardial salvage (Bøtker et al., 2010). This effect was notably more pronounced in patients with totally occluded vessels and in those with infarctions in the left anterior descending artery (Bøtker et al., 2010). In the largest remote ischaemic conditioning trial to date, the CONDI-2/ERIC-PPCI, which included 5401 ST-elevation myocardial infarction (STEMI) patients undergoing primary percutaneous coronary intervention, no significant reduction was seen in cardiac death or heart failure hospitalisations at 12 months (Hausenloy et al., 2019). Additionally, in a pre-planned cardiovascular magnetic resonance sub-study of this trial with 169 STEMI patients, remote ischaemic conditioning was observed to be associated with a lower incidence of microvascular obstruction and higher left ventricular ejection fraction (LVEF) on the acute scan of anterior STEMI patients when compared with control, but it failed to reduce myocardial infarct size or LVEF at six months (Francis et al., 2021). Interestingly, in a meta-analysis involving patients with stable coronary artery disease undergoing elective percutaneous coronary intervention, RIPC was shown to significantly lower the perioperative incidence of myocardial infarction and contrast-induced kidney injury, with this effect being more pronounced in male patients (Pei et al., 2014). Additionally, a few other meta-analyses encompassing patients undergoing coronary angiography and percutaneous coronary intervention have revealed that RIPC substantially lowered the risk of contrast-induced nephropathy, especially in those at moderate to high risk (Deng et al., 2020; Pranata et al., 2020) and may lead to a decrease in mortality, rehospitalisation rates, the need for haemodialysis and the incidence of other major adverse events (Pranata et al., 2020). Overall, while RIPC shows promise in certain areas, its varying effectiveness across different clinical scenarios is not consistent. Such discrepancies raise questions about the translatability of animal model findings to human treatments, an area ripe for further exploration.

2.3.2. Remote Ischaemic Preconditioning and Arterial Stiffness

Arterial stiffness, indicated by reduced arterial wall elasticity, limits the arteries' capacity for expansion and contraction. It stems from both functional changes,

such as altered vascular smooth muscle cell dynamics, and structural modifications, including variations in elastin, collagen fibres and extracellular matrix components (Sena et al., 2022). Concurrently, endothelial dysfunction results in decreased production of vasodilators like NO, leading to a disturbance in vascular homeostasis (Herrera-Zelada et al., 2021). NO also enhances microvascular function by improving red blood cell (RBC) deformability, facilitates blood flow through capillaries and optimises gas exchange (Grau et al., 2016). This function of NO in enhancing microcirculatory flow complements mechanisms that ameliorate arterial stiffness and vascular health, as observed in RIPC interventions, which boost NO availability to better prepare the vasculature for managing ischaemic events (Grau et al., 2016). This is corroborated by a randomised controlled trial involving 129 patients undergoing coronary angiography, where RIPC was found to significantly enhance coronary microcirculation function and increase coronary blood flow, as evidenced by the quantitative flow ratio and index of microcirculatory resistance (Z.-Z. Zhao et al., 2023). Deficiency in NO exacerbates arterial stiffness, creating a feedback loop that accelerates the progression of cardiovascular diseases and often precedes the appearance of symptoms and clinical diagnosis (Heitzer et al., 2001; Mitchell et al., 2010).

Accurate assessment of arterial stiffness and endothelial dysfunction are important in cardiovascular research, utilising methods like pulse wave velocity (PWV) and pulse wave analysis for comprehensive evaluation and improved risk prediction (McEniery et al., 2006; Ohkuma et al., 2017; Sena et al., 2022). Stiffening of the large arteries accelerates the pulse wave from the left ventricle, leading to an earlier return of the reflected pressure wave (H.-L. Kim, 2023). This premature return increases pressure during left ventricular ejection, raising the afterload on the left ventricle and reducing coronary artery perfusion pressure during diastole (H.-L. Kim, 2023). Both PWV and pulse wave analysis, which allow for the measurement of central blood pressures and augmentation index (AIx), can be performed non-invasively using specialised devices that register the aortic pulse wave indirectly through peripheral arteries (Butlin et al., 2013). PWV is assessed between the carotid and femoral arteries and is considered the gold standard for measuring arterial stiffness (Van Bortel et al., 2012). Increased arterial stiffness accelerates PWV, making pulse waves travel and reflect more quickly, which in turn raises systolic blood pressure (Laurent et al., 2006). The AIx, which represents the difference between the second and first systolic peaks in the aortic pressure waveform in percentages, measures the impact of the reflected pulse wave on central pulse pressure, with higher AIx values linked to an increased risk of LEAD (Ismaeel et al., 2018). Several factors can affect AIx, including age, gender, heart rate, blood pressure, arterial stiffness and lifestyle habits such as physical activity and smoking (Janner et al., 2012; Pichler et al., 2016). An increase in heart rate generally results in a lower AIx, which is why AIx@75 is used to standardise this measurement to a heart rate of 75 beats per minute. AIx typically increases with age due to the stiffening of arterial walls, and women often have higher AIx values compared to men, potentially due to hormonal differences and variations in arterial structure (Pichler et al., 2016).

Higher blood pressure and arterial stiffness increase AIx by enhancing pulse wave reflection, while regular exercise and a healthy diet can lower AIx (Pichler et al., 2016).

Prior to the initiation of the trial (I), the impact of RIPC on PWV or augmentation indices had not been studied. Assessing the impact of interventions like RIPC in humans should be most effectively achieved by focusing on the peripheral limb vasculature, where the direct non-invasive and precise measurement of its effects is feasible (Sena et al., 2022). Integrating measures such as the large artery elasticity index (C1), including the aorta and its major branches, with the compliance of small artery elasticity index (C2), and combining these with established techniques like central PWV, may enhance the comprehensive assessment of arterial health even more (Woodman et al., 2005). This approach not only captures distinct aspects of arterial stiffness across various segments of the vascular system, but also facilitates a deeper understanding of the mechanisms affecting arterial stiffness and the effects of therapeutic interventions, thus improving the evaluation of vascular function and health (Woodman et al., 2005).

The underlying mechanisms of RIPC to reduce arterial stiffness involve releasing endothelial-derived factors that alter vascular tone such as NO, endothelial dependent hyperpolarisation factors and arachidonic acid metabolites like prostacyclin and thromboxane A2, along with superoxide anions (Aggarwal et al., 2016; Herrera-Zelada et al., 2021). In RIPC, NO production is primarily driven by inducible NOS and endothelial NOS, which are activated in different phases of protection: endothelial NOS responds to early-phase shear stress during reperfusion, while inducible NOS activation occurs in the late phase (Aggarwal et al., 2016). Also activation of neuronal NOS has been described (Varga et al., 2019). Despite the short half-life of NO, increased bioavailability is achieved during ischaemic periods with circulating nitrite, a more stable by-product of NO oxidation that can be converted back to NO in response to hypoxia or ischaemic stress by haemoglobin or myoglobin, thus extending its beneficial effects beyond localised areas (Rassaf et al., 2014).

RIPC has been shown to enhance endothelial reactivity in the context of IRI, as it not only augments flow-mediated dilation in both its immediate and extended phases of protection, but also significantly improves remote microvascular endothelial function (Loukogeorgakis et al., 2005; Moro et al., 2011; Rytter et al., 2020). However, the response by vasculature can vary based on the chosen method of measurement and clinical situation (Sena et al., 2022; Wahlstrøm et al., 2023). In healthy adults, RIPC shows no immediate improvement in arterial stiffness (Müller et al., 2019), whereas in patients with coronary heart disease, it reduces central systolic blood pressure and central pulse pressure, indicating less pressure from pulse wave reflection in the central arteries (Zagidullin et al., 2016). Thus, RIPC offers a promising approach to enhance vascular health, particularly improving endothelial function and potentially reducing arterial stiffness, to better manage and treat cardiovascular disease.

2.3.3. Potential Confounders of Remote Ischaemic Preconditioning

Reliable demonstration of the effects of RIPC in humans is challenging. Translation of findings from animal studies to clinical practice is complicated due to the invasive nature of these studies and impracticality of application of direct preconditioning in humans. Lastly, confounding factors like comorbidities and co-medications augment the complexity of this translation.

The timing of RIPC is crucial, particularly in the context of counteracting clinically induced IRI scenarios. Performing RIPC too early or too late could miss the optimal window for maximum clinical benefit (Lang & Kim, 2022; Loukogeorgakis et al., 2005). Furthermore, the efficacy of RIPC timing can vary depending on the clinical context. For instance, RIPC administered just before anaesthesia in vascular surgery might not yield the same results as when performed immediately before coronary angiography (Pranata et al., 2020; Stather et al., 2019).

Medications can significantly influence RIPC's signalling pathways. Certain drugs like statins activate pathways such as the RISK pathway, while others like β-blockers, sulfonylureas and propofol can inhibit the signal transduction (Hausenloy et al., 2016; Pierce et al., 2017; X. Wang et al., 2023; Zhou et al., 2013). For example, atorvastatin combined with RIPC has shown a synergistic effect in protecting against IRI, characterised by reduced levels of inflammatory and cardiac biomarkers like TNF-α, cTnI, IL-6, creatine kinase-myocardial band (CK-MB) and CRP, and increased NO levels (El Desoky et al., 2016). These drug interactions make interpreting RIPC's net effects complex, especially in patients in ICU or general anaesthesia, where multiple medications might be used.

Moreover, various diseases impact the effectiveness of RIPC (Trachte et al., 2021). Diabetes mellitus, for instance, impairs activation of neural and intracellular pathways and lowers the threshold for MPTP opening (Hausenloy et al., 2016; Jensen et al., 2012; Miki et al., 2012). Additionally, factors such as age and sex have been shown to influence the outcomes of RIPC in some studies (Heinen et al., 2018; Moro et al., 2011). The heterogeneity of patient populations in terms of pathologies and comorbidities has been a major point of criticism in large-scale RIPC clinical trials (Heusch & Gersh, 2016, 2020). As the advancements in patient care have also reduced major clinical events, isolating the distinct effects of RIPC from numerous confounding factors has become increasingly challenging.

2.4. Remote Ischaemic Preconditioning and Lower Extremity Artery Disease

2.4.1. Lower Extremity Artery Disease, Risk Factors and Consequences

LEAD, also referred to as lower extremity PAD, is a manifestation of systemic atherosclerosis that is often underdiagnosed. LEAD patients face functional impairments, diminished quality of life, chronic limb-threatening ischaemia and are at a heightened risk for any cardiovascular event or mortality (Aboyans et al., 2018). Over the past 30 years, the incidence of LEAD has risen by more than 70%, now affecting upwards of 113 million people globally (GBD 2019 Peripheral Artery Disease Collaborators, 2023). This disease exhibits a higher prevalence in women, especially in ageing populations, though it is also associated with greater mortality and more years of life lost in men (GBD 2019 Peripheral Artery Disease Collaborators, 2023). Modifiable risk factors, including smoking, hypertension, dyslipidaemia, diabetes and kidney failure, account for about 70% of LEAD's global impact, with inflammatory conditions further contributing to an increased risk (Aboyans et al., 2018; Chuang et al., 2016; GBD 2019 Peripheral Artery Disease Collaborators, 2023).

The overall changes in LEAD induce a proinflammatory, procoagulant and prothrombotic state, which collectively contribute to vascular remodelling, diminished tissue perfusion and, at the macrovascular level, the formation of plaque and increased arterial stiffness (Poledniczek et al., 2023). Arterial stiffness, a critical metric in vascular health assessment, serves as a significant predictor of future cardiovascular events and overall vascular ageing (H.-L. Kim, 2023). The initiation of LEAD's pathogenesis is characterised by the impairment and deterioration of the endothelial glycocalyx, a protective coating composed of a network of membrane-bound proteoglycans and glycoproteins, covering the luminal surface of endothelial cells that line the blood vessels, by inflammation in atheroprone vessel regions (Poledniczek et al., 2023; Reitsma et al., 2007). This damage facilitates the interaction and migration of inflammatory cells to the vessel walls and allows low-density lipoproteins (LDL) to accumulate in the subendothelial space (Mitra et al., 2017). Oxidation, enzymatic cleavage and aggregation of LDL promotes inflammatory cascade, monocyte recruitment and their transformation into foam cells in the intima, forming the foundation of arterial plaque (Mitra et al., 2017). With the expansion of lesions, the production of the extracellular matrix transitions towards an increase in collagen, resulting in the hardening of the vessel walls (Mitra et al., 2017). The degradation of the glycocalyx and vascular inflammation lead to endothelial dysfunction, marked by a reduced release of vasodilative substances, especially NO (Poledniczek et al., 2023). Endothelial dysfunction in peripheral arteries is an early factor in LEAD progression, closely mirroring dysfunction in coronary artery endothelium (Anderson et al., 1995; Brunner et al., 2005). Since NO inhibits excessive smooth muscle cell proliferation and leukocyte adhesion, its diminished availability also

facilitates the progression of atherosclerosis (Mitra et al., 2017; Poledniczek et al., 2023).

The presence of higher levels of inflammatory markers has been associated with an increased likelihood of prevalent LEAD (Cauley et al., 2016). In LEAD, particularly in cases of critical limb ischaemia, the production of ROS and OxS significantly increases under chronic IRI conditions, which further aggravates the endothelial cell damage (Apichartpiyakul et al., 2022; Poledniczek et al., 2023). It is noteworthy that at signalling levels, the production of ROS is advantageous, enhancing ischaemic angiogenesis and the activation of cell survival pathways, which in turn improves tissue resilience against the prolonged negative impacts of IRI (Yu et al., 2019). However, prolonged IRI settings with excessive ROS formation can cause microcirculatory alterations that include endothelial cell death and weakened endothelial barriers (Poledniczek et al., 2023). IRI also limits the availability of capillaries during reperfusion, thus hindering nutrient transport and intensifying damage to muscle fibres, which contributes to a progressive decline in muscle functionality (Poledniczek et al., 2023; Steven et al., 2017). These alterations are observable as modifications in microvascular flow, notably indicated by an increased pulse amplitude (Young et al., 2021; Yu et al., 2019). This growing body of evidence was further reinforced in a cohort study which demonstrated that microvascular disease, either on its own or combined with peripheral artery disease, significantly amplifies the risk of amputation (Beckman et al., 2019).

Optimal medical management of LEAD includes addressing cardiovascular risk factors with integrated strategies that combine pharmacological treatments and non-pharmacological interventions (Aboyans et al., 2018). Interventions aimed at reducing arterial stiffness and improving endothelial function may improve functional performance in individuals with LEAD (Coutinho et al., 2011). The choice between surgical and endovascular revascularisation treatments is influenced by the anatomical location and severity of arterial lesions (Aboyans et al., 2018). Additionally, hybrid procedures that combine elements of both endovascular techniques and open surgery are also a viable option.

2.4.2. Role of Remote Ischaemic Preconditioning in the Management of Lower Extremity Artery Disease

Several studies have indicated that repeated sessions of RIPC over weeks can enhance walking distances, adjust sympathovagal balance and promote healing of chronic lower extremity ulcers (Balin & Kıvrak, 2019; J. Epps et al., 2016; Gardner et al., 2020; Shaked et al., 2015). Additionally, improvements in blood pressure, including anti-hypertensive effects on systolic, diastolic and mean arterial pressures have been described (J. Epps et al., 2016; W. Guo et al., 2021). This is thought to be facilitated by enhanced endothelium-dependent vasodilation, attributed to increased production of NO and a higher count of endothelial progenitor cells, that supports the repair of damaged endothelium (Kimura et al., 2007). A review comparing the benefits and harms of RIPC versus no RIPC in

individuals undergoing elective major vascular and endovascular surgery, which included 1295 patients from 14 trials, showed no significant differences in perioperative mortality, myocardial infarction, renal impairment, stroke, length of hospital stay and operating time (Liang et al., 2023). However, the applicability of this study to LEAD patients receiving endovascular treatment is limited, as the meta-analysis predominantly included patients undergoing either endovascular aortic procedures or open surgery for LEAD (Liang et al., 2023). To address these knowledge gaps effectively, future research should prioritise the delineation of RIPC's mechanistic pathways in the context of LEAD-specific interventions like lower limb DSA and endovascular treatment. It is crucial to investigate the effects of RIPC on arterial stiffness, endothelial function and overall vascular health to substantiate its utility in clinical practice. Establishing a comprehensive understanding of RIPC's impact could significantly advance treatment strategies and improve outcomes for patients suffering from LEAD.

2.5. Risks Associated with Digital Subtraction Angiography and Endovascular Treatment

2.5.1. Digital Subtraction Angiography and Endovascular Interventions: Diagnostic and Therapeutic Roles in Vascular Medicine

DSA is a diagnostic imaging method that enhances the visibility of blood vessels by digitally subtracting non-contrasted images from those with contrast material, thereby accentuating vascular structures and identifying abnormalities. For the effective planning of revascularisation treatments, DSA is frequently essential, either to guide percutaneous peripheral interventional procedures or to identify viable arteries for distal bypass grafting (Aboyans et al., 2018).

PTA is a minimally invasive endovascular procedure that involves inflating a balloon on a catheter inside narrowed or blocked arteries to restore blood flow, often with the option of placing a stent to keep the vessel open (Beckman et al., 2021). PTA is associated with fewer immediate complications and lower costs when compared to open surgery (Jongsma et al., 2020). The most frequent complications arising from endovascular treatments include site hematomas, distal embolisation, arterial dissection and pseudoaneurysms (Jongkind et al., 2010). However, studies extending beyond five years indicate that arteries treated with endovascular methods exhibit a higher risk of restenosis, suggesting that, in the long term, these treatments may be less effective than open surgical approaches (Beckman et al., 2021). Continued advancements in endovascular treatments and procedural techniques have the potential to enhance long term outcomes (Abdoli et al., 2020).

The healing process following a vascular intervention encompasses four phases, each potentially contributing to restenosis (Beckman et al., 2021). Immediate response features platelet deposition and neutrophil recruitment and is

followed by an acute inflammatory phase, where growth factors and cytokines trigger cell proliferation and migration (B. Wu et al., 2017). Subsequently, the resolution phase diminishes inflammation and leads to tissue remodelling, the final phase where tissue matrix deposition stabilises the vessel (B. Wu et al., 2017). Current antirestenosis drugs primarily focus on targeting proliferation and inflammation; however, the ideal approach to prevent restenosis would involve using cytostatic and antithrombotic treatments that would also promote endothelial regrowth, attenuate vascular smooth muscle cell activation, and accelerate the resolution of inflammation, thus ensuring complete vascular healing (Beckman et al., 2021).

2.5.2. Potential Organ Damage by Digital Subtraction Angiography and Endovascular Treatment and the Role of Remote Ischaemic Preconditioning in Risk Mitigation

Both DSA and endovascular inteventions utilise iodinated contrast media for visualisation, presenting a range of potential complications from mild and temporary to severe and life-threatening (J. Singh & Daftary, 2008). These complications may arise from allergic reactions or direct effects of the contrast media leading to reduced perfusion and ischaemia in hypoxia-sensitive tissues like the renal medulla (Geenen et al., 2013). Following peripheral balloon angioplasty and stent implantation, a measurable inflammatory vascular response is evident through elevated serum acute-phase reactants, indicating that these interventions can trigger a systemic inflammatory response (Schillinger et al., 2002).

RIPC demonstrates diverse effects across various clinical settings involving stent implantation. It has been shown to significantly reduce myocardial injury and the incidence of myocardial infarction following percutaneous coronary interventions in patients receiving drug-eluting stents, highlighting its protective potential (S. J. Luo et al., 2013). However, its benefits are not uniformly observed; for instance, it did not significantly impact myocardial biomarkers or myocardial infarction rates in elderly patients with coronary heart disease and diabetes (Xu et al., 2014). Similarly, while considered safe in carotid artery stenting, RIPC did not consistently prevent the development of cerebral infarction lesions, as evidenced by mixed results in lesion reduction and no significant impact on clinical outcomes like transient ischaemic attacks and strokes post-stenting (Asadi et al., 2022; W. Zhao et al., 2017). In contrast, another study highlighted that RIPC could enhance myocardial protection by increasing intracoronary nitric oxide levels, suggesting a potential mechanism for reducing myocardial damage during stent placement (Arroyo-Martínez et al., 2016). However, evidence is lacking on the effects of RIPC in patients with LEAD undergoing stenting. Overall, while RIPC shows potential benefits in specific contexts, its effectiveness is determined by patient-specific factors and the nature of the intervention, which adds depth and complexity to our understanding of its clinical utility.

Studies such as those by Soleymani et al. have demonstrated that while RIPC shows promise theoretically, its practical application does not significantly impact the occurrence of AKI or change renal biomarkers in low-risk patients undergoing coronary angiography (Soleymani et al., 2018). In the broader context of coronary interventions, RIPC has been shown with a high degree of certainty to mitigate the risk of contrast-induced nephropathy, offering significant protective benefits (Deng et al., 2020; Pranata et al., 2020; Zhou et al., 2016). RIPC potentially shields the kidneys by triggering distant tissues to release damage-associated molecular patterns, which upon being filtered by the kidneys, activate receptors on tubule cells to prompt defence mechanisms including energy conservation and cell-cycle pause to fortify the kidneys against future inflammatory or ischaemic challenges (Zarbock & Kellum, 2016).

Studies indicate that the incidence of AKI in patients receiving percutaneous interventions for LEAD is approximately 10% (Prasad et al., 2019). The risk of AKI and its associated mortality significantly increases in patients with pre-existing chronic kidney disease, a prevalent condition among those with LEAD (GBD 2019 Peripheral Artery Disease Collaborators, 2023; Nijssen et al., 2018). Furthermore, factors like reduced haemoglobin levels, use of larger volumes of contrast media and diabetes further heighten the risk of these complications (Morabito et al., 2012). In light of recent research, the application of RIPC, although shown to mitigate contrast-induced nephropathy in coronary procedures, may not offer the same protective effects in lower limb inteventions, particularly in patients with pre-existing renal conditions where the incidence of complications remains significant (Roy et al., 2021). This discrepancy highlights the need for tailored preventative strategies based on patient-specific risk factors and underlying health conditions, emphasising a more personalised approach to managing the risks associated with iodinated contrast media.

