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LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original publications that are referred to in the text by their Roman numerals:

I Kepler T, Kuusik K, Lepner U, Starkopf J, Zilmer M, Eha J, Lieberg J, Vähi M, Kals J. The Effect of Remote Ischaemic Preconditioning on Arterial Stiffness in Patients Undergoing Vascular Surgery: A Randomised Clinical Trial. Eur J Vasc Endovasc Surg 2019;57:868–875.

II Kepler T, Kuusik K, Lepner U, Starkopf J, Zilmer M, Eha J, Vähi M, Kals J. Remote ischaemic preconditioning attenuates cardiac biomarkers during vascular surgery: a randomised clinical trial. Eur J Vasc Endovasc Surg 2020;59:301–308.

III Kasepalu T, Kuusik K, Lepner U, Starkopf J, Zilmer M, Eha J, Vähi M, Kals J. Remote Ischaemic Preconditioning Reduces Kidney Injury Biomarkers in Patients Undergoing Open Surgical Lower Limb Revascularisation: A Randomised Trial. Oxid Med Cell Longev 2020:7098505.

IV Kasepalu T, Kuusik K, Lepner U, Starkopf J, Zilmer M, Eha J, Vähi M, Kals J. Remote ischaemic preconditioning influences the levels of acylcarnitines in vascular surgery: a randomised clinical trial. Nutr Metab (Lond). 2020;17:76.

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ABBREVIATIONS

AAA	abdominal aortic aneurysm
ACs	acylcarnitines
AIx	augmentation index
AIx@75	augmentation index corrected for a heart rate of 75 beats/minute
AKI	acute kidney injury
ANOVA	analysis of variance
ASA	American Society of Anaesthesiologists' (ASA) physical status classes
ATP	adenosine triphosphate
CIN	contrast-induced nephropathy
BNP	brain natriuretic peptide
BCAA	branched chain amino acids
C1	large artery elasticity index
C2	small artery elasticity index
eGFR	estimated glomerular filtration rate
eNOS	endothelial nitric oxide synthase
HDL	high-density lipoprotein
HIF-1α	hypoxia inducible factor-1α
Hs-TnT	high sensitivity troponin T
IL	interleukin
IPC	ischaemic preconditioning
IQR	interquartile range
I/R	ischaemia-reperfusion
JAK-STAT	Janus kinase/signal transducers and activators of transcription
KIM-1	kidney injury molecule-1
LCACs	long chain acylcarnitines
LDL	low-density lipoprotein
L-FABP	liver-type fatty acid-binding protein
MANOVA	multivariate analysis of variance
MAP	mean arterial pressure
MCACs	medium chain acylcarnitines
MMP8	matrix metallopeptidase 8
MPO	myeloperoxidase
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NGAL	neutrophil gelatinase-associated lipocalin
NO	nitric oxide
NT-proBNP	N-terminal pro b-type natriuretic peptide
OxLDL	oxidized low-density lipoprotein
OxS	oxidative stress
O-GlcNAc	O-linked β-N-acetylglucosamine
PAD	peripheral artery disease
PCI	percutaneous coronary intervention

PI-3	phosphoinositide 3-kinase
PSBP	peripheral systolic blood pressure
PTCA	percutaneous transluminal coronary angioplasty
RIPC	remote ischaemic preconditioning
RIPostC	remote ischaemic postconditioning
RIPerC	remote ischaemic periconditioning
ROS	reactive oxygen species
PWV	pulse wave velocity
SD	standard deviation
SCACs	short chain acylcarnitines
STATx	Signal transducer and activator of transcription x
TNF-α	tumour necrosis factor alpha
TnT	troponin T
TRPV1	transient receptor potential cation channel subfamily V member 1
VEGF	vascular endothelial growth factor

1. INTRODUCTION

Vascular surgery is considered an intermediate-to-high-risk surgery as the risk for 30-day cardiovascular mortality or myocardial infarction is estimated to be greater than 5% in peripheral revascularisation surgery and open aortic aneurysm repair and 1–5% in carotid endarterectomy (Boersma et al. 2005). Additionally, vascular surgery increases the risk for renal insufficiency as postoperative acute kidney injury (AKI) has been found to occur in 12.7–49% of patients undergoing vascular surgery (Adalbert et al. 2013; Huber et al. 2016). Patients undergoing vascular surgery usually have multiple comorbidities resulting from systemic atherosclerosis. Coexisting diseases combined with major tissue trauma increase the peri-operative risk, mainly in terms of cardiovascular events. Moreover, during revascularisation surgery, ischaemia-reperfusion (I/R) injury and rhabdomyolysis are inevitable, which increases the risk for kidney injury. Consequently, there arises the need for an effective risk reduction strategy.

The protective effects of short nonlethal episodes of ischaemia on dog heart were first described decades ago (Murry et al. 1986) and this phenomenon was called ischaemic preconditioning (IPC). Later, the cardioprotective effects of ischaemic precondition to distant tissues were described in terms of the discovery that ischaemia episodes in one vascular bed offers protection also to another vascular bed (Przyklenk et al. 1993), which was the first published evidence of remote ischaemic preconditioning (RIPC). The effect of IPC on different organs has been widely studied in animals and since 2006 also in humans (Cheung et al. 2006). The exact mechanisms of ischaemic conditioning are not known, but during the last decade multiple pathways and biochemical markers involved in achieving the effect of ischaemic conditioning have been discovered. Most generally, humoral and neural pathways are believed to be involved (Aimo et al. 2015). Nevertheless, there are still uncertainties about the conditions under which RIPC has a protective effect and about whether it should be performed routinely. The metabolism of the human body and the heterogeneity between individuals may also alter the effect of RIPC. Metabolomic profiling of RIPC, i.e. studying the systemic effect of RIPC on low-molecular weight metabolites in humans may therefore help to understand better the mechanisms and potential targets of ischaemic conditioning and to find out what is essential for achieving the protective effects of RIPC. Consequently, it is possible to select eligible patients for RIPC.

The present research was undertaken to assess the effect of RIPC on arterial stiffness (i.e. hypertension mediated organ damage), on heart and kidneys and on acylcarnitines' metabolism in patients undergoing vascular surgery, as well as to gain a better understanding of the mechanisms of RIPC and of the factors ensuring its protective effects.

2. REVIEW OF THE LITERATURE

2.1. Protective effects of remote ischaemic preconditioning

2.1.1. Ischaemia-reperfusion injury

In the case of prolonged ischaemia, accumulation of lactic acid and depletion of ATP occurs, which leads to failure of Na^+–K^+ and Ca^{2+} pumps. This results in an intracellular increase of Ca^{2+} and Na^+ and a decrease of K^+, which can lead to cell swelling decline in cellular integrity. Even though reperfusion of the ischaemic tissue is necessary to prevent irreversible ischaemic damage, reperfusion itself paradoxically also promotes further damage and dysfunction, which is called reperfusion injury. In the event of restoring blood flow to ischaemic tissue, oxygen is provided along with reactive oxygen species (ROS). In ischaemic tissues, there is a lack of antioxidative agents, which causes oxidative stress. This leads to inflammatory response, endothelial dysfunction, and to DNA and other damage of cellular structure. The state of reperfusion damage may persist for several days (Wu et al. 2018). The damage is not only local; there has been found to occur also systemic stress response (Karg et al. 1997; García-de-la-Asunción et al. 2012). It has been established that reperfusion causes microvascular dysfunction both in local and distant tissues (Carden and Granger 2000) and restoration of the blood flow in lower limbs leads to kidney, lung and liver damage (Yassin et al. 2002). The target of RIPC is a situation where I/R injury is present or there is a risk for it. In addition to preconditioning, ischaemic conditioning has the potential for organ protection, being applied not only prior to ischaemia, but also after ischaemia and before reperfusion (remote ischaemic periconditioning, RIPerC) and similarly during reperfusion (remote ischaemic postconditioning, RIPostC).

2.1.2. Study targets

The effect of RIPC on heart has been most intensively studied in heart-related procedures like cardiac surgery and percutaneous transluminal coronary angioplasty (PTCA), where I/R injury is direct and often inevitable (Piper et al. 1998). However, during cardiac surgery, secondary damage to the kidneys (Wang and Bellomo 2017) or primary damage due to contrast dye in the case of PTCA (Geenen et al. 2013) may occur and the nephroprotective effects of RIPC are of great interest. Also, there is a risk for acute kidney and heart damage in other major surgeries such as vascular surgery (Flu et al. 2010; Hobson et al. 2018). Nowadays, the range of organs studied for the protective effects of RIPC is wide. Increasingly more studies focus on the effects of RIPC on lungs (Weber et al. 2018), liver (Ruan et al. 2016; Koh et al. 2019), brain (Lv et al. 2020) and intestines (Hummitzsch et al. 2019) – everywhere where I/R damage plays an important role. One of the new directions is transplantation surgery involving

liver (Jung et al. 2020), heart (Wang et al. 2019a) and kidney (Veighey et al. 2019). In addition to elective procedures, studies on RIPC have been shifted to emergency situations such as myocardial infarction (Liu et al. 2016) and stroke (Che et al. 2019). Possibly, interest in other acute ischaemic situations such as mesenteric ischaemia or pulmonary embolism might be increasing. Also, the effect of chronic ischaemic conditioning has been studied (Tong et al. 2019). Nevertheless, the number of studies focusing on the effect of RIPC in vascular surgery has been limited and the studies tend to be rather small or involving important confounders.

2.1.3. Arteries and cardiovascular diseases

2.1.3.1. Endothelial function and arterial stiffness

Arterial stiffening is considered a characteristic of hypertension-mediated organ damage in the latest version of the ESC/ESH guidelines for arterial hypertension (Williams et al. 2018). Three studies have focused on the effects of RIPC on arterial stiffness parameters. The first study (Zagidullin et al. 2016) found that RIPC reduced MAP, augmentation index (AIx), and systolic blood pressure in patients with stable angina pectoris and linked the effect to improvement in endothelial function. Another study (Kuusik et al. 2019) demonstrated that RIPC reduced significantly AIx and blood pressure in patients undergoing angiographic procedures on the lower limb. However, the last study (Müller et al. 2019) did not find any effect of RIPC on arterial stiffness, blood pressure or heart rate in young healthy adults. This can be explained by the assumption that the effect of RIPC may be revealed only when damage, in this case, arterial stiffening, is present, but young healthy adults presumably have normal arteries.

Multiple factors have been found to contribute to the mechanisms of RIPC, with some being directly connected to endothelial function. It was recently shown that RIPC reduces vasoconstriction induced by acetylcholine in patients undergoing elective coronary angiography (Corcoran et al. 2018). The positive effect of RIPC on vasodilatation in the microvasculature has also been described (Lang et al. 2019). In addition, several studies have demonstrated the role of nitric oxide (NO), an endothelium-derived relaxing factor, in the mechanisms of RIPC in non-clinical settings (Chen et al. 2005; Kang et al. 2013). Moreover, in a systematic review, it was emphasized that RIPC promotes endothelial function and has a long-term effect on lowering systolic and diastolic pressures and MAP (Epps et al. 2016). Additionally, the beneficial effects of RIPC on arterial compliance can be linked to increased numbers of endothelial progenitor cells and elevated expression of vascular endothelial growth factor (Liu et al. 2013), but also to activation of the parasympathetic nervous system (Donato et al. 2016).

2.1.3.2. Peripheral artery disease and cerebrovascular disease.

Although intermittent claudication and other clinical forms of peripheral artery diseases (PAD) can be viewed as chronic preconditioning, an additional effect of RIPC has deserved attention. It has been found that four weeks of daily RIPC prolongs the walking distance and relieves claudication symptoms in patients with intermittent claudication (Balin and Kıvrak 2019). Additionally, RIPC demonstrated a positive effect on the initial claudication distance (Saes et al. 2013) and ankle-brachial index in patients with PAD (Shahvazian et al. 2017). However, when performed only before treadmill walking test, RIPC has shown contradictory results as Delagarde et al. did not find any effect of it on claudication (Delagarde et al. 2015).

More studies have been published about the effect of RIPC in cerebrovascular diseases. It has been found that RIPC offers neuroprotection in reducing infarction size and improving neurological recovery after brain injury following hypothermic circulatory arrest (Jensen et al. 2011) and ischaemic stroke (Ren et al. 2008; Du et al. 2020) in animals. In addition, RIPC enhances collateral circulation (Zhang et al. 2019). Protection from ischaemia and cell membrane preservation up to 2 days following RIPC has been described in patients with aneurysmal sub-arachnoid haemorrhage (Gonzalez et al. 2013). Furthermore, daily RIPC has been found to reduce the incidence of recurrent stroke from 26.9% to 7.9% during 300 days, to increase cerebral perfusion in patients with symptomatic athero-sclerotic stenosis of the intracranial arteries (Meng et al. 2012) and to improve neurological outcome after stroke (England et al. 2017). Also, in the case of carotid endarterectomy, RIPC has been shown to reduce the size of brain lesions (Zhao et al. 2017).

2.1.3.3. Heart diseases

The best studied organ regarding the effect of RIPC is possibly the heart and relevant studies tend to be larger and to have more statistical power than those addressing other organs. There are multiple positive results from animal studies and from the proof of concept studies. In multiple trials on animals, RIPC has reduced the size of infarct (Gho et al. 1996; Tang et al. 1999; Schoemaker and van Heijningen 2000; Liem et al. 2002; Weinbrenner et al. 2002; Konstantinov et al. 2005; Wolfrum et al. 2005; Zhang et al. 2006; Dong et al. 2018; Lieder et al. 2018; Bunte et al. 2019). However, there are other potential positive effects of RIPC on the heart. For example, RIPC has been found to reduce reperfusion-related arrhythmias (Dow et al. 2012; Jang et al. 2017), but also atrial fibrillation in non-ischaemic conditions (Han et al. 2016). Also, RIPC has been found to be useful in heart transplantation, as it reduces I/R injury in the brain-dead donor heart (Konstantinov et al. 2005). The effect of RIPC in ischaemic heart diseases is quite well studied. Still, besides consistent positive results from animal studies, those from clinical studies are somewhat contradictory. Although there are some larger RCTs that have described the cardioprotective effect of RIPC in patients

under elective percutaneous coronary intervention (PCI) (Bøtker et al. 2010; Pryds et al. 2016), three largest trials in patients undergoing coronary artery bypass surgery found no clinically relevant cardioprotection by RIPC (Hong et al. 2014; Hausenloy et al. 2015; Meybohm et al. 2015). The effect of RIPerC and RIPostC has been more thoroughly studied in patients with ST-segment elevation myocardial infarction (STEMI). Attenuation of cardiac damage has been described in patients undergoing primary PCI (Crimi et al. 2013; Cao et al. 2018) and higher rate of resolution of ST-segment elevation has been described in the case of thrombolysis (Ghaffari et al. 2018). Moreover, RIPerC has appeared to be feasible and safe in patients with STEMI and has not been found to prolong bedside time. Patients with STEMI have even been suggested as an ideal group for RIPC utilization (Martin-Gill et al. 2016).

The threat of cardiac damage is prevalent also in non-cardiac surgeries, especially in high risk procedures such as vascular surgery (Flu et al. 2010). RIPC has been shown to reduce the leakage of troponin and the incidence of myocardial infarction in patients undergoing open abdominal aortic aneurysm (AAA) repair surgery (Ali et al. 2007). In contrast, neither of the more recent two larger trials reported the benefit from RIPC to patients undergoing open AAA repair, carotid endarterectomy or surgical lower limb revascularisation (Healy et al. 2015; Garcia et al. 2016). Still, in a larger trial with patients undergoing emergency hip fracture surgery, RIPC was found to reduce the risk of myocardial injury and infarction (Ekeloef et al. 2019). Summing up, although many studies have dealt with the cardioprotective effects of RIPC, it needs to be clarified who exactly will benefit from this procedure in terms of cardioprotection.

