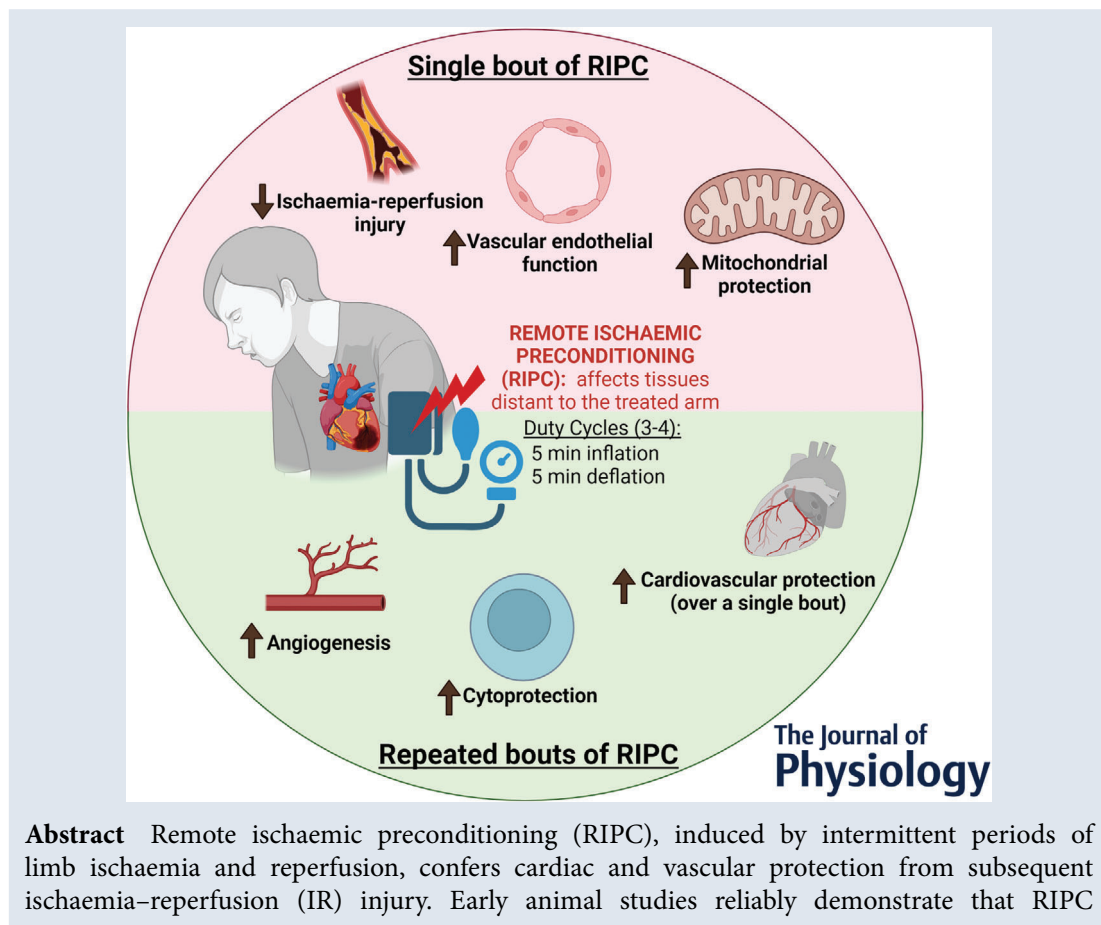TOPICAL REVIEW

Remote ischaemic preconditioning – translating cardiovascular benefits to humans

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Abstract Remote ischaemic preconditioning (RIPC), induced by intermittent periods of limb ischaemia and reperfusion, confers cardiac and vascular protection from subsequent ischaemia–reperfusion (IR) injury. Early animal studies reliably demonstrate that RIPC