2.6. Metabolomics and its Implication in Remote Ischaemic Preconditioning and Lower Extremity Artery Disease

Metabolomics systematically studies low molecular weight metabolites, including amino acids, peptides, lipids, carbohydrates and nucleic and fatty acids (Paapstel & Kals, 2022). Metabolomics, by simultaneously evaluating alterations in hundreds of metabolites, constructs profiles associated with diseases, capturing the dynamic metabolic alterations linked to disease states and providing insights into underlying mechanisms and potential therapeutic interventions (Fitzpatrick & Young, 2013; Paapstel & Kals, 2022). In the setting of inflammatory disorders such as LEAD, metabolomics enhances understanding of pathophysiology, discriminates among patient populations, tracks therapeutic outcomes and facilitates the identification of novel biomarkers (Kouassi Nzoughet et al., 2017; McGranaghan et al., 2021; Ross, 1999; Z. Wang et al., 2011). For example, in patients with symptomatic LEAD, metabolomics profiling identified elevated

serum concentrations of key metabolites such as lactate, free carnitine, phenylalanine, tyrosine, aspartate, glutamate, glycine, histidine, leucine, methionine, serine and ornithine, while levels of pyruvate, citrate, α-ketoglutarate, aconitate and cysteine were found to be lower compared to healthy controls (Zagura et al., 2015). Additionally, recent research demonstrates that increased branched-chain amino acid (BCAA) levels provoke OxS and inflammatory responses in endothelial cells, leading to enhanced expression of adhesion molecules and subsequent endothelial dysfunction, thereby establishing a critical connection between elevated BCAA concentrations and increased cardiovascular risk by promoting the adhesion of inflammatory cells to the endothelial lining, which compromises vascular health (Zhenyukh et al., 2018). Furthermore, elevated levels of oxidative phosphorylation intermediates in LEAD patients, such as acylcarnitines, suggest impaired mitochondrial metabolism, which hinders ATP production and enhances ROS generation, potentially leading to endothelial dysfunction by diminishing NO bioactivity through mitochondrial-derived oxidants (Signorelli et al., 2020).

As emphasised in the mechanisms chapter, in the organs subjected to ischaemic preconditioning a more gradual reduction in ATP levels has been demonstrated, along with a slower increase in lactate and milder acidosis (X. Yang et al., 2010; Yellon et al., 1993). In patients undergoing vascular surgery, including those with LEAD, RIPC markedly influenced the acylcarnitine profile, with notable reductions in metabolites, such as propionylcarnitine, indicating potential mitochondrial protection through metabolic modulation (Kasepalu et al., 2020a). Additionally, in the same cohort, there was a significant correlation between the kynurenine/tryptophan ratio and markers of cardiac and renal injury, highlighting the potential protective role of this pathway in cardio- and nephroprotection (Eerik et al., 2022). Evidence from Günaydin et al. suggests that RIPC also enhances anaerobic glycolysis in patients undergoing coronary artery surgery (Günaydin et al., 2000). Additionally, metabolomics provides insights into subtle cellular changes induced by RIPC, extending beyond the detection capabilities of conventional biomarkers for organ damage. Furthermore, it facilitates the identification of circulating biomarkers related to NO bioavailability, such as L-arginine (Arg), asymmetric dimethylarginine (ADMA) (an endogenous inhibitor of all isoforms of NOS) and the Arg/ADMA ratio, explaining the vascular response mechanisms across various clinical contexts (Böger et al., 1997; Hulin et al., 2020; Lüneburg et al., 2014; Wahlstrøm et al., 2023).

To effectively harness the therapeutic potential of RIPC in LEAD patients undergoing lower limb DSA and endovascular treatment, future research should focus on how RIPC alters systemic metabolomics related to oxidative stress, inflammation and organ damage. Detailed metabolomic profiling before and after RIPC will help identify crucial biomarkers and underlying mechanisms that mitigate vascular injury. Such insights are essential for developing targeted interventions that improve patient outcomes in vascular health. This approach promises to refine therapeutic strategies and enhance our understanding of RIPC's systemic benefits in LEAD patients.

2.6.1. Structural and Functional Roles of Phosphatidylcholines and Lysophosphatidylcholines

Lysophosphatidylcholines (LysoPCs) and phosphatidylcholine (PCs) are phospholipids that serve as essential components of cell membranes and play crucial roles in various cellular functions (Polonis et al., 2020). PCs modulate membrane fluidity and participate in cell signalling; they also are important components of the lung surfactant and regulate lipid homeostasis in the liver (Agudelo et al., 2020; Furse & de Kroon, 2015). PCs are primarily synthesised in the endoplasmic reticulum through two main pathways: the cytidine 5-diphosphocholine (CDP-choline) pathway and the phosphatidylethanolamine methyl transferase (PEMT) pathway (Louise, 2022). PC is composed of a glycerol backbone, two fatty acid chains, a phosphate group and a choline molecule. A fatty acyl residue, connected by an ester bond, is represented by an "a" in the name of PC. If the fatty alcohol residue in the sn-1 position is present instead of a fatty acyl residue, the name of the PC includes an "e." The first number in the names of PC and lysoPC indicates the total number of carbon atoms, while the second number after the colon represents the number of double bonds. PCs undergo hydrolysis by phospholipase A2 (PLA2), resulting in the formation of lysoPCs and fatty acids (Kennelly et al., 2018). LysoPCs are also formed by lecithin cholesterol acyltransferase (LCAT) activity in HDL and oxidation of LDL (Rao et al., 2013). Unlike PC, lysoPC contains a glycerol backbone with only one fatty acid chain, a phosphate group and a choline molecule. LysoPCs play a role in various physiological processes, including cell signalling, inflammation and immune responses. The role of lysoPC in clinical settings is conflicting, as both its pro- and anti-inflammatory properties have been reported previously (J. Y. Kim et al., 2013; Law et al., 2019; Paapstel & Kals, 2022). Understanding the biochemical pathways and functions of PCs and LysoPCs enhances our ability to pinpoint therapeutic targets and develop strategies for intervention in diseases characterised by lipid metabolism disorders.

2.7. Summary of the Literature Review

RIPC has shown considerable promise as a non-invasive intervention in the management of LEAD by mitigating the effects of IRI. Studies have consistently highlighted RIPC's ability to preserve NO bioavailability essential for regulating vascular tone, maintaining endothelial function and inhibiting platelet aggregation, critical areas compromised in LEAD due to reduced NO availability. This preservation is predominantly facilitated through the NO/PKG pathway, which involves phosphorylation of downstream targets crucial for mitochondrial function, reducing OxS and enhancing endothelial cell survival. Additional pathways like RISK and SAFE further enrich RIPC's protective mechanism, providing a complex network of interactions crucial for its effectiveness.

While these findings have established a robust foundation for RIPC's utility, they also illuminate several significant gaps in our understanding, which the current research aims to address:

- Firstly, the precise molecular intermediaries within these pathways and their interactions remain largely unidentified. This gap in knowledge suggests that a deeper exploration into the molecular dynamics initiated by RIPC could significantly enhance the predictability and effectiveness of treatments. Thus, our first specific aim is to evaluate the effect of RIPC on arterial stiffness in patients undergoing procedures like DSA and endovascular interventions, which are common in LEAD management. Arterial stiffness is a known consequence of altered vascular dynamics and structural changes in the vascular matrix, which RIPC might ameliorate by improving endothelial function and microcirculatory flow through enhanced NO bioavailability.
- Secondly, while the reduction of OxS and inflammation through RIPC has been observed, the extent and nature of these effects are not fully quantified in clinical settings particularly in LEAD patients undergoing DSA and endovascular treatment. Therefore, our second aim is to assess RIPC's impact on organ damage, OxS and inflammation, providing a clearer picture of its systemic effects and potential for reducing periprocedural complications.
- Lastly, the emerging field of metabolomics offers a new lens through which to view the biochemical changes induced by RIPC. By investigating these systemic metabolomic alterations, we can identify novel biomarkers and therapeutic targets that could further refine RIPC protocols and personalise therapy based on individual metabolic responses. Hence, our third aim is to unravel these potential systemic metabolomic alterations in LEAD patients undergoing lower limb procedures.

Through these specific aims, this study seeks to close the critical gaps identified in the literature, thereby advancing our understanding of RIPC's mechanisms and enhancing its application in clinical practice. Each aim not only addresses a direct need highlighted by previous studies but also builds on the existing knowledge to push the boundaries of how RIPC can be utilised to improve patient outcomes in LEAD. This integrative approach ensures that our research is both grounded in empirical evidence and geared towards tangible clinical advancements.

3. AIMS OF THE STUDIES

The general aim is to identify novel specific targets and pathways that may be associated with the protective effects of RIPC intervention, thereby advancing the understanding of the mechanisms by which RIPC might improve outcomes for patients with LEAD.

Specific aims:

- To evaluate the effect of RIPC on arterial stiffness in patients with LEAD undergoing lower limb DSA and endovascular treatment.
- To assess the effect of RIPC on organ damage, oxidative stress and inflammation in patients with LEAD undergoing lower limb DSA and endovascular treatment.
- To unravel the potential systemic metabolomic alterations induced by RIPC in patients with LEAD undergoing lower limb DSA and endovascular treatment.

4. SUBJECTS AND METHODS

4.1. Study Population

The study recruited hospitalised patients who had previously been diagnosed with symptomatic LEAD. These patients were scheduled to undergo lower limb DSA and/or endovascular interventions. Enrolment was conducted in a non-consecutive manner at the Department of Vascular Surgery, Tartu University Hospital, Estonia, from February 2016 to March 2019. To participate, all individuals were required to provide written informed consent in their native language, ensuring their voluntary and informed participation.

The study excluded individuals under the age of 18, those with an estimated glomerular filtration rate less than 30 ml/min/1.73 m^2 measured at hospital admission, and individuals simultaneously participating in other clinical trials. Patients with upper limb conditions that would limit the use of a cuff were also excluded. Other exclusion criteria were those with an active malignant tumour (either in remission for less than five years or receiving ongoing treatment), documented allergic reactions to iodinated contrast agents, acute infections (body temperature 38 ºC or higher, CRP 50 mg/L or higher) and cardiac rhythm abnormalities (atrial fibrillation, frequent supraventricular and ventricular complexes). Participants were also excluded if they were receiving home-based oxygen treatment, were unable to lie supine for 40 minutes, had undergone vascular surgery in the axillary region, or had documented upper limb deep vein thrombosis.

4.2. Study Design and Eligibility

This study was conducted as a single-centre, prospective, randomised, double-blinded and sham-controlled clinical trial. The study was approved by the Research Ethics Committee of the University of Tartu (protocol nr. 252/M-24) on 10.11.2015 and was registered in the ClinicalTrials.gov database prior to the study (ID NCT02700958).

4.3. Methods

4.3.1. The Remote Ischaemic Preconditioning and Sham Interventions

The RIPC and sham interventions were administered by the study director, who is also the author of this academic thesis, using a standard calibrated blood pressure cuff on the participant's upper arm. In the RIPC intervention, the cuff was inflated to 200 mmHg for a period of five minutes. However, if the participant's systolic blood pressure exceeded 180 mmHg prior to the intervention, the cuff

was inflated to a level 20 mmHg above the systolic pressure. The sham intervention was conducted by inflating the cuff to 20 mmHg for five minutes. Both the RIPC and sham interventions consisted of a cycle of inflation and deflation, repeated four times, with a five-minute perfusion period in between each cycle to ensure normal blood flow (Zarbock et al., 2015).

4.3.2. Digital Subtraction Angiography and Endovascular Treatment

Although the catheterisation laboratory's procedure schedule was planned in advance, the exact start times for DSA or endovascular treatments could be unpredictable. Additionally, unexpected emergencies necessitated schedule adjustments, resulting in delays between the initiation of the RIPC or sham interventions and the subsequent DSA or endovascular inteventions. To maintain consistency, interventions were applied as close as possible prior to the subsequent DSA or endovascular intevention. All DSA and endovascular interventions were performed under local anaesthesia with lidocaine. No alterations were made to the standard treatment protocols of the patients in either the RIPC or the sham intervention groups because of their participation in this study. All medical interventions were carried out according to the established clinical guidelines and best practices (Aboyans et al., 2018; European Stroke Organisation et al., 2011).

4.3.3. Randomisation

The randomisation sequence was generated using the WINPEPI computer program (Version 11, Abramson J.H., Jerusalem, Israel) prior to the onset of the study. The stratified permuted-block randomisation technique was utilised, forming six strata by combining age (≥75 or <75 years) and the latest available estimated glomerular filtration rate (≥90, 60–89, or 30–59 ml/min/1.73 m^2). Block sizes were set to randomly alternate between 2 and 4 to reduce predictability and balance treatment allocation within each stratum. The generated sequences were manually sealed into opaque envelopes by an independent third party, with each envelope labelled with the stratum number and sequence order. Immediately before the initiation of the intervention, the study director opened the envelope corresponding to the participant's age and estimated GFR at hospital admission.

4.3.4. Blinding

To ensure blinding of the participants, the cuff's pressure gauge was concealed during the intervention. The medical personnel in charge of treatment of the participant were informed about the patient's consent to participate in the trial but were kept blinded to the specific intervention applied. Furthermore, the personnel tasked with evaluating arterial stiffness and peripheral and central haemodynamics, as well as those involved in the collection and analysis of biomarker

levels from blood and urine samples, operated in a blinded manner, without any knowledge of the assigned intervention.

4.3.5. Outcomes

The primary study outcomes included changes in the augmentation index (AIx), AIx adjusted for a heart rate of 75 (AIx@75) and carotid-femoral pulse wave velocity (PWV). Following a power analysis, based on the initial 30 participants treated as a pilot study (15 patients in both the RIPC and sham groups), AIx@75 was selected as the primary outcome.

4.3.5.1. Parameters of arterial stiffness and peripheral and central haemodynamics – Paper I

Arterial stiffness and haemodynamic profile were assessed through a combination of measures, including PWV, AIx, AIx@75, small and large artery elasticity indices (C1 and C2, respectively) and central and peripheral diastolic and systolic blood pressure. Baseline measurements were taken by the study director in the evening before the onset of the study. Follow-up measurements were taken by study group member 24 hours after DSA and endovascular intervention. Patients were in a resting state and had abstained from eating and smoking for at least three hours prior to the assessments.

Augmentation indices and PWV were derived non-invasively using the Sphygmocor XCEL PWA & PWV device (AtCor Medical, software version 1.2, Sydney, Australia). To assess augmentation indices, the machine captured the brachial waveform using a partially inflated cuff. This data was then processed by the SphygmoCor generalised transfer function to generate the central aortic waveform. The AIx, AIx@75, central blood pressure, pulse pressure and mean arterial pressure were subsequently calculated. If the variance between the first two measurements exceeded 2 units (%) for AIx or 1 unit (%) for AIx@75, a third measurement was performed and the median value of the three measurements was taken as the final value. If the variability of the augmentation indices remained within the acceptable range, the mean values were computed. Likewise, the mean values of blood pressure and heart rates were utilised for subsequent calculations. For the assessment of PWV, simultaneous recordings of pulse waves were conducted from the carotid and femoral arteries. This was achieved through the partial inflation of a cuff placed on the patient's thigh, in combination with the use of applanation tonometry on the carotid artery. The measurement process was repeated if the discrepancy between two separate readings exceeded 0.5 metres per second.

The elasticity indices of large and small arteries were obtained for radial arteries using the HDI/PulseWave CR-2000 Research CardioVascular Profiling System (Hypertension Diagnostics, Inc., Eagan, MN, USA). Two measurements with a maximum difference of 2 in C2 and 3 in C1 were acquired. The mean value was then used in the final analysis.

4.3.5.2. Biochemical analysis of blood and urine – Paper II

Blood and urine samples were collected in the morning before the intervention and 24 hours after, ensuring that the patient had fasted for at least three hours. These samples were tested for cardiac and renal biomarkers, OxS and inflammation.

These samples were examined at the United Laboratories of Tartu University Hospital, Tartu, including high-sensitivity CRP (hs-CRP), NT-proBNP, CK-MB, hs-TnT, urea, creatinine, cystatin C, beta-$_2$ microglobulin (B2M), cholesterol, high-density lipoprotein (HDL), LDL, triglycerides and neutrophil gelatinase-associated lipocalin (NGAL). For the analysis of OxS and inflammation biomarkers like hs-CRP, IL-18, oxidised low-density lipoprotein (ox-LDL), adiponectin and MPO serum was extracted from the collected blood samples. These serum samples were subsequently stored at -80 °C until they could be measured in the Department of Biochemistry at the University of Tartu. For the analysis of isoprostanes, urinary creatinine, liver-type fatty acid-binding protein (L-FABP) and kidney injury molecule-1 (KIM-1), urine samples were kept in similar conditions until the samples were evaluated at the same department.

4.3.5.3. Targeted serum metabolite profiling – Paper III

Quantitative and targeted metabolomics assays were performed using the AbsoluteIDQ p180 kit (Biocrates Life Sciences AG, Innsbruck, Austria). The analytical procedure was carried out according to the manufacturer's standard protocol at the laboratory of the Department of Biochemistry, University of Tartu, Tartu. Measurements were made using a QTRAP 4500 (ABSciex, Framingham, MA, USA), linked to an Agilent 1260 series HPLC (Agilent Technologies, Santa Clara, CA, USA) with a C18 column and flow injection analysis.

4.3.6. Statistical Analysis

4.3.6.1. Sample size calculation – Papers I, II and III

Prior to initiation of the trial, the impact of RIPC on PWV or augmentation indices (AIx and AIx@75) had not been studied. The necessary sample size was determined by initially recruiting 15 patients for each of the RIPC and sham groups. Anticipating a 5% effect size difference in AIx@75 with use of a one-tailed Welch's t-test, a need for at least 47 patients in each group was established. This number would achieve an 80% statistical power with a significance level (alpha) of 0.05. A power analysis was not performed for metabolomic and organ damage markers.

4.3.6.2. Statistical analysis of non-metabolomics data – Papers I and II

Continuous variables between two groups were analysed using the two-sample t-test when the data followed a normal distribution, and the Wilcoxon rank-sum test was used when the distribution was non-normal. For assessing changes within a single group, the paired t-test was applied to normally distributed data, whereas the Wilcoxon signed-rank test was utilised for data with non-normal distributions. To assess the normality of data distribution, we employed the Kolmogorov-Smirnov test. Categorical variables were compared with the Pearson's chi-squared test. For statistical analysis of multiple repeated measures, one-sided or two-sided analysis of variance (ANOVA) was used, where appropriate. A p-value of less than 0.05 was considered to indicate significance in all tests. In our statistical analysis, we refined the AIx using a multivariable regression model that accounted for group assignment as a variable. We controlled for covariates including mean arterial pressure, sex, age, heart rate and height to evaluate their influence on the outcome, given their established roles as significant covariates (Janner et al., 2012; Pichler et al., 2016).

To evaluate the impact of RIPC and stent placement on arterial stiffness indices (AIx and AIx@75), we employed a two-way ANOVA to assess interaction and main effects of treatment and stent placement. This was complemented by calculating Cohen's d to quantify the effect sizes, enabling a direct comparison of the intervention's impact across groups and time points. These analyses enabled the determination of statistically and clinically relevant changes attributable to the interventions.

For the comparison of haemodynamic and arterial stiffness parameters, we strictly employed the per-protocol (PP) analysis. This approach ensured that our evaluations were based on adherence to the treatment protocol, thereby giving us an accurate measure of the intervention's effectiveness under ideal circumstances.

In analysing organ damage, inflammation and OxS biomarkers, we employed both intention-to-treat (ITT) and PP analyses. The ITT analysis, which considers both known and unknown factors, was completed using the arithmetic mean imputation method for any missing outcomes. No adjustments to baseline were deemed necessary for this analysis. The PP analysis was then conducted, with an additional comparison being provided by adjusting individual markers to their corresponding baseline values using a linear regression model, which included these baseline values as covariates to further reinforce the findings from the ITT analysis.

4.3.6.3 Statistical analysis of metabolomics data – Paper III

To address inter-kit variability, a common challenge in metabolomics caused by minor variations in reagents or manufacturing changes, batch correction techniques were applied to ensure data consistency and accuracy across different batches. This ensured that observed differences in the data were due to experimental conditions rather than discrepancies in the kits, thereby enhancing the reliability of the results across various kit batches.

When analysing the metabolomic profile, a PP approach was used. Metabolites that had less than 67 percent of valid results, including instances of excessive missing concentration values or values below the lower limit of detection, were excluded from the analysis. After data cleaning, missing metabolites were imputed using the random forest method with the missForest package (Stekhoven & Bühlmann, 2012) in R version 4.2.2 (R Core Team, Vienna, Austria).

Given the non-parametric nature of the metabolomics data, non-parametric tests were employed to ensure the validity of the analysis in the presence of multiple outliers and deviations from normality, as these are better handled by non-parametric methods. The RIPC and sham groups were compared using the Mann-Whitney U test. To account for multiple comparisons, we utilized the Benjamini-Hochberg procedure to manage the false discovery rate effectively where appropriate. For assessing correlations between significant metabolites and significant haemodynamic, organ damage, inflammation and OxS markers from Papers I and II, Spearman's correlation coefficient was employed. Analyses were performed using SPSS version 25 (IBM Corporation, Armonk, NY, USA).

5. RESULTS

In total, 127 eligible non-consecutive patients were invited to participate in the trial. Of these, 54 were allocated to the RIPC group and 57 to the sham group and were included in the ITT analysis (Figure 1). Eleven patients declined to participate and five were taken to angiography before randomisation. Primary outcome data were obtained from 47 patients in the RIPC group and 55 in the sham group. Due to the inability to obtain necessary blood samples, two patients were excluded, leaving 100 (90%) patients in the PP analysis of the secondary endpoints.