2.1.4. Kidney injury

One of the organs of interest for application of RIPC is the kidney due to its sensitivity to ischaemic injury. Ischaemia can occur in various surgeries; e.g. AKI has been found to affect 12.7% of patients undergoing lower limb revascularisation surgery (Adalbert et al. 2013) and even up to 30% patients requiring cardiac surgery (Hobson et al. 2009). In addition, contrast-induced nephropathy (CIN) is caused by medullary ischaemia and is a threat to many patients who need an exam with contrast medium, e.g. PCI. According to a meta-analysis, RIPC was found to reduce AKI in patients requiring cardiac surgery (Zhang et al. 2016; Zhou et al. 2017) and in those undergoing PCI (Wang et al. 2017b). In a large meta-analysis including studies on patients undergoing cardiac and vascular surgery, AKI was reduced by RIPC, but the statistical heterogeneity of these studies was high (Li et al. 2017). The nephroprotective effects of RIPC have also been studied in patients undergoing non-cardiac vascular surgery. Although early studies reported a nephroprotective effect (Ali et al. 2007; Walsh et al. 2009), the last 3 large studies report no benefit (Healy et al. 2015; Garcia et al. 2016; Thomas et al. 2016). Also, there is an increasing need for methods to reduce CIN and RIPC has been even more studied in the light of this response. In a recent meta-

analysis, it was concluded that RIPC is an effective procedure to reduce the risk of CIN in patients undergoing PCI or coronary angiography, who have moderate-to-high risk for developing CIN (Pranata et al. 2020). However, it was found earlier that RIPC did not have any additional effect in preventing development of CIN (Ghaemian et al. 2018). Regarding a more recently emerged area of interest, i.e. transplantation medicine, RIPC improved long-term renal function after living-donor kidney transplantation, when administered before the induction of anaesthesia (Veighey et al. 2019).

2.1.5. The most effective protocol for RIPC and its timing

No optimal RIPC protocol has been established to this date. Usually, a protocol consisting of 3 or 4 cycles of 5-minute ischaemia is used. However, application of such a protocol has led to both positive and negative outcomes. Some studies with longer or shorter episodes or with a different number of cycles have had positive outcomes. Most meta-analyses have reached the conclusion that an optimal RIPC protocol is yet to be established. The first animal study (Murry et al. 1986) and a human study (Cheung et al. 2006), which assessed the effect of ischaemic preconditioning used a protocol of 5 min in a quadruple. It has been concluded that 4 to 6 RIPC cycles provided significant cardioprotection and that one- and two-hind-limb preconditioning are equally protective (Johnsen et al. 2016). In a meta-analysis, it was established that an ischaemia episode should last at least 5 minutes, but no conclusions were drawn regarding the number of cycles (Pei et al. 2014). In 1993, it was reported that the effect of RIPC can be observed up to 24 hours after the preconditioning stimulus (Kuzuya et al. 1993). Two “windows” of the effect of preconditioning have been described: the first lasting a few hours after conditioning and the second appearing 24 hours after conditioning (Loukogeorgakis et al. 2005). The second “window” has been reported to last 24 to 72 hours after the preconditioning stimulus (Baxter et al. 1997). Daily ischaemic preconditioning for a longer period has also been studied and the protocols are similar to ischaemic conditioning with one or two sessions (Epps et al. 2016; Maxwell et al. 2019). No complications of noninvasive ischemic conditioning have been reported and it is well-tolerated by patients (Anttila et al. 2016; Guo et al. 2019).

2.2. Mechanisms of RIPC

2.2.1. Humoral factors of RIPC

2.2.1.1. Nitric oxide (NO)

Ischaemic conditioning performed directly or remotely has been described to induce vasodilatation (Enko et al. 2011) and to preserve endothelial function (Kharbanda et al. 2001, 2002; Loukogeorgakis et al. 2005). The role of NO, the signalling molecule, which is iconic of endothelial function, has been studied in the protective mechanisms of RIPC in different organs. RIPC was found to increase the levels of intracoronary NO and was associated with cardiac salvage (Arroyo-Martínez et al. 2016). Also, RIPC was reported to reduce liver (Abu-Amara et al. 2011; Duan et al. 2017), brain (Vlasov et al. 2005) and kidney (Jung et al. 2019) I/R injury by affecting the eNOS-NO pathway.

2.2.1.2. Anti-apoptotic and anti-inflammatory effects

I/R injury leads to cell death: during ischaemia, both necrosis and apoptosis occur, but reperfusion enhances apoptosis even more (Eefting et al. 2004). Ischemic conditioning has demonstrated anti-apoptotic effects by inhibition of endogenous mitochondrial pathways (Lv et al. 2020) and reducing apoptosis by activation of phosphoinositide 3-kinase (PI-3)/Akt signalling (Li et al. 2011). Inflammation is the major response of the human body to tissue damage and has an important role in I/R injury (Chen et al. 2017). As a protective mechanism, it often helps to eliminate the cause of injury, yet it has harmful effects itself. To avoid its unnecessary damage, inflammatory response can be modulated (Yang et al. 2018; Zapata-Chavira et al. 2019).

One of the effects of RIPC is inducing a decrease in inflammation mediators, among which hypoxia inducible factor-1α (HIF-1α) plays a key role in this context (Yang et al. 2018). Ischaemic conditioning has also been found to reduce the levels of proinflammatory cytokines (e.g. IL-1β, IL-6, TNF-α) and to increase the levels of anti-inflammatory cytokines IL-10 (Kim et al. 2014; Xia et al. 2019) and hence to reduce neutrophil activation. Suppression of inflammation leads to suppression of reactive oxygen species (ROS), which ameliorates oxidative status. In addition, the cardioprotective effects of RIPC have also been linked to modulation of autophagy (Billah et al. 2020). The organ protective effect of RIPC has been associated with both inducing (Rohailla et al. 2014; Wang et al. 2014) and inhibiting (Gurusamy et al. 2009; Huang et al. 2010) autophagy. Inhibition of the proapoptotic- and inflammatory pathways has been associated with activation of the JAK2/STAT3 pathway (Cheng et al. 2014).

2.2.1.3. Anti-oxidative stress

Along with inflammatory response to tissue damage caused by medical procedures and diseases, oxidative stress (OxS) is induced, which enhances total tissue damage. There is evidence that the protective effects of RIPC are mediated through opposing OxS (Arvola et al. 2016; Motomura et al. 2017). RIPC has been shown to have antioxidative effects in renal I/R injury, which is linked to preventing damage to the kidneys (Hussein et al. 2016; Dong et al. 2018; Hu et al. 2018). In patients with STEMI undergoing PCI, RIPostC has been found to increase the levels of powerful anti-oxidants such as glutathione peroxidase and superoxide dismutase, as well as to increase total antioxidant capacity (Lotfollahi et al. 2016). This probably plays an important role in the salvage of the myocardium.

2.2.1.4. Mitochondria

I/R injury is associated with impairment of mitochondrial function. In addition, inflammatory response promotes catabolic pathways, which increases the load on mitochondria as they occupy a principal position in energy metabolism. Cardioprotection by RIPC has been shown to occur along with improved mitochondrial function (Gedik et al. 2017; Paez et al. 2019) in animals. Also, evidence suggests that preservation of mitochondrial respiration is achieved by RIPC in humans (Slagsvold et al. 2014) and that cardioprotection is related to mitochondrial salvage (Kleinbongard et al. 2018).

2.2.1.5. Platelets

Platelets have major a role in thrombosis, haemostasis, atherogenesis and inflammation, and have also been found to be important players in various types of cardiovascular diseases (Malchow et al. 2017). Platelets also participate in the protective mechanism of IPC. Specifically, a crucial role has been assigned to serotonin, which is released by activated platelets and activates the VEGF/IL-10/MMP8 pathway (Oberkofler et al. 2014). Also, platelet-derived microparticles have been found to mediate the cardioprotective effects of RIPC (Ma et al. 2015). The importance of platelets has been demonstrated by the finding that thrombocytopenia and the lack of platelet serotonin abolishes the protective effect of RIPC against hepatic I/R injury (Oberkofler et al. 2014). On the other hand, RIPC had no effect on hemostasis or fibrinolysis, or on the occurrence of thromboembolic complications in cancer patients who underwent surgery (Krag et al. 2019).

2.2.1.6. Transcription – JAK-STAT pathway

The JAK-STAT signalling pathway is involved in many processes in the human body, e.g. cell death and inflammation. Also, it has been found to be activated in both the first and the second windows of RIPC (Hattori et al. 2001; Xuan et al. 2001). Among the STAT proteins, the one essential for achieving the effect of RIPC is STAT3 (Oshima et al. 2005; Butler et al. 2006) and STAT5 (Heusch et al. 2012; Bibli et al. 2014). STAT3 has been found to have a key role in apoptosis (Yuan et al. 2004). In the second window, the main effect of activating STAT3 is most probably reduction in cell apoptosis (Bolli 2000). STAT5 is believed to induce cardioprotection in the second window by promoting angiogenesis, releasing survival signals and inhibiting apoptosis (Chen et al. 2018). However, the effect in the first window is not so clear. It has been suggested that the effect in the first window may be evoked by posttranslational protein modification, activation of supplementary intracellular kinases (Hattori et al. 2001), upregulation of superoxide dismutase (S et al. 2001) and upregulation of inducible NO synthase (Bolli 2000). RIPC has also been found to reduce the activity of Nf-κb, and hence reduces the inflammatory response by reducing the transcription of major pro-inflammatory cytokines (Cheng et al. 2014).

2.2.2. Neural pathways and RIPC

2.2.2.1. Sympathetic and parasympathetic nervous systems

The autonomic nervous system consists of sympathetic and parasympathetic divisions that function in opposition to each other controlling all autonomic functions, such as respirations, cardiac and vasomotor actions. The sympathetic division is involved in actions requiring quick response, the so called „fight or flight“ response, while the parasympathetic division acts when the body is at rest („rest and digest“ activities). Chronic sympathetic overactivity is related to multiple cardiovascular diseases (Fisher et al. 2009). The importance of afferent neural pathways in cardioprotection has been proved on animal models, where sectioning of peripheral limb nerves or vagus nerve abolished the protective effect of RIPC (Donato et al. 2013). In the same study the electrical stimulation of vagus nerve was shown to mimic the cardioprotective effect of RIPC and administration of the antimuscarinic drug atropine was shown to reduce that effect, indicating the involvement of vagus nerve and muscarinic receptors (parasympathetic nervous system) in the mechanism of RIPC.

2.2.2.2. Opioid receptors

Opioid receptors are widely expressed in brain, spinal cord and multiple peripheral organs and are known for their role in pain modulation. During RIPC procedure, endogenous opioids such as endorphins, enkephalins and dynorphins are released

to cause activation of opioid receptors that are found to be involved in mediating the protective effect of RIPC (Aulakh et al. 2017). Opioids released into the bloodstream act on myocardial opioid receptors that possibly also mediate the cardioprotective effects (Randhawa and Jaggi 2017b).

2.2.2.3. Adenosine and bradykinin

Adenosine is a nucleoside that is found in different forms in all cells of the human body. It is locally released during RIPC and stimulates sensory nerve endings and activates the neural pathway, which leads to the cardioprotective effect, possibly by activating the myocardial adenosine receptors (Dong et al. 2004). Intraarterial infusion of adenosine has produced cardioprotection similar to RIPC, supporting its importance in the mechanism (Liem et al. 2002).

Bradykinin is a proinflammatory peptide that acts on endothelial receptors, causing vasodilatation. Similarly to adenosine, bradykinin is released locally during RIPC procedure and participates in activating afferent neural pathways that evoke the cardioprotective effect (Ng et al. 2019). Likewise, its intraarterial infusion has been shown to induce cardioprotection (Schoemaker and van Heijningen 2000).

2.2.2.4. TRPV1 channels

Transient receptor potential vanilloid 1 (TRPV1) channels are a part of the transient receptor potential family of ion channels. These channels are expressed in sensory nerve cells, innervating cardiovascular system, and are most often associated with sensation of temperature and pain. They are also involved in regulating calcium signalling, which makes them important in multiple cellular processes (Aneiros et al. 2011). Activation of TRPV1 channels in RIPC is thought to be involved in mediating the cardioprotective effect through stimulating the release of calcitonin gene-related peptide and substance P (Randhawa and Jaggi 2017a). Activation of TRVP1 channels has also some anti-inflammatory effects: it reduces the generation of free radicals, inhibits the production of inflammatory cytokines and recruitment of inflammatory cells, as well as stimulates the production of anti-inflammatory cytokines (Bujak et al. 2019). Administration of the TRPV1 agonist capsaicin has been shown to enhance the protective effect of RIPC, while its antagonist capsazepine seems to abolish it (Randhawa and Jaggi 2017a).

2.3. Confounders to ischaemic conditioning

2.3.1. Age

As multiple studies have reported no effect of ischaemic conditioning, a range of possible confounders have been highlighted, which may explain overall neutral results. One of these confounders is age. It has been suggested that the release of cardioprotective humoral factor(s) into the blood may be age-dependent (Heinen et al. 2018). Although aged patients are at greater risk for many diseases and complications and need protective measures, the effect of RIPC may be reduced or abolished with aging. In animal studies, the cardioprotective effect of RIPC has been found to disappear in aged rats (Behmenburg et al. 2017). Also, plasma from aged preconditioned volunteers did not have any protective effects on rat heart, while plasma from young preconditioned volunteers was found to initiate cardioprotection (Heinen et al. 2018). Interestingly, plasma from the young had also an effect on aged heart. Additionally, in a meta-analysis, the nephroprotective effect of RIPC was found to be more pronounced in younger patients (Zhou et al. 2017). However, a large-scale clinical trial, which failed to show the effectiveness of RIPC in heart surgery patients, did not suggest that age is a confounding factor (Hausenloy et al. 2015).

2.3.2. Gender

Gender has been suggested to be a potential confounder, however human studies assessing differences in the effect of RIPC between the genders are lacking. In animal studies, females have been found to experience a weaker (Penna et al. 2009; Ciocci Pardo et al. 2018) or no effect (Dow and Kloner 2007; Sachdeva et al. 2014; Heinen et al. 2018) of RIPC. Yet, a recent study on rat hearts demonstrated no difference in cardioprotection by RIPC between the genders (Lieder et al. 2019). In addition, a retrospectively analysed human study did not reveal any differences in the effect of IPC in cardioprotection (Kleinbongard et al. 2016).

2.3.3. Diabetes

There is some evidence that diabetes might decrease the effect of RIPC. Moretti et al. found that the nephroprotective effect of RIPC is not present in diabetic patients (Moretti et al. 2018). Additionally, RIPC showed no protection in hyperglycaemic endothelial cells in vitro (Schenning et al. 2015) and no cardioprotection in diabetic rats compared to normoglycaemic rats (Wider et al. 2018). Also, it has been suggested that diabetes reduces the cardioprotective effect of RIPostC, but does not completely abolish it (Oosterlinck et al. 2013). Jensen et al. showed that diabetes abolishes the cardioprotective effect of RIPC already at the cost of chronic activation of cardioprotection by circulating O-linked β-N-

acetylglucosamine (O-GlcNAc) with no additional effect by RIPC in diabetic patients (Jensen et al. 2013). Nevertheless, diabetic patients with concomitant PAD have experienced positive effects of RIPC (Shahvazian et al. 2017) and improvement of endothelial function (Maxwell et al. 2019). Moreover, a meta-analysis showed that RIPC even tended to strengthen its nephroprotective effect in these trials with a high percentage of participants with diabetes mellitus (Hu et al. 2016).