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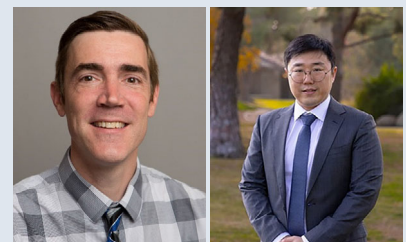
attenuated infarct size and preserved cardiac tissue. However, translating these adaptations to clinical practice in humans has been challenging. Large clinical studies have found inconsistent results with respect to RIPC eliciting IR injury protection or improving clinical outcomes. Follow-up studies have implicated several factors that potentially affect the efficacy of RIPC in humans such as age, fitness, frequency, disease state and interactions with medications. Thus, realizing the clinical potential for RIPC may require a human experimental model where confounding factors are more effectively controlled and underlying mechanisms can be further elucidated. In this review, we highlight recent experimental findings in the peripheral circulation that have added valuable insight on the mechanisms and clinical benefit of RIPC in humans. Central to this discussion is the critical role of timing (i.e. immediate *vs.* delayed effects following a single bout of RIPC) and the frequency of RIPC. Limited evidence in humans has demonstrated that repeated bouts of RIPC over several days uniquely improves vascular function beyond that observed with a single bout alone. Since changes in resistance vessel and microvascular function often precede symptoms and diagnosis of cardiovascular disease, repeated bouts of RIPC may be promising as a preclinical intervention to prevent or delay cardiovascular disease progression.

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Abstract figure legend Explanation of the effects of single bout as opposed to repeated bouts of remote ischaemic preconditioning.

Introduction

Approximately 70% of deaths from cardiovascular disease (CVD) are due to ischaemic heart disease and stroke (Roth et al., 2020). If not a lethal outcome, these events often result in profound tissue damage due to both ischaemia and subsequent reperfusion injury. Paradoxically, it has been demonstrated in animal models that one of the most powerful stimuli for protection from ischaemia–reperfusion (IR) injury is to administer brief periods of mild cardiac ischaemia interspersed with reperfusion periods on the coronary artery directly (i.e. 'preconditioning' the heart) prior to an ischaemic event (Murry et al., 1986). However, administering such a procedure in humans is invasive and impractical for routine clinical use. Alternatively, periods of short ischaemia and reperfusion can be applied to more accessible areas, such as a peripheral limb, and confer protection from IR injury in distant tissues and organs (e.g. the heart and brain) by neural and humoral mechanisms (Gho et al., 1996; Oxman et al., 1997; Przyklenk et al., 1993) (Fig. 1). This phenomenon, called remote ischaemic preconditioning (RIPC), performed before IR injury robustly attenuates infarct size in animal models (Bromage et al., 2017) and preserves conduit artery endothelial function from IR injury in humans (Kharbanda et al., 2002). RIPC is typically evoked by administering 3–4 cycles of ∼5 min limb ischaemia, by inflating a cuff to a suprasystolic pressure, followed by ∼5 min of deflation and reperfusion. Although this intervention may also be beneficial when administered after IR injury (i.e. 'postconditioning'), the focus of this review will be on preconditioning, or when RIPC is then followed by clinical or experimentally induced IR injury.

Mechanisms of RIPC and windows of protection in animal models

Much of what is currently known about the underlying mechanisms and duration of protective effects following RIPC have been inferred from animal models that examined infarct damage following direct cardiac preconditioning. These studies consistently show a biphasic pattern of myocardial protection (Baxter et al., 1997; Kuzuya et al., 1993; Murry et al., 1986). The *first window* of protection is transient, reaching a peak ∼2–3 h after preconditioning and quickly dissipating by ∼4 h post. The delayed or *second window* of protection gradually starts 24 h after preconditioning, peaks ∼48 h post, and persists for another ∼2–3 days. However, it is important to ascertain whether this long duration of protection for cardiomyocytes also occurs with RIPC. Although there are reports to the contrary (Ren et al., 2008), the duration and biphasic pattern of protective effects following RIPC, in both the myocardium and the vasculature, appear to be similar to direct cardiac preconditioning (Kim et al., 2021; Loukogeorgakis et al., 2005; Przyklenk et al., 2003).