The median time from the beginning of intervention to the beginning of DSA or DSA-endovascular intervention was 80 minutes (IQR 60–118) in the RIPC group and 79 minutes (IQR 64–112) in the sham group (p=0.38). There was no significant difference between the RIPC and the sham intervention regarding the time spent for DSA and endovascular intervention (p=0.11) or the time from the beginning of intervention to the time blood samples were collected (23 h 49 min and 24 h 13 min; p=0.18, respectively). Of the patients included in the final analysis, 21 patients (44.7%) from the RIPC group and 22 patients (40.0%) from the sham group received only DSA (p=0.78), and both DSA and endovascular intervention were administered to 26 patients (55.3%) in the RIPC group and 33 patients (60.0%) in the sham group (p=0.78). At least one stent was placed to 22 (47.8%) patients in the RIPC group and to 30 (55.6%) patients in the sham group (p=0.44).

In the ITT population, we observed no significant difference in the baseline characteristics (Table 1). The baseline values for both the primary outcome and the haemodynamic parameters did not significantly differ in the PP analysis (Table 2). However, in the PP analysis of the population undergoing analysis of organ damage, inflammation, OxS biomarkers and metabolomic profiles, the baseline values for NT-proBNP and NGAL were significantly higher in the RIPC group (Table 3). Additionally, baseline measurements revealed significant differences in the levels of specific metabolites (glutamate, kynurenine-to-tryptophan ratio and PC ae C30:2), but these differences became non-significant after adjusting for multiple testing.

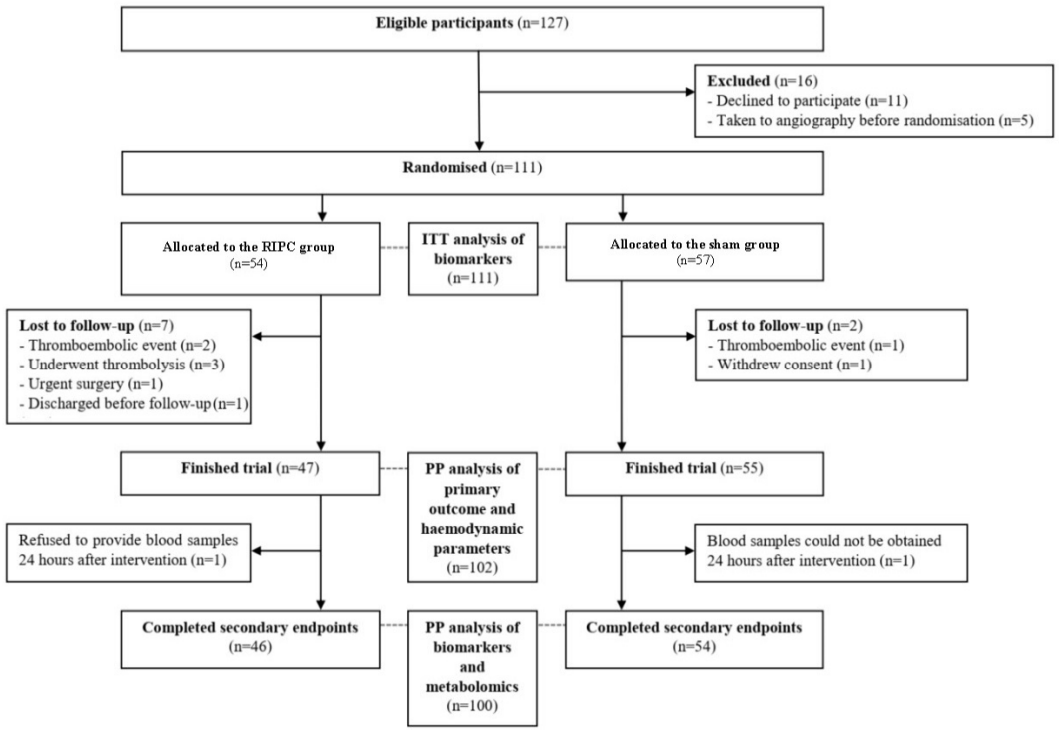


Figure 1. Flow diagram of the patient enrolment. ITT: intention-to-treat; PP: per-protocol

Table 1. Baseline characteristics of the study population included in the intention-to-treat analysis.

	RIPC (n=54)		Sham (n=57)		p-value
Characteristics	**Mean/ median**	**SD/(IQR)**	**Mean/ median**	**SD/(IQR)**	
Demographic					
Male (n)	39 (72.2%)		48 (84.2%)		0.19
Mean age (y)	65.5	±10.1	65.3	±11.9	0.90
Weight (kg)	76.6	±17.5	78.2	±17.1	0.62
Body mass index (kg/m^2)	25.4	(23.0–30.0)	25.7	(23.5–29.4)	0.79
Renal function at inclusion					
eGFR <90 (ml/min/1.73 m^2) (n)	30 (55.6%)		32 (56.1%)		1.0
60–89 (ml/min/1.73 m^2) (n)	20 (37.0%)		20 (35.1%)		
30–59 (ml/min/1.73 m^2) (n)	10 (18.5%)		12 (17.5%)		
History of smoking (n) †	42 (77.8%)		42 (73.7%)		0.78
Concomitant diseases					
Stage of LEAD III or more ‡	27 (50.0%)		27 (47.4%)		0.93
Stage of LEAD III (n) ‡	11 (20.4%)		10 (17.5%)		
Stage of LEAD IV (n) ‡	16 (29.6%)		17 (29.8%)		
Diabetes (n)	12 (22.2%)		15 (26.3%)		0.78
Hypertension (n) ◊	35 (64.8%)		31 (54.4%)		0.36
Medications					
ACE inhibitors (n)	20 (37.0%)		16 (28.1%)		0.31
ARB (n)	14 (25.9%)		11 (19.3%)		0.40
Calcium channel blockers (n)	18 (33.3%)		17 (29.8%)		0.69
Beta blockers (n)	13 (24.1%)		15 (26.3%)		0.79
Diuretics (n)	18 (33.3%)		14 (24.6%)		0.31
Antiagregants (n)	29 (53.7%)		26 (45.6%)		0.39
Anticoagulants (n)	1 (1.9%)		2 (3.5%)		0.59
Naftidrofuryl/pentoxifylline (n)	37 (68.5%)		36 (63.2%)		0.55
Statins (n)	20 (37.0%)		16 (28.1%)		0.38
Insulin therapy (n)	7 (13.0%)		8 (14.0%)		0.87
Oral antidiabetic agents (n)	4 (7.4%)		7 (12.3%)		0.39
PSBP (mmHg)	144.3	±21.9	139.9	±18.3	0.25
PDBP (mmHg)	78.0	±11.8	75.9	±9.8	0.32
Heart rate (bpm)	66.1	±10.2	67.6	±10.3	0.46
WBC (10^9/L)	7.04	±2.00	6.99	±1.79	0.90
RBC (10^{12}/L)	4.57	±0.44	4.60	±0.44	0.75
HGB (g/L)	136.1	±16.8	141.6	±14.4	0.07
Hct (%)	40.3	±6.5	42.0	±3.9	0.10
PLT (10^9/L)	252.5	±85.3	231.5	±56.2	0.13
hs-TnT (ng/L)	9.9	(6.8–15.7)	11.2	(6.6–17.6)	0.70
CK-MB mass (µg/L)	1.8	(1.5–2.6)	1.9	(1.6–2.8)	0.39
NT-proBNP (pg/mL)	168	(93–448)	94	(50–376)	0.13
hs-CRP (mg/L)	2.54	(1.65–6.15)	3.02	(1.51–5.26)	0.84
Glucose (mmol/L)	5.1	(4.8–5.7)	5.3	(4.9–6.2)	0.23

	RIPC (n=54)		Sham (n=57)		p-value
Characteristics	**Mean/ median**	**SD/(IQR)**	**Mean/ median**	**SD/(IQR)**	
Creatinine (μmol/L)	76	(65–87)	78	(67–92)	0.90
Urea (mmol/L)	5.0	(4.3–6.6)	5.6	(4.4–6.6)	0.54
Cystatine C (mg/L)	1.11	(0.96–1.36)	1.10	(0.93–1.31)	0.81
Cholesterol (mmol/L)	4.74	±1.38	4.83	±1.39	0.73
HDL (mmol/L)	1.19	(0.97–1.56)	1.10	(0.92–1.43)	0.24
LDL (mmol/L)	2.76	(2.10–3.67)	3.01	(2.07–3.90)	0.70
TG (mmol/L)	1.33	(1.05–1.96)	1.43	(1.12–1.98)	0.45
B2M (μg/L)	2470	(2042–2870)	2180	(1870–2780)	0.14
eGFR (mL/min/1.73 m^2)	86	(71–95)	91	(68–100)	0.39
Adiponectine (ng/mL)	5808	(3419–8507)	5619	(3327–7654)	0.66
MPO (ng/mL)	58.7	(33.5–85.3)	52.5	(30.7–88.8)	0.54
NGAL (ng/mL)	81.8	(63.5–101.5)	71.9	(65.0–83.2)	0.08
Ox-LDL (U/L)	56.0	(45.7–73.7)	65.5	(44.0–79.3)	0.29
KIM-1 (pg/mL)	1406	(738–2354)	1440	(839–2407)	0.93
L-FABP (ng/mL)	0.85	(0.67.1.52)	0.87	(0.62–1.51)	0.86
Isoprostane/creatinine ratio (ng/mg)	41.0	(33.1–50.1)	45.5	(32.9–61.0)	0.32
IL-18 (pg/mL)	276	(231–361)	283	(201–348)	0.86

† – current and ex-smokers; ‡ – Stage of lower extremity artery disease by Fontaine's classification; ◊ – on medication; y – years of age; RIPC – remote ischaemic preconditioning; SD – standard deviation; IQR – interquartile range; eGFR – estimated glomerular filtration rate; LEAD – lower extremity artery disease; ACE – angiotensin-converting enzyme; ARB – angiotensin receptor blocker; PSBP – peripheral systolic blood pressure; PDBP – peripheral diastolic blood pressure; WBC – white blood cells; RBC – red blood cells; HGB – haemoglobin; Hct – haematocrit; PLT – platelet; hs-TnT – high-sensitivity troponin T; CK-MB – creatine kinase-myocardial band; NT-proBNP – N-terminal pro b-type natriuretic peptide; hs-CRP – high-sensitivity C-reactive protein; HDL – high-density lipoprotein; LDL – low-density lipoprotein; TG – triglycerides; B2M – beta-2 microglobuline; MPO – myeloperoxidase; NGAL – neutrophil gelatinase-associated lipocalin; Ox-LDL – oxidised low-density lipoprotein; KIM-1 – kidney injury molecule-1; L-FABP – liver-type fatty acid-binding protein; IL – interleukin.

Table 2. Baseline characteristics of the study population included in the per-protocol analysis of primary outcome and haemodynamic parameters.

	RIPC (n=47)		Sham (n=55)		p-value
Characteristics	**Mean/ median**	**SD/(IQR)**	**Mean/ median**	**SD/(IQR)**	
Demographic					
Male (n)	33 (70.2%)		48 (87.3%)		0.060
Mean age (y)	66.1	±10.2	65.1	±11.4	0.66
Weight (kg)	75.7	±17.5	78.4	±16.8	0.44
Body mass index (kg/m^2)	25.6	(22.8–30.3)	25.7	(23.5–29.4)	0.69
Concomitant diseases					
History of smoking (n) †	36 (76.7%)		41 (74.5%)		0.99
Stage of LEAD III or more ‡	24 (51.1%)		26 (47.3%)		0.86
Stage of LEAD III (n) ‡	9 (19.1%)		10 (18.2%)		
Stage of LEAD IV (n) ‡	15 (31.9%)		16 (29.1%)		
Diabetes (n)	11 (23.4%)		14 (25.5%)		0.75
eGFR <90 (ml/min/1.73 m^2) (n)	28 (59.6%)		31 (56.4%)		0.90
60–89 (ml/min/1.73 m^2) (n)	19 (40.4%)		20 (36.4%)		
30–59 (ml/min/1.73 m^2) (n)	9 (19.1%)		11 (20.0%)		
Hypertension (n) ◊	33 (74.5%)		29 (56.4%)		0.11
Statin usage (n)	19 (42.6%)		16 (29.1%)		0.32
Haemodynamic parameters					
PSBP (mmHg)	144.0	±22.0	138.7	±17.1	0.19
PDBP (mmHg)	77.1	±12.0	75.7	±9.7	0.54
CSBP (mmHg)	132.2	±19.2	126.3	±13.8	0.084
CDBP (mmHg)	78.1	±11.9	76.8	±9.8	0.54
MAP (mmHg)	97.9	±13.3	94.7	±10.7	0.19
PPP (mmHg)	65	(53.5–79.0)	62	(54.5–69.0)	0.20
CPP (mmHg)	53	(42.5–65.7)	47	(42.2–54.2)	0.086
CPP/PPP	0.81	±0.05	0.79	±0.06	0.084
AIx (%)	36.6	±11.8	33.9	±11.8	0.25
AIx@75 (%)	31.8	±11.8	30.0	±11.8	0.45
Heart rate (1/min)	65.2	±9.8	67.3	±10.4	0.29
C1 (ml/mmHg × 10) *	11.7	(9–13.72)	12.2	(8.75–14.75)	0.33
C2 (ml/mmHg × 100) *	2.7	(1.45–4.08)	3.2	(2.35–4.08)	0.50
SVR (dyn × s × cm^{-5}) *	1564	(1326–1945)	1588	(1404–1831)	0.87
PWV (m/s) *	8.8	(8–10.2)	8.9	(7.7–10.0)	0.67

† – current and ex-smokers; ‡ – Stage of lower extremity artery disease by Fontaine's classification; ◊ – on medication; y – years of age; RIPC – remote ischaemic preconditioning; SD – standard deviation; IQR – interquartile range; LEAD – lower extremity artery disease; eGFR – estimated glomerular filtration rate; PSBP – peripheral systolic blood pressure; PDBP – peripheral diastolic blood pressure; CSBP – central systolic blood pressure; CDBP – central diastolic blood pressure; MAP – mean arterial pressure; PPP – peripheral pulse pressure; CPP – central pulse pressure; AIx – augmentation index; AIx@75 – augmentation index adjusted for a heart rate of 75; C1 – large artery elasticity index; C2 – small artery elasticity index; SVR – systemic vascular resistance; PWV – pulse wave velocity.

Table 3. Baseline characteristics of the study population included in the per-protocol analysis of organ damage, inflammation, oxidative stress biomarkers and metabolomic profile.

	RIPC (n=46)		Sham (n=54)		p-value
Characteristics	**Mean/ median**	**SD/(IQR)**	**Mean/ median**	**SD/(IQR)**	
Demographic					
Male (n)	33 (71.7%)		47 (87.0%)		0.10
Mean age (y)	66.1	±10.3	65.0	±11.4	0.61
Weight (kg)	75.2	±17.3	78.0	±16.7	0.42
Body mass index (kg/m^2)	25.4	(22.7–30.0)	25.3	(23.5–29.4)	0.66
Renal function at inclusion					
eGFR <90 (ml/min/1.73 m^2) (n)	27 (58.7%)		30 (55.6%)		0.91
60–89 (ml/min/1.73 m^2) (n)	19 (41.3%)		20 (37.0%)		
30–59 (ml/min/1.73 m^2) (n)	8 (18.5%)		10 (18.5%)		
History of smoking (n) †	35 (76.1%)		41 (75.9%)		1
Concomitant diseases					
Stage of LEAD III or more ‡	23 (50.0%)		25 (46.3%)		0.87
Stage of LEAD III (n) ‡	9 (19.6%)		10 (18.5%)		
Stage of LEAD IV (n) ‡	14 (30.4%)		15 (27.8%)		
Diabetes (n)	10 (21.7%)		13 (24.1%)		0.97
Hypertension (n) ◊	32 (69.6%)		28 (51.9%)		0.11
Medications					
ACE inhibitors (n)	18 (39.1%)		14 (25.9%)		0.16
ARBs (n)	12 (26.1%)		11 (20.4%)		0.50
Calcium channel blockers (n)	18 (39.1%)		15 (29.8%)		0.23
Beta blockers (n)	12 (26.1%)		12 (22.2%)		0.65
Diuretics (n)	16 (34.8%)		12 (22.2%)		0.16
Antiagregants (n)	24 (52.2%)		26 (48.1%)		0.69
Anticoagulants (n)	1 (2.2%)		1 (1.9%)		0.91
Naftidrofuryl/pentoxifylline (n)	33 (71.7%)		35 (64.8%)		0.46
Statins (n)	18 (39.1%)		16 (29.6%)		0.39
Insulin therapy (n)	6 (13.0%)		8 (14.8%)		0.80
Oral antidiabetic agents (n)	3 (6.5%)		5 (9.3%)		0.62
PSBP (mmHg)	144.2	±22.2	138.5	±17.2	0.17
PDBP (mmHg)	77.3	±12.1	75.7	±9.8	0.48
Heart rate (bpm)	65.2	±9.9	67.0	±10.2	0.37
WBC (10^9/L)	6.80	±1.74	6.99	±1.79	0.69
RBC (10^{12}/L)	4.56	±0.42	4.58	±0.44	0.92
HGB (g/L)	135.9	±16.3	141.6	±14.7	0.08
Hct (%)	41.0	±4.3	42.0	±4.0	0.21
PLT (10^9/L)	232.5	(187.8–280.2)	219.5	(190.2–264.2)	0.49
hs-TnT (ng/L)	9.9	(6.9–16.8)	11.1	(6.5–16.8)	1.0
CK-MB mass (µg/L)	1.8	(1.5–2.8)	2.0	(1.6–3.1)	0.32
NT-proBNP (pg/mL)	200	(92–463)	89	(45–292)	0.037
hs-CRP (mg/L)	2.23	(1.52–5.24)	2.81	(1.51–4.91)	0.57
Glucose (mmol/L)	5.1	(4.8–5.7)	5.3	(4.8–6.2)	0.51
Creatinine (μmol/L)	78	(65–92)	77	(67–92)	0.80
Urea (mmol/L)	5.0	(4.4–6.6)	5.5	(4.4–6.6)	0.76
Cystatine C (mg/L)	1.11	(0.97–1.36)	1.09	(0.93–1.26)	0.45
Cholesterol (mmol/L)	4.66	±1.38	4.85	±1.42	0.52

Characteristics	RIPC (n=46)		Sham (n=54)		p-value
	Mean/ median	SD/(IQR)	Mean/ median	SD/(IQR)	
HDL (mmol/L)	1.17	(0.96–1.55)	1.12	(0.94–1.45)	0.58
LDL (mmol/L)	2.70	(2.07–3.63)	3.02	(2.05–3.91)	0.50
TG (mmol/L)	1.30	(0.98–2.06)	1.43	(1.1–1.98)	0.36
B2M (μg/L)	2470	(2080–2840)	2145	(1830–2750)	0.06
eGFR (mL/min/1.73 m^2)	84	(68–94)	91	(69–100)	0.17
Adiponectine (ng/mL)	6322	(3769–8523)	5541	(3327–9406)	0.48
MPO (ng/mL)	57.8	(32.4–81.8)	51.4	(30.2–81.6)	0.58
NGAL (ng/mL)	82.1	(65.5–103.0)	71.8	(61.12–80.7)	0.019
Ox-LDL (U/L)	55.6	(45.3–71.1)	65.1	(43.5–79.3)	0.21
KIM-1 (pg/mL)	1392	(733–2215)	1455	(870–2432)	0.42
L-FABP (ng/mL)	0.85	(0.61–1.43)	0.81	(0.62–1.42)	0.74
Isoprostane/creatinine ratio (ng/mg)	40.8	(30.2–50.5)	44.6	(32.9–61.0)	0.29
IL-18 (pg/mL)	269	(230–364)	280	(196–334)	0.83

† – current and ex-smokers; ‡ – Stage of lower extremity artery disease by Fontaine's classification; ◊ – on medication; y – years of age; RIPC – remote ischaemic preconditioning; SD – standard deviation; IQR – interquartile range; eGFR – estimated glomerular filtration rate; LEAD – lower extremity artery disease; ACE – angiotensin-converting enzyme; ARB – angiotensin receptor blocker; PSBP – peripheral systolic blood pressure; PDBP – peripheral diastolic blood pressure; WBC – white blood cells; RBC – red blood cells; HGB – haemoglobin; Hct – haematocrit; PLT – platelet; hs-TnT – high-sensitivity troponin T; CK-MB – creatine kinase-myocardial band; NT-proBNP – N-terminal pro b-type natriuretic peptide; hs-CRP – high-sensitivity C-reactive protein; HDL – high-density lipoprotein; LDL – low-density lipoprotein; TG – triglycerides; B2M – beta-2 microglobuline; MPO – myeloperoxidase; NGAL – neutrophil gelatinase-associated lipocalin; Ox-LDL – oxidised low-density lipoprotein; KIM-1 – kidney injury molecule-1; L-FABP – liver-type fatty acid-binding protein; IL – interleukin.

5.1. Effects of Remote Ischaemic Preconditioning on Arterial Stiffness (Paper I)

RIPC had a significant effect on the AIx, showing a decrease of 5.46% compared to a decrease of 1.45% in the sham group (p=0.045) (Figure 2). This remained significant after adjustment for diabetes (p=0.047). After adjusting AIx for mean arterial pressure, sex, age, heart rate and height, the difference between the RIPC and sham groups was not statistically significant (p=0.11). No significant change was observed in AIx@75 (p=0.071) (Figure 2) or PWV (p=0.74) (Table 4) between groups.

Only in the RIPC group, AIx (p=0.001), AIx@75 (p=0.002), mean arterial pressure (p=0.014) and peripheral (p=0.023) and central systolic blood pressure (p=0.006) were significantly reduced 24 hours after DSA and endovascular intervention compared to the baseline measurements (Table 4). Both in the RIPC and sham groups, a significant change in carotid femoral PWV, central blood pressure and peripheral pulse pressure was observed 24 hours after DSA.