2.3.4. Anaesthesia and the role of propofol

The most widely discussed confounder to the effect of RIPC is supposedly propofol. Two large studies with patients undergoing cardiac surgery blamed propofol for their negative results (Hausenloy et al. 2015; Meybohm et al. 2015). Also, a meta-analysis revealed that the protective effect of RIPC against AKI was present only in a subgroup of the studies where propofol was not used (Pierce et al. 2017). Loss of cardioprotection due to the use of propofol has also been reported in animal trials (Behmenburg et al. 2018; Bunte et al. 2019). In addition to propofol, sevofluran was also found to abolish the effect of RIPC in one study (Cho et al. 2019). However, isoflurane anaesthesia showed cardioprotective effects, while propofol did not (Kottenberg et al. 2012). Contrary to the above results, RIPC offered a positive effect on leakage of TnT in patients who were under propofol-anaesthesia compared to patients under volatile anaesthesia in a systematic review; still, the number of patients who received volatile anaesthesia was limited (Benstoem et al. 2018). It has been shown that propofol abolishes the effect of RIPC only if administered before ischaemia, but not immediately after it (Chen et al. 2020). Some possible explanations for propofol's neutralising effect have been suggested. As propofol has a phenolic structure similar to that of the natural antioxidant α-tocopherol (Vasileiou et al. 2009) it has also antioxidant properties (Gan et al. 2015). It has been proposed that the free radical scavenging ability of propofol might attenuate the effects of RIPC (Kottenberg et al. 2014). Additionally, propofol has been demonstrated to interfere with cardioprotection by blocking the activation of the TRPV1 channel in cases of remote preconditioning of trauma via surgical incision (Yu et al. 2019). Since RIPC has also been found to activate TRPV1 channels (Kleinbongard et al. 2017), which contributes to myocardial salvage from ischemia-reperfusion injury (Heymann et al. 2017), propofol is likely to abolish the cardioprotective effect of RIPC by blocking the activation of these channels.

2.3.5. Regular medications

Quite few studies have assessed possible confounding medications affecting RIPC. It has been described that beta-blockers may attenuate the cardioprotective effect of RIPC (Zhou et al. 2013). Statins have been found to have synergistic cardioprotective effects with RIPC through reducing the levels of pro-inflammatory and cardiospecific enzymes (El Desoky et al. 2016; Cho et al. 2019).

2.4. Metabolomics

Metabolites are essential for normal functioning of cells and their flux is influenced by internal and external stimuli. Metabolomics (comprehensive analysis of metabolites) is an innovative way to study the mechanism and objectives of ischaemic conditioning. During recent years, metabolites, including those with low-molecular weight, have been increasingly used as diagnostic and prognostic tools in clinical science. For example, in addition to traditional risk factors for atherosclerotic cardiovascular disease, the metabolome at large has been progressively studied. The metabolomic signatures differ between patients with advanced atherosclerosis and clinically healthy controls (Zagura et al. 2015). In particular, the levels of acylcarnitines (ACs) are different in patients with various stages of PAD and in those without PAD (Ismaeel et al. 2019). There are limited number of studies that have assessed the effect of RIPC on low-molecular weight metabolites. The effect of RIPC on amino acids and biogenic amines that are mainly involved in energy metabolism has been described (Chao de la Barca et al. 2016; Olenchock et al. 2016; Wang et al. 2018). The effect of RIPC is thought to be attained via multiple combined factors. Although there are some data about the actions of RIPC their exact mechanisms, including the actions on the metabolome, are still unknown.

2.4.1. Acylcarnitines

ACs are esters of L-carnitine and fatty acids and due to the existence of different fatty acids (Wishart et al. 2018), a large set of ACs, generally divided into short, medium and long chain ACs (denoted as SCACs, MCACs and LCACs), can be produced. For the transportation of fatty acids into mitochondria for beta-oxidation, the coenzyme A group is attached and is displaced further by carnitine to form acylcarnitine. The latter is able to enter the mitochondrial matrix, where it can be broken down by carnitine palmitoyl transferase II to release activated fatty acid to enter the process of beta-oxidation (McGill et al. 2014). It is crucial to produce LCACs as the mitochondrial inner membrane is impenetrable for long chain fatty acids. The increase of LCACs is most commonly associated with metabolic disorders such as mitochondrial dysfunction and genetically determined enzyme deficiencies. Based on previous studies, decrease of the levels of ACs

can be associated with preserved mitochondrial function (Bjørndal et al. 2018), whereas their increase has been linked to increased mortality in patients with chronic heart failure (Reuter and Evans 2012) and to worse prognosis in those with IgA nephropathy (Makrecka-Kuka et al. 2017). Several carnitine esters and members of the ACs' family are elevated in patients with PAD (Ismaeel et al. 2019). Nevertheless, the impact of ACs in clinical practice is unknown as relevant studies are lacking. Considering the results of the studies published on ACs, the knowledge of the ACs profile may facilitate assessment of the patients' general metabolic milieu, mitochondrial functioning and prognosis.

Plasma ACs have a different origin. The main precursors of SCACs are branched chain amino acids (BCAAs), but some SCASs are also produced by the catabolism of glucose and some triglycerides. MCACs and LCACS are only derived from fatty acid metabolism, whereas carnitine is required for transporting long-chain fatty acids into mitochondria (Makrecka-Kuka et al. 2017). Plasma SCASs have been found to be released from the liver (Schooneman et al. 2015), MCACs, from the skeletal muscles and liver (Xu et al. 2016) and LCACs from the heart (Makrecka-Kuka et al. 2017). Summing up, although the exact origin of plasma ACs is not clear, basing on assessment of the whole ACs spectrum, conclusions can be drawn about whole-body acylcarnitine metabolism.

2.5. Summary of the literature review

RIPC is an experimental procedure in which short episodes of ischaemia are induced in order to offer protection to the tissues of distant organs. Protective effects of RIPC for heart, kidneys and other organs, as well as to tissues sensitive to ischaemia have been described in procedures where ischaemia-reperfusion injury occurs. Nevertheless, the results are still quite contradictory. The exact mechanisms of RIPC are unknown, but multiple neural and humoral pathways along with biochemical markers involved in achieving the effect of RIPC have been discovered during recent decades. However, still little is known about the effects of RIPC on arterial stiffness and metabolomics, and only a few studies have assessed the impact of RIPC on vascular surgery. The most effective protocol of RIPC has not yet been established, although protocols consisting of 3–4 cycles of 5-minute ischaemia are most commonly used. It is not clear who and under what circumstances will benefit the most from RIPC, but some possible confounders have been described: age, gender, concomitant diseases and certain medications.

3. AIMS OF THE THESIS

The general aim of this thesis was to evaluate the effect of RIPC on multiorgan (arteries, heart, kidneys) salvage and on acylcarnitines' metabolism in association with vascular surgery and to find out associations between the achieved effect and the clinical characteristics of the patients.

Specific aims:

1. To evaluate the effects of RIPC on arterial stiffness in patients undergoing vascular surgery
2. To assess the effect of RIPC on the cardiospecific biomarkers and to establish clinical characteristics associated with these biomarkers in patients undergoing vascular surgery
3. To estimate the effect of RIPC on kidney biomarkers in patients undergoing lower limb revascularisation surgery
4. To measure the effect of RIPC on acylcarnitines' metabolism in patients undergoing vascular surgery

4. SUBJECTS AND METHODS

4.1. Study group

Patients undergoing open surgery for repair of infra-renal AAA or surgical lower limb revascularisation surgery (for intermittent claudication or critical limb ischaemia, common femoral artery endarterectomy, aorto(bi)femoral or femoro-popliteal or femorotibial or iliofemoral bypass surgery) or carotid endarterectomy (for symptomatic or asymptomatic carotid stenosis) were enrolled non-consecutively. Signed informed consent was obtained from each patient. The study was carried out at the Department of Vascular Surgery, Clinic of Surgery, Tartu University Hospital, from January 1, 2016 to February 8, 2018.

The exclusion criteria were the following: age under 18 years, pregnancy, known malignancy in the past 5 years, permanent atrial fibrillation or flutter, symptomatic upper limb atherosclerosis, the need for oxygen therapy at home, estimated preoperative glomerular filtration rate (eGFR) < 30 ml/min/1.73 m2, myocardial infarction in the past month, previous history of upper limb vein thrombosis or vascular surgery in the axillary region, and inability to follow the study regimen.

4.2. Study design and eligibility

This was a one-centre, prospective, randomised double-blinded sham-controlled clinical trial. The study was approved by the Research Ethics Committee of the University of Tartu (protocol nr 252/M-24), and was registered in the ClinicalTrials.gov database (NCT02689414).

4.3. Methods

4.3.1. Intervention

The protocol of RIPC consisted of four 5-minute episodes of ischaemia, which is one of the most commonly described protocols in previous studies (Zarbock and Kellum 2016; Zhou et al. 2017). The intervention was performed with a blood pressure cuff on an upper arm. Ischaemia was achieved by raising cuff pressure to 200 mm Hg or, when the patient's blood pressure exceeded 180 mm Hg, to a value that was 20 mm Hg higher than the value of his/her systolic blood pressure. Patients' blood pressure was measured at the same time on the contralateral arm with the blood pressure cuff or directly via a cannula needle in an artery. In the sham group, the pressure in the cuff was equal to venous pressure (10–20 mm Hg). Between all episodes, there was a 5-minute period of reperfusion. The intervention was started along with preparation for anaesthesia in the operating

theatre. Participation in the study did not influence any other aspects of treatment (surgery, anaesthesia or medication use). The author of this academic dissertation who was the principal investigator and was responsible for enrolment of patients, for performing the intervention and for data storage.

4.3.2. Randomisation

Randomisation was accomplished using a block design with block size 2 or 4 and stratification. The WINPEPI (PEPI-for-Windows) computer program was used to generate a random allocation sequence. Patients were stratified by surgery (aneurysm repair or other), age (under or over 65 years) and the American Society of Anaesthesiologists' (ASA) physical status classes 2, 3 or 4. By using these differentia, 16 strata were created. The allocation letter was kept in an opaque sealed envelope and opened immediately before the intervention when patients arrived in the operating theatre. Randomisation and the opaque sealed envelopes were arranged by a third party. Patients were randomly allocated at a ratio of 1:1 in parallel to the RIPC or the sham group.

4.3.3. Blinding

The patient, patient's physician, surgeon, anaesthesiologist and everyone else in the surgical team were blinded to study intervention. The principal investigator (T.K.) was responsible for performing the intervention and while keeping the scale of the manometer concealed throughout the intervention. In principle, patients were able to feel pressure in the cuff, but their group affiliation and the pressure required to achieve preconditioning was not revealed to them. The statistician was blinded to the meaning of group affiliation.

4.3.4. Outcomes

The co-primary outcomes were augmentation index (AIx) and carotid-femoral pulse wave velocity (PWV).
The secondary outcomes were:

- arterial elasticity indices
- central and peripheral blood pressure and heart rate
- cardiac biomarkers: high sensitivity troponin T (hs-TnT) and N-terminal pro b-type natriuretic peptide (NT-proBNP);
- traditional biomarkers of renal function: urea and creatinine;
- novel biomarkers of renal function: neutrophil gelatinase-associated lipocalin (NGAL), liver-type fatty acid binding protein (L-FABP), kidney injury molecule-1 (KIM-1), cystatine C, beta-2 microglobulin;

- markers of inflammation and oxidative stress: oxidized low-density lipoprotein (oxLDL), interleukin-18 (IL-18), myeloperoxidase (MPO), isoprostanes
- acylcarnitines
- complications of remote ishcaemic preconditioning: upper-extremity deep vein thrombosis, acute upper limb ischaemia.

4.3.4.1. Biochemical analysis of blood and urine

Blood samples were collected in the morning of surgery, and at 2 hours, 8 hours and at approximately 24 hours of surgery, and urine samples were collected in the morning of surgery and 24 hours after surgery. The last blood and urine collection was set as close as possible to 24 hours after surgery provided that the patient had fasted for at least 3 hours. Analysis of hs-TnT, NT-proBNP, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, creatinine, urea, beta-2 microglobulin, cystatin C, hs-CRP and complete blood count were analysed in the United Laboratories of Tartu University Hospital. Serum for analysis of oxLDL, IL-18, MPO, NGAL was extracted after centrifugation and stored in the refrigerator at –80 °C until measurements in the laboratory of the Department of Biochemistry, University of Tartu. Urine samples for analysis of isoprostanes, creatinine, L-FABP and KIM-1 were also centrifuged and stored in the refrigerator at –80 °C and analysed in the same laboratory.

4.3.4.2. Targeted serum metabolite profiling

The levels of low-molecular weight metabolites were analysed using the AbsoluteIDQp180 kit (Biocrates Life Sciences AG, Innsbruck, Austria). The analytical procedure was performed according to the manufacturer's standard protocol in the laboratory of the Department of Biochemistry, University of Tartu. In brief, for targeted analysis of metabolites, the internal standard was pipetted onto a 96-well extraction plate and 10 µL of serum was added to each well. Drainage was achieved with nitrogen and derivatisation was performed with phenyl isothiocyanate. Measurements were accomplished with QTRAP 4500 (ABSciex, USA) coupled to an Agilent 1260 series HPLC (USA) using the C18 column and flow injection analysis. The vendor's software with internal standards' intensities was used to calculate the concentrations of metabolites.

4.3.4.3. Parameners of arterial stiffness and peripheral and central haemodynamics

Arterial stiffness was evaluated with measurements of carotid-femoral PWV, AIx and small and large artery elasticity indices. PWV and AIx were measured with Sphygmocor XCEL PWA and PWV (AtCorMedical, Sydney, Australia) pre-

operatively and approximately 24 hours postoperatively. The measurements were carried out in the resting state with the patient having fasted and not having smoked for at least three preceding hours. For AIx assessment, the brachial waveform was captured by the machine inflating the cuff partially. The central aortic waveform and pulse wave analysis were generated by SphygmoCor Brachial GTF. The AIx, AIx@75, central blood pressure, pulse pressure, and mean arterial pressure were provided. Two or three measurements were performed. If the difference between two measurements was greater than 2 units (%) in the case of AIx and/or 1 unit (%) in the case of AIx@75, a third measurement was performed and the median value of three measurements was used for final analysis. The median value was to reduce the possible impact of one dissimilar measurement. Otherwise, the mean value of two measurements was used. For blood pressure and heart rate, the mean value of two or three measurements was used in the final analysis.

For PWV assessment, femoral and carotid pulse waves were recorded simultaneously. The femoral waveform was captured by the machine inflating the cuff partially over the thigh. The carotid waveform was captured by applanation tonometry. At least two quality PWV measurements with a maximum difference of 0.5 m/s were obtained and the mean value of these measurements was used for the final analysis.

The elasticity indices of large (C1) and small (C2) arteries were obtained non-invasively using the HDI/PulseWave CR-2000 research CardioVascular Profiling System (Hypertension Diagnostics, Inc., MN, USA). At least two quality measurements were required with a maximum difference of 2 in C2 and 3 in C3. The mean value of these measurements was used for the final analysis.

4.3.5. Statistical analysis

Sample size calculation was performed based on one of the co-primary outcomes – AIx@75. As no previous studies had dealt with the effect of RIPC on AIx@75 or other parameters of vascular stiffness, no data required for calculation of sample size were available. Hence, sample size was calculated based on the data of the first 30 participants. Also, as the postulated prediction was unidirectional, one-tailed Welch's t test was used for calculation of sample size. The desired study power was set at 80% and the magnitude of the effect to be obtained was 5% of the difference in AIx@75 between the study group and the sham group. The calculated sample size was 44 for each group.