Furthermore, each window of protection induces different signalling pathways (Fig. 2).

There are mechanistic similarities between direct preconditioning and RIPC but they notably differ based on the delivery of a 'trigger' signal, communicated through neural or humoral pathways, that activates mechanisms of cytoprotection. A neuronal origin of RIPC is demonstrated by the elimination of the protective effects of RIPC after administration of a ganglionic blocker (Gho et al., 1996). A humoral method of delivery is evidenced with reduced infarct damage in rabbits after receiving a transfusion of blood from humans that underwent RIPC (Shimizu et al., 2009). Additionally, perfusates from preconditioned hearts depleted of exosomes failed to elicit cardioprotection, thereby indicating an important role of exosomes in the interorgan communication that occurs with RIPC (Giricz et al., 2014).

Upon delivery of the signal to vital target tissues, the protective pathways of RIPC are complex and heavily dependent upon the timing from the last RIPC bout. Other review papers more thoroughly address the protective mechanisms (Heusch et al., 2015; Kleinbongard et al., 2017). Briefly, there are multiple endogenous substances that act on cardiomyocytes to trigger cytoprotective responses such as adenosine, bradykinin, opioids and calcitonin gene-related peptide (CGRP). During the first window, receptor binding results in opening of ATP-sensitive potassium (K_{ATP}) channels on the sarcolemma and mitochondria via direct stimulation by intracellular kinases or indirectly through increased nitric oxide (NO) production and a subsequent increase in reactive oxygen species. Reactive oxygen species may also inhibit the opening of the mitochondrial permeability transition pore, which is critical to the early preconditioning effects (Lim et al., 2007). The second or delayed window may be mediated by the transcriptional nuclear factor κB and synthesis of anti-oxidants and inducible nitric oxide synthase as well as regulation of anti-apoptotic molecules. Although the precise mechanisms of RIPC remain unclear, animal models have provided much of our knowledge on how RIPC may protect the heart and brain in humans.
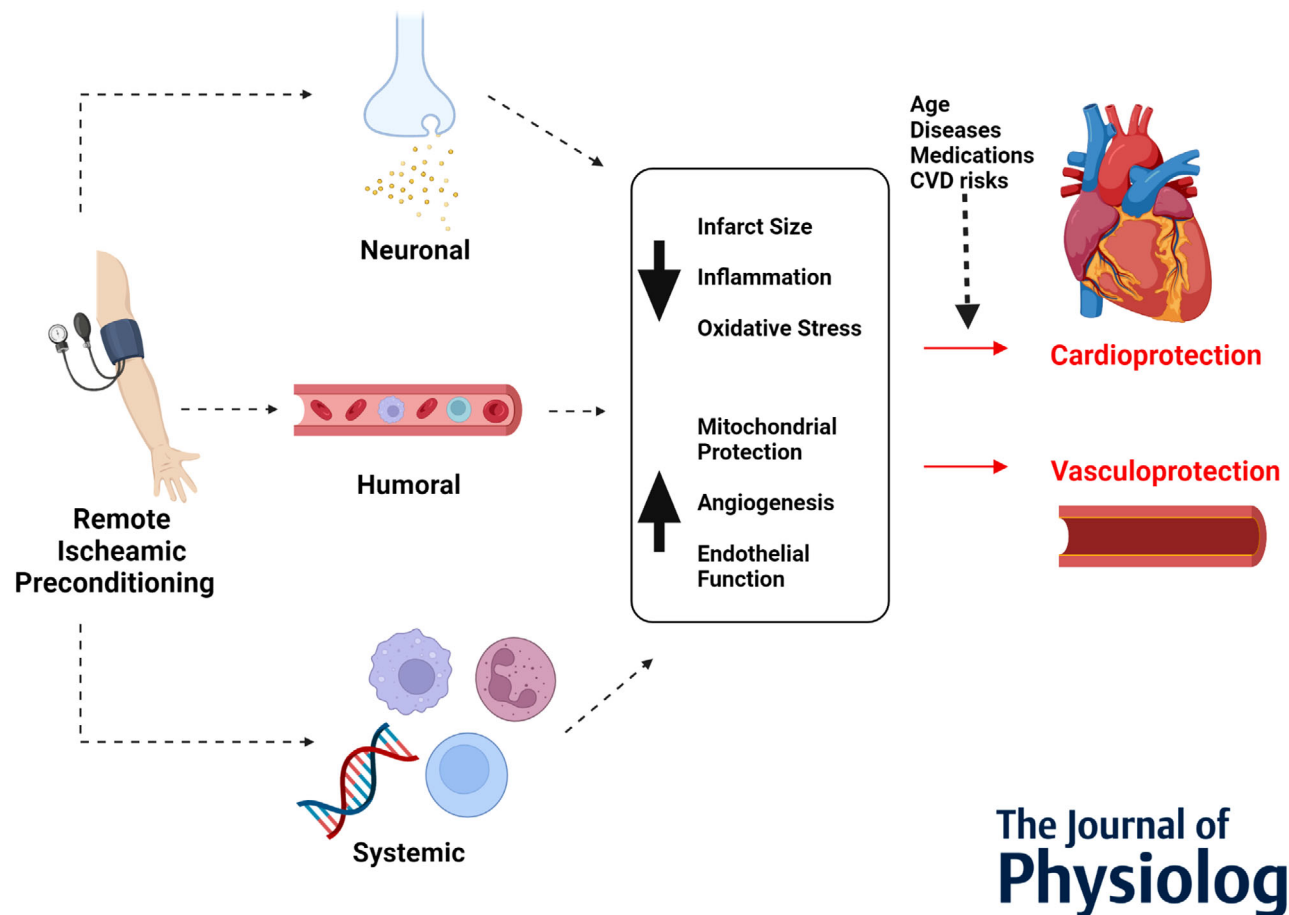