When compared to the sham control, a significant decrease in AIx (p=0.002) and AIx@75 (p=0.003) was observed in the RIPC group after stenting. These

decreases correspond to large effect sizes with Cohen's d values of -0.91 for AIx and -0.69 for AIx@75, indicating substantial reductions. Conversely, such changes were not observed in patients who did not receive stents, where the effects were minimal (AIx p=0.60; AIx@75 p=0.46) and Cohen's d values were small, at 0.15 for AIx and 0.21 for AIx@75 (Figure 3).

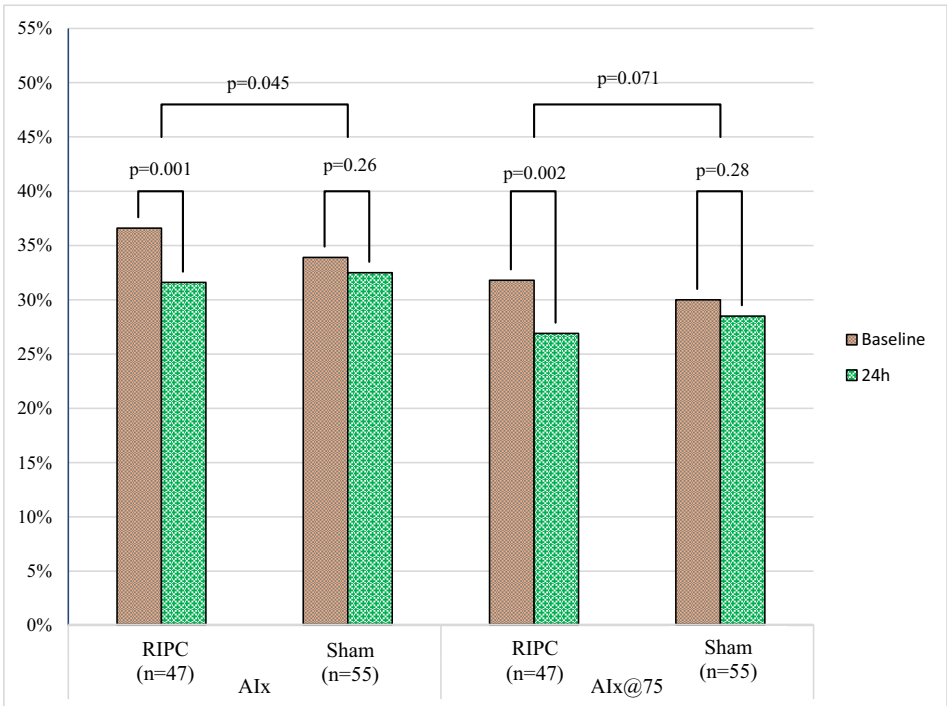


Figure 2. Comparison of augmentation index and heart rate-adjusted augmentation index at 75 bpm, before and 24 hours after DSA and endovascular intervention, in the RIPC and sham groups, expressed in percentages. Data were analysed using two-sample t-tests to compare differences between groups at each time point, and paired t-tests to assess changes within groups over time. P-values displayed above the bars indicate statistical significance of the changes, with $p < 0.05$ considered statistically significant.

Table 4. Changes from baseline in haemodynamic parameters 24 hours after the RIPC and sham interventions.

	RIPC (n=47)			Sham (n=55)			p-value
	Mean/ median	SD/(IQR)	p-value	Mean/ median	SD/(IQR)	p-value	
PSBP	-6.23	±18.21	0.023	-3.02	±13.78	0.11	0.32
PDBP	-1.81	±7.16	0.090	-0.69	±7.89	0.52	0.46
CSBP	-6.92	±16.42	0.006	-2.72	±11.72	0.091	0.15
CDBP	-1.81	±7.07	0.086	-0.55	±7.88	0.61	0.40
MAP	-3.70	±9.95	0.014	-1.04	±9.81	0.44	0.18
PPP	-4.43	±12.62	0.032	-2.33	±8.35	0.044	0.36
CPP	-5.11	±13.69	0.006	-2.17	±6.35	0.014	0.14
CPP/PPP	0.04	±0.10	0.008	0.02	±0.09	0.21	0.22
Heart rate	1.15	±8.41	0.35	-0.28	±9.93	0.84	0.44
C1*	0.3	(-1.5–2.3)	0.18	0.03	(-2.09–1.5)	1	0.32
C2*	-0.1	(-0.8–0.43)	0.34	-0.45	(-1.1–0.55)	0.053	0.37
SVR*	103.5	(-186.5–312.0)	0.28	-9.25	(-143.0–139.5)	0.94	0.29
PWV*	-0.3	(-0.85–0.1)	0.016	-0.35	(-0.95–0.4)	0.037	0.74

Change was calculated by subtracting the baseline measurement from the final measurement. * – non-normal distribution (median and IQR are presented instead of mean and ±SD); PSBP – peripheral systolic blood pressure; PDBP – peripheral diastolic blood pressure; CSBP – central systolic blood pressure; CDBP – central diastolic blood pressure; MAP-mean arterial pressure; PPP – peripheral pulse pressure; CPP – central pulse pressure; C1 – large artery elasticity index; C2 – small artery elasticity index; SVR – systemic vascular resistance; PWV – pulse wave velocity.

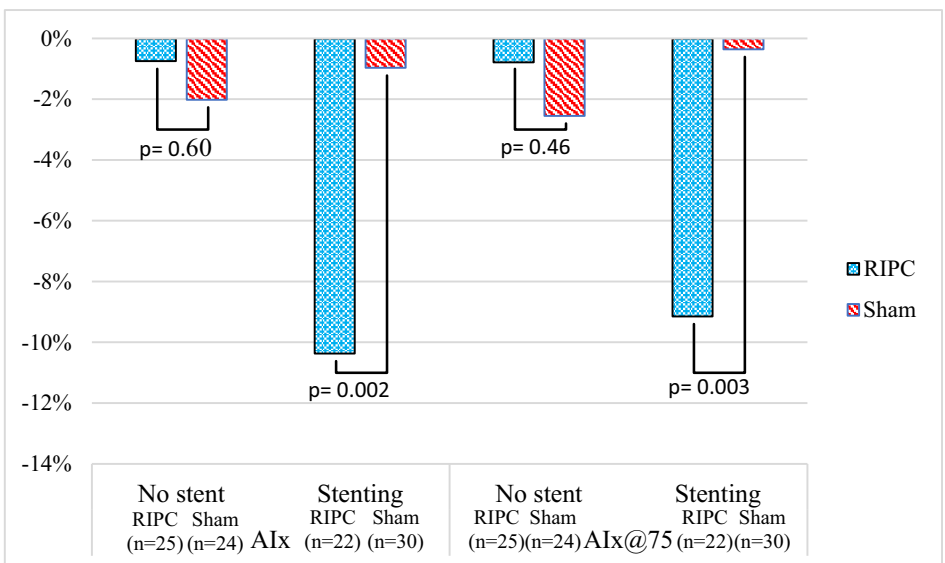


Figure 3. Impact of the RIPC and Sham procedures on changes in augmentation index (AIx) and augmentation index normalised to a heart rate of 75 (Alx@75) with and without stenting. Values are expressed as percentages. Change was calculated by subtracting the baseline measurement from the final measurement.

5.2. Effects of Remote Ischaemic Preconditioning on Preventing Organ Damage, Inflammation and Oxidative Stress (Paper II)

5.2.1. Changes in Oxidative Stress and Inflammation Markers

In the ITT analysis, hs-CRP levels did not significantly differ between the RIPC and the sham groups (p=0.45) (Figure 4a). A significant increase in hs-CRP levels was observed in both the RIPC ($p < 0.0001$) and sham groups ($p = 0.030$) 24 hours after DSA or endovascular treatment. Upon adjusting the PP analysis to baseline values, this increase remained significant only in the RIPC group (p=0.002) and not in the sham group (p=0.40). Notably, hs-CRP levels were significantly elevated in patients who had received stents in both the RIPC (p=0.036) and sham groups (p<0.0001) (Table 2).

In the ITT analysis, a significant increase in IL-18 levels occurred only in the sham group (p=0.020) and not in the RIPC group (p=0.88) (Figure 4b). There were no significant changes in IL-18 or ox-LDL levels when the RIPC group was compared to the sham group.

We did not see a significant change in the levels of urinary isoprostanes corrected for creatinine in the RIPC vs. the sham group ($p = 0.79$), but a significant decrease in the isoprostanes-creatinine ratio was noted only in the RIPC group both in the ITT ($p = 0.008$) and in PP ($p = 0.008$) analysis (Figure 4d).

ITT analysis revealed a significant increase in adiponectin levels only in the sham group (p=0.040). A significant difference in adiponectin levels was seen between the RIPC group and the sham group both in the ITT (p=0.020) and PP analysis (p=0.028) (Figure 4e).

MPO levels were significantly increased in both groups 24 hours after intervention ($p = 0.007$ in the RIPC group; p=0.015 in the sham group), but there was no significant difference between the groups (p=0.48) in the ITT analysis. Similar changes occurred also in the PP population before and after adjusting to baseline values (Figure 4f).

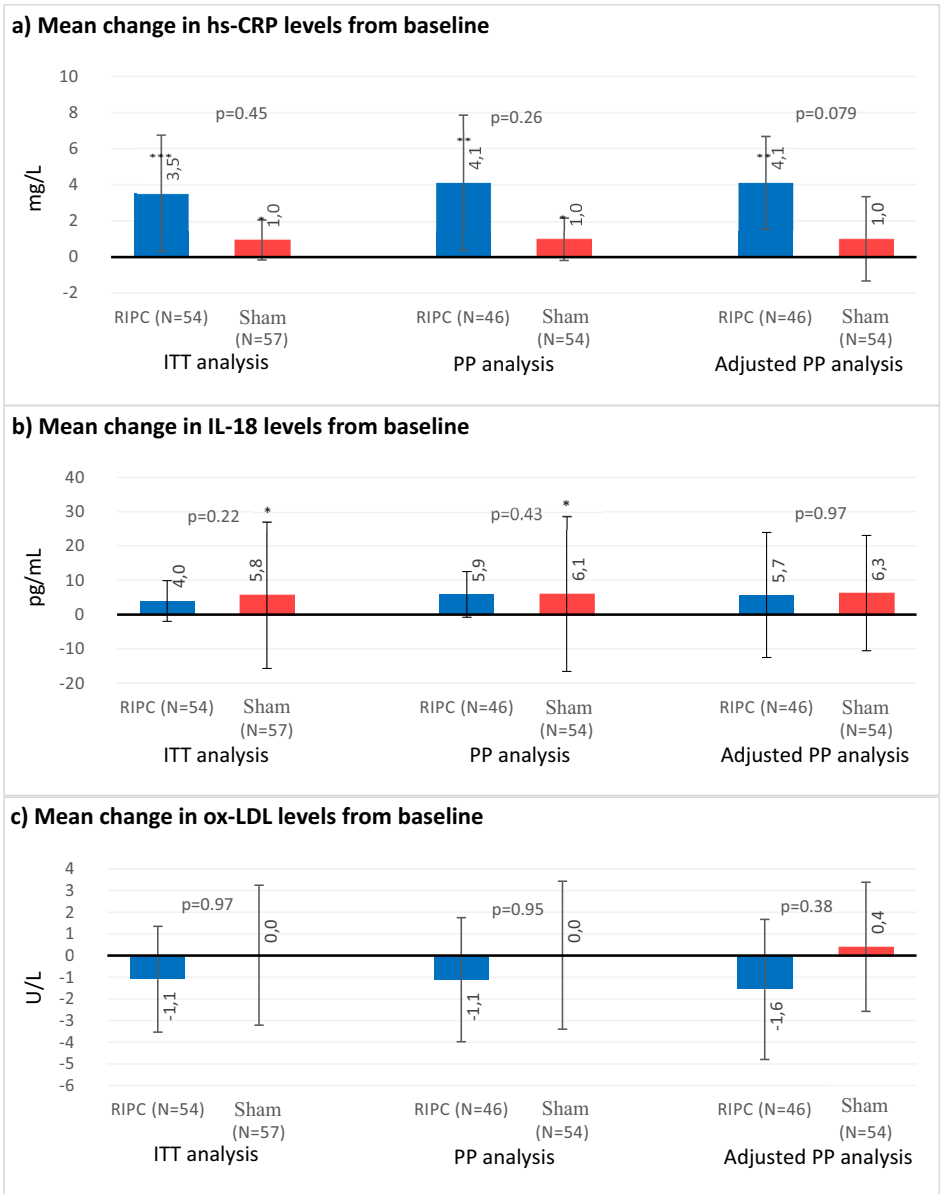


Figure 4. Continued

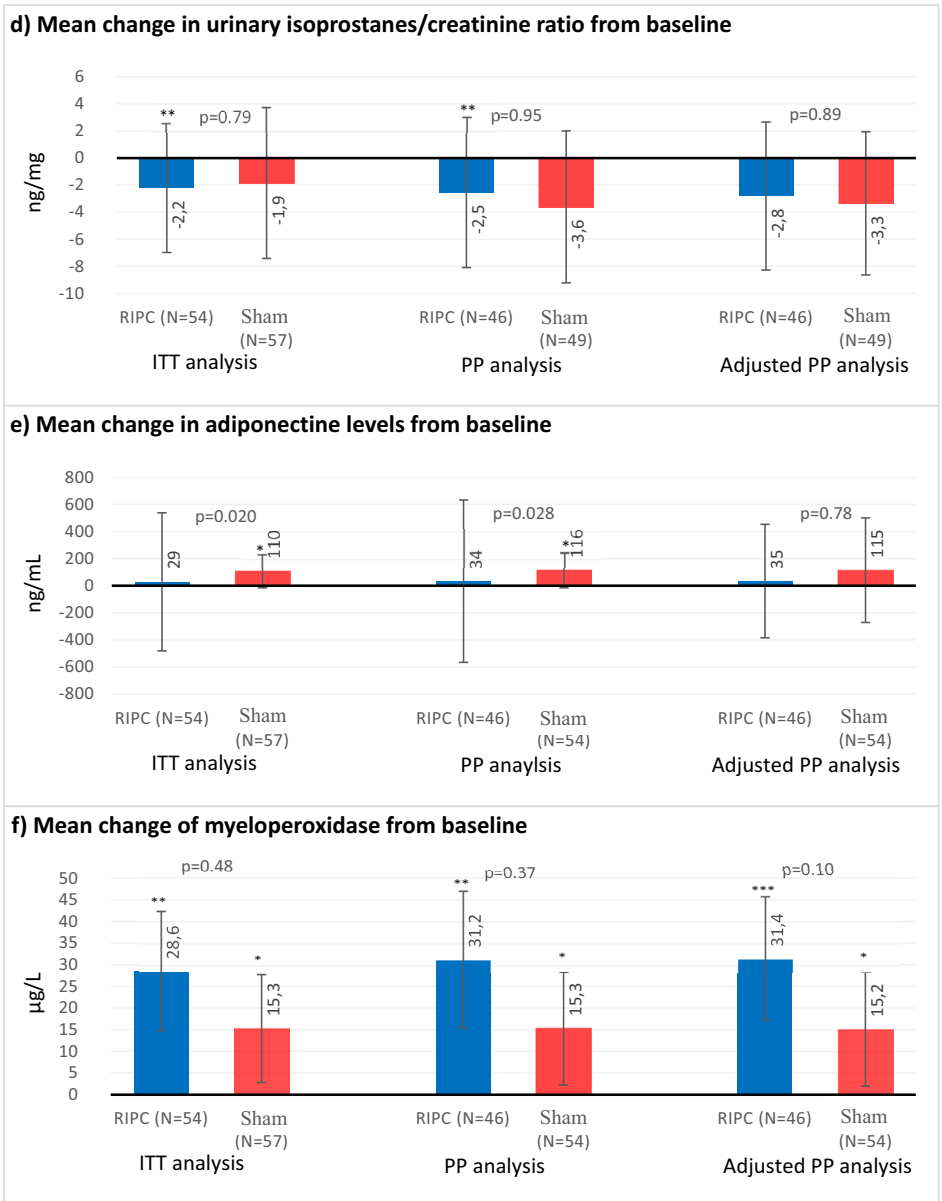


Figure 4. Mean changes of serum and urinary oxidative stress and inflammation biomarkers. The error bars represent the confidence interval for the mean. *-p<0.05; **p<0.01; ***p<0.0001 for the within subgroup changes. Results were derived using two-sample t-tests to compare differences between the RIPC and sham groups for normally distributed data, while the Wilcoxon rank-sum test was used for non-normally distributed data. Adjustments for the PP analysis were made using a linear regression model, incorporating baseline values as covariates. *p<0.05; **p<0.01; ***p<0.001 indicate levels of statistical significance for comparisons between RIPC and sham groups.

Table 5. Mean change of organ damage biomarkers in the groups of the per protocol population with respect to stenting.

	RIPC			Sham			p-value+
	p-value	Stent		p-value	Stent		
		No	Yes		No	Yes	
hs-TnT (ng/L)	0.51	-0.09	-0.15	0.48	-0.31	-0.54	0.56
CK-MB mass (μg/L)	0.65	-0.45	-0.08	0.26	-0.36	-0.56	0.33
NT-proBNP (pg/mL)	0.66	-54.0	-39.6	0.34	-51.0	-23.0	0.87
hs-CRP (mg/L)	0.036	0.97	7.56	<0.0001	-0.93	2.57	0.059
Glucose (mmol/L)	0.76	0.25	0.38	0.88	-0.09	0.31	0.62
Creatinin (μmol/L)	0.46	1.46	3.95	0.47	3.33	1.37	0.80
Urea (mmol/L)	0.59	0.07	0.31	0.039	0.35	-0.07	0.76
Cystatin-C (mg/L)	0.20	0.01	0.03	0.49	0.00	-0.02	0.19
B2M (μg/L)	0.20	14.2	142.3	0.69	-13.8	-3.7	0.10
eGFR (mL/min/1.73 m^2)	0.47	-0.8	-3.1	0.37	-2.9	-1.2	0.95
Adiponectin (ng/mL)	1	268	-221.2	0.44	204	46	0.72
IL-18 (pg/mL)	0.24	0.7	11.6	0.88	7.5	5.0	1
MPO (ng/mL)	0.53	23.3	39.9	0.075	0.5	27	0.087
NGAL (ng/mL)	0.90	9.9	11.6	0.38	5.4	5.1	0.089
Ox-LDL (U/L)	0.89	-1.8	-0.4	0.47	-1.0	0.8	0.67
KIM-1 (pg/mL)	0.56	1569	1741	0.44	881	645	0.17
L-FABP (ng/mL)	0.72	-0.06	-0.04	0.084	0.11	-0.14	0.81
Isoprostanes/creatinine (ng/mg)	0.026	-7.8	3.5	0.005	-11.6	2.1	0.34

Data are presented as means. Change was calculated by subtracting the baseline measurement from the final measurement. + – Between groups comparison adjusted to stenting; hs-TnT – high-sensitivity troponin T; CK-MB – creatine kinase-myocardial band; NT-proBNP – N-terminal pro b-type natriuretic peptide; hs-CRP – high-sensitivity C-reactive protein; B2M – beta-$_2$ microglobulin; eGFR – estimated glomerular filtration rate; IL – interleukin; MPO – myeloperoxidase; NGAL – neutrophil gelatinase-associated lipocalin; Ox-LDL – oxidized low-density lipoprotein; KIM-1: kidney injury molecule-1; L-FABP: liver-type fatty acid-binding protein.

5.2.2. Changes in the Cardiac Biomarkers

There was no significant difference between the RIPC and the sham group in mean changes of hs-Troponin-T (p=0.25) and NT-proBNP (p=0.24) levels (Figure 5a, 5c). A significant decrease in CK-MBm occurred in both the RIPC (-0.26 μg/L; p=0.009) and sham (-0.45 μg/L; p<0.0001) groups and was greater in the sham group (p=0.047). This difference, however, was not found either before (p=0.061) or after adjusting to baseline values (p=0.36) in the PP analysis (Figure 5b).