Categorical variables were compared with Pearson's chi-square test. The difference of continuous variables between two groups were compared using the two sample t-test for data with a normal distribution or with the Wilcoxon rank sum test in the case of a data with a non-normal distribution. The significance of change of continuous variables within one group was calculated with the paired T-test for data with normal distribution or Wilcoxon signed rank test in the case of a data with non-normal distribution. For statistical analysis of multiple

repeated measures, analysis of variance (ANOVA) or multivariate analysis of variance (MANOVA) for repeated measures was used. For assessing correlations between cardiac enzymes and other clinical parameters, Spearman's correlation coefficient was employed. All tests were performed as two-way tests. Data with a normal distribution is presented as mean and standard deviation (SD) and data with a non-normal distribution is presented with median and interquartile range.

For correction for multiple comparisons, Benjamini-Hochberg procedure was used to control false discovery rate. A p value of < 0.05 was considered to be indicative of statistical significance. Kolmogorov-Smirnov test was used for testing the normality of distribution.

Statistical analysis was performed by a qualified statistician of the University of Tartu.

5. RESULTS

Ninety-eight patients were recruited and randomised into study groups. After dropouts, the data of 45 patients in the RIPC group and 48 patients in the sham group was used in the final data analysis. The detailed patient flow is shown in Figure 1. The reason for not measuring arterial stiffness twice was the patient's stay in the intensive care unit. In 42 patients (93%) of the RIPC group and in 45 (96%) patients of the sham group, blood was collected at all time points: preoperatively and at 2 hours, 8 hours and 24 hours postoperatively. In 3 patients of the RIPC group and in 2 patients of the sham group, blood collection was missed either at 2 hours or 8 hours after surgery. The median time from the end of the intervention to the beginning of surgery did not differ significantly (p=0.057) between the RIPC (36 min, IQR 21–46 min) and the sham group (25 min, IQR 15–38 min). The change in eGFR through all time points was similar between the groups (p=0.369). The median change in eGFR from baseline to 24 hours postoperatively was 2 mL/min/1.73m^2 (IQR –3–5 mL/min/1.73m^2;

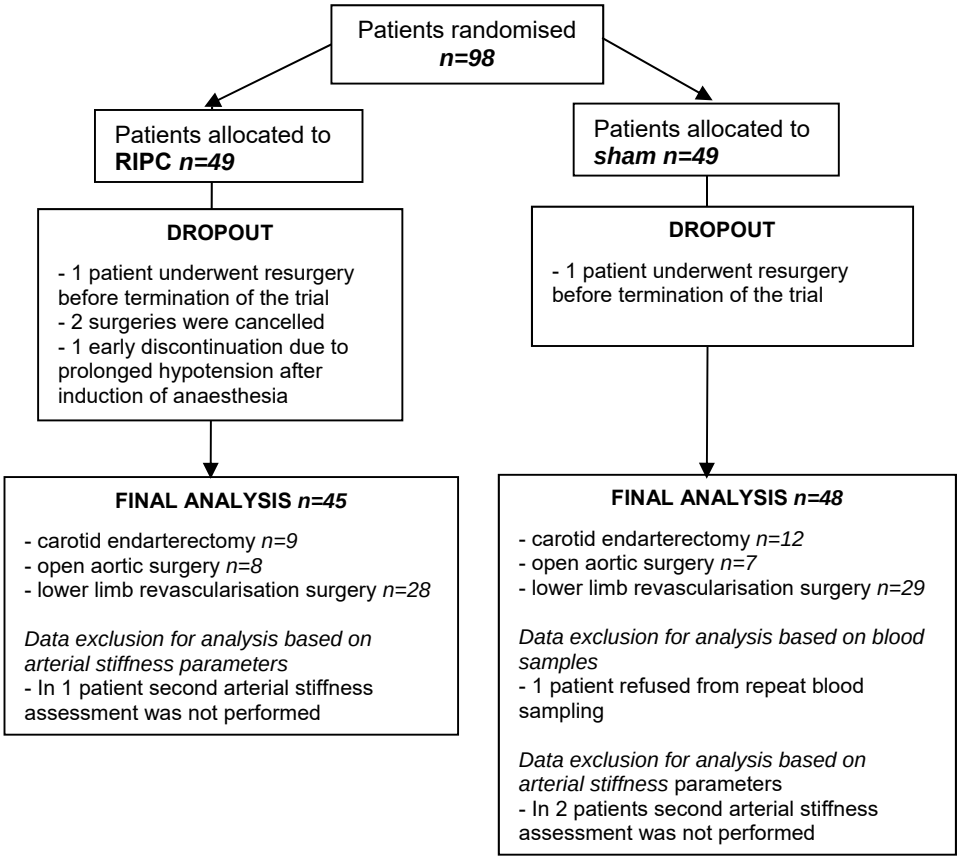

Figure 1. Patients' flow chart for the remote ischaemic preconditioning (RIPC) group and the sham group in vascular surgery

p=0.401) in the RIPC group and 1 ml/min/1.73m^2 (IQR –8–5 mL/min/1.73m^2; p=0.393) in the sham group with no significant difference between the groups (p=0.348). The baseline characteristics of the two groups were similar (Table 1) and there were no significant differences between the groups regarding the characteristics of surgery (Table 2). No patient experienced complications of RIPC (upper-extremity deep vein thrombosis, acute upper limb ischaemia) nor considered the procedure intolerable.

Table 1. Baseline characteristics of the vascular surgery patients allocated to remote ischaemic preconditioning (RIPC) or sham procedure

Variable	RIPC (n=45)	SHAM (n=47)	p-value
Age, years (SD)	67 (±9)	66 (±10)	0.577
Male, n (%)	36 (80)	32 (68)	0.288
BMI, kg/m2 (SD)	26.3 (±6.4)	26.5 (±6.7)	0.840
ASA 2, n (%)	18 (40)	19 (40)	1
ASA 3, n (%)	20 (44)	22 (47)	0.986
ASA 4, n (%)	7 (16)	6 (13)	0.933
ACEI or ARB, n (%)	21 (47)	30 (64)	0.148
Calcium channel blockers, n (%)	9 (20)	17 (37)	0.135
Beta-blockers, n (%)	11 (24)	19 (40)	0.158
Statins, n (%)	13 (29)	14 (30)	1
Diabetes, n (%)	5 (11)	8 (17)	0.607
Myocardial infarction, n (%)	8 (18)	3 (6)	0.172
Stroke, n (%)	10 (22)	12 (26)	0.899
Smoker (current or ex-smoker), n (%)	40 (89)	42 (89)	1
PSBP, mm Hg (SD)	143 (±18)	141 (±16)	0.537
PDBP, mm Hg (SD)	78 (±11)	79 (±11)	0.674
CSBP, mm Hg (SD)	131 (±15)	130 (±14)	0.634
CDBB, mm Hg (SD)	79 (±11)	81 (±11)	0.522
MAP, mm Hg (SD)	99 (±12)	100 (±11)	0.678
Heart rate, bpm (SD)	66 (±9)	67 (±11)	0.754
Cholesterol	5.0 (4.2–5.7)	5.0 (3.9–5.6)	0.793
LDL	3.4 (8.1–10.4)	3.3 (2.5–3.8)	0.500
HDL	1.1 (0.9–1.4)	1.1 (1.0–1.3)	0.311
Triglycerides	1.6 (1.3–1.8)	1.5 (1.2–2.0)	0.787
AIx, % (SD)	36 (±11)	34 (±13)	0.467
AIx@75, % (SD)	32 (±11)	30 (±13)	0.517
PWV, m/s (IQR)	9.4 (8.1–10.4)	8.7 (7.9–9.8)	0.233

BMI – body mass index; ASA – American Society of Anaesthesiologists' physical status score; ACEI – angiotensin-converting-enzyme inhibitor; ARB – angiotensin II receptor blocker; PSBP – peripheral systolic blood pressure; PDBP – peripheral diastolic blood pressure; CSBP – central systolic blood pressure; CDBP – central diastolic blood pressure; MAP – mean arterial blood pressure; AIx – augmentation index; AIx@75 – augmentation index corrected for a heart rate of 75 beats per minute; PWV – pulse wave velocity; SD – standard deviation; IQR – interquartile range

Table 2. Characteristics of vascular surgery of patients allocated to remote ischaemic preconditioning (RIPC) or sham procedure

	RIPC (n=45)	SHAM (n=47)	p-value
General anaesthesia, n (%)	24 (53)	28 (60)	0.6941
Spinal anaesthesia, n (%)	21 (47)	18 (38)	0.548
Intravenous anaesthesia, n (%)	0	1 (2)	1
Administration of propofol, n (%)	19 (42)	26 (55)	0.295
Carotid endarterectomy, n (%)	9 (20)	12 (26)	0.701
Lower limb revascularisation, n (%)	28 (62)	28 (60)	0.963
Open aortic aneurysm repair, n (%)	8 (18)	7 (15)	0.927
Infusion during surgery, ml (IQR)	1200 (1000–2100)	1350 (1000–1600)	0.858
Blood loss during surgery, n (%; average ml)	8 (18; 1157)	13 (28; 754)	0.379
Duration of surgery, min (IQR)	108 (89–135)	112 (84–156)	0.827
Hospitalisation to 3rd level intensive care unit, n (%; average days)	1 (2; 4)	4 (9; 2.25)	0.384

IQR ─ interquartile range

5.1. Effect of RIPC on arterial stiffness (Paper I)

5.1.1. Comparison between the groups: effects of RIPC

The baseline values of arterial stiffness parameters did not differ between the groups (Table 1). There were no significant differences between the RIPC and sham groups, regarding changes 24 h post-operatively, in AIx (p=0.828), AIx@75 (p=0.837), PWV (p = 0.701), C1 (p=0.785), C2 (p=0.635), or mean arterial pressure (MAP, p=0.676).

5.1.2. Post-operative changes within the RIPC and the sham group

There were significant improvements at approximately 24 h post-operatively in AIx, AIx@75, and C2 within both groups, but changes of PWV and C1 were not significant (Figure 2). In the RIPC group, changes of the arterial stiffness parameters were as follows: PWV mean change –0.3 m/s, (SD 1.7); AIx median change –5.8 %, (IQR –15–0); AIx@75 median change –2.5%, (IQR –9.8–0.5); C1 median change 0.5 mL/mmHg × 100, (IQR –1.6–3.7; C2 median change 0.7 mL/mmHg × 100, (IQR 0–3.4). In the sham group, the changes in the arterial stiffness parameters were the following: mean change in PWV –0.4 m/s, (SD 1.4); median change in AIx –5.5%, (IQR –13.5–0.5); median change in AIx@75 –2%, (IQR –10.0–1.0); median change in C1 0.1 mL/mmHg × 10, (IQR –1.7–2.5); median change in C2 0.85 mL/ mmHg × 100, (IQR 0–2.2). All haemodynamic changes were statistically significant in both groups (Figure 3).

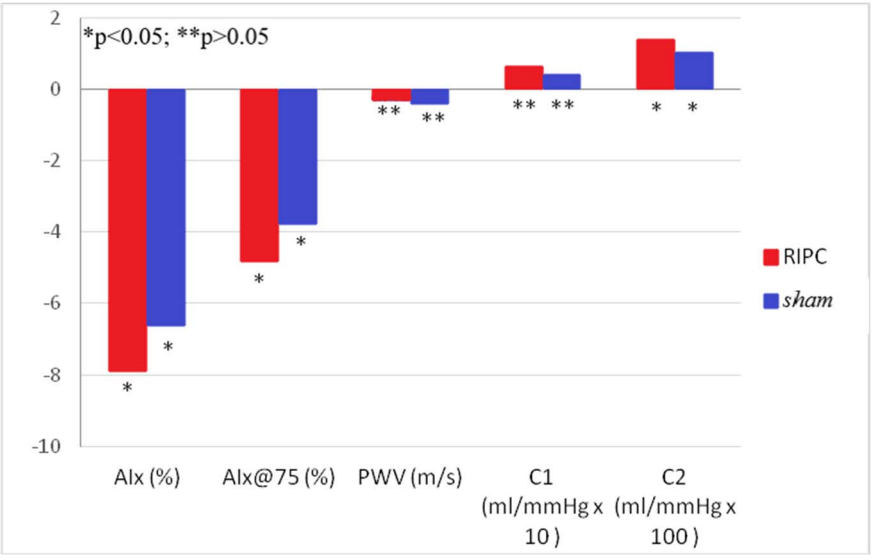

Figure 2. Changes in the arterial stiffness parameters in the remote ischaemic preconditioning (RIPC) group and in the sham group in vascular surgery. AIx – augmentation index (%); AIx@75 – augmentation index corrected for heart rate of 75 beats per minute (%); PWV – pulse wave velocity (m/s); C1 – large artery elasticity index; C2 – small artery elasticity index.

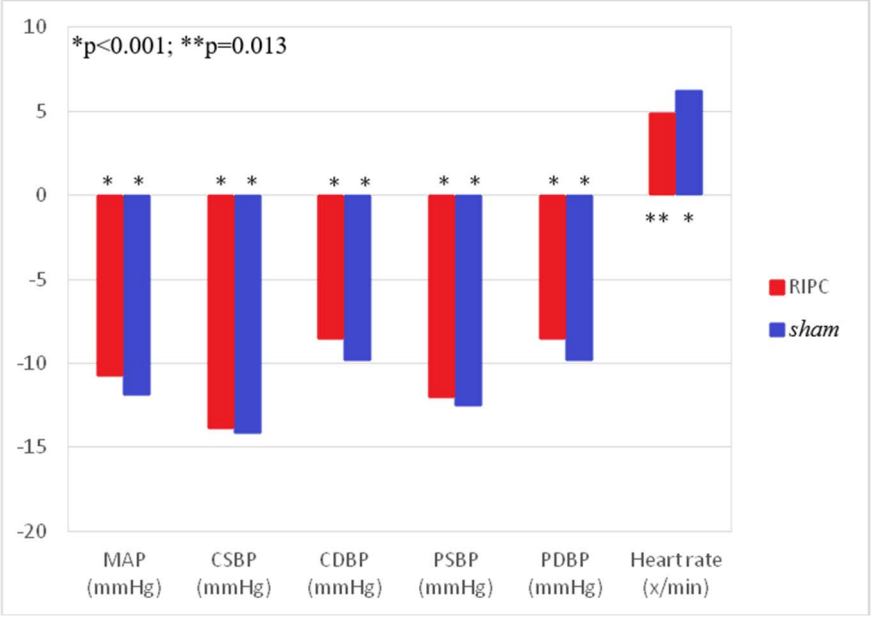

Figure 3. Changes in the haemodynamic parameters in the remote ischaemic preconditioning (RIPC) group and in the sham group in vascular surgery. MAP – mean artrial pressure; CSBP – central systolic blood pressure; CDBP – central diastolic blood pressure; PSBP – peripheral systolic blood pressure; PDBP – peripheral diastolic blood pressure.

5.2. Effect of RIPC on cardiac damage biomarkers (Paper II)

5.2.1. Hs-Troponin T

The baseline hs-TnT did not differ statistically (p=0.900) between the RIPC group (median 10.4 ng/L IQR 7.1–14.7 ng/L) and the sham group 10.5 ng/mL (IQR 7.2–15.3 ng/L). Hs-TnT increased significantly more in the sham group compared to the RIPC group during follow-up at 2 hours, 8 hours and 24 hours after surgery (p<0.001, Figure 4). Also, the difference in the peak change of hs-TnT between the RIPC group (median change 0.6 ng/L; IQR –0.7–2.1 ng/L) and the sham group (median change 2 ng/L; IQR 0.9–6.2 ng/L) was statistically significant (p=0.003). With regard to within-group differences, the increase of hs-TnT from the baseline to its maximum value was statistically significant in both groups (p<0.001).