Figure 1. Schematic representation of the triggers and cardiovascular benefits of remote ischaemic preconditioning

Figure 2. Proposed mechanism of remote ischaemic preconditioning for a single bout, both first and second windows of protection, and following repeated bouts

AA, arachidonic acid; ACh, acetylcholine; CaN, calcineurin; CGRP, calcitonin gene-related peptide; CGRP-R, calcitonin gene-related peptide receptor; COX, cyclooxygenase; CYP, cytochrome P450; EDHF, endothelium-derived hyperpolarizing factor; ERK1/2, extracellular signal-regulated kinases 1 and 2; eNOS, endothelial nitric oxide synthase; HIF-1α, hypoxia-inducible factor 1α; HSP, heat shock protein; iNOS, inducible nitric oxide synthase; IP$_3$, inositol 1,4,5-trisphosphate; LOX, 5-lipoxygenase; MAPK, mitogen activated protein kinase; MnSOD, manganese superoxide dismutase; mPTP, mitochondrial permeability transition pore; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor κB; NO, nitric oxide; PGI$_2$, prostaglandin I$_2$; PI3K, phosphoinositide 3-kinase; PIP$_2$, phosphatidylinositol 4,5-bisphosphate; PKA, protein kinase A; PKC, protein kinase C; PKG, protein kinase G; PLA2, phospholipase A2; ROS, reactive oxygen species; sGC, soluble guanylate cyclase; TRPV, transient receptor potential cation channel subfamily V; VEGFR2, vascular endothelial growth factor receptor 2.