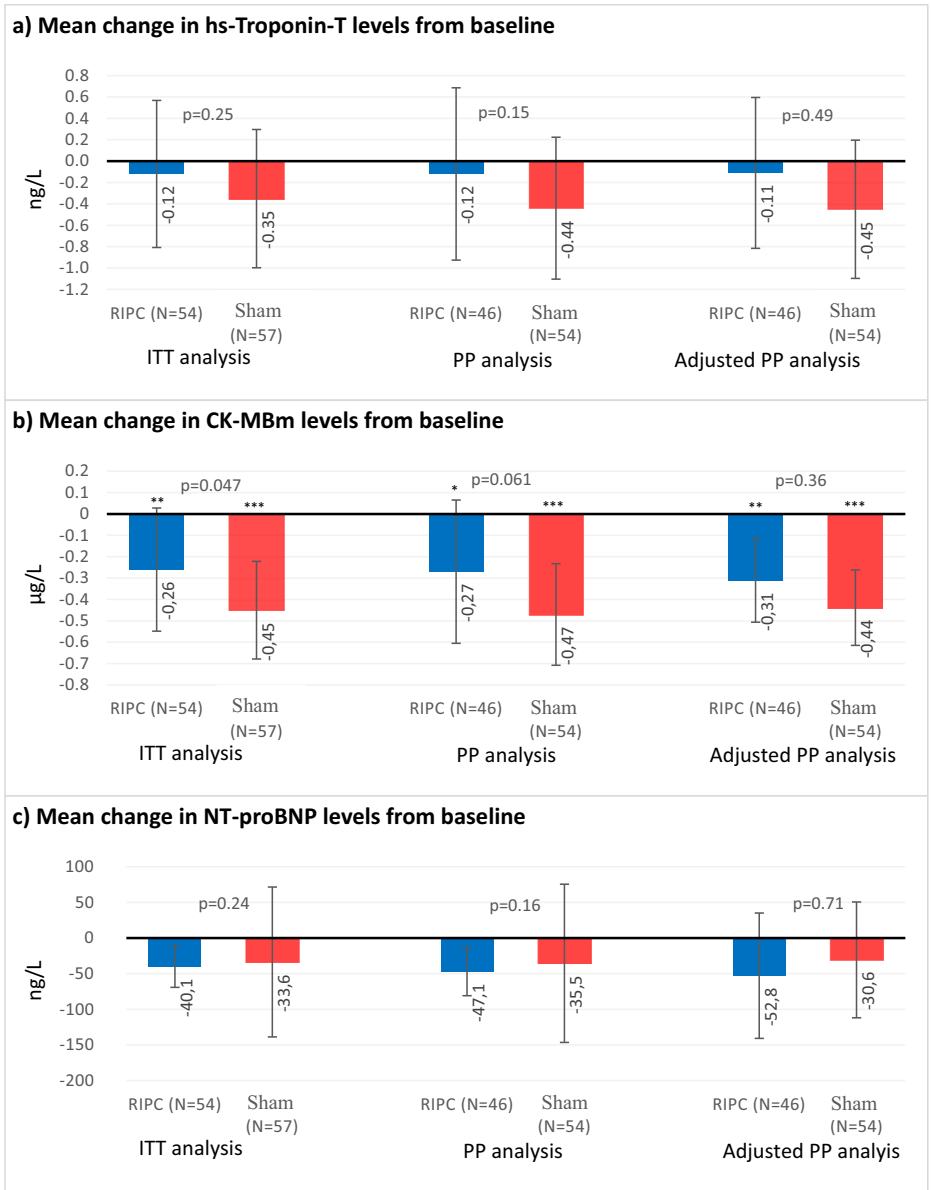
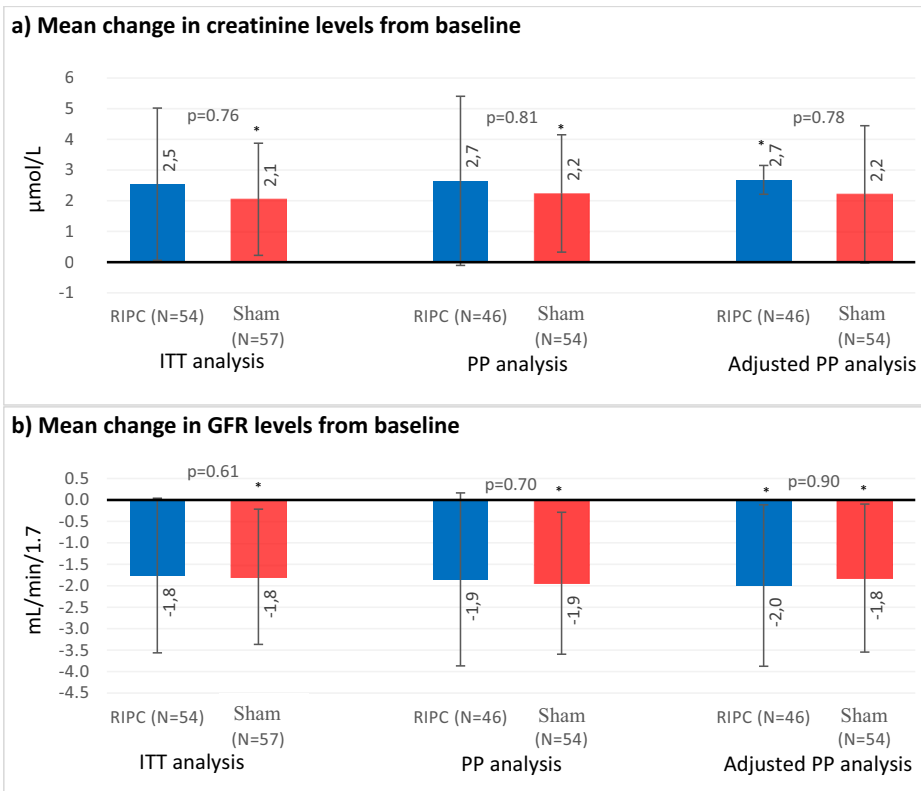


Figure 5. Mean changes of cardiac biomarkers in serum. The error bars represent the confidence interval for the mean. *-p<0.05; **p<0.01; ***p<0.0001 for the within subgroup changes. Results were derived using two-sample t-tests to compare differences between the RIPC and sham groups for normally distributed data, while the Wilcoxon rank-sum test was used for non-normally distributed data. Adjustments for the PP analysis were made using a linear regression model, incorporating baseline values as covariates. *p<0.05; **p<0.01; ***p<0.001 indicate levels of statistical significance for comparisons between RIPC and sham groups.

5.2.3. Changes in Kidney Function Markers in Serum

The increase in creatinine levels was seen in both the RIPC (p=0.050) and sham (p=0.032) groups. RIPC did not significantly reduce the rise in creatinine (p=0.76) levels compared to the sham group (p=0.76) (Figure 6a). No significant change was noted in eGFR (p=0.61), urea (p= 0.95), B2M (p=0.34) or cystatine C (p=0.24) levels (Figure 6b - 6e). A decrease in eGFR was revealed in the sham group in the ITT analysis (-1.79 mL/min/1.73 m^2; p=0.024). This finding was supported by the PP analysis (-1.94 mL/min/1.73 m^2; p=0.015). After adjusting to baseline values, the decrease in eGFR levels from baseline was evident in both groups: -1.99 mL/min/1.73 m^2 in the RIPC group (p=0.040) and -1.82 mL/ min/1.73 m^2 in the sham group (p=0.042) (Figure 6b).

In the ITT analysis, a significant increase in NGAL levels was seen both in the RIPC (8.6 ng/mL; p=0.002) and in the sham group (5.1 ng/mL; p=0.002); however, this change was not significant between the groups (p=0.24) and remained also insignificant in the PP analysis (p=0.11). After adjustment to baseline values, a significant increase in NGAL levels in the RIPC vs. sham group was noted for the PP population (p=0.023) (Figure 6f).



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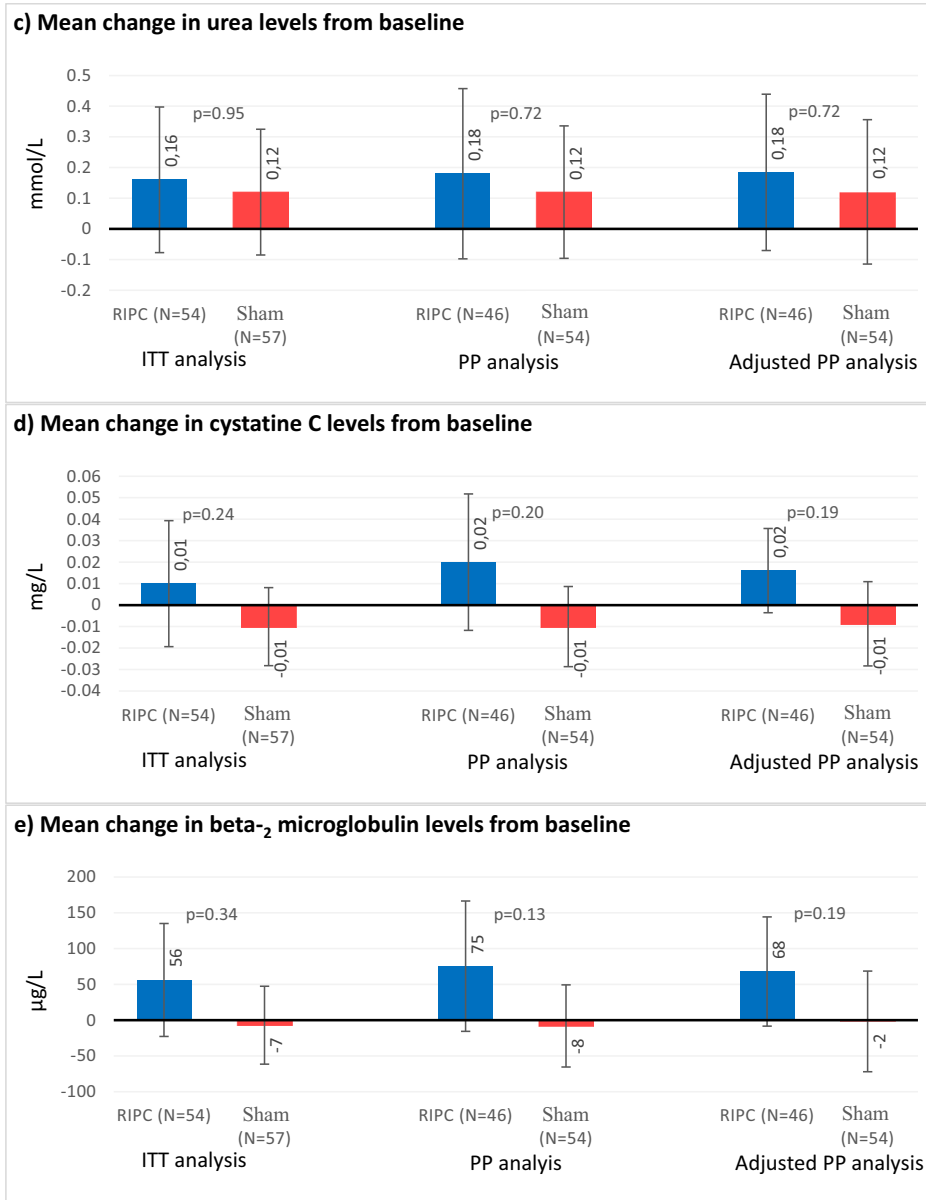

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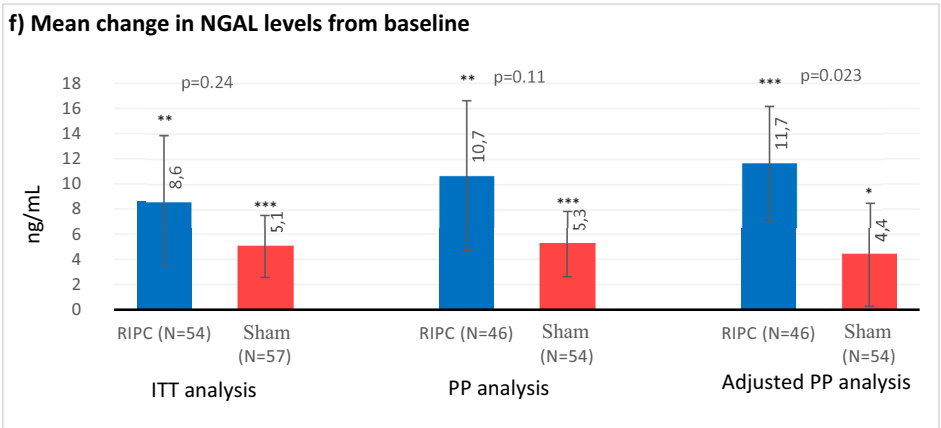

Figure 6. Mean changes of serum kidney biomarkers. The error bars represent the confidence interval for the mean. *p<0.05; **p<0.01; ***p<0.0001 for the within subgroup changes. Results were derived using two-sample t-tests to compare differences between the RIPC and sham groups for normally distributed data, while the Wilcoxon rank-sum test was used for non-normally distributed data. Adjustments for the PP analysis were made using a linear regression model, incorporating baseline values as covariates. *p<0.05; **p<0.01; ***p<0.001 indicate levels of statistical significance for comparisons between RIPC and sham groups.

5.2.4. Changes in Kidney Injury Markers in Urine

A significant increase in KIM-1 levels was found in the RIPC group (p=0.011) but not in the sham group (p=0.092). Similar changes were also revealed in the PP analyses before and after adjusting to baseline values. There were no significant differences in KIM-1 (p=0.14) or L-FABP (p=0.20) levels between the RIPC group and the sham group (p=0.14) in the ITT analysis (Figure 7).

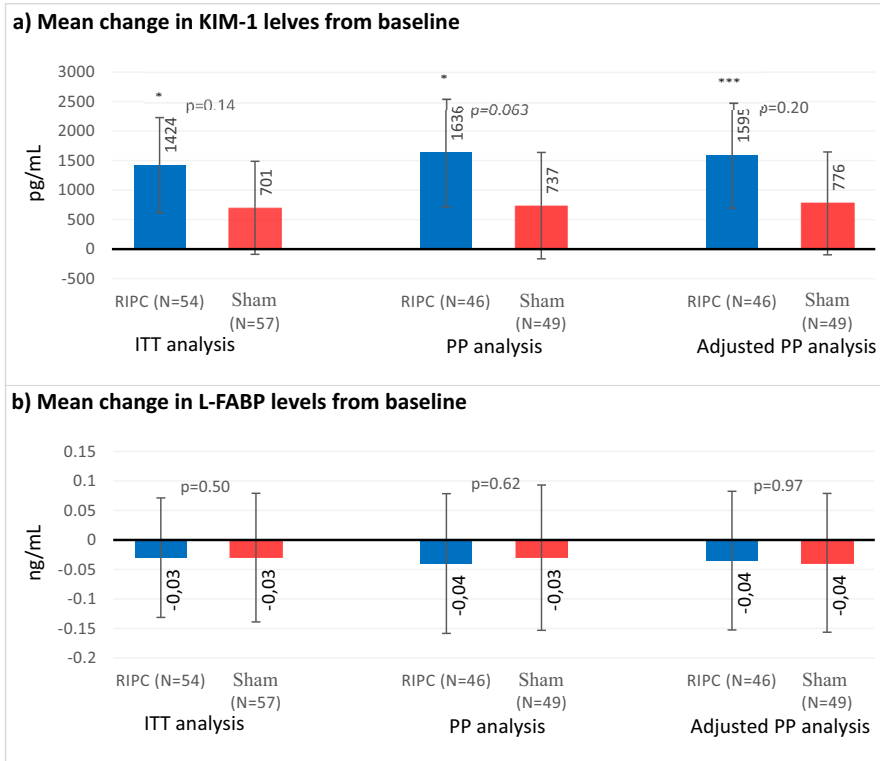

Figure 7. Mean change of kidney biomarkers in urine. The error bars represent the confidence interval for the mean. *p<0.05; **p<0.01; ***p<0.0001 for the within subgroup changes. Results were derived using two-sample t-tests to compare differences between the RIPC and sham groups for normally distributed data, while the Wilcoxon rank-sum test was used for non-normally distributed data. Adjustments for the PP analysis were made using a linear regression model, incorporating baseline values as covariates. *p<0.05; **p<0.01; ***p<0.001 indicate levels of statistical significance for comparisons between RIPC and sham groups.

5.3. Effects of Remote Ischaemic Preconditioning on the Serum Metabolome (Paper III)

To assess the effect of intervention, we calculated changes in metabolite concentrations. Significant changes in the concentrations of certain metabolites, including glutamate, taurine, ADMA to Arg ratio, lysoPC a C24:0, lysoPC a C28:0, lysoPC a C26:1, PC aa C38:1, PC ae C30:2 and PC ae C44:3, were found between the groups (Figure 8).

To unravel possible underlying reasons for the observed changes between the groups, further changes in the metabolite levels within each intervention group were analysed. Decreases in the concentrations of all the aforementioned metabolites were observed in the sham group, while metabolite levels in the RIPC group remained stable (Table 6).

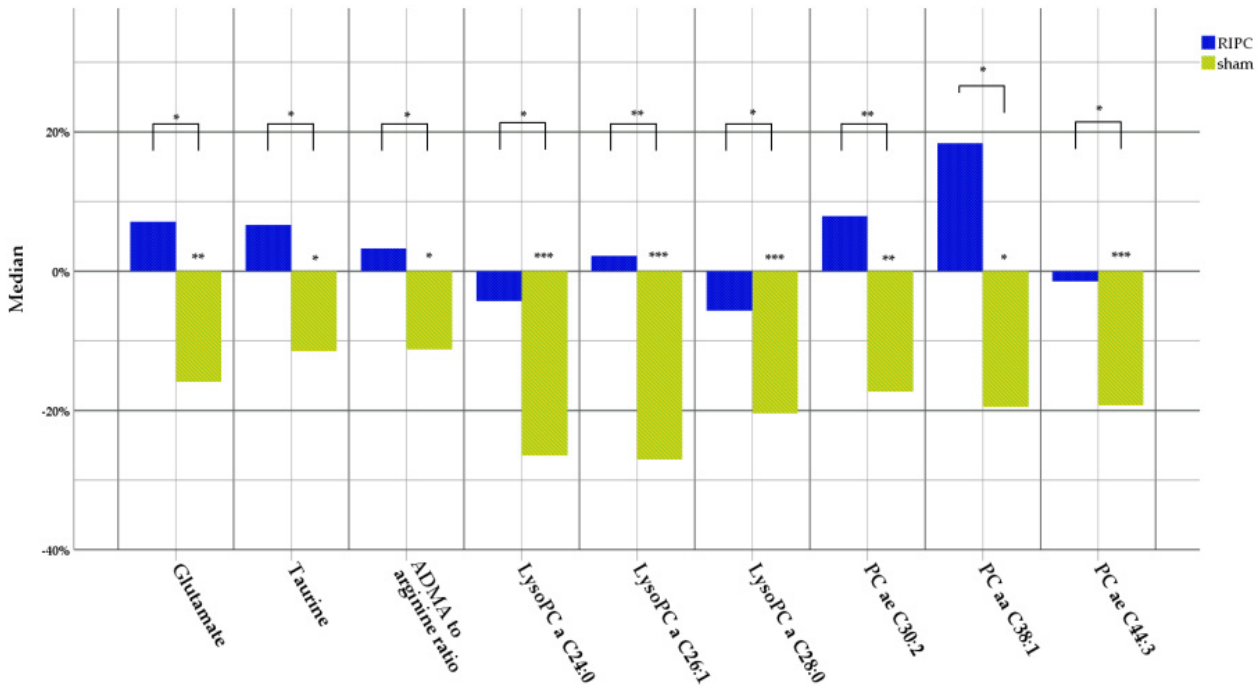

Figure 8. Changes in serum metabolite levels 24 h post-intervention in the remote ischaemic preconditioning (RIPC) and sham groups. Changes in metabolite concentrations were calculated by subtracting the baseline measurement values from the 24 h measurement values and are represented as percentages from baseline measurement. All statistical analyses reflecting changes between the groups were conducted using the Mann–Whitney U test. Only the results that are statistically significant, following the application of the Benjamini-Hochberg method for multiple comparison correction, are provided. ADMA – asymmetric dimethylarginine; LysoPC – lysophosphatidylcholine; PC – phosphatidylcholine; a – acyl; aa – diacyl; ae – acyl-alkyl. * $p < 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

Table 6. Significant changes within the study groups based on metabolite levels in serum 24 h after intervention.

	Group	Change	SD/(IQR)	p-value
ADMA	Sham	-0.075	(-0.367 – -0.217)	0.005
Citrulline-to-arginine ratio	Sham	-0.042	(-0.157 – -0.074)	0.003
Citrulline	Sham	-5.3	(-25.2 – -14.6)	0.003
Glutamate	Sham	-18.0	(-66.3 – -30.3)	0.004
Total dimethylamide	Sham	-0.173	(-0.876 – -0.530)	0.001
Tyrosine	RIPC	-9.8	(-32.5 – -12.9)	0.004
Tyrosine-to-phenylalanine ratio ♦	Sham	0.08	±0.20	0.004
Tyrosine-to-phenylalanine ratio ♦	RIPC	0.12	±0.19	<0.001
LysoPC a C16:0	Sham	-25.0	(-89.6 – -39.7)	0.003
LysoPC a C17:0	Sham	-0.59	(-1.91 – -0.73)	<0.001
LysoPC a C18:0	Sham	-4.9	(-15.3 – -5.6)	<0.001
LysoPC a C18:0	RIPC	-6.2	(-20.2 – -7.8)	0.002
LysoPC a C18:1	Sham	-8.8	(-23.3 – -5.7)	< 0.001
LysoPC a C18:1	RIPC	-7.07	(-26.1 – -11.9)	0.001
LysoPC a C18:2	Sham	-16.1	(-48.4 – -16.2)	<0.001
LysoPC a C18:2	RIPC	-13.0	(-50.4 – -24.3)	<0.001
LysoPC a C20:3	Sham	-0.94	(-2.74 – -0.87)	<0.001
LysoPC a C20:3	RIPC	-1.0	(-3.5 – -1.4)	0.001
LysoPC a C20:4	Sham	-2.5	(-7.5 – -2.5)	<0.001
LysoPC a C20:4	RIPC	-3.3	(-11.3 – -4.7)	0.002
LysoPC a C24:0	Sham	-0.21	(-0.73 – -0.32)	0.001
LysoPC a C26:1	Sham	-0.25	(-1.04 – -0.55)	0.001
LysoPC a C28:0	Sham	-0.23	(-0.86 – -0.39)	0.001
PC ae C30:0	Sham	-0.026	(-0.132 – -0.081)	0.005
PC ae C30:2	Sham	-0.027	(-0.123 – -0.069)	0.002
PC ae C34:3	RIPC	-0.75	(-2.30 – -0.80)	0.001
PC ae C42:1	Sham	-0.094	(-0.323 – -0.136)	0.005
PC ae C44:3	Sham	-0.041	(-0.154 – -0.071)	0.001

The reported results include only the statistically significant findings that have been adjusted for multiple comparisons using the Benjamini–Hochberg method. The changes in metabolite concentrations were calculated by subtracting the baseline measurement values from the 24 h measurement values and are given in units of micromolar (μM). Median values are shown if not otherwise indicated. ♦—mean changes and standard deviations are given. SD – standard deviation; IQR – interquartile range; ADMA – asymmetric dimethylarginine; LysoPC – lysophosphatidylcholine; PC – phosphatidylcholine; a – acyl; aa – diacyl; ae – acyl-alkyl.

Table 7. Correlations between significant metabolic changes in serum from Paper III and notable haemodynamic and biochemical changes from Papers I and II.