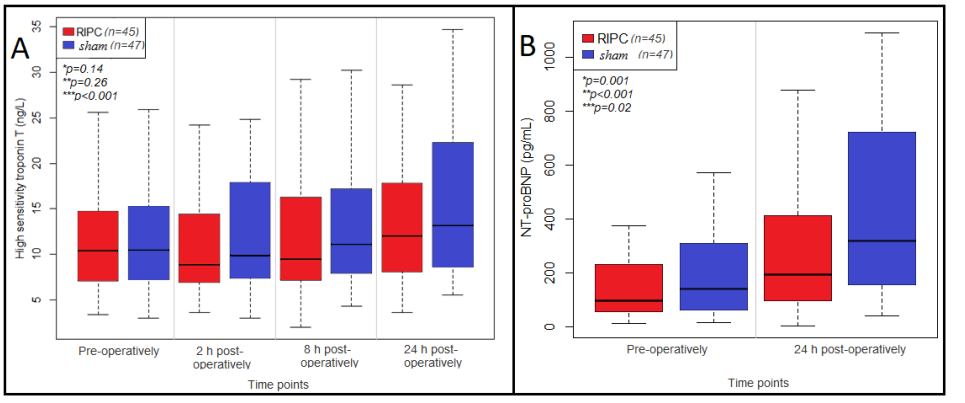

Figure 4. Changes in cardiac biomarkers in the remote ischaemic preconditioning (RIPC) group and in the sham group in vascular surgery **A** Changes in high-sensitivity troponin T across the time points **B** Changes in N-terminal pro b-type natriuretic peptide (NT-proBNP); *p-value for the change in the RIPC group; ** p-value for the change in the sham group;***p-value for the changes between the groups

5.2.2. NT-proBNP

The baseline median NT-proBNP value was 99 pg/mL (IQR 56─232 pg/mL) in the RIPC group and 143 pg/mL (IQR 63─312 pg/mL) in the sham group (p=0.419). A significant increase of NT-proBNP was noted both in the RIPC (median increase 51 pg/mL, IQR –12–196 pg/mL) and sham groups (median increase 144 pg/mL, IQR 17–318 pg/mL, Figure 4). The difference in the increase between the groups was statistically significant (p=0.020).

5.2.3. Cardiac complications

Two patients were diagnosed with decompensation of heart failure and a new onset of atrial fibrillation was diagnosed in one of them. In the other patient, a marked increase of hs-TnT (596 ng/L) was detected, but there were no ECG changes, nor did the patient have angina. Both of these patients were from the sham group. No patient was diagnosed with perioperative myocardial infarction. Four patients in the sham group and one patient in the RIPC group were hospitalised to a 3rd level intensive care unit after surgery (p=0.384). There were no statistically significant differences between the groups regarding the characteristics of surgery (Table 2).

5.2.4. Correlations between cardiac biomarkers and clinical parameters (Table 3)

Positive correlation between gender (male<female) and increase of hs-TnT (ρ=0.36; p=0.015) and NT-proBNP (ρ=0.38; p=0.010) was established in the RIPC group. There was also correlation between increase of hs-TnT and baseline AIx (ρ=0.29; p=0.051) in the RIPC group. Negative correlation (ρ=–0.30; p=0.042) occurred between pretreatment with statins and increase of hs-TnT in the RIPC group. There was positive correlation (ρ=0.46; p=0.027) between increase of NT-proBNP and increase of PWV in the sham group. Positive correlation occurred between administration of propofol and increase of NT-proBNP both in the RIPC group (ρ=0.46; p=0.001) and in the sham (ρ=0.37; p=0.012) group. Negative correlation was found between type of anaesthesia (general<spinal) and increase in NT-proBNP both in the sham (ρ=–0.34; p=0.019) and RIPC group (ρ=–0.36; p=0.016). Also, negative correlation occurred between hs-TnT and eGFR both in the RIPC group (ρ=–0.47, p=0.001) and in the sham group (ρ=–0.37, p=0.010)

Table 3. Spearman's correlation coefficients between high-sensitivity troponin T, NT-proBNP and other clinical markers for patients undergoing vascular surgery, who were allocated to the remote ischaemic preconditioning (RIPC) or the sham group,

	Hs-TnT				NT-proBNP			
	RIPC (n=45)	**p***	**SHAM (n=47)**	**p***	**RIPC (n=45)**	**p***	**SHAM (n=47)**	**p***
Age	0.20	0.177	–0.02	0.909	–0.03	0.840	–0.09	0.536
Gender	**0.36**	0.015	0.07	0.628	**0.38**	0.010	0.04	0.800
Current or past smoker	0.07	0.638	–0.04	0.795	–0.12	0.451	0.18	0.242
eGFR	**–0.47**	0.001	**–0.37**	0.010	–0.25	0.097	–0.02	0.875
Blood loss	0.03	0.870	0.23	0.130	0.02	0.879	0.19	0.206
Infusion	–0.11	0.469	0.11	0.486	0.10	0.508	0.18	0.238
Anaesthesia (general<spinal)	–0.25	0.092	–0.07	0.642	**–0.36**	0.016	**–0.34**	0.019
Administration of propofol	0.25	0.101	0.07	0.633	**0.46**	0.001	**0.37**	0.012
Baseline AIx	0.29	0.051	0.16	0.288	0.16	0.280	–0.01	0.952
Change in AIx	0.09	0.580	–0.25	0.106	–0.13	0.411	–0.13	0.400
Baseline Aix@75	0.24	0.109	0.11	0.478	0.10	0.504	0.09	0.573
Change in Aix@75	0.24	0.120	–0.22	0.149	–0.09	0.545	0.02	0.890
Baseline PWV	–0.03	0.852	0.08	0.638	–0.10	0.560	–0.01	0.967
Change in PWV	–0.11	0.523	–0.07	0.691	0.21	0.216	**0.36**	0.027
Baseline MAP value	–0.03	0.859	0.002	0.986	–0.17	0.269	0.15	0.325
Change in MAP	–0.05	0.758	–0.15	0.345	0.25	0.106	0.05	0.771
ACEI/ARB	–0.08	0.583	–0.06	0.687	–0.10	0.516	0.22	0.147
Ca-channel blockers	–0.04	0.802	–0.02	0.892	–0.04	0.933	–0.01	0.794
Beta-blockers	0.13	0.382	–0.16	0.299	0.06	0.670	–0.12	0.435
Statins	**–0.30**	0.042	–0.12	0.409	0.08	0.622	0.06	0.690
Antiagregants	–0.01	0.964	–0.10	0.515	–0.002	0.991	0.15	0.333
Diabetes	–0.04	0.790	0.08	0.607	–0.03	0.859	–0.24	0.109
Myocardial infarction	–0.07	0.661	–0.14	0.355	–0.11	0.473	–0.20	0.188

eGFR – estimated glomelural filtration rate; AIx – augmentation index; AIx@75 – augmentation index corrected for a heart rate of 75 beats per minute
PWV – pulse wave

5.3. Effect of RIPC on kidney biomarkers in the subgroup of patients undergoing lower limb revascularization surgery (Paper III)

5.3.1. The effect of RIPC on kidney biomarkers in serum and urine

Creatinine increased significantly more in the sham group than in the RIPC group (p=0.021, Figure 5) through all time points (from baseline to 2, 8 and 24 hours after surgery). The eGFR decreased significantly more in the sham group (p=0.015, Figure 5). There was no significant change in urea between the groups through all time points (p=0.067, Figure 5). The change in cystatin C and beta-2 microglobulin was significantly different between the groups (p-values 0.021 and 0.024, respectively, Figure 5). Creatinine, cystatin C, and beta-2 microglobulin increased and eGFR decreased significantly more in the sham group than in the RIPC group through all time points (p= 0.021, p=0.024, p=0.021, and p=0.015, respectively, Figure 5). The change in NGAL levels did not differ between the groups (p=0.174). When comparing the two timepoints, baseline and 24 hours after surgery, the increase in creatinine, eGFR, urea, cystatin C and beta-2 microglobulin was significantly smaller in the RIPC group (p=0.003, p=0.005, p=0.041, p=0.022, p=0.033, respectively, Table 4). In 4 patients (14%) from the sham group and in 1 (4%) patient from the RIPC group, AKI could be diagnosed based on the KDIGO's criteria, however, the difference was insignificant (p=0.604)

The level of KIM-1 in urine increased significantly both in the RIPC group (change median 930, IQR –81–3078, p=0.001) and in the sham group (change median 1238, IQR -124–3097, p=0.006), but there was no difference between the groups (p=0.935). The change in urinary L-FABP level was not statistically significant in either group, nor was there any difference between the groups in this respect (p=0.710, Table 4).

There was a significant increase in creatinine (p=0.011), eGFR (p=0.007), cystatin C (p=0.038) and beta-2 microglobulin (p=0.013) in the sham group through all time points (Figure 5). There was a significant decrease in urea (p=0.050, Figure 5) and a significant increase in NGAL (p=0.001) in the RIPC group through all time points. The change in creatinine, urea, cystatin C and beta-2 microglobulin was not statistically significant in the RIPC group (p>0.05) and the change in urea and NGAL was not statistically significant in the sham group (p>0.05) (Table 4).

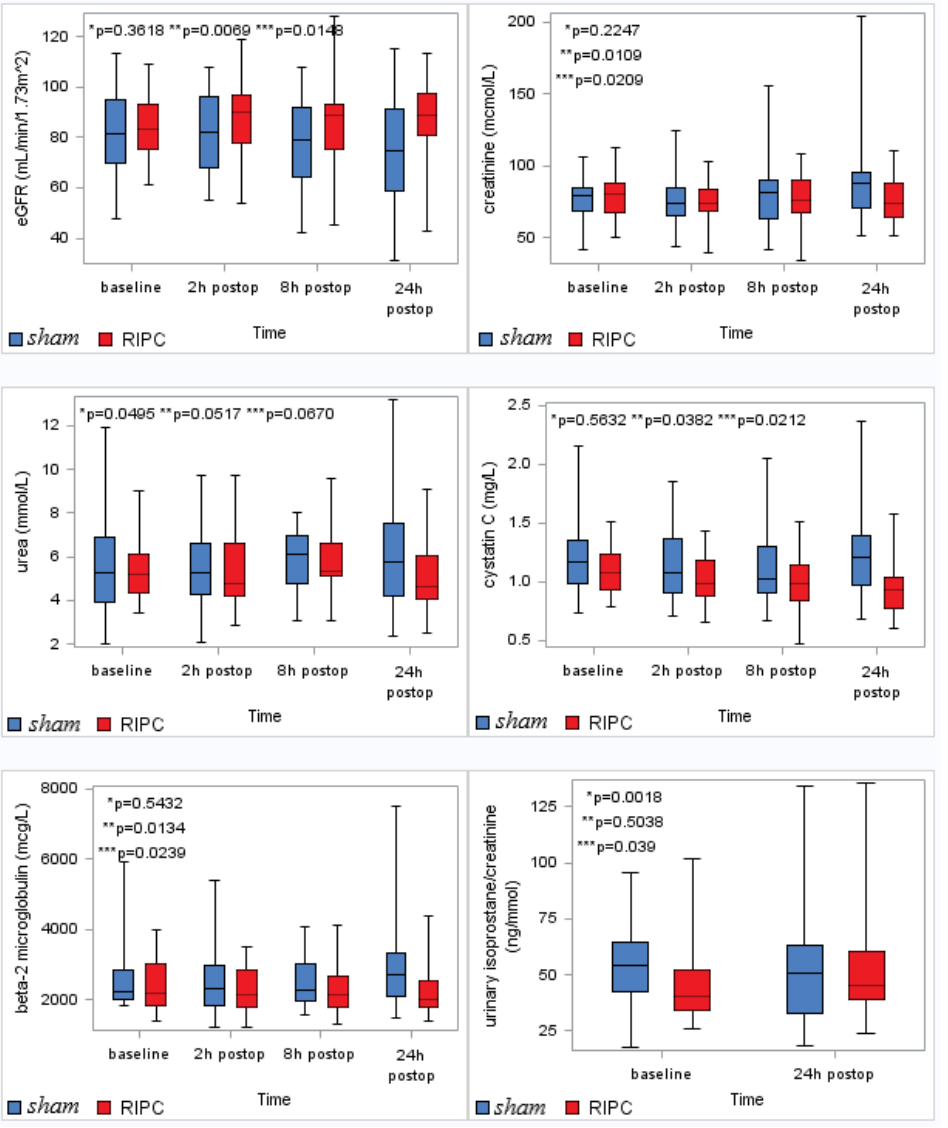

Figure 5. Statistically significant changes between the sham group and the remote ischaemic preconditioning (RIPC) group in creatinine, estimated glomerular filtration rate (eGFR), urea, cystatin C, beta-2 microglobulin and in the ratio of urinary isoprostanes to creatinine in patients undergoing lower limb revascularisation surgery. *p-value for the change in the RIPC group; ** p-value for the change in the sham group; ***p-value for the changes between the groups

Table 4. Changes in the kidney and oxidative stress biomarkers from baseline to 24 hours after vascular surgery in the remote ischaemic preconditioning (RIPC) group and in the sham group

	RIPC			SHAM			RIPC vs SHAM p-value
	Baseline	24 h after surgery	p-value	Baseline	24 h after surgery	p-value	
Serum creatinine (µmol/L)	80 (68–88)	74 (64–88)	**0.030**	79 (69–85)	88 (71–95)	**0.024**	**0.003**
eGFR (mL/min/1,73m^2)	84 (75–93)	89 (81–98)	**0.037**	82 (70–95)	75 (59–92)	**0.016**	**0.005**
Serum urea (mmol/L)	5.2 (4.4–6.2)	4.7 (4.1–6.1)	0.222	5.3 (4.0–6.9)	5.8 (4.2–7.6)	0.075	**0.041**
Serum cystatin C (mg/L)	1.1 (0.9–1.2)	0.9 (0.8–1.0)	**0.0002**	1.2 (1.0–1.4)	1.2 (1.0–1.4)	0.690	**0.022**
Serum beta-2 microglobulin (µg/L)	2195 (1820–3005)	2000 (1775–2520)	0.184	2215 (2010–2850)	2720 (2100–3325)	0.062	**0.033**
Serum NGAL (ng/mL)	79 (68–94)	100 (82–118)	**<0.0001**	82 (71–106)	106 (80–166)	**<0.0001**	0.407
Urinary KIM-1 (pg/mL)	1343 (871–2046)	2689 (1324–4793)	**0.001**	1428 (776–2352)	3025 (1185–4629)	**0.006**	0.935
Urinary L-FABP (ng/ml)	1.2 (0.9–1.8)	1.2 (0.9–1.4)	0.640	1.0 (0.9–1.4)	1.0 (0.9–1.6)	0.814	0.710
Serum Adiponectin (ng/mL)	5712 (3037–9063)	5179 (2651–7214)	**<0.0001**	5129 (2708–7244)	4697 (2604–6431)	**<0.0001**	0.744
Serum IL-18 (pg/mL)	294 (229–388)	277 (236–365)	**<0.0001**	292 (236–371)	265 (230–359)	**<0.0001**	0.794
Serum oxLDL (U/L)	67 (55–84)	52 (44–67)	**<0.0001**	60 (49–74)	47 (40–59)	**<0.0001**	0.942
Serum hs-CRP	3.5 (1.4–13.5)	43.9 (18.5–77.9)	**<0.0001**	3.9 (2.5–8.0)	62.1 (29.8–80.9)	**<0.0001**	0.209
Urinary isoprostanes/ creatinine (ng/mmol)	40 (34–52)	45 (39–60)	**0.002**	54 (43–64)	51 (33–63)	0.504	**0.039**

eGFR – estimated glomerular filtration rate; NGAL – neutrophil gelatinase-associated lipocalin; oxLDL – oxidized low-density lipoprotein; hs-CRP – high-sensitivity C-reactive protein; KIM-1 – kidney injury molecule 1; L-FABP – liver-type fatty acid-binding protein

5.4. Effect of RIPC on the levels of acylcarnitines (Paper IV)

5.4.1. Changes in the levels of ACs in the RIPC and sham groups (Table 5)

A significant decrease ($p<0.05$) was detected both in the sham and the RIPC group in the levels of the following ACs: C2, C8, C10, C10:1, C12, C12:1, C14:1, C14:2, C16, C16:1, C18, C18:1, C18:2. In the sham group, there was a significant increase ($p<0.05$) in the levels of C0 (carnitine) and a significant decrease in the level of C18:1-OH. In the RIPC group, a significant decrease ($p<0.05$) was noted in the levels of C3-OH, C3-DC (C4-OH), C6:1, C9, C10:2. There was a significant difference between the groups regarding the changes in C3-OH ($p=0.023$): there was a significant decrease (–0.007 µmol/L, SD 0.020 µmol/L, $p=0.023$) in the RIPC group and an insignificant increase (0.002 µmol/L, SD 0.015 µmol/L, $p=0.481$) in the sham group.