From animal model to human clinical research model

Inasmuch as RIPC has consistently demonstrated protective cardiovascular effects in various animal models, the reliable appearance of these effects in humans remains uncertain. Multiple large clinical studies found that markers of cardioprotection, such as troponin concentration and infarct size, and functional clinical outcomes (e.g. mortality from cardiovascular causes, incidence of myocardial infarction or stroke, duration of hospital stay) were not improved in individuals receiving RIPC prior to surgery (Hausenloy et al., 2015; Meybohm et al., 2015; Rahman et al., 2010). In the vasculature, a meta-analysis showed no benefit of RIPC administered prior to vascular surgery (Stather et al., 2019). However, these studies typically administered RIPC immediately prior to surgery, and thus may have missed the optimal timing of cardiovascular protection following RIPC. One study that administered RIPC during patient transport to the hospital, thereby increasing the latency between RIPC and surgery to ∼2 h, demonstrated an attenuation of myocardial infarct size (Botker et al., 2010). Another potential explanation for the lack of positive clinical outcomes with RIPC is the anaesthesia agent used during surgery. Most patients received propofol, which has been shown to abolish the protective effects of RIPC (Behmenburg et al., 2018; Kottenberg et al., 2012). Propofol suppresses β-adrenoreceptor responsiveness (Zhou et al., 1999), and may interrupt a neurogenic pathway during the first window of protection. Collectively, these negative findings highlight the need for more controlled human studies to ascertain underlying mechanisms and what factors affect the efficacy of RIPC.

Translating the promising effects of RIPC from animal models to humans may require an experimental model where confounding factors can be more effectively controlled so that the underlying mechanisms of RIPC can be elucidated. Multiple factors have been identified that alter the cardioprotective effects of RIPC (Table 1). In addition to propofol (Kottenberg et al., 2012), other medications such as beta-blockers may attenuate the effects of RIPC (Zhou et al., 2013), whereas statins may work synergistically with RIPC to further increase NO-mediated dilatation and blunt markers of inflammation (El Desoky et al., 2016). Factors such as age and sex may also affect RIPC. Older adults exhibited less improvement in vascular function following either after a single bout or after multiple bouts of RIPC (Moro et al., 2011; van den Munckhof et al., 2013). Although sex differences have not been adequately described in humans, no differences in the reduction in infarct size were observed between male and female mice; however, females appeared to be less reliant on humoral factors to confer protective effects (Heinen et al., 2018). The cardio-vascular protective effects of RIPC may also be blunted or preserved depending on comorbidities or CVD risk factors that are present (Maxwell et al., 2019; Seeger et al., 2016; Trachte et al., 2021). Lastly, considering the ischaemia and reperfusion experienced during exercise, aerobic fitness or prior physical activity may alter the efficacy of RIPC. This topic regarding the potentially additive or overlapping adaptations of exercise and RIPC is effectively addressed in recent review papers (Thijssen et al., 2018, 2022). Collectively, multiple factors have been identified that potentially affect the clinical use and efficacy of RIPC.

In addition to determining the importance of confounding factors, multiple effects of RIPC observed in animal models have not been verified in humans such as the timing of the windows of cardiovascular protection as well as their underlying humoral and neural mechanisms. Directly assessing coronary vascular mechanisms in humans is methodologically challenging. However, the peripheral limb vasculature is a suitable model to translate findings from an animal to a human model because it is an accessible and representative vascular bed for non-invasive, *in vivo* measurements using various techniques such as ultrasound to assess flow-mediated dilatation (FMD), laser-based skin microvascular measurements, and venous occlusion plethysmography (Joannides et al., 2006; Low et al., 2020). These methods are able to test various signalling mechanisms by examining vascular responsivity to perturbations such as reactive hyperaemia after arterial occlusion, thermal stimuli and localized drug infusion. Blunted responses to these stimuli in the skin microvasculature are an indicator of systemic vascular impairment and preclinical CVD progression (Abularrage et al., 2005; Khan et al., 2008; Mitchell et al., 2005; Minson, 2010). Considering that ischaemic heart disease and stroke are mainly initiated by vascular dysfunction (Gori et al., 2007), measurements in the peripheral limb vasculature may provide a more sensitive and accessible method of evaluating the effects of RIPC in humans.