		Glutamate		Taurine		ADMA to arginine ratio		LysoPC a C24:0		LysoPC a C28:0		LysoPC a C26:1		PC aa C38:1		PC ae C30:2		PC ae C44:3	
		RIPC	Sham	RIPC	Sham	RIPC	Sham	RIPC	Sham	RIPC	Sham	RIPC	Sham	RIPC	Sham	RIPC	Sham	RIPC	Sham
AIx	Spearman's rho	-0.01	-0.20	0.15	-0.27	0.07	-0.04	0.06	-0.14	0.04	-0.09	0.12	-0.05	-0.06	0.06	-0.01	-0.04	0.19	0.07
	p-value	0.97	0.14	0.32	0.051	0.65	0.79	0.69	0.33	0.77	0.53	0.45	0.73	0.71	0.67	0.94	0.77	0.20	0.62
AIx@75	Spearman's rho	-0.04	-0.28	0.23	-0.25	0.11	-0.04	0.05	-0.24	0.04	-0.19	0.10	-0.17	-0.01	-0.04	0.08	-0.10	0.13	-0.03
	p-value	0.80	0.039	0.12	0.07	0.48	0.76	0.76	0.09	0.82	0.16	0.52	0.21	0.97	0.79	0.60	0.48	0.38	0.84
MAP	Spearman's rho	-0.18	-0.12	0.04	0.02	-0.13	-0.07	0.36	-0.08	0.29	-0.23	0.31	-0.18	0.25	0.05	0.05	-0.22	<-0.01	0.07
	p-value	0.23	0.41	0.81	0.89	0.38	0.64	0.014	0.56	0.051	0.06	0.039	0.19	0.10	0.71	0.75	0.10	0.99	0.60
PWV	Spearman's rho	-0.01	-0.09	-0.11	-0.01	0.01	0.01	-0.14	0.07	-0.16	-0.07	-0.07	-0.04	0.06	0.12	-0.15	-0.01	-0.06	0.07
	p-value	0.93	0.52	0.47	0.94	0.95	0.96	0.36	0.62	0.29	0.60	0.63	0.80	0.70	0.38	0.32	0.93	0.68	0.63
PSBP	Spearman's rho	-0.15	0.06	0.03	0.05	-0.04	-0.04	0.36	0.03	0.35	-0.16	0.40	-0.10	0.23	0.01	0.06	-0.12	0.04	0.12
	p-value	0.32	0.69	0.87	0.77	0.78	0.75	0.015	0.84	0.02	0.24	0.006	0.47	0.13	0.94	0.70	0.38	0.79	0.39
CSBP	Spearman's rho	-0.14	-0.04	0.04	0.03	-0.07	-0.04	0.36	-0.02	0.33	-0.19	0.41	-0.12	0.19	0.07	0.05	-0.14	0.10	0.13
	p-value	0.37	0.78	0.78	0.85	0.67	0.76	0.015	0.86	0.024	0.17	0.005	0.39	0.22	0.63	0.72	0.30	0.51	0.34
Adiponectin	Spearman's rho	-0.34	0.22	-0.14	-0.14	-0.32	-0.21	0.12	0.14	0.05	0.12	0.09	0.15	-0.18	-0.07	0.22	0.09	-0.24	-0.06
	p-value	0.021	0.12	0.37	0.30	0.032	0.13	0.42	0.33	0.74	0.40	0.56	0.30	0.23	0.64	0.14	0.50	0.11	0.66
hs-TnT	Spearman's rho	-0.02	0.02	0.20	0.10	-0.21	0.07	0.17	<-0.01	0.19	-0.07	0.11	-0.07	0.24	0.01	0.18	0.09	0.10	-0.13
	p-value	0.90	0.88	0.19	0.47	0.17	0.59	0.27	0.99	0.20	0.61	0.48	0.60	0.11	0.94	0.24	0.51	0.51	0.35
CK-MB	Spearman's rho	-0.20	-0.11	-0.15	0.11	-0.10	-0.03	0.03	-0.19	-0.14	-0.24	-0.12	-0.17	-0.17	-0.09	-0.10	-0.07	-0.19	-0.04
	p-value	0.18	0.44	0.31	0.42	0.51	0.85	0.83	0.18	0.36	0.09	0.45	0.36	0.26	0.52	0.51	0.61	0.20	0.78
NT-proBNP	Spearman's rho	-0.09	-0.08	-0.09	-0.04	-0.07	-0.17	0.02	-0.03	-0.04	-0.09	0.03	-0.01	0.09	-0.16	-0.03	0.02	-0.06	0.12
	p-value	0.56	0.56	0.58	0.76	0.66	0.22	0.90	0.98	0.80	0.54	0.83	0.92	0.54	0.26	0.85	0.87	0.71	0.37
Creatinine	Spearman's rho	-0.30	-0.21	-0.19	-0.17	0.04	-0.07	-0.01	0.08	-0.04	0.20	-0.12	0.18	-0.19	0.18	-0.15	0.05	<0.01	-0.11
	p-value	0.041	0.12	0.20	0.23	0.80	0.59	0.91	0.56	0.82	0.14	0.44	0.21	0.20	0.19	0.31	0.74	0.99	0.44
eGFR	Spearman's rho	0.26	0.20	0.22	0.16	0.05	0.08	<0.01	-0.12	<0.01	-0.25	0.08	-0.21	0.11	-0.19	0.08	-0.09	-0.03	0.08
	p-value	0.08	0.15	0.14	0.26	0.77	0.59	0.99	0.40	1	0.07	0.58	0.14	0.45	0.16	0.59	0.52	0.85	0.58
Urea	Spearman's rho	-0.06	-0.03	0.07	-0.10	0.09	0.23	0.28	-0.13	0.23	-0.06	0.16	-0.08	0.08	0.20	0.20	0.01	0.12	-0.12
	p-value	0.71	0.81	0.65	0.46	0.56	0.09	0.05	0.35	0.13	0.65	0.28	0.58	0.58	0.16	0.18	0.95	0.43	0.38

Correlation coefficients of Spearman's rho are represented. ADMA – asymmetric dimethylarginine; LysoPC – lysophosphatidylcholine; PC – phosphatidylcholine; a – acyl; aa – diacyl; ae – acyl-alkyl AIx – augmentation index; AIx@75 – augmentation index adjusted for a heart rate of 75; MAP – mean arterial pressure; PWV – pulse wave velocity; PSBP – peripheral systolic blood pressure; CSBP – central systolic blood pressure; hs-TnT – high-sensitivity troponin T; CK-MB – creatine kinase-myocardial band; NT-proBNP – N-terminal pro b-type natriuretic peptide; eGFR – estimated glomerular filtration rate.

6. DISCUSSION

6.1. Effects of Remote Ischaemic Preconditioning on Arterial Stiffness in Patients Undergoing Lower Limb Digital Subtraction Angiography and Endovascular Interventions (Paper I)

This study has evaluated for the first time the effect of the RIPC procedure on arterial stiffness and haemodynamic parameters in patients with LEAD undergoing DSA and endovascular procedure (I). Although RIPC significantly decreased AIx, these changes were primarily observed 24 hours post-procedure (I), coinciding with the activation of the second window of protection (Loukogeorgakis et al., 2005). However, AIx@75 and carotid femoral PWV did not show significant reduction (I). In the RIPC group, significant decreases in AIx and AIx@75 were observed after stenting compared with the sham group intervention (I). AIx, AIx@75, mean arterial pressure and peripheral and central systolic blood pressure were significantly reduced 24 h after DSA only in the RIPC group (I).

The timing of the RIPC intervention in relation to the onset of DSA and endovascular intervention is important (Loukogeorgakis et al., 2005), especially since the first window of protection coincided with when patients underwent DSA and endovascular therapy (I). During this period, IRI due to contrast media and acute inflammation from procedural damage to the vascular wall and revascularisation of peripheral tissues exacerbate chronic IRI in LEAD patients, causing increased ROS and OxS levels with endothelial damage and microcirculatory alterations (Geenen et al., 2013; Ismaeel et al., 2018; Poledniczek et al., 2023; Schillinger et al., 2002). RIPC is also important in the second window of protection, which usually begins 24 hours after the initial ischaemic preconditioning (Lang & Kim, 2022). During this phase, the transcription and synthesis of proteins that protect against ischaemic damage occur, potentially contributing to long-term haemodynamic improvements (Hausenloy & Yellon, 2010; Lang & Kim, 2022). This delayed phase involves the activation of pathways that could modify vascular response to stress, potentially enhancing the benefits observed in arterial stiffness and overall vascular function (Loukogeorgakis et al., 2005). Our results suggest that the protective effects of RIPC might be optimally harnessed if aligned closely with procedural timings (I), to fall within this therapeutic window.

Even though PWV, defined as the speed of travel of the pulse wave along an arterial segment, has been considered a gold standard for measuring arterial stiffness (Van Bortel et al., 2012), occlusive lesions are potentially present in patients with LEAD and might cause false measurement results (Husmann et al., 2015). Instead, surrogate markers for arterial stiffness, for example central systolic blood pressure, pulse pressure and AIx, which provides additional information about pulse wave reflection (Husmann et al., 2015), can be used to describe the possible

effect RIPC may impose on arterial stiffness and the haemodynamic profile. AIx, defined as the ratio of augmentation pressure (the difference between the first and second systolic peaks of the arterial waveform) to pulse pressure as a percentage, has been independently associated with LEAD (Zahner et al., 2017), severity of the lower limb perfusion impairment (Mosimann et al., 2012) and shorter walking distance (Brewer et al., 2007). Considering that patients with LEAD often have concomitant atherosclerotic lesions, including coronary artery disease, they are at great risk of cardiovascular events (Weber et al., 2010). It has been shown that higher AIx is accompanied by an increase in central systolic blood pressure and a decrease in diastolic pressure because the pulse wave, reflected from stiff arteries and atherosclerotic lesions and arriving during systole, leads to higher myocardial oxygen demand, reduced coronary blood flow and increased afterload, ultimately resulting in myocardial ischaemia and heart failure (Husmann et al., 2015; Palombo & Kozakova, 2016). Hence, it is reasonable to assume that the reduction of AIx, as well as that of peripheral and central systolic pressure and mean arterial blood pressure (I), should be beneficial to patients with LEAD undergoing DSA and endovascular intervention.

It has been reported that exposure to contrast media during lower limb DSA and endovascular intervention reduces renal function and increases the risk of long-term cardiovascular events and mortality (Schillinger et al., 2001; Sigterman et al., 2016). RIPC has been shown to protect against contrast-induced AKI through increased generation of NO (Zarbock & Kellum, 2016). Moreover, increased inflammatory response and OxS as a result of IRI following revascularisation has been shown to cause vascular dysfunction (Ismaeel et al., 2018). RIPC imposes a biphasic pattern against IRI induced endothelial dysfunction (Laude et al., 2002), with the first wave of protection lasting up to two hours and the second window of protection covering 24–48 h after the onset of the stimulus (Loukogeorgakis et al., 2005). Previously, the effect of the acute phase effect of RIPC on arterial stiffness and the haemodynamic profile was shown by Zagidullin et al., where a significant reduction in peripheral and central systolic pressure, as well as in augmentation pressure was noted in coronary heart disease patients, with a possible explanation for the improvement in endothelial function (Zagidullin et al., 2016). As similar haemodynamic changes were observed in the present study 24 h after applying the preconditioning stimulus (AIx, AIx@75, mean arterial pressure and peripheral and central systolic blood pressure were significantly reduced in the RIPC group) (I), this suggests that RIPC also provides improvement in the haemodynamic profile in the delayed phase of protection. As most of the patients usually leave hospital one day after successful endovascular therapy, the high rate of readmissions because of procedural complications (Secemsky et al., 2018) might be avoided by reversing the pathophysiological responses to lower limb DSA and endovascular interventions beyond hospital stay. Measurement of arterial stiffness and haemodynamic profile after the endovascular intervention may provide a better insight into the overall vascular function, thereby having a major clinical impact, including improved risk prediction and possibly also earlier commencement of treatment.

The exact mechanisms of RIPC and how it affects the vasculature are complex. RIPC reduces platelet activation, which is present during limb ischaemia (Burdess et al., 2010) and has been proposed to be one of the contributors to IRI (Pedersen et al., 2011). In addition, RIPC downregulates pro-inflammatory genes (Konstantinov et al., 2004) and has been shown to improve endothelial function when measured with flow-mediated dilatation (Loukogeorgakis et al., 2005). By modifying smooth muscle tone of the vascular wall with different mediators, such as NO produced by the functional endothelium, RIPC has vasodilating effects on the peripheral vasculature (Aggarwal et al., 2016). This may reduce reflection and augmentation of the pulse from peripheral sites as seen in the RIPC group in the present study (I).

A significant difference was currently noted between the RIPC group and the sham group in AIx and AIx@75 after stenting (I). Even though a definite causal link cannot be established based on this study (I), one of the possible explanations why the observed changes occurred in this group might be improved endothelial function (Loukogeorgakis et al., 2005), because augmentation indices decreased only in the group subjected to RIPC and stenting, not in the same settings in the sham group or in patients who had received only RIPC without stenting (I). Alterations in the arterial wall due to stressors or interventions typically involve complex biochemical processes and cell signalling pathways, which are unlikely to manifest immediately (Evans et al., 2022). It can be hypothesised that in RIPC preconditioned vessels, vasodilation in peripheral muscular arteries reduced pulse wave reflection by improving endothelial function (Rytter et al., 2020), which subsequently led to a decrease in augmentation indices 24 hours post-angiography (I). As RIPC has been shown not to immediately affect the arterial wall's structural composition (Zagidullin et al., 2016), it is clear why no reduction was observed in PWV. To our knowledge, this is the first observation of RIPC's effect on AIx and AIx@75 in patients undergoing stenting (I). These findings suggest that RIPC may offer a protective vascular effect in stented patients, potentially leading to better long-term cardiovascular outcomes by reducing arterial stiffness. This could have significant implications for the management and treatment protocols in patients undergoing stenting, particularly those at high risk of cardiovascular complications.

Even though in animal models RIPC has been shown to have clear beneficial effects, clinical trials have failed to establish a reduction in major adverse cardiac and cerebral events, often because of the possible heterogeneity of the study population (Addison et al., 2003; Chen et al., 2005; Gho et al., 1996; Hausenloy et al., 2015; Heusch & Gersh, 2016; Meybohm et al., 2018; Takaoka et al., 1999). Different comorbidities may also affect the effect of RIPC (Trachte et al., 2021). Diabetes has been a major confounding factor in many clinical studies as it may induce both autonomic and peripheral neuropathy (J. A. Epps & Smart, 2016; Miki et al., 2012). The effect of RIPC on diabetic patients might be reduced as both the first and second RIPC windows are dependent on the presence of intact neural pathways (J. A. Epps & Smart, 2016).

6.2. The Role of Remote Ischaemic Preconditioning in Preventing Organ Damage, Inflammation and Oxidative Stress During Lower Limb Angiography (Paper II)

RIPC failed to improve the profile of renal and cardiac biomarkers of patients with LEAD peri-procedurally (II). On the other hand, it was shown that RIPC significantly limits the increase in adiponectin levels and may affect the decrease in CK-MB levels 24 hours after DSA and endovascular interventions (II). These finding align with the onset of RIPC's second window of protection, where de novo protein synthesis could mediate cytoprotective effects (Hausenloy & Yellon, 2010), potentially explaining the observed biochemical changes.

6.2.1. The Effect of Contrast Media and Revascularisation on Organ Damage

Deterioration in renal function often affects the removal of biomarkers and their levels (Mizdrak et al., 2022). The risk for significant reduction in renal function after endovascular interventions in patients with LEAD has been estimated to be around 10% and can be even higher in patients with the more advanced disease (Prasad et al., 2016). According to a study by Sigterman *et al.*, average reduction in eGFR one year after endovascular intervention in symptomatic LEAD patients was 8.6 mL/min/1.73 m^2, suggesting long-term loss of kidney function (Sigterman et al., 2016).

One possible explanation for the observed decline in renal function could be the impact of contrast media administered during DSA and endovascular intervention (II). Contrast media have been shown to directly exert a cytotoxic effect on renal tubular cells and to indirectly induce tubular hypoxia by reducing the renal blood flow and by increasing oxygen demand in the medulla (Geenen et al., 2013). Mitochondrial damage and rise in oxygen demand enhances ROS formation, which further damages renal tubular cells through IRI when oxygen supply is improved (Geenen et al., 2013; Heyman et al., 2010). However, as a significant decrease in renal function has also been shown to ensue when contrast media is not administered during angiography (Ghumman et al., 2017), other possible mechanisms reducing renal function have to be considered. Patients with LEAD often have several concurrent comorbidities that may be exacerbated and may play a role in how organ damage manifests itself after angiographic procedure. For example, heart failure, an important risk factor of kidney injury (Damman et al., 2014), may through reduced cardiac output and venous congestion reduce perfusion of the kidneys, thus activating renin-angiotensin-aldosterone-system (RAAS) and triggering a proinflammatory state (Chahal et al., 2020). Other such common comorbidities are diabetes, hypertension, chronic kidney disease, renal artery atherosclerosis (estimated to be present in 30–40% of patients with LEAD) and acute infections that may directly or through medical therapy play a significant role (Krasinski et al., 2020).

Following limb revascularisation, IRI has been shown to occur as an immediate reaction to improved oxygenation as ROS generation is induced in skeletal muscle due to an imbalance within the antioxidant system and dysfunctional mitochondria (Ismaeel et al., 2018). OxS, however, induces an inflammatory response that enhances leucocyte recruitment, adhesion and activation and is followed by a release of proinflammatory cytokines into the systemic circulation (Ismaeel et al., 2018). Systemic inflammatory reactions may explain elevated levels of cardiovascular complications and mortality, but also markedly increased major adverse limb events following endovascular inteventions (Baumgartner et al., 2018; Ismaeel et al., 2018). This is supported by the fact that high baseline CRP values have been shown to be predictive for the risk of secondary interventions, such as open surgical procedures (Bleda et al., 2015). In our study, significantly higher levels of hs-CRP were observed in patients who received stents following the endovascular intervention, indicating an acute inflammatory response (Table 5) (II). Despite this, RIPC did not effectively mitigate the increase in the overall inflammatory response, which was assessed using a variety of biomarkers such as hs-CRP, IL-18, ox-LDL, urinary isoprostanes corrected for creatinine and MPO levels, with no significant differences noted between the RIPC and sham groups (II).

6.2.2. Oxidative Stress and Inflammatory Response

In our study, no statistically significant differences were observed in OxS markers between the RIPC and Sham groups (II). Notable was the decrease in the isoprostanes-creatinine ratio in the RIPC group (Figure 4d) (II). This marker, indicative of lipid peroxidation, vasoconstriction and platelet aggregation (Davì Giovanni & Patrono Carlo, 2007), suggests a potential reduction in OxS exclusively in the RIPC group (II). OxS and inflammation are well-known contributors to the formation and progression of atherosclerosis, leading to increased synthesis of proinflammatory cytokines and the oxidation of proteins and lipids in the vascular wall (Kattoor et al., 2017). Accordingly, markers for OxS and inflammation have been highlighted as potential diagnostic and evaluative tools for LEAD (Fort-Gallifa et al., 2016; Kremers et al., 2020).

MPO, an enzyme largely produced by activated neutrophils and macrophages, is mainly considered to be a regulator of inflammatory response (Ndrepepa, 2019). In our study, hs-CRP and MPO levels increased significantly in both study groups 24 hours after endovascular intervention (II). Intriguingly, even though there was no statistically significant difference between the groups, the increase in inflammatory markers was more pronounced in the RIPC group (II). Controversial at first sight, increased inflammatory response following RIPC has also been reported earlier (Albrecht et al., 2012; Billah et al., 2019). Albrecht et al. found that RIPC procedure vs. control increased MPO activity in the right atrial cardiac tissue and upregulated serum cytokines in patients who had undergone cardiopulmonary bypass, while a concurrent decrease occurred in troponin T levels (Albrecht et al., 2012). The authors suggested that even though deleterious

in chronic excess, increased neutrophil numbers at an early time point in a short time frame may be not associated with negative outcome. Rather, it may positively influence the affected tissue during the initial reperfusion phase as both pro- and anti-inflammatory cytokine functions may be needed to precondition the target organ (Albrecht et al., 2012). This is further supported by our findings (II). The levels of IL-18, a well-known proinflammatory cytokine associated with atherosclerosis, coronary artery disease and myocardial IRI (Silvis et al., 2021), increased only in the sham group and not in the RIPC group (II). This indicates that although there was increased inflammatory response after RIPC procedure, it is not necessarily associated with increased cardiovascular risk. Rather, the contrary is likely, since proinflammatory cytokine downstream of the inflammatory pathway did not increase following RIPC procedure (II).

6.2.3. Adiponectin

In our study, a significant rise in adiponectin levels was noted only in the control group and not in the RIPC group (II). Adiponectin, a high concentration plasma protein that is primarily produced in the adipose tissue, has been proposed to exert an anti-inflammatory and anti-apoptotic effect and to increase insulin sensitivity (Fang & Judd, 2018; Z. V. Wang & Scherer, 2016). Through binding to the membrane-bound protein T-cadherin present in the vasculature, including endothelium and smooth muscle cells, adiponectin has been shown to also play a critical role in revascularisation after chronic ischaemia and to protect against neointimal and atherosclerotic plaque formation (Fujishima et al., 2017; Parker-Duffen et al., 2013). However, contrary to the findings in cellular and animal models, high levels of adiponectin have been shown to independently predict both all-cause and cardiovascular mortality in many different clinical settings, including patients with coronary artery disease, chronic kidney disease and LEAD (Dieplinger et al., 2009; Menzaghi & Trischitta, 2018). The reasons for these controversial results are still unknown. Relative resistance to adiponectin in metabolically active organs, including the vasculature and heart and the possible role of adiponectin as a marker for increased natriuretic peptides, due to their strong correlation with it, have been suggested in some studies (Dieplinger et al., 2009; Menzaghi & Trischitta, 2018; Y. Wang et al., 2017). Since no statistically significant changes were observed in NT-proBNP levels across either study group, the increase in adiponectin levels cannot be attributed to NT-proBNP alterations (II). Although adiponectin levels rose in both the ITT and PP analyses, this effect was not sustained after adjusting for baseline levels in the PP population, suggesting that initial differences might have influenced the findings (II). However, given that randomisation aims to equalise any inherent disparities at recruitment, the variations noted in the ITT analysis are likely indicative of a genuine effect of RIPC.