Table 5. Changes in the levels of acylcarnitine esters from baseline to 24 hours after vascular surgery in the remote ischaemic preconditioning (RIPC) group and in the sham group. Data are shown as mean change ± standard deviation.

	SHAM		RIPC		RIPC vs SHAM p-value
	Change (µmol/L)	p-value	Change (µmol/L)	p-value	
C0	5.094 (±14.137)	**0.017**	4.211 (±14.795)	0.063	0.706
C2	–1.137 (±3.082)	**0.015**	–1.258 (±2.862)	**0.005**	0.791
C3	0.011 (±0.313)	**<0.001**	–0.078 (±0.385)	**<0.001**	0.243
C3-DC	–0.003 (±0.026)	0.394	–0.008 (±0.027)	**0.049**	0.723
C3-OH	0.002 (±0.015)	0.481	–0.007 (±0.02)0	**0.023**	**0.023**
C3:1	–0.000 (±0.015)	0.956	–0.002 (±0.017)	0.458	0.608
C4	0.017 (±0.111)	**<0.001**	–0.013 (±0.117)	**<0.001**	0.204
C4:1	0.002 (±0.015)	0.268	–0.003 (±0.018)	0.223	0.098
C5	0.004 (±0.116)	**<0.001**	–0.037 (±0.153)	**<0.001**	0.055
C5-DC (C6-OH)	0.001 (±0.030)	0.847	0.003 (±0.024)	0.427	0.724
C5-M-DC	0.011 (±0.036)	0.050	0.008 (±0.039)	0.187	0.399
C5-OH (C3-DC-M)	–0.002 (±0.016)	0.468	–0.003 (±0.018)	0.350	0.677
C5:1	0.001 (±0.014)	0.782	–0.004 (±0.022)	0.244	0.058
C5:1-DC	0.003 (±0.014)	0.093	–0.002 (±0.016)	0.530	0.182
C6 (C4:1-DC)	–0.007 (±0.040)	0.242	–0.008 (±0.042)	0.203	0.764
C6:1	–0.000 (±0.001)	0.871	–0.001 (±0.002)	**0.048**	0.107
C7-DC	–0.002 (±0.012)	0.183	–0.002 (±0.011)	0.215	0.903
C8	–0.053 (±0.109)	**0.002**	–0.052 (±0.085)	**<0.001**	0.966
C9	0.000 (±0.019)	0.864	–0.008 (±0.021)	**0.023**	0.059
C10	–0.103 (±0.166)	**<0.001**	–0.115 (±0.183)	**<0.001**	0.935

	SHAM		RIPC		RIPC vs SHAM p-value
	Change (µmol/L)	p-value	Change (µmol/L)	p-value	
C10:1	–0.030 (±0.054)	**<0.001**	–0.033 (±0.043)	**<0.001**	0.686
C10:2	–0.002 (±0.021)	0.442	–0.009 (±0.021)	**0.007**	0.145
C12	–0.026 (±0.062)	**<0.001**	–0.026 (±0.048)	**<0.001**	0.840
C12-DC	–0.007 (±0.026)	0.070	0.000 (±0.027)	0.914	0.180
C12:1	–0.024 (±0.050)	**0.002**	–0.033 (±0.034)	**<0.001**	0.216
C14	–0.005 (±0.023)	**<0.001**	–0.002 (±0.032)	**<0.001**	0.904
C14:1	–0.026 (±0.046)	**<0.001**	–0.022 (±0.027)	**<0.001**	0.938
C14:1-OH	0.000 (±0.010)	0.826	–0.001 (±0.011)	0.510	0.518
C14:2	–0.011 (±0.015)	**<0.001**	–0.007 (±0.014)	**0.002**	0.153
C14:2-OH	–0.000 (±0.022)	0.938	0.002 (±0.013)	0.254	0.972
C16	–0.036 (±0.040)	**<0.001**	–0.029 (±0.035)	**<0.001**	0.367
C16-OH	–0.002 (±0.012)	0.325	–0.003 (±0.017)	0.289	0.760
C16:1	–0.012 (±0.018)	**<0.001**	–0.010 (±0.017)	**<0.001**	0.648
C16:1-OH	–0.003 (±0.009)	0.054	–0.003 (±0.011)	0.072	0.826
C16:2	–0.003 (±0.018)	0.310	–0.002 (±0.017)	0.412	0.935
C16:2-OH	–0.001 (±0.008)	0.435	–0.000 (±0.009)	0.876	0.891
C18	–0.494 (±0.787)	**<0.001**	–0.619 (±0.990)	**<0.001**	0.803
C18:1	–0.057 (±0.051)	**<0.001**	–0.050 (±0.043)	**<0.001**	0.453
C18:1-OH	–0.006 (±0.014)	**0.005**	–0.000 (±0.016)	0.924	0.098
C18:2	–0.015 (±0.015)	**<0.001**	–0.018 (±0.016)	**<0.001**	0.454

5.4.2. Correlations between change in hs-TnT and changes in the levels of ACs (Table 6)

In the RIPC group, there were significant positive correlations between change in hs-TnT and change in C4 (ρ=0.38, p=0.01), C10 (ρ=0.38, p=0.010), C10:1 (ρ=0.38, p=0.010), C12:1 (ρ=0.31, p=0.037), C18:1 (ρ=0.32, p=0.030) and C18-OH (ρ=0.35, p=0.019). In the sham group, there was a significant negative correlation between change in hs-TnT and change in C5-OH (ρ= –0.34, p=0.021). No other significant correlations were observed between changes in the levels of hs-TnT and ACs in the sham group.

5.4.3. Correlations between change in NT-proBNP and changes in the levels of ACs (Table 6)

In the RIPC group, a significant positive correlation occurred between change in NT-proBNP and change in C16:2 (ρ=0.34, p=0.021). In the sham group, a significant positive correlation occurred between change in NT-proBNP and change in C18 (ρ=0.31, p=0.031) and a significant negative correlation occured between change in NT-proBNP and change in C16:1 (ρ= –0.35, p=0.016)

Table 6. Correlations between cardiac biomarkers (i.e.high sensitivity troponin T and NT-proBNP) and acylcarnitines for vascular surgery patients who were allocated to the remote ischaemic preconditioning (RIPC) or sham procedure

	Hs-TnT				NT-proBNP			
	RIPC (n=45)	**p***	**SHAM (n=47)**	**p***	**RIPC (n=45)**	**p***	**SHAM (n=47)**	**p***
C0	–0.08	0.608	–0.13	0.360	0.10	0.516	0.01	0.928
C2	0.20	0.190	–0.07	0.638	0.03	0.855	0.18	0.236
C3	0.202	0.184	0.20	0.18	–0.08	0.608	–0.04	0.765
C3-DC	0.08	0.613	–0.01	0.935	0.13	0.411	0.06	0.748
C3-OH	0.11	0.467	<0.01	0.983	–0.06	0.705	–0.01	0.958
C3:1	0.06	0.706	0.05	0.730	0.18	0.246	–0.16	0.283
C4	**0.377**	**0.01**	–0.05	0.734	0.10	0.532	–0.26	0.081
C4:1	0.15	0.322	–0.04	0.764	0.18	0.230	–0.03	0.844
C5	0.207	0.172	–0.07	0.630	0.04	0.770	0.07	0.621
C5-DC	0.18	0.230	–0.29	0.050	0.15	0.320	–0.16	0.276
C5-M-DC	–0.08	0.588	–0.10	0.489	<0.01	0.976	–0.05	0.739
C5-OH	0.13	0.379	**–0.34**	**0.021**	–0.06	0.719	0.14	0.359
C5:1	–0.14	0.356	–0.02	0.874	0.13	0.403	–0.13	0.402
C5:1-DC	0.07	0.647	–0.14	0.352	0.15	0.318	–0.19	0.208
C6	0.24	0.119	0.04	0.788	–0.05	0.725	–0.01	0.960
C6:1	0.19	0.206	0.08	0.581	0.16	0.309	–0.02	0.909
C7-DC	0.07	0.639	–0.08	0.580	0.18	0.241	0.28	0.055
C8	0.26	0.087	–0.15	0.323	0.24	0.112	0.05	0.754
C9	0.26	0.086	–0.01	0.964	0.09	0.575	–0.02	0.904
C10	**0.38**	**0.010**	–0.12	0.416	0.30	0.049	–0.10	0.519
C10:1	**0.38**	**0.010**	–0.12	0.404	0.18	0.225	–0.12	0.405
C10:2	0.04	0.795	–0.08	0.595	0.08	0.587	0.06	0.667
C12	0.17	0.272	0.05	0.754	0.02	0.888	0.08	0.603
12-DC	–0.07	0.644	–0.10	0.50	–0.06	0.719	–0.17	0.251
C12:1	**0.31**	**0.037**	–0.06	0.672	0.18	0.232	–0.04	0.797
C14	0.06	0.691	–0.02	0.915	0.25	0.100	0.03	0.824
C14:1	0.14	0.349	–0.11	0.444	0.15	0.311	–0.05	0.741
C14:1-OH	0.10	0.531	–0.19	0.207	0.25	0.100	–0.208	0.160
C14:2	0.22	0.140	–0.07	0.656	0.13	0.405	–0.23	0.120
C14:2-OH	–0.15	0.327	–0.09	0.559	–0.09	0.552	–0.05	0.757
C16	–0.11	0.478	–0.08	0.584	0.06	0.691	–0.03	0.855
C16-OH	–0.22	0.147	0.10	0.516	0.09	0.554	0.08	0.587
C16:1	–0.15	0.341	–0.25	0.090	0.10	0.500	**–0.35**	**0.016**
C16:1-OH	–0.10	0.521	0.24	0.112	0.19	0.217	0.14	0.364
C16:2-OH	0.12	0.451	–0.13	0.370	0.13	0.396	–0.02	0.909
C18	0.05	0.745	–0.11	0.476	0.17	0.268	**0.315**	**0.031**
C18:1	**0.32**	**0.030**	–0.15	0.300	0.15	0.329	–0.13	0.389
C18-OH	**0.35**	**0.019**	–0.07	0.628	0.12	0.431	–0.27	0.07
C18:2	0.16	0.297	<0.01	0.995	0.14	0.359	–0.04	0.785

6. DISCUSSION

6.1. Effect of RIPC on arterial stiffness during vascular surgery

Studies that have assessed the effect of RIPC on arterial stiffness are limited while no study has assessed the effect of vascular surgery on arterial stiffness. As RIPC has been proven to have positive effects on endothelial function and on AIx (Zagidullin et al. 2016; Kuusik et al. 2019), its positive effect on arterial stiffness could be expected.

Until today, evidence about the clinical significance of RIPC is lacking as most studies have either not had clinically relevant outcomes or have not presented positive results regarding clinical measures. PWV, the "gold standard" parameter of measuring arterial stiffness, has been shown to predict cardiovascular events and all-cause mortality (Vlachopoulos et al. 2010). If RIPC alters positively PWV, its clinical value would be evident. However, as PWV is a relatively constant parameter, it may need daily preconditioning for a longer period to reveal the true effect of RIPC. As RIPC has produced a positive effect on AIx (Zagidullin et al. 2016; Kuusik et al. 2019), which is more labile parameter of arterial stiffness, then its effect on PWV is even more likely. Considering that our study (Paper I) demonstrated no effect of RIPC on any parameter of arterial stiffness, its true effect on arterial stiffness is hard to evaluate based on the present study. This can be due to a number of confounders that originate primarily from surgery and anaesthesia. Vascular surgery as a high-risk procedure is in needs protective measures. Until our study was performed, it was completely unknown how vascular surgery affects arterial stiffness and whether its change is related to cardiovascular events. Based on our study (Paper I), vascular surgery has a positive impact on decreasing arterial stiffness parameters measured approximately 24 hours after surgery. However, the decline in arterial stiffness 24 hours after surgery reflects not only the effects of vascular manipulation, but also the impact of anaesthesia and perioperative medication, as well as the impact of acute stress from the trauma caused by surgery itself. Yet the results from the Paper I, indicating that surgery and anaesthesia with medication may affect arterial stiffness and haemodynamics, have some clinical relevance. Perioperative monitoring of arterial stiffness, an independent predictor of cardiovascular events and mortality, may facilitate guided treatment and prevention of the cardiovascular risks of patients undergoing vascular surgery. Perioperative increase of central haemodynamics and arterial stiffness may result in elevated cardiac afterload and reduced coronary perfusion, which presumably leads to a greater risk of cardiovascular events (myocardial infarction, heart failure, stroke, etc.).

Perioperative leakage of cardiac enzymes may be associated with cardiovascular events as it has been linked to increased risk for 30-day mortality (Redfern et al. 2011; Vascular Events In Noncardiac Surgery Patients Cohort Evaluation (VISION) Study Investigators et al. 2012). Nevertheless, there was no correlation

between leakage of cardiac enzymes and change in the parameters of arterial stiffness in our study (Paper II). Although our study may have been too small to evaluate correlations between clinical outcomes and changes in the parameters of arterial stiffness, it is still possible to draw some conclusions. Notably, changes in the parameters of arterial stiffness are not associated with cardiac damage (Paper II). However, there was positive correlation between baseline AIx and increase of hs-TnT in the RIPC group, which indicates that the protective effect of RIPC is more pronounced in patients with lower baseline AIx (Paper II). This finding may imply a stronger effect of RIPC in young participants, as younger people principally have better arterial compliance and hence lower AIx. Although in our study there were no correlations between leakage of cardiac enzymes and age, the weight of person's vascular age on the effect of RIPC may be more substantial than that of his/her biological age.

In longer term, the material and location of vascular graft may have a crucial impact on arterial stiffness, in particular, on PWV. Finally, the long-term effect of vascular surgery on arterial stiffness may be completely different from the immediate effect, which needs additional studies.