Single bout of RIPC in humans. In human conduit arteries, a single bout of RIPC improves endothelial function (Moro et al., 2011) and confers ischaemic tolerance from IR injury (Kharbanda et al., 2001; Loukogeorgakis et al., 2005). Similar to animal studies, the protocol of eliciting RIPC in humans often consists of three to four periods of 5 min of limb cuff inflation (∼200 mmHg) and ischaemia followed by 5 min of deflation and reperfusion. Reducing the amount of limb tissue that is ischaemic (i.e. arm *vs.* leg) and fewer periods of ischaemia and reperfusion reduces the protective effects of RIPC in the vasculature (Loukogeorgakis et al., 2007). Nevertheless, it appears that four periods in a single arm or

Table 1. Confounding factors of remote ischaemic preconditioning in humans

Study	Confounder	Patients	Cycles	Period	Outcomes
Moro et al. (2011)	Age + hypertension	Young ($n = 10$) Older (73 years, $n = 10$) Hypertensive older (69 years, $n = 10$)	3×5 min	Seven days (seven bouts)	• ↓Improvement in FMD (absolute) in older hypertensive compared to young group • Similar increase in FMD (as percentage change from pre-RIPC values) between groups
Kottenberg et al. (2012)	Propofol	Isoflurane RIPC ($n = 20$) Isoflurane no RIPC ($n = 19$) Propofol RIPC ($n = 14$) Propofol no RIPC ($n = 19$)	3×5 min	Single bout	• Propofol abolished the attenuation in serum troponin I concentration with RIPC after CABG
Zhou et al. (2013)	Beta-blocker	Meta-analysis (15 trials with 1155 patients)			• ↓Attenuation of markers of myocardial injury after RIPC in patients on beta-blocker
van den Munckhof et al. (2013)	Age (men only)	Young ($n = 15$) Older (68–77 years, $n = 15$)	3×5 min	Single bout of IPC	• IPC preserved FMD after IR injury in young, but IPC did not prevent endothelial IR injury in older adults
Seeger et al. (2016)	Heart failure	Heart failure ($n = 15$) Healthy control ($n = 19$)	3×5 min	Single bout of IPC	• IPC preserved FMD in age- and sex-matched controls but did not prevent endothelial IR injury in heart failure group
Heinen et al. (2018)	Sex	Young male ($n = 10$) Older male ($n = 10$) Young female ($n = 10$) Older female ($n = 10$)	3×5 min	Single bout	• RIPC plasma from young males reduced infarct size in rat hearts • RIPC plasma from young females did not induce humoral cardioprotective effects
Maxwell et al. (2019)	Diabetes	Diabetics RIPC ($n = 11$) Diabetics no RIPC ($n = 10$)	4×5 min	Seven days	• RIPC enhanced peripheral vascular endothelial function
Trachte et al. (2021)	CVD risk factors	Health control ($n = 12$) High CVD risk ($n = 10$)	3×5 min	Single bout	• High CVD risk group show blunted protective effects of RIPC from IR injury

Remote ischaemic preconditioning cycles are provided in periods of occlusion and reperfusion. Abbreviations: CABG, coronary artery bypass graft surgery; CVD, cardiovascular disease; FMD, flow mediated dilatation.

leg is sufficient to maximize the effects of a single session of RIPC.

Animal infarct data emphasize that timing is critical to realizing the protective effects following a single bout of RIPC (Baxter et al., 1997; Kuzuya et al., 1993; Murry et al., 1986). The extent of IR injury in animal models was attenuated when RIPC occurred either immediately prior or 24–72 h prior to myocardial infarction, but IR damage was not affected when RIPC occurred 3 or 12 h prior to infarction (Baxter et al., 1997; Kuzuya et al., 1993). Few studies have examined the duration and timing of the protective effects of RIPC in humans. One study demonstrated that vascular IR injury induced by 15 min of continuous limb occlusion markedly reduced endothelial function assessed by brachial FMD; however, FMD was preserved 20 min, 24 h and 48 h, but not 4 h, after a single RIPC bout (Loukogeorgakis et al., 2005). Furthermore, a single bout of RIPC increased acetylcholine and local

heating-mediated cutaneous vasodilatation responses 48 h after but not 72 h or 1 week after an RIPC bout (Kim et al., 2021). Collectively, these findings suggest that a similar biphasic pattern of vascular protection occurs in humans, and the second window peaks ∼48 h after RIPC and lasts for ∼3 days.