Under increased inflammatory and OxS conditions, production of adiponectin in skeletal muscle, liver and cardiomyocytes is upregulated (Delaigle et al., 2004; Massip-Salcedo et al., 2008; Niedziela et al., 2020). It has been shown that it

accumulates in damaged tissues as the result of leakage from the damaged endothelial barrier (Shibata et al., 2007). Massip-Salcedo et al. have previously shown that adiponectin levels increased after IRI in steatotic rat livers compared to the sham group. When ischaemic preconditioning stimulus was applied under the same conditions, adiponectin levels were significantly lower (Massip-Salcedo et al., 2008). In addition, with preconditioning stimulus, reduction of OxS markers and reduced hepatic injury was also described. Even though serum adiponectin levels were higher in the ischaemia-reperfusion group, no correlation between circulating adiponectin levels and hepatic adiponectin levels was described by the authors (Massip-Salcedo et al., 2008). Possible reasons for the observed serum adiponectin levels in our study might be the relatively modest preconditioning stimulus, the low sample size or the lack of involvement of skeletal muscle in inflammatory and OxS conditions represented by the patients with LEAD in our study (II). Whether this translates into lower cardiovascular and all-cause mortality in the RIPC vs. sham groups, due to the difference in adiponectin levels, is yet to be answered in future studies.

6.2.4. The Effect of Remote Ischaemic Preconditioning on Kidneys

We found a significant increase in creatinine levels in both the RIPC and sham groups (II). Although according to the ITT analysis eGFR decreased only in the sham group (Figure 6), we cannot assert that RIPC mitigated this reduction as no statistically significant differences were found between the groups in any of the performed analyses (II). Significant enhancements in medullary and cortical oxygenation following the RIPC procedure have been demonstrated in humans (Siedek et al., 2018). Moreover, a previous large meta-analysis on the effects of RIPC on renal biomarkers and kidney function presented promising results (Pranata et al., 2020). Nonetheless, conflicting findings often arise from the heterogeneity of study designs and populations, with suggestions that low-risk patients and procedures may not produce the differences expected in higher or intermediate-risk scenarios (Zarbock & Kellum, 2016). As eGFR and creatinine are considered relatively delayed indicators of acute changes in kidney function, we included some proposed earlier markers of organ damage in this study (Mizdrak et al., 2022).

In our study, both study groups showed an increase in NGAL levels, albeit without significant differences between the RIPC and sham groups in the ITT analyses (II). Following adjustment for baseline values, a significant rise in serum NGAL levels was observed in the RIPC group patients who completed the study compared to the sham group (II). Additionally, although no differences were observed between the groups, a significant increase in urinary KIM-1 was noted exclusively in the RIPC group, not in the sham group (II). However, no changes were observed in urinary L-FABP levels (II), which are released from the proximal tubule under conditions of OxS and ischaemia (Menez & Parikh, 2019). Serum NGAL, an early indicator of kidney injury, is released from tubular

epithelium into distal nephrons following toxic or ischaemic injury (Mishra et al., 2003). NGAL is also produced by neutrophils, epithelial cells, the liver and within atherosclerotic plaques (L. Guo et al., 2018). KIM-1, another marker found to increase significantly in the RIPC group, signals renal tubular damage and is released from proximal tubules; it not only plays a role in the regeneration and repair of tubular epithelial cells but also offers protective effects in the early stages of kidney injury (Song et al., 2019). As the levels of only KIM-1 and NGAL were elevated, not that of L-FABP (II), the changes noted in the RIPC group are unlikely to be due to extensive damage to proximal tubules but rather indicate modifications in the inflammatory response that might be beneficial.

6.2.5. Cardiac Biomarkers

We did not find any significant difference between the sham and the RIPC groups for the cardiac markers for hs-TnT or NT-proBNP 24 hours after any of the DSA and endovascular interventions (II). However, a significant decrease of CK-MB mass was noted in the control group in the ITT analysis. As CK-MB mass is considered to be a less sensitive marker for cardiac damage than hs-troponin T and is known to be elevated also in skeletal muscle diseases, the change of CK-MB mass in both groups might indicate general improvement in skeletal muscle health after DSA and endovascular intervention under conditions of improved blood flow. Moreover, as the difference between the groups was revealed only in the PP analysis, not in ITT analysis, other possible underlying conditions could have influenced these findings. This might explain why after adjusting to baseline values, there was no significant difference between the RIPC group and the sham group in CK-MB mass (II).

6.3. The Role of Remote Ischaemic Preconditioning in Serum Metabolome in Lower Extremity Artery Disease Patients (Paper III)

The most noteworthy observation from the current study was a ‘stabilisation’ effect in the RIPC group, where RIPC was able to maintain serum levels of various metabolites despite ischaemia-reperfusion injuries (III). This suggests that RIPC could enhance metabolic control, potentially leading to improved clinical outcomes in patients with LEAD.

6.3.1. Taurine

Changes in taurine levels between the groups in our study were primarily due to its decreased levels in the sham group, while the levels in the RIPC group remained stable (III). Endogenous taurine is primarily synthesised in the liver, and its biosynthesis varies between individuals in relation to the nutritional state, amount of protein intake and availability of cysteine as the substrate (Duszka,

2022). Taurine inhibits atherogenesis by lowering cholesterol levels and protects endothelial cells from OxS (Baliou et al., 2021). It stabilises the membrane´s potential through interference with Na^+/K^+-ATPase and counteracts ischaemic oxidative damage by reducing intracellular calcium levels (Baliou et al., 2021). Furthermore, taurine deficiency has been associated with muscle atrophy, renal dysfunction, myocardial failure and cardiomyopathy (Baliou et al., 2021; Duszka, 2022). The decrease in taurine levels after DSA and peripheral percutaneous transluminal angioplasty with DSA might have been caused by several factors in the sham group, such as reduced biosynthesis in the body, genetic factors affecting taurine synthesis, or transportation (Duszka, 2022).

Taurine plays a crucial role in various biological functions, including membrane stabilisation, osmoregulation, calcium homeostasis and antioxidant activity (Duszka, 2022). It has been shown to improve vascular function and arterial stiffness across various populations (Paapstel & Kals, 2022). Although no significant correlation was found between taurine levels and arterial stiffness profiles (Table 7) (III), there was an improvement in the haemodynamic profile observed in the RIPC group (I). The observed 'stabilisation' of taurine levels implies a potential role for RIPC in preserving taurine concentrations, which could help to alleviate the health concerns associated with taurine deficiency (Qaradakhi et al., 2020). This hints at a possible function of taurine in mediating the protective effects of RIPC in patients with LEAD.

6.3.2. Asymmetric Dimethylarginine and L-arginine

Although in the current trial no significant changes in ADMA or Arg levels between the study groups were found, there was a difference in the ADMA/Arg ratio (III). Total dimethylarginine (DMA), ADMA, citrulline/Arg ratio and citrulline levels decreased only in the sham group (III). These findings indicate that the likely cause of the observed changes in the ADMA/Arg ratio between the study groups was mainly due to the decrease in ADMA levels in the sham group. The decrease in ADMA levels in the sham group could be attributed to changes in ADMA synthesis and removal processes, involving factors such as enzymatic activity of dimethylaminohydrolase and changes in renal function (Hulin et al., 2020). However, without direct measurements of these enzymatic activities, the exact mechanisms underlying these observed changes remain speculative.

Previous studies have suggested that RIPC might modify the citrulline-NO cycle by increasing the activity of argininosuccinate synthetase 1 and decreasing arginase activity, which could lead to an increased Arg/Orn ratio and elevated Arg levels that can be directly used for NO production (Barca et al., 2016). However, since ADMA, a well-known inhibitor of all three isoforms of NOS (Hulin et al., 2020), decreased only in the sham group, the RIPC group might have relatively low NOS activity and NO production compared to the sham group (III). The decrease in circulating citrulline levels further emphasises this, as citrulline is converted to Arg in proximal kidney tubule cells (Maric et al., 2021), indicating improved citrulline delivery to the kidney and Arg turnover in patients

in the sham group. The unaffected ADMA/Arg ratio could suggest that RIPC plays a role in maintaining this ratio, potentially affecting NO production and could contribute to a healthy endothelial function.

Previous metabolomics research has shown that deficiency of tetrahydrobiopterin (BH4), an essential cofactor of NOS, is common in symptomatic LEAD patients due to its depletion caused by OxS and inflammation (Ismaeel et al., 2019). These reduced levels of BH4 can impair production of NO, leading to endothelial dysfunction (L. Li et al., 2011). BH4 is also a cofactor for phenylalanine hydroxylase, which converts phenylalanine to tyrosine (Ismaeel et al., 2019). A decreased tyrosine-to-phenylalanine ratio has been associated with OxS, inflammation and a decreased ankle brachial index (Ismaeel et al., 2019). In our study, both groups exhibited a similar increase in the tyrosine-to-phenylalanine ratio. This suggests greater availability of BH4 not only for the phenylalanine hydroxylase but also for the NOS. Furthermore, the changes observed might suggest a decrease in OxS in general, as well as an enhancement in blood supply following the DSA and endovascular interventions.

6.3.3. Glutamate

Data on metabolic changes in human skeletal muscles after IRI are scarce. Glutamate serves as an intersection between carbohydrate and amino acid metabolism, while also acting as a source for all precursors of intestinal citrulline synthesis (Maric et al., 2021). A significant decrease in glutamate levels during maximal ischaemia and 24 hours after lower limb surgery (-29% and -51%, respectively) has been described earlier, indicating that glutamate usage is upregulated both during and after ischaemia (Westman et al., 2007). Elevated glutamate levels following RIPC have been previously observed in both human and animal studies, implying an enhancement in energy metabolism to accommodate increased demand (Barca et al., 2016; Eerik et al., 2022; Shen et al., 2017). Our results align with these findings as a significant change in glutamate levels was noted between the groups, with levels only reduced in the sham group (III). A reduction in glutamate levels could indicate its uptake by cell, as glutamate can be readily utilised through alpha-ketoglutarate in the Krebs cycle as an energy source. Stabilisation of glutamate levels in the context of RIPC suggests an ability to regulate and balance the metabolic demand, potentially preventing excessive utilisation of glutamate as an energy source. The observed changes underline the interactions of metabolic pathways and enzymes in amino acids, providing further insights into the mechanisms through which RIPC operates.

6.3.4. Lysophosphatidylcholines and Phosphatidylcholines

In our study significant changes were only seen in long and very long chain lysoPCs (C24:0, C26:1 and C28:0) and PCs (C30:2, C38:1 and C44:3) between the groups (III). Notably, the significant changes in lysoPCs and PCs did not involve highly unsaturated forms, likely due to their use for beta-oxidation

necessitating more complex pathways (Fillmore et al., 2014; Tahri-Joutey et al., 2021). As the changes occurred mainly in the sham group (III), this could reflect an increased rate of catabolism and a more efficient removal of lysoPCs from the bloodstream and into a variety of tissues (Stegemann et al., 2014). This suggests more balanced energy usage as a consequence of the RIPC procedure. However, as the circulating levels of lysoPCs are determined by a combination of their production, clearance and degradation, we could not establish the direct cause of the changes found in this study since specific enzyme activities were not measured.

We found that lysoPC and PC levels remained stable in the RIPC group, suggesting the role of RIPC in preserving membrane integrity and function (III). Contrarily, similar patients undergoing vascular surgery did not exhibit significant changes in lysoPCs and PCs (Eerik et al., 2022). This may be due to the greater metabolic disturbance from open surgery compared to the relatively less invasive stent placement procedure (Eerik et al., 2022). As the research of lysoPCs used as signal molecules is still scarce, further studies are needed to fully comprehend the changes and pathways seen in the RIPC group.

6.3.5. Adiponectin and Its Effect on the Metabolomic Profile

RIPC significantly limits the increase in adiponectin levels (II). Adiponectin has been shown to mediate insulin sensitising effects mainly by suppressing gluconeogenesis in the liver and by stimulating fatty acid oxidation (L. Luo & Liu, 2022). In addition, through suppressing the endoplasmic reticulum, adiponectin has been shown to induce autophagy in skeletal muscle cells (Ahlstrom et al., 2017). Although autophagy is essential for maintaining cellular homeostasis, excessive and dysregulated autophagy in acute situations may contribute to further muscle deterioration as it is upregulated already within two hours after ischaemia (Jeong et al., 2020; Scalabrin et al., 2022). A reduction in several metabolites observed in the metabolomic profile of the sham group (III) could suggest an elevated uptake and utilisation of these metabolites, likely in response to increased energy demands in IRI conditions (M.-Y. Wu et al., 2018). However, the metabolite levels in the RIPC group remained stable compared to baseline (III). Similar results have been obtained previously on rodents, where ischaemic preconditioning attenuated brain ischaemia-reperfusion damage by limiting hyper-glycolysis and affecting the expression of genes associated with energy metabolism (Geng et al., 2019; Stenzel-Poore et al., 2003). Improvement in energy usage following RIPC has also been shown in humans, indicating preserved mitochondrial function after an injurious event and suggesting improved tolerance of IRI (Gonzalez et al., 2013). The unchanged adiponectin levels in the RIPC group in the current study (III) might imply that RIPC prevents the excessive upregulation of autophagy and catabolic activity, thereby contributing to the maintenance of metabolic balance. As a result, RIPC may potentially mitigate the adverse effects associated with medical interventions and improve the metabolic profile of patients with LEAD.

6.4. Limitations (Papers I, II and III)

The limitations of the current studies should be acknowledged for a comprehensive interpretation of the findings. Firstly, the study's sample size might have constrained the ability to detect subtler differences between the study groups, particularly concerning the associations between RIPC and AIx, as well as other haemodynamic parameters and biomarkers. While a power analysis was conducted and the group sizes for AIx@75 were calculated accordingly, power analyses for other findings in this research were not performed. Future studies may require a larger sample size to robustly confirm the associations reported here. Furthermore, the relatively light pressure applied with a blood pressure cuff during the sham intervention, as compared to the more intensive pressure used in the RIPC procedure, might have allowed participants to deduce their assigned treatment, potentially undermining the blinding integrity of the study.

Secondly, the study design may have influenced the outcomes. The significant decrease of peripheral and central pulse pressure and PWV observed in both the control and RIPC groups could potentially be attributed to physiological circadian changes or to differences in the time of the day, when the baseline and second measurements were undertaken. Additionally, there is evidence suggesting that endovascular treatment may also have influenced the outcomes by potentially reducing arterial stiffness (Kaczmarczyk et al., 2023), which could have also contributed to the results observed.

Thirdly, the timing between the RIPC or sham interventions and subsequent DSA or endovascular treatments was not standardised, varying due to the unpredictable scheduling of procedures and urgent clinical demands, with a median interval of about 80 minutes that was inconsistent across the study but showed no significant differences between the RIPC and sham groups. This variation represents a potential confounding factor that could influence the efficacy and outcome measures of RIPC, suggesting the need for future studies to systematically assess and control for this variable to determine its impact on the results.

Furthermore, the potential impact of the disease stage, lesion location and specific treatments during angiography were not individually assessed in this study, and therefore the analyses were not adjusted accordingly. Hence, the effect of RIPC might vary across these factors, potentially influencing the observed results. Additionally, it is likely that confounding factors such as comorbidities, concurrent medications and unmeasured variables like neuropathy in diabetics may have impacted the results, particularly the metabolomics data.

Finally, the study period was relatively short, with a follow-up period of only 24 hours, which limited the ability to determine long-term effects or sustained relationships of the studied biomarkers in the RIPC and sham groups. Therefore, our conclusions predominantly pertain to the acute effects following the RIPC procedure post-endovascular treatment. The observed metabolic changes induced by RIPC may not become directly translated to clinical benefits, but further

research is necessary to solidify the link between these metabolic alterations and improved clinical outcomes in patients with LEAD.

6.5. Potential Applications and Implications

Studying RIPC, a therapeutic approach that seeks to limit organ damage following ischaemia and its subsequent restoration (reperfusion), has potential implications for patients with LEAD undergoing DSA and endovascular interventions. Mitigating IRI to preserve vascular haemodynamics is crucial for enhancing interventional treatments for LEAD (Poledniczek et al., 2023). By promoting a protective response against IRI and inflammation, RIPC may facilitate minimisation of complications, such as in-stent restenosis, associated with endovascular therapy in patients with LEAD (Beckman et al., 2021). Even reduction of hospital readmission rates may be possible as a consequence.

RIPC has demonstrated potential in stabilising metabolites during IRI, suggesting its ability to enhance metabolic control (III). This aspect is integral to its potential role in mitigating adverse effects associated with medical interventions, such as angiography, angioplasty and stent placement. For instance, the preservation of taurine concentrations by RIPC could alleviate health concerns linked to its deficiency, including muscle atrophy, renal dysfunction, myocardial failure and cardiomyopathy (Qaradakhi et al., 2020). This evidence builds a direct link between RIPC's intervention and its potential for personalised medical approaches, where individual metabolic profiles can guide treatment decisions, thus elevating the standards of patient care. Moreover, RIPC also plays a potential role in regulating adiponectin response (II), a protein with anti-inflammatory and anti-apoptotic effects (Fang & Judd, 2018; Z. V. Wang & Scherer, 2016). This regulation could lead to a decrease in adiponectin levels, a significant revelation as high levels of adiponectin predict mortality in various clinical settings, including LEAD (Dieplinger et al., 2009; Menzaghi & Trischitta, 2018). This regulatory role, in conjunction with RIPC's potential in managing levels of OxS and inflammatory markers as the key drivers of atherosclerosis (Signorelli et al., 2020), again emphasises its multifaceted clinical potential.

The implications of RIPC on patients with LEAD are further highlighted by its impact on AIx reduction (I), which has been independently associated with LEAD severity and walking distance (Mosimann et al., 2012). However, the effectiveness of RIPC might vary depending on the presence of certain comorbidities, like diabetes (J. A. Epps & Smart, 2016). As the protective windows of RIPC are dependent on intact neural pathways (Aulakh et al., 2017), its effectiveness might be reduced in patients with diabetes-induced neuropathy (Jensen et al., 2012). Understanding these mechanisms could pave the way for novel therapeutic strategies in LEAD and possibly other vascular diseases.

Taking into consideration these factors, the potential of RIPC as a therapeutic intervention not only opens the possibility of a more personalised approach to management of LEAD but also has broader implications on various medical

practices. With its capacity to minimise complications and stabilise metabolic profiles, RIPC could potentially make healthcare more efficient and cost-effective.

6.6. Conclusions

1. RIPC markedly reduced the AIx in patients with LEAD who undergo DSA and endovascular treatments on the lower limbs. In particular, RIPC considerably improved AIx, AIx@75, mean arterial pressure, as well as peripheral and central systolic blood pressure 24 hours post-procedure, with the most significant effects observed in patients who were intervened by stenting. However, RIPC did not substantially impact carotid femoral pulse wave velocity, suggesting that its influence on arterial stiffness might be measure-specific.
2. Although RIPC did not significantly improve the renal and cardiac biomarker profiles peri-procedurally, it has the potential to modulate the inflammatory and oxidative stress response. Specifically, RIPC significantly restricts the rise in adiponectin levels and may influence the decrease of CK-MB mass levels 24 hours after DSA and endovascular intervention. This suggests a possible tempering effect of RIPC on IRI stimuli and inflammation.
3. RIPC demonstrates a 'stabilisation' effect on the metabolomic alterations in patients with LEAD who undergo DSA and endovascular treatment. This stabilisation, marked by the maintenance of low-molecular weight metabolite serum levels amidst ischaemia-reperfusion injuries, suggests an enhancing role of RIPC in metabolomic control, which could potentially lead to improved clinical outcomes. These insights into RIPC’s metabolomic impacts provide a novel understanding of how RIPC might be used to mitigate metabolomic disruptions in patients with LEAD who undergo DSA and endovascular therapy. These findings pave the way for the development of personalised approaches in management of LEAD based on metabolic changes.

6.7. Future Directions

1. **Broader Application and Combination Therapies:** Whilst RIPC has demonstrated effectiveness in improving haemodynamic parameters for LEAD patients undergoing lower limb DSA and endovascular treatment interventions, future studies should evaluate its utility across different vascular regions and in combination with other therapeutic modalities. This expansion could help determine if RIPC’s benefits extend to various cardiovascular conditions and if combined therapies could enhance overall treatment efficacy.

2. **Long-Term Cardiovascular Outcomes:** Given the short-term benefits observed in AIx and blood pressure, there is a critical need to explore the long-term effects of RIPC on cardiovascular health in LEAD patients. Longitudinal studies assessing the sustained benefits or potential risks associated with RIPC could provide deeper insights into its capacity to enhance long-term outcomes and reduce cardiovascular events.
3. **Expanded Biomarker Analysis:** Although no significant improvements were noted in renal and cardiac biomarkers in this study, incorporating a wider range of biomarkers related to oxidative stress and inflammation could yield more comprehensive insights into the biochemical pathways influenced by RIPC. Understanding these pathways is crucial to enhance the predictability and effectiveness of RIPC treatments.
4. **Metabolomic Profiling and Pathway Analysis:** Building on the evidence that RIPC stabilises metabolomic alterations, future research should thoroughly profile changes in a broader array of metabolites. This could help identify specific metabolic pathways affected by RIPC and lead to targeted therapeutic approaches. Additionally, integrating studies on how pathological, genetic and pharmacological factors influence RIPC outcomes could significantly refine therapeutic strategies.
5. **Clinical Trials for Standardisation and Optimisation:** Conducting randomised controlled trials to evaluate the clinical efficacy and safety of RIPC in a diverse LEAD patient population is paramount. Such trials should focus on standardising protocols and determining the most effective timing and frequency for RIPC interventions, considering individual patient characteristics such as genetic predispositions and existing comorbidities that may impact RIPC efficacy.
6. **Personalised Medicine Approaches:** The insights gained from RIPC's influence on metabolomics underscore the potential for personalised medicine in managing LEAD. Future research should concentrate on developing personalised treatment plans based on individual metabolomic profiles to optimise therapeutic outcomes. This approach could include refining the timing and protocols for RIPC, with an emphasis on personalised strategies that enhance efficacy based on individual patient characteristics.
7. **Integration with Other Therapies:** Investigating how RIPC can be synergistically combined with pharmacological treatments or other non-invasive therapies is another critical area for future research. This exploration could open new avenues for comprehensive management strategies that enhance treatment efficacy and patient outcomes in vascular health.