6.2. Impact of RIPC in cardioprotection: from clinically non-substantial changes to clinically relevant effects

Cardioprotective effects of ischaemic conditioning is the most widely studied (Annachhatre and Annachhatre 2019) issue in this field. There are multiple animal studies, which have demonstrated that RIPC reduces the size of myocardial infarction(Gho et al. 1996; Tang et al. 1999; Schoemaker and van Heijningen 2000; Liem et al. 2002; Weinbrenner et al. 2002; Konstantinov et al. 2005; Wolfrum et al. 2005; Zhang et al. 2006; Dong et al. 2018; Lieder et al. 2018; Bunte et al. 2019). These data, when translated to humans, may help to maintain physical capability and also quality of life. Unfortunately, human studies showing a positive effect of RIPC on cardiac enzymes or size of myocardial infarction, with simultaneous assessment of quality of life, are missing. A large study reported no clinical effect (including quality of life) in patients undergoing cardiac surgery (Hausenloy et al. 2015). However, the above trial found no effect of RIPC on enzymatic cardiac damage. There are several "proof of concept" studies on humans that have obtained positive results (Hausenloy et al. 2007; Venugopal et al. 2009; Wang et al. 2019b). Many studies on RIPC, including our study, have used cardiospecific biomarkers as primary or secondary outcome. In recent years, more studies have concentrated on the clinical benefit of RIPC (Hausenloy et al. 2015; Meybohm et al. 2015). Studies focusing on clinical outcomes have mostly obtained neutral results (Hausenloy et al. 2015; Meybohm et al. 2015). Similarly, a number of meta-analyses showed no effect of RIPC on all-cause mortality, myocardial infarction, renal failure, stroke or length of ICU or hospital stay in

patients undergoing cardiovascular surgery (Haji Mohd Yasin et al. 2014; Remote Preconditioning Trialists' Group et al. 2014; Pierce et al. 2017). Also, one meta-analysis has presented positive effects on cardiospecific biomarkers, but not on clinical outcomes (Xie et al. 2018). Despite the fact that according to some meta-analyses, RIPC reduces the incidence of myocardial infarction (Pei et al. 2014; Wang et al. 2017a), definite final conclusions about its clinical benefit regarding cardioprotection cannot be drawn.

Postoperative leakage has also some clinical value in predicting vascular and non-vascular 30-day mortality. It has been reported that the higher is post-operative leakage of TnT, the higher is mortality (Redfern et al. 2011; Vascular Events In Noncardiac Surgery Patients Cohort Evaluation (VISION) Study Investigators et al. 2012). Likewise, micro-leakage of TnT predicts mortality and subsequent myocardial infarction in patients undergoing elective PCI (Milani et al. 2009). Also, postoperative elevation of BNP or NT-proBNP has been associated with increased risk for mortality, myocardial infarction and cardiac failure (Rodseth et al. 2013). Based on the above findings, we can assume that the effect of RIPC in reducing cardiospecific biomarkers may have some clinical value. Still, there is no proof that reducing perioperative or -procedural leakage of cardiospecific biomarkers leads to better long-term outcomes. Leakage of cardiac biomarkers may merely have a predictive value without affecting the clinical result.

In our study (Paper II), RIPC displayed its cardioprotective effects by reducing the leakage of hs-TnT and NT-proBNP in association with vascular surgery. However, our study was probably too small to detect any significant differences in clinical outcomes in the acute postoperative period. Still, as the increase of NT-proBNP was significantly higher in the sham group, RIPC might have an effect in lowering the rate of perioperative acute heart failure. This is supported by the fact that two cases of acute heart failure occurred in the sham group but none in the RIPC group (Paper II). Still, a larger study is needed to evaluate the relevance of this observation, as well as the overall effect of RIPC on the cardiovascular system. A negative correlation between change in hs-TnT and change in eGFR occurred in both groups while the correlation was stronger in the RIPC group (Paper II). Therefore, the increase of hs-TnT may have been more likely connected with the decrease in kidney function in the RIPC group than in the sham group.

According to Cochrane systematic review RIPC reduces leakage of TnT but does not have any impact on clinical outcomes assessed as the composite endpoint (including all-cause mortality, myocardial infarction, or stroke) in cardiac surgery (Benstoem et al. 2018). Negative results from studies may be explained with presence of important confounders (e.g propofol anaesthesia) and the hetero-geneous nature of patient groups. Furthermore, the heterogeneity of individual studies is a major drawback in meta-analysis. In addition, the majority of studies assessing cardioprotection have been conducted on patients undergoing cardiac surgery or elective PCI, while only some studies have focused on patients with acute diseases. For example, remote ischaemic conditoning has been found to

reduce leakage of cardiospecific biomarkers and to preserve left ventricular contractility (Cao et al. 2018), as well as to reduce cardiac mortality and hospitalization for heart failure (Gaspar et al. 2018) in patients with STEMI. This suggests that the effect of RIPC may be more pronounced in specific circumstances, e.g. in type I acute myocardial infarction. Moreover, animal studies are usually performed in the case of acute occlusion of coronary artery and many of them have shown that IPC reduces infarction size (Gho et al. 1996; Tang et al. 1999; Schoemaker and van Heijningen 2000; Liem et al. 2002; Weinbrenner et al. 2002; Konstantinov et al. 2005; Wolfrum et al. 2005; Zhang et al. 2006; Dong et al. 2018; Lieder et al. 2018; Bunte et al. 2019). However, additional, preferably homogeneous, large studies are needed to verify these assumptions. It should be clarified which patients would experience a strong protective effect of RIPC. The results of Paper II suggest that the effect of RIPC is stronger in those vascular surgery patients who are treated with statins, but also in patients with lower augmentation index, and hence with better arterial compliance, and in male patients. There is also similar proof from previous studies (El Desoky et al. 2016; Cho et al. 2019).

In conclusion, the clinical benefit of RIPC regarding heart is yet to be established. Future research should be more concentrated on patient-related outcomes as the protective concept of ischaemic conditioning has been quite well proved.

6.3. Role of RIPC in nephroprotection: a strategy to reduce acute kidney injury in lower limb revascularisation surgery?

AKI is a possible complication of many acute diseases and medical procedures. Vascular surgery is high-risk surgery in which postoperative AKI has been found to occur in 12.7–49% of patients (Adalbert et al. 2013; Huber et al. 2016). In revascularisation, serious I/R injury and clinically insignificant rhabdomyolysis are inevitable and increase the risk for kidney injury. Perioperative AKI is related to higher cardiovascular morbidity, mortality, risk for chronic kidney injury and longer hospitalisation. Moreover, elevation of creatinine level even below the AKI criteria has been linked to adverse outcome both in cardiac (Lassnigg et al. 2008) and non-cardiac surgery (Kork et al. 2015). Nonetheless, AKI has still remained an underdiagnosed and undertreated problem and effective strategies to avoid or reduce AKI are lacking. In this light, RIPC has emerged as a possible strategy to reduce AKI. A relatively large study whose primary outcome was postoperative renal function showed that RIPC reduces the rate of AKI within the first 72 h after surgery, the need for kidney replacement therapy and the intensive care unit stay in patients requiring cardiac surgery (Zarbock et al. 2015). Additionally, long-term kidney protection and improved renal recovery in the case of AKI were reported in a follow-up analysis (Zarbock et al. 2017). Two large studies, RIPHeart (Meybohm et al. 2015) and ERICCA (Hausenloy et al. 2015),

where postoperative kidney function was a secondary outcome and propofol anaesthesia was used, no benefit of RIPC on kidney function was reported in patients undergoing elective coronary artery bypass grafting surgery with or without valve surgery. However, in a meta-analysis, RIPC reduced the incidence of AKI in studies where propofol was not administrated (Pierce et al. 2017). Hence, it seems that propofol may be an important confounder to nephroprotection by RIPC. In our study, RIPC showed a nephroprotective effect by lowering kidney injury biomarkers in a subgroup analysis of patients undergoing lower limb revascularisation surgery (Paper III). However, in the whole study group, no significant effect of RIPC was observed however. This may have been due to the administration of propofol, as carotid endarterectomy and abdominal aortic aneurysm repair were both performed under general anaesthesia, propofol being the most common anaesthetic. Also, propofol was administered to 25% of patients undergoing lower limb revascularisation surgery in the RIPC group. Because of this, the true effect of RIPC might have been underestimated. It was not possible to accurately evaluate the presence of AKI according to the KDIGO's criteria (Khwaja 2012) that require AKI to be diagnosed on the basis of creatinine and urine output changes within 7 days. Our trial, however, lasted only 24 hours after surgery (Paper III). Still, in a subgroup analysis of patients undergoing lower limb revascularisation surgery AKI was present in 4 patients (14%) in the sham group and in 1 patient (4%) in the RIPC group during the follow-up period, yet the difference was not statistically significant (Paper III). However, it is likely that more patients developed AKI in the post-trial period. Moreover, we excluded patients with eGFR <30 mL/min/1.73m^2, who were at greater risk for AKI. In the earlier studies, one of the exclusion criteria was also eGFR <30 mL/min/1.73m^2 (Hausenloy et al. 2015; Meybohm et al. 2015). At the same time, studies assessing the nephroprotective effect of RIPC in patients with more severe chronic kidney disease are missing.

Taken together, RIPC might be a clinically valuable option for vascular surgery patients to reduce the incidence of AKI, especially as well-established procedures for decreasing perioperative AKI are lacking. Reduction in perioperative AKI could shorten hospital stay and improve prognosis. Furthermore, RIPC is a low-cost procedure and, according to our findings, it is easily applicable and well tolerated by patients, facilitating its usage in clinical practice.

6.4. Shifts in acylcarnitines' metabolism after RIPC: a novel insight into potential mechanisms/targets of RIPC?

Unfortunately, no studies have evaluated the effect of RIPC on carnitine (C0) level and on the profile of all acylcarnitines (C2-C18). Still, relevant conclusions can be drawn based on our study IV). Paper IV reports the positive effect of RIPC in lowering the levels of several ACs in patients undergoing vascular surgery, which is also an evidence of its effect on the ACs' profile in plasma. According

to previous studies, decrease of the levels of ACs can be associated with preserved mitochondrial function (Bjørndal et al. 2018). The knowledge of the ACs profile may facilitate assessment of patients' general metabolic milieu, mitochondrial functioning and prognosis. As ACs have a different origin in plasma, plasma ACs does not directly reflect whole-body acylcarnitine metabolism (Schooneman et al. 2015), but it allows to make some conclusions about its metabolism in certain organs. Plasma SCASs have been found to be released from liver (Schooneman et al. 2015), while plasma MCACs are released from skeletal muscles and liver (Xu et al. 2016) and LCACs, from heart (Makrecka-Kuka et al. 2017). It should be noted that the hepatoprotective effect of RIPC has been reported previously (Kanoria et al. 2017; Wu et al. 2019). In our study, there occurred an increase of some SCACs levels in the sham group and no increase of ACs in the RIPC group (Paper IV).

Evidently, the stress caused by surgery enhances the catabolism of BCCA in liver in order to produce additional metabolic energy, which is accompanied by an increase of plasma SCACs. RIPC has been found to intensify hepatic oxygenation and hepatic microcirculation via the activation of eNOS (Abu-Amara et al. 2011), as well as to increase the expression of protector proteins including heme oxygenase 1 (Cornide-Petronio et al. 2019), which preserves mitochondrial functionality. Hence, by using RIPC, the produced SCACs can be spent more efficiently. Considering the above findings, it can be supposed that RIPC might have a protective effect on liver.

In addition, we currently found a significant difference in the levels of C3-OH between the RIPC and the sham group (Paper IV). Surgery increases the demand for metabolic energy and may also cause elevated level of ketone bodies leading to production of C3-OH. The above significant difference could be explained by a more effective elimination of this SCAC or by a lesser need for ketone bodies in case RIPC is applied. Moreover, Paper IV describes positive correlations between decrease of cardiac biomarkers (NT-proBNP and hsTnT) and decrease of several ACs in the RIPC group. This allows to suggest cardioprotective effects based on shifts in the levels of ACs. However, further studies are needed to corroborate these assumptions.

Summing up, according to our study, it can be concluded that RIPC has an effect on the levels of ACs and can therefore have protective effects on mitochondria in patients undergoing vascular surgery. Further research is needed to make more definite conclusions about the effect of RIPC on the levels of ACs.

6.5. Possible causes of contradictory results from human studies

While animal studies have consistently reported positive effects of RIPC, studies on humans have shown more contradictory results. Animal studies are easier to perform in a uniform manner while human studies involve many confounders

which are difficult to avoid; also, human study populations tend to be more heterogeneous. The present study was faced with similar issues. Long-term research of the effects of ischaemic conditioning with varying results allows to suggest that ischaemic conditioning has beneficial effects only under certain conditions and in certain patients. Various factors have been found to mitigate or abolish the effect of ischaemic conditioning (e.g. propofol, gender, age, diabetes etc.) (Penna et al. 2009; Oosterlinck et al. 2013; Pierce et al. 2017; Zhou et al. 2017; Behmenburg et al. 2018; Heinen et al. 2018; Moretti et al. 2018; Wider et al. 2018; Bunte et al. 2019). Also, as the effect of RIPC may vary in high and low risk patients, this may explain in part the contradictory results. All these confounders may have played a certain role in the results of our study: propofol usage, inclusion of diabetic patients and absence of limitations regarding gender and age. Most human studies have failed to avoid these possible confounders, first and foremost usage of propofol for anaesthesia (Hausenloy et al. 2015; Meybohm et al. 2015). Yet as propofol is a standard drug for sedation worldwide, it would be complicated to apply propofol-free sedation in clinical practise. Hopefully more homogenous or larger studies will be conducted in future. This would help to clarify the true effect of RIPC, as well as the issue of suitability of patients to be selected for RIPC.

At the same time, there is a possible cause of negative results which could be easier to modified. Only a few papers have evaluated different protocols of RIPC (Pei et al. 2014; Johnsen et al. 2016). Usually, protocols consisting of 2–8 cycles of ischaemia lasting 2–10 minutes have been applied to one or two limbs (Johnsen et al. 2016). However, an early RIPC study reported positive results with only one 15-minute cycle of RIPC (Gho et al. 1996). The delayed effect of RIPC (Kim et al. 2017) is relatively less studied than the acute effect. The delayed phase and the acute phase of RIPC have been compared with contradictory results (Dow et al. 2012; Rohailla et al. 2014; Varga et al. 2019). Unfortunately, not all protocols of RIPC may be equally favourable. The timing of conditioning and the protocol of the current study may have had an impact on the results. Once the most beneficial protocol is developed, it will be possible to evaluate the true effect of RIPC.

6.6. Limitations

There are several limitations associated with this series of studies. First, the main limitation is the small size of the study group, which made assessment of the clinical outcomes very difficult. Power calculation was based on AIx@75 and an effect size of 5 units (%) was chosen as possible meaningful change and only post-hoc power calculations were carried out basis of the other outcomes. Unfortunately, there is no solid proof or recommendations regarding what change in AIx@75 is relevant. It may be that a smaller change than 5 units (%) of AIx@75 is clinically important and, to reach statistical significance, a larger study group would have been necessary. Also, power calculation did not take account the possible confounders, that were present in this study, which can impact the results. Second, negative results of primary outcomes may lessen the importance

of secondary outcomes. Yet as the secondary outcomes may not have been clinically or pathophysiologically connected with primary outcomes, the positive results of the secondary outcomes may be relevant and the effect of RIPC on the biomarkers' level may have some clinical value as discussed above.

Third, there are multiple possible confounders in this study. The main confounder to the effect of RIPC is the use of propofol anaesthesia (Behmenburg et al. 2017; Bunte et al. 2019), which probably weakened the effect of RIPC. Also, the true effect of RIPC may have been attenuated by recruiting diabetic patients as diabetes has also been described as an important confounder to its effect (Jensen et al. 2013; Oosterlinck et al. 2013). Moreover, there was considerable heterogeneity among the study population, with one group of patients, having occlusive arterial disease and the others having aneurysmal disease. This may have led to different impacts due to clamping and reperfusion in the recruited patients. In the case of lower limb occlusive disease, the effect of clamping and reperfusion is weaker than it is during aortic aneurysm repair surgery where arteries are unobstructed. So a more systemic effect can be achieved in the case of aortic aneurysm. Also, patients with claudication are believed to have chronic ischaemic conditioning, which may have reduced the effect of RIPC intervention. Additionally, the involved surgeries were different in other aspects, such as duration and preparation for anaesthesia and surgery. This resulted in different periods from intervention to I/R, as the intervention was performed after the patient had arrived in the operating theatre. It is possible that the patient experienced a different effect of RIPC namely due to these temporal variations. Still, as the "first window" of the effect of RIPC is known to last approximately a few hours, one can not expect significant differences here. Also, clamping time and location varied among surgeries and I/R injuries and so the patients may have experienced different severities of ischaemia-reperfusion injury. In addition, the material of vascular graft may also have an impact, in particular, on pulse wave travelling. Yet, it is not clear how large the impact of a few-dozens-centimetre graft is among other contributing factors. Moreover, it is even unknown how different grafts affect the pulse wave. Finally, the peripheral graft type could potentially also influence the parameters of arterial stiffness.

6.7. Concluding remarks and future perspectives

RIPC is a promising method to be used in clinical practise in order to ameliorate clinical outcome. The present thesis provides proof that RIPC reduces cardiac and kidney damage in vascular surgery, and that its effects are achieved through changes in the metabolism of ACs. Considering perspectives, long-term follow-up of the same cohort could describe the effect of RIPC on total mortality, cardiovascular morbidity and mortality, and major adverse limb events. Analysis of changes in other metabolites, in addition to ACs, could offer knowledge about the exact mechanisms of RIPC. A new larger study that presupposes international collaboration could enlighten the clinical benefits of RIPC and would specify who

can benefit the most from RIPC. Additionally, it should be clarified if it is possible to enhance the effect of RIPC by using some medications, supplements or other strategies.

6.8. Conclusions

1. There were no significant differences between the RIPC group and the sham group in terms of the postoperative changes in augmentation indices, pulse wave velocity and indices of arterial elasticity. Hence, RIPC had no effect on arterial stiffness in patients undergoing vascular surgery. Significant post-operative improvements in the augmentation indices and small artery elasticity within both groups indicate that the surgical procedure itself may acutely ameliorate arterial stiffness, while these changes may be related to the systemic stress response to surgery, anaesthesia and perioperative medications.
2. There was significantly higher leakage of high hs-TnT and NT-proBNP in the sham group compared to the RIPC group. Accordingly, RIPC offers cardio-protection by significant reduction in leakage of cardiac biomarkers hs-TnT and NT-proBNP in patients undergoing vascular surgery. Additionally, since negative correlation between increase of cardiac biomarkers and pretreatment with statins and positive correlations of increase of cardiac biomarkers with gender (male<female) and baseline augmentation index were observed in the RIPC group, it can be concluded that the effect of RIPC may be stronger in patients, who receive statins, particularly in those with lower arterial stiffness, and in males. RIPC may thus serve as a protective measure against peri-operative cardiac damage.
3. In patients undergoing surgical revascularisation of lower limb, serum creatinine, cystatin C and beta-2 microglobulin increased and eGFR decreased across all time points significantly more in the sham group than in the RIPC group. Hence, RIPC reduces leakage of the biomarkers indicative of kidney injury and renal function in these patients and could be a protective measure against acute kidney injury.
4. The pattern of acylcarnitines differed between the RIPC group and the sham group, yet there was no statistically significant difference in the changes of most acylcarnitines. RIPC, but not sham, application decreased the levels of certain short and medium chain acylcarnitines in the RIPC group, which was not noted in the sham group. There was a statistically significant difference between the groups regarding changes in C3-OH: a decrease in the RIPC group and an increase in the sham group. Thus, RIPC has an effect on the levels of ACs and may therefore have protective effects on mitochondria in vascular surgery patients.

SUMMARY IN ESTONIAN

Kaugisheemilise eelkohastamise mõju elundikahjustusele ja atsüülkarnitiinide ainevahetusele veresoontekirurgias

Veresoontekirurgia on kõrge riskiga kirurgia, kuna patsiendid on süsteemsest ateroskleroosist tulenevalt mitmete kaasuvate haigustega. See suurendab haigetel operatsiooniaegsete ja -järgsete tüsistuste esinemise tõenäosust. Veresoontekirurgias olulist rolli mängiva isheemia-reperfusioonikahjustuse vähendamise võimalusena on viimase 30 aasta jooksul uuritud isheemilist eelkohastamist. Viimase erivorm, kaugisheemiline eelkohastamine (KIE), seisneb lühiajalistes eemal paikneva koe (nt. ülajäseme) isheemia episoodide tekitamises, mille eesmärgiks on kaitse loomine sihtelundile. Enim uuritud sihtelundid on süda ja neerud, kuid põhimõtteliselt võib selleks olla ükskõik milline isheemiatundlik kude. Mitmetes senistes uuringutes on ilmnenud KIE kaitsev toime südame- ja neerukahjustusele, kuid on leitud ka KIE mittetoimimist. Enamik KIE uuringuid on läbi viidud patsientidel, kellel on teostatud kas lahtine südameoperatsioon või perkutaanne pärgarterite angioplastika. Veresoontekirurgias on KIE efekti vähem uuritud. Kuigi on tuvastatud mitmeid KIE efektis osalevaid tegureid, siis täpsed KIE toimemehhanismid ei ole teada. Avastatud on ka KIE toimet vähendavaid mõjureid, sealhulgas näiteks vanus, suhkruhaigus ja anesteetikum propofool.

Käesolev doktoritöö kirjeldab KIE mõju arterite jäikusele, südame- ja neerukahjustusele ning atsüülkarnitiinide ainevahetusele veresoontekirurgia patisentidel. Juhuslikustatud topeltpimendatud kontrollitud kliinilisse uuringusse kaasati 98 uuritavat. Peale väljalangemist jäi KIE gruppi 45 ja kontrollgruppi (*sham* ehk imitatsioon) 48 patsienti. Menetluseks oli operatsioonieelselt vererõhu mansetiga tehtud 4 tsüklit 5-minutilist ülajäseme isheemiat (mansetis rõhk vähemalt 200 mmHg) 5-minutiliste reperfusiooni pausidega ning kontrollrühmas rakendati ülajäsemele isheemia asemel mansetis venoosset rõhku (10–20 mmHg).

Arterite jäikusele KIE-l mõju ei leitud, küll aga paranesid operatsioonijärgselt mõlemas uuringugrupis märgatavalt arterite jäikuse parameetrid. Need akuutsed muutused arterite jäikuses on tingitud nii operatsioonitraumast, anesteesiast kui ka manustatud ravimitest. Uuringu tulemusena esines oluline gruppide vaheline erinevus postoperatiivselt südamekahjustuse markerite (kõrgtundlik troponiin T ja NT-proBNP) muutustes – KIE grupis oli markerite tõus väiksem. Lisaks oli KIE grupis negatiivne seos südamekahjustuse markerite taseme tõusu ja eelneva statiinravi vahel ning positiivne seos südamekahjustuse marketite tõusu ja soo (mees<naine) ning algtaseme augmentatsiooni indeksi vahel. Sellest võib järeldada, et KIE vähendab perioperatiivset südamekahjustust veresoontekirurgilistel operatsioonidel ning KIE toime on tugevam statiinravil ning meessoost ja elastsemate arteritega haigetel. Täiendavalt oli KIE grupis väiksem neerukahjustuse markerite tõus alajäseme revaskulariseerival operatsioonil käinud patsientidel, mis näitab KIE neerukahjustustust vähendavat toimet. Lisaks oli KIE-l

positiivne toime atsüülkarnitiinide tasemele, mis viitab mitokondrite kaitsele ja avardab teadmisi ka KIE toimemehhanismidest ja sihtmärkidest.

Kokkuvõtvalt on KIE potentsiaalseks menetluseks neeru- ja südamekahjustuse vähendamiseks veresoontekirurgias. KIE toime on seotud mitme kliinilise teguriga ja ka positiivsete muutustega atsüülkarnitiinide ainevahetuses. Kliinilise kasutuse tõhususe täpsustamiseks on vajalikud suuremad ja homogeensema uuringugrupiga uuringud.

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PUBLICATIONS

CURRICULUM VITAE

I General information

Name: Teele Kasepalu (maiden name Kepler)
Birth date: 17.04.1990
Citizenship: Estonia
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Education: level of education (Institution, study years):
Cardiology residency (University of Tartu, 2019–…)
Doctoral studies (University of Tartu, Institute of Clinical Medicine, 2015–...)
Bachelor's and Master's Integrated degree (University of Tartu, Medicine, 2009–2015)
Secondary education (Hugo Treffner Gymnasium, 2006–2009)
Primary education (Tartu Kivilinna School, 1997–2006)

Languages: Estonian, English, Russian

Professional experience:

2017–2019	University of Tartu, Institute of Clinical medicine, Department of Surgery, junior research fellow
2017–...	Hospital of Pärnu, Emergency Department, general practitioner
2018–...	Hospital of Viljandi, Emergency Department and Department of Internal medicine, general practitioner
2015–2018	Hospital of Põlva, Department of Internal medicine, general practitioner

II Research

Main fields of research:
Remote ischaemic preconditioning in patients undergoing vascular surgery

A list of publications:

Kepler T., Kuusik K., Lepner U., Starkopf J., Zilmer M., Eha J., Lieberg J., Vähi M., Kals J. The Effect of Remote Ischaemic Preconditioning on Arterial Stiffness in Patients Undergoing Vascular Surgery: A Randomised Clinical Trial. Eur J Vasc Endovasc Surg. 2019;57:868–875.

Kepler T., Kuusik K., Lepner U., Starkopf J., Zilmer M., Eha J., Vähi M., Kals J. Remote ischaemic preconditioning attenuates cardiac biomarkers during vascular surgery: a randomised clinical trial. Eur J Vasc Endovasc Surg. 2020;59:301–308.

Kasepalu T., Kuusik K., Lepner U., Starkopf J., Zilmer M., Eha J., Vähi M., Kals J. Remote Ischaemic Preconditioning Reduces Kidney Injury Biomarkers in Patients Undergoing Open Surgical Lower Limb Revascularisation: A Randomised Trial. Oxid Med Cell Longev. 2020:7098505.

Kasepalu T., Kuusik K., Lepner U., Starkopf J., Zilmer M., Eha J., Vähi M., Kals J. Remote ischaemic preconditioning influences the levels of acyl-carnitines in vascular surgery: a randomised clinical trial. Nutr Metab (Lond). 2020;17:76.

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(6):875–882.

III Teaching work

Conducting seminars and practical trainings in biochemistry for the second year medical students

IV Professional development

Participation in conferences:

2016 „*Arterial Hemodynamics: past, present and future"* London, England
2016 Spring workshop of Estonian Society of Cardiology, Tallinn, Estonia
2017 Workshop of Estonian Society of Cardiology, lectures for residents, Tartu, Estonia
2017 „*Clinic 2017*" Tartu, Estonia
- oral presentation „*Remote ischaemic preconditioning in vascular surgery*"

2018 „*Clinic 2018*" Tartu, Estonia
2018 Scientific conference of the Faculty of Medicine
- oral presentation „*Remote ischaemic preconditioning reduces peri-operative kidney damage in vascular surgery*"

2018 „*ARTERY 2018*" Guimarães, Portugal
- poster presentation „*Remote ischaemic preconditioning attenuates kidney injury perioperatively in patients undergoing surgical lower limb revascularisation"*

2018 „*Arterial Stiffness and Vascular Aging*", Vilnius, Lithuania
2019 „*ARTERY 2019"* Budapest, Hungary
- poster presentation „*Remote ischaemic preconditioning attenuates cardiac biomarkers during vascular surgery: a randomised clinical trial*"

2019 European Society of Hypertension Summer School, Vravrona, Greece
- oral presentation „*The effect of remote ischaemic preconditioning on arterial stiffness in vascular surgery*"

2020 25th Anniversary conference of Estonian Society of Hypertension, Tallinn, Estonia
- oral presentation „*Renal denervation*"

ELULOOKIRJELDUS

I Üldinformatsioon

Nimi: Teele Kasepalu (end. Kepler)
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Haridus: haridustase (institutsioon, õppeaastad):
Doktoriõpe (Tartu Ülikool, kliinilise meditsiini instituut, 2015–...)
Integreeritud bakalaureuse ja magistriõpe (Tartu Ülikool, arstiteaduskond, 2009–2015)
Keskharidus (Hugo Treffneri Gümnaasium, 2006–2009)
Põhiharidus (Tartu Kivilinna Kool, 1997–2006)

Keeled: eesti, inglise, vene

Erialane töökogemus:

2017–2019	Tartu Ülikool, kliinilise meditsiini instituut, kirurgiakliinik, nooremteadur
2015–...	SA Pärnu Haigla, erakorralise meditsiini osakond, üldarst
2018–...	SA Viljandi Haigla, erakorralise meditsiini osakond ja sisehaiguste osakond, üldarst
2015–2018	AS Põlva Haigla, sisehaiguste osakond, üldarst

II Teadustöö

Publikatsioonid:

Kepler, T., Kuusik, K., Lepner, U., Starkopf, J., Zilmer, M., Eha, J., Lieberg, J., Vähi, M., Kals, J. The Effect of Remote Ischaemic Preconditioning on Arterial Stiffness in Patients Undergoing Vascular Surgery: A Randomised Clinical Trial. Eur J Vasc Endovasc Surg. 2019;57:868–875.

Kepler, T., Kuusik, K., Lepner, U., Starkopf, J., Zilmer, M., Eha, J., Vähi, M., Kals, J. Remote ischaemic preconditioning attenuates cardiac biomarkers during vascular surgery: a randomised clinical trial. Eur J Vasc Endovasc Surg. 2020; 59:301–308.

Kasepalu, T., Kuusik, K., Lepner, U., Starkopf, J., Zilmer, M., Eha, J., Vähi, M., Kals, J. Remote Ischaemic Preconditioning Reduces Kidney Injury Biomarkers in Patients Undergoing Open Surgical Lower Limb Revascularisation: A Randomised Trial. Oxid Med Cell Longev. 2020:7098505.

Kasepalu, T., Kuusik, K., Lepner, U., Starkopf, J., Zilmer, M., Eha, J., Vähi, M., Kals, J. Remote ischaemic preconditioning influences the levels of acyl-carnitines in vascular surgery: a randomised clinical trial. Nutr Metab (Lond). 2020;17:76.

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(6):875–882.

III Õppetöö

2. aasta arstiteaduskonna üliõpilastele biokeemia seminaride ja praktikumide läbiviimine

III Erialaline täiendus

Osalemine konverentsidel:

2016 „*Arterial Hemodynamics: past, present and future"* London, Inglismaa
2016 Eesti Kardioloogide Seltsi kevadine koosolek, Tallinn, Eesti
2017 Kardioloogia loengud residentidele, Tartu, Eesti
2017 „*KLIINIK 2017*" Tartu, Eesti
- suuline ettekanne „*Kaugisheemiline eelkohastamine veresoontekirurgias*"

2018 „*KLIINIK 2018*" Tartu, Eesti
2018 Arstiteaduskonna aastapäeva teaduskonverents
- suuline ettekanne *" Kaugisheemiline eelkohastamine vähendab veresoontekirurgias perioperatiivset neerukahjustust*"

2018 „*ARTERY 2018*" Guimarães, Portugal
- posterettekanne „*Kaugisheemiline eelkohastamine vähendab perioperatiivset neerukahjustust alajäseme revaskulariseerivatel operatsioonidel*"

2018 „*Arterial Stiffness and Vascular Aging*", Vilnius, Leedu
2019 „*ARTERY 2019"* Budapest, Ungari
- posterettekanne „*Kaugisheemiline eelkohastamine vähendab kardiomarkerite leket veresoonteoperatsioonidel: randomiseeritud kliiniline uuring*"

2019 Euroopa Hüpertensiooniühingu Suvekool, Vravrona, Kreeka,
- suuline ettekanne „*Kaugisheemilise eelkohastamise mõju arterite jäikusele veresoontekirurgias*"

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