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8. SUMMARY IN ESTONIAN

Kaugisheemilise eelkohastamise mõju arterite jäikusele, organkahjustusele ja metaboloomsele profiilile alajäseme arterite haigusega patsientidel

Kirjanduse ülevaade

Isheemia korral väheneb kudedes rakkude hapnikuga varustatus kiiresti ja nad lülituvad ümber anaeroobsele ainevahetusele, mille tagajärjel tekib rakus energia puudus. See on rakusurmale eelnev seisund juhul kui verevarustust ei taastata. Kuigi vereringe taastamine ehk reperfusioon on edasise koekahjustuse vältimise seisukohalt kriitiline, tekib reperfusiooni tingimustes rakkudes oksüdatiivne stress. Oksüdatiivsest stressist tulenevad muutused võivad põhjustada rakus täieliku energia ammendumise ning seeläbi koekahjustuse ala vahetu suurenemise. Sellist protsessi nimetatakse isheemia-reperfusioonikahjustuseks (IRK). IRK on meditsiinis sage probleem. Seda esineb näiteks südame- ja veresoonteoperatsioonide/protseduuride käigus, samuti mitmete ägedate isheemiliste sündroomide korral, kus isheemiale tundliku koe verevarustus täielikult katkeb. Seejärel võib verevarustuse taastumine tekitada ägeda elundikahjustuse.

Kaugisheemilise eelkohastamise (KIE) kasutamine on kõige efektiivsem olukordades, kus isheemia tekkimise aeg on ette teada, nagu operatsioonid ja protseduurid. KIE rakendamine võib langetada meditsiiniliste protseduuridega seonduvaid riske, pakkudes võimaluse vähendada kudede kahjustusi. KIE protseduur hõlmab korduvaid lühiajalisi isheemia ja reperfusiooni tsükleid eemalasetsevas koes, eelistatult ülajäsemes, eesmärgiga kaitsta isheemiatundlikku elundit või kudet IRK tagajärgede eest. KIE tekitab rakkudes ja kudedes soodsaid kohastumuslikke muutuseid kasutades selleks keha füsioloogilisi mehhanisme. Need muutused hõlmavad mitmesuguseid molekulaarseid ja rakulisi protsesse, mille tulemusel väheneb oksüdatiivne stress, põletikuline vastus pidurdub ning ainevahetus ja energia tootmine optimeeritakse. KIE toime avaldub ajaliselt kahes faasis. Esimeses faasis käivitatakse lühiajaliste isheemia-reperfusioonitsüklitega kiire kaitsev toime. See hõlmab mitmesuguseid molekulaarseid muutusi, nagu valkude fosforüleerimine, mis aitavad kaitsta rakke ja kudesid otsese isheemia eest. Kiire faas kestab tavaliselt vaid mõne tunni. Teine faas on hilisem ja kestab tavaliselt 24 kuni 72 tundi pärast KIE teostamist. Selles faasis käivituvad geeniekspressiooni muutused, mis aitavad sünteesida uusi valke ja ensüüme, et tugevdada kudede vastupanuvõimet pikaajalistele isheemilistele sündmustele ning parandada kudede taastumisvõimet.

Alajäseme arterite haigusega (AAH) patsientidel, kellel on tavapopulatsiooniga võrreldes oluliselt kõrgem suremus südame-veresoonkonna haigustesse (SVH), esineb IRK igapäevaselt näiteks vahelduva lonkamise käigus. Haiguse arenedes tekivad jala rahuolekuvalud ning haavandid, mis võivad jäseme vere-

varustuse täielikul katkemisel viia vajaduseni jalg amputeerida. Digitaalne subtraktsioon angiograafia (DSA) ja endovaskulaarsed raviprotseduurid on AAH patsientide raviks olulised meetodid. Nende protseduuridega kaasnevad täiendavad riskid, sealhulgas kontrastainest tingitud neerukahjustus ja veresoone seina laiendamise või stentimise järgne ülemäärane põletikuvastus, mille risk tuleks pikaajaliste ravitulemuste parandamiseks maandada.

Suurte ja väikeste arterite funktsiooni langus eelneb sageli SVH kliiniliste sümptomite tekkele. Arterite elastsuse suurendamine on üks KIE potentsiaalsetest kasulikest mõjudest, mis tuleneb selle võimest parandada endoteeli funktsiooni ja suurendada lämmastikoksiidi (NO) tootmist. KIE järgselt on täheldatud ka mikrotsirkulatsiooni paranemist, mis on SVH ennetamise ja ravi seisukohast oluline. Varasemad uuringud on näidanud, et KIE mõju arterite jäikusele võib parandada veresoonte tervist vähendades arterite seina jäikust ja suurendades veresoonte endoteeli reaktiivsust. Seetõttu on KIE-uuringud kriitilise tähtsusega AAH patsientidel, kus läbi verevarustuse parandamise ja põletikulise aktiivsuse kontrolli saame parandada kliinilisi tulemusi ning haigete prognoosi.

KIE SVH vastast kaitset on korduvalt näidatud loomkatsetes. Muuhulgas on KIE vähendanud müokardiinfarkti ulatust, parandanud neuroloogilist taastumist pärast ajuisheemiat ja vähendanud ägeda neerukahjustuse tekke riski. Suuremates kliinilistes uuringutes on aga tulemused olnud enamasti negatiivsed. Seni suurimates KIE uuringutes, ERICCA, mis kaasas 1612 aortokoronaarse šunteerimise patsienti, ja RIPHeart, mis kaasas 1403 südamekirurgia patsienti, ei esinenud olulist erinevust KIE ja kontrollgrupi vahel surma, müokardiinfarkti, insuldi ja ägeda neerupuudulikkuse tekke osas. Üheks võimalikus negatiivse tulemuse põhjuseks võib olla patsientide suur varieeruvus. Näiteks võivad KIE toimet oluliselt mõjutada kaasuvad seisundid, nagu diabeet ja kasutatavad ravimid, nagu statiinid või beetablokaatorid. Samuti on oluline märkida, et KIE toime ajastamine, eriti seoses operatsiooni või muu meditsiinilise sekkumisega, on olulise tähtsusega, et potentsiaalset kasu ja negatiivseid mõjusid optimeerida.

Metaboloomika on meetod, mis pakub üksikasjalikku ülevaadet madalmolekulaarsete metaboliitide profiilidest ja muutustest. Selle abil saab tuvastada uusi biomarkereid, mis võivad tulevikus ennustada arterite jäikuse teket ning prognoosida terapeutiliste sekkumiste pikaajalisi mõjusid. Metaboloomika annab seega väärtuslikku sisendi ka personaalmeditsiini arengusse, võimaldades individuaalse sekkumise optimeerimist AAH patsientide ravis.

Uurimistöö eesmärgid

Doktoritöö eesmärgiks oli kirjeldada KIE mõju AAH patsientidel alajäseme DSA ja endovaskulaarse raviprotseduuri järgselt arterite jäikuse, neeru- ja südamekahjustuse markerite, põletiku ja oksüdatiivsele stressi ning seerumi metaboloomi abil.

Uurimistöö spetsiifilised eesmärgid

– Hinnata KIE mõju arterite jäikusele AAH patsientidel alajäseme DSA ja endovaskulaarse raviprotseduuri järel.
– Hinnata KIE mõju organkahjustustele, oksüdatiivsele stressile ja põletikule AAH patsientidel alajäseme DSA ja endovaskulaarse raviprotseduuri järel.
– Selgitada KIE võimalikke süsteemseid metaboolseid muutusi AAH patsientidel alajäseme DSA ja endovaskulaarse raviprotseduuri järel.

Uuritavad ja meetodid

Uuritavateks olid hospitaliseeritud patsiendid, kellel oli varasemalt diagnoositud sümptomaatiline AAH. Kõik osalejad pidid andma kirjaliku nõusoleku osalemiseks oma emakeeles. Patsiendid olid haiglasse tulnud plaanilises korras alajäseme DSA või endovaskulaarse raviprotseduuri teostamiseks. Uuringus osalejate värbamine toimus ajavahemikus veebruar 2016 kuni märts 2019 Tartu Ülikooli Kliinikumi Veresoontekirurgia osakonnas.

Uuringu väljaarvamiskriteeriumiteks olid vanus alla 18 eluaasta, hinnanguline glomerulaarfiltratsiooni kiirus haiglasse saabumisel alla 30 ml/min/1.73m^2 või samaaegne osalemine mõnes muus kliinilises uuringus. Lisaks olid väljaarvamiskriteeriumiteks aktiivne pahaloomuline kasvaja, dokumenteeritud allergiline reaktsioon joodile, äge infektsioon ning südame rütmihäired. Lisaks välistati patsiendid kellel õlavarre seisund ei võimaldanud vererõhumanseti kasutamist ning need, kes vajasid kodust hapnikuravi või ei suutnud pikali asendis olla vähemalt 40 minutit.

Uuringu esmaseks tulemiks oli hinnata arterite jäikuse parameetri, südamelöögisagedusele kohandatud augmentatsiooni indeksi (AIx@75), muutust.

Uuringu teisesed tulemid:
Arterite jäikuse ja hemodünaamika profiil:

- Karotiid-femoraalne pulsilaine leviku kiirus
- Augmentatsiooni indeks
- Suurte ja väikeste arterite elastsusindeksid
- Tsentraalne ja perifeerne süstoolne ning diastoolne vererõhk

Biokeemiline analüüs verest ja uriinist:

- Südame ja neerude biomarkerid:
  - C-reaktiivne valk (kõrgtundlik)
  - B-tüüpi natriureetilise propeptiidi N-fragment
  - Kreatiini kinaasi MB isoensüümi mass
  - Troponiin T (kõrgtundlik)
  - Uurea
  - Kreatiniin
  - Tsüstatiin C
  - Beeta-$_2$ mikroglobuliin
- Lipiidide profiil

    - Kolesterool
    - Kõrge tihedusega lipoproteiin (HDL)
    - Madala tihedusega lipoproteiin (LDL)
    - Triglütseriidid
- Neutrofiilide želatinaasiga seotud lipokaliin
- Oksüdatiivse stressi ja põletiku biomarkerid:
    - Oksüdeeritud madala tihedusega lipoproteiin
    - Adiponektiin
    - Müeloperoksidaas
- Uriini biomarkerid:
    - Isoprostaanid
    - Kreatiniin uriinis
    - L-FABP
    - KIM-1

Seerumi metaboliitide profiili määramine:

- Kvantitatiivsed metaboloomika analüüsid AbsoluteIDQ p180 komplektiga, et mõõta ja analüüsida erinevaid metaboliite, mis on seotud SVH ja metaboolsete häiretega.

KIE-rühma patsientidele teostati kokku neljal korral järjest õlavarrele asetatud vererõhu mansetiga viie minuti pikkune isheemia episood. Selle tarvis tõsteti vererõhumansetis rõhk vähemalt 200 mmHg. Kontrollgrupis tekitati õlavarrele asetatud vererõhumansetiga 20 mmHg rõhk samuti neljal korral järjest viieks minutiks. Niisuguseid tsükleid viidi igal patsiendil kokku läbi neli korda. Igale viie minuti pikkusele manseti täitmisele järgnes viieminutiline reperfusiooni periood.

Uurimistöö tulemused

Topeltpimendatud kontrollitud kliinilisse uuringusse kaasati kokku 111 AAH patsienti, kes jaotati juhuslikustamise alusel KIE-rühma või kontrollrühma. Uuringu esmase tulemi saavutas 47 patsienti KIE-rühmas ja 55 patsienti kontrollrühmas.

1. KIE-rühmas paranesid märkimisväärselt arterite jäikuse parameetrid, AIx ja AIx@75, kuid olulist mõju karotiid-femoraalsele pulsilaine levikukiirusele ei avaldunud. KIE rühmas vähenes oluliselt keskmine arteriaalne rõhk ning perifeerne ja tsentraalne süstoolne vererõhk 24 tundi peale protseduuri. Muutused olid oluliselt enam väljendunud KIE-rühma patsientidel, kes olid endovaskulaarse protseduuri käigus saanud revaskulariseeriva ravi eesmärgil stendi.
2. Neeru- ja südamekahjustuse biomarkerite profiil KIE-järgselt oluliselt ei muutunud. KIE-rühmas esinesid muutused põletikulist aktiivsust ja oksüdatiivse stressi vastust peegeldavates biomarkerites. Olulise tulemusena nägime KIE võimet piirata adiponektiini taseme tõusu ööpäev pärast DSA ja endovaskulaarse raviprotseduuri teostamist. Need muutused viitavad KIE võimalikule IRK leevendavale mõjule.

3. Täheldasime olulist KIE metoboloomset profiili stabilisiseerivat toimet DSA ja endovaskulaarse raviprotseduuri järgselt. Rühmadevaheline erinevus tulenes peamiselt kontrollrühma metaboliitide kontsentratsiooni langusest. Kokku esines KIE- ja kontrollrühma vahel oluline erinevus üheksas seerumi metaboliidis (glutamaat, tauriin, asümmeetrilise dimetüülarginiini ja arginiini suhe, lysoPC a C24:0, lysoPC a C28:0, lysoPC a C26:1, PC aa C38:1, PC ae C30:2, PC ae C44:3). KIE-rühmas nende metaboliitide kontsentratsioon oluliselt 24-tunni jooksul ei muutunud.

Arutelu

Käesolev uurimistöö keskendus KIE mõjudele AAH patsientidel kasutades selleks topeltpimendatud kontrollitud kliinilist uuringut. Uuringu tulemused näitasid, et KIE suudab märkimisväärselt parandada arterite jäikuse parameetreid, nagu AIx ja AIx@75, kuigi karotiid-femoraalsele pulsilaine levikukiirusele olulist mõju ei avaldunud. Huvitav oli leid, et KIE-rühma patsientidel, kellele paigaldati endovaskulaarse ravi käigus stent, vähenes oluliselt keskmine arteriaalne rõhk ning perifeerne ja tsentraalne süstoolne vererõhk 24 tundi pärast protseduuri. Need muutused võivad olla seotud vasodilatatsiooniga, mida vahendavad KIE tagajärjel endoteeli poolt toodetud vasoaktiivsed ained nagu NO. KIE võib parandada endoteeli funktsiooni, mis aitab kaasa arterite elastsuse tõusule ja vererõhu langusele. Lisaks võib KIE-st tulenev positiivne mõju süsteemsele arterite jäikusele olla tingitud sellest, et protseduur vähendas pulsilaine tagasipeegeldumist perifeeriast, mis põhjustas AIx ja AIx@75 väärtuste languse. See viitab KIE võimalikule rollile mitte ainult arterite jäikuse vähendamises, vaid ka vererõhu langetamises, mis on SVH riskide langetamise seisukohast oluline.

Uuring näitas, et neeru- ja südamekahjustuse biomarkerite profiil ei muutunud KIE-rühmas oluliselt võrreldes kontrollrühmaga. Siiski esinesid mõningased muutused põletikulise aktiivsuse ja oksüdatiivse stressi vastust peegeldavates biomarkerites. Eriti märkimisväärne oli KIE võime piirata adiponektiini taseme tõusu 24 tundi pärast DSA ja endovaskulaarset raviprotseduuri. Adiponektiini taseme piiramine võib olla seotud KIE-indutseeritud tsütoprotektiivsete mõjudega, mis tekivad valkude de novo sünteesi kaudu KIE kaitse teises faasis. See viitab võimalusele, et KIE kaudu saavutatud vaskulaarsed ja metaboolsed muutused aitavad vähendada SVH tüsistuste riski. KIE võime leevendada IRK on krooniliste vaskulaarsete ja metaboolsete haigustega patsientide puhul olulise tähtsusega.

Uuringus täheldati, et KIE-rühmas stabiliseerusid metaboliidide tasemed pärast DSA ja endovaskulaarse raviprotseduure. Seda erinevalt kontrollrühmast, kus mitmete metaboliidide tasemed, muuhulgas tauriini, arginiini ja erinevate fosfolipiidide (lysoPC ja PC) tasemed märkimisväärselt langesid. See võib viidata suurenenud metaboolsele stressile ja energia tarbimisele kontrollrühmas, rõhutades nende metaboliitide olulisust veresoonte tervise hindamisel ja haiguste patogeneesis. Nähtud muutused viitavad sellele, et KIE võib mängida olulist rolli metaboolse tasakaalu säilitamisel ja kaitsevõime tugevdamisel isheemiliste

sündmuste korral. Tauriini stabiliseerumine KIE-rühmas võib aidata leevendada tauriini puudusest tingitud tervisemuresid, nagu lihasatroofia ja kardiomüopaatia, mis on AAH patsientidel oluline probleem. KIE sekkumise kaudu paraneb ka raku metaboolne kontroll, mis võib aidata kaasa paremate kliiniliste tulemuste saavutamisele AAH patsientidel.

Uuringu peamiseks puuduseks oli suhteliselt väike valim, mis võib vähendada võimet tuvastada väiksemaid erinevusi KIE ja kontrollrühma vahel, eriti seoses AIx ja teiste hemodünaamika parameetrite ning biomarkeritega. Lisaks võisid suhteliselt suured ajalised erinevused rühmade sees KIE teostamise ning DSA ja endovaskulaarse ravi vahel mõjutada tulemusi. Samuti ei arvestatud analüüsi teostamisel AAH haiguse staadiumite ja kahjustuse asukohaga, mis võivad samuti mõjutada KIE mõju.

Kokkuvõtvalt näitavad uuringu tulemused, et KIE on potentsiaalselt tõhus strateegia arterite jäikuse vähendamisel. KIE võib samuti aidata kaasa metaboloomse profiili ja põletikulise seisundi paranemisele pärast vaskulaarseid protseduure. Need tulemused pakuvad väärtuslikku teavet edasisteks kliinilisteks uuringuteks ja võivad aidata kujundada tulevikus KIE kasutamise strateegiaid SVH ravis.

Uurimistöö järeldused

- KIE on ohutu ja kergesti rakendatav meetod, mis vähendab IRK ning võib seeläbi leevendada diagnostikast ja ravitegevusest tulenevaid riske AAH patsientidel. Eriti võib KIE oluliselt parandada arterite jäikust patsientidel, kellele paigaldati endovaskulaarse raviprotseduuri käigus stent. Selline hemodünaamiliselt positiivne tulemus soodustab ravi muutumist personaalsemaks ja seeläbi ka efektiivsemaks.
- KIE kasutamine vähendab põletikulist aktiivsust ja oksüdatiivse stressi taset ning võib seeläbi langetada AAH patsientide SVH riske.
- KIE stabiliseerib AAH patsientidel periprotseduraalsed metaboloomse profiili muutused. Täiendavad uuringud on vajalikud, et täpsustada KIE efektiivsust erinevates AAH patsientide diagnoosigruppides ja määratleda kõige asjakohasemad biomarkerid, mis aitaksid mõista ja jälgida KIE mõju. Tuleviku uurimissuunad peaksid keskenduma ka KIE pikaajalise mõju uurimisele SVH ja kliiniliste tulemuste osas.

ACKNOWLEDGEMENTS

This research was financially supported by grants from the Estonian Research Council (PRG no. 1437, PUT no. 1169, PRG no. 435 and PRG no. 2078).

This thesis owes its completion to a wide array of individuals who generously dedicated their time, assistance and support throughout its development. I extend my deepest gratitude to my team of academic and medical advisors and supervisors.

I owe a profound debt of gratitude to Prof. Jaak Kals, my principal supervisor, for his unwavering encouragement, support and faith in me. His exceptional diplomacy and interpersonal skills have taught me how to manage challenging and contentious situations with respect and poise. His support during tough times has helped me to navigate through the complexities of both academic and personal challenges. For this, I am eternally grateful.

It is with immense pleasure that I extend my heartfelt gratitude to

- Prof. Mihkel Zilmer for his boundless optimism, profound knowledge and exceptional ability to clarify complex relationships within the human body, which have significantly enhanced my research experience;
- Prof. Jaan Eha for his dedicated support, invaluable insights, constructive criticism, robust academic guidance and consistent attention to my resources and needs, all of which have been indispensable to my journey;
- Prof. Alan Altraja and Prof. Vallo Volke for their critical review of this thesis;
- Mrs. Mare Vähi for invaluable statistical support;
- Mrs. Ester Jaigma for the meticulous linguistic refinement of my manuscripts;
- Mrs. Sille Tamm for her positivity, indispensable support, ability to seamlessly address every issue with care and always finding perfect solutions;
- Kalle Kilk and the staff of the Department of Biochemistry for explaining the complexities of biochemistry and biomolecular research methods and analysis;
- Dr. Teele Kasepalu for co-developing and improving the design and helping to push through the routine of nights and days of clinical trial research;
- Members of the Endothelial Research Centre for generously offering their support and enriching academic discussions over the years;
- Dr. Kaido Paapstel, my friend and mentor, whose invaluable ideas have enriched my research and inspired me. Your encouragement was there when I needed it the most;
- My beloved parents, Endel and Silja; my siblings, Eveli and Silver; and their families. Your love and belief in me have been the cornerstone of my strength. You have provided a refuge through both the sorrows and successes of this journey;
- I am profoundly grateful to all the individuals who consented to participate in this research, making the execution of these studies feasible.
- And to my wife, Heleen, who has been a constant source of support and inspiration, the shoulder I lean on, enduring alongside me through our most challenging times. She is the keeper of our flame, while I pursued this research. Her love has been the compass guiding me.

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