Based on the robust reduction in infarct size in RIPC-treated animals, multiple clinical studies incorporated a single bout of RIPC immediately prior to cardiac surgery to reduce IR injury (Hausenloy et al., 2015; Meybohm et al., 2015; Rahman et al., 2010; Stather et al., 2019). Although markers of cardiac damage were reduced (Hausenloy et al., 2015; Thielmann et al., 2010), none of these studies resulted in a reduction in IR injury or an improvement in clinical outcomes. In contrast, one study showed a reduction in infarct damage from 13 to 9% when RIPC was administered while in the ambulance during patient transport to the hospital (Botker et al., 2010). Consequently, the longer duration between RIPC and surgery may have resulted in more optimal alignment with the peak protective effects of the first window. Capturing this brief window requires more precise timing and may be less efficacious than if surgery was performed during the lengthier delayed window. These clinical studies further underscore the importance of the timing of RIPC; however further studies in humans are needed to reveal more precisely when a single bout of RIPC should occur to optimally reduce IR injury.

Potential mechanisms of single bout RIPC on vascular function in humans. Many of the underlying mechanisms that are thought to confer vasculoprotection following a single RIPC bout have not been directly tested in humans. Similar to animal work in cardiomyocytes, the importance of both neuronal and humoral pathways has been demonstrated in the human vasculature. Ganglionic blockade with intravenous trimethaphan infusion abolished the protective effect of RIPC in mitigating vascular IR injury (Loukogeorgakis et al., 2005). Although trimethphan may have non-specific effects that alter vasomotor function (i.e. affecting histamine release or blocking α-adrenergic receptors), this finding highlights the role of the autonomic nervous systems in mediating the protective effects of RIPC. However, although there are reports to the contrary (Angius et al., 2022; Mulliri et al., 2016), a single bout of ischaemic preconditioning did not alter sympathetic activity, assessed by microneurography, or reduce haemodynamic responses to static handgrip exercise or subsequent post-exercise ischaemia (Incognito et al., 2017). Furthermore, heart rate variability measures, assessed by symbolic dynamics and power spectral density analysis, at multiple time points in the delayed window of protection indicate no change in sympathovagal balance following a single bout of RIPC (Gardner et al., 2020;

Zagidullin et al., 2016). Interestingly, only repeated bouts of RIPC over several days altered sympathovagal balance (Gardner et al., 2020).

Another potential neuronal mechanism of RIPC may be the activation of sensory afferents by transient receptor potential vallinoid-1 (TRPV1) channel activation, which elicits the antidromic release of paracrine mediators such as CGRP (Moseley et al., 2020). Although this hypothesis is supported in animals, there is no direct evidence in humans. In the skin microvasculature, TRPV1 strongly contributes (i.e. as much as 50%) to the initial axon reflex-mediated dilatation to localized heating (Wong & Fieger, 2010). A single bout of RIPC did not affect this initial response to heating (Kim et al., 2021); however, the protocol used ($T_{loc} = 42°C$) typically elicits an axon reflex reponse that approximates ∼60–70% of maximum dilatation. Thus, a 'ceiling effect' may have prevented the detection of RIPC-mediated changes in the initial peak of the local heating response (Choi et al., 2014). Both capsaicin-sensitive sensory afferents and the vagus nerve may release humoral factors following RIPC (Pickard et al., 2016), and thus the neural and hormonal mechanisms of RIPC are likely interdependent.

Endothelial cells are uniquely sensitive to IR injury, and therefore their function is critical to the protective effects of RIPC. Primary mediators of endothelium-dependent vasodilatation include nitric oxide (NO), endothelial dependent hyperpolarization factors (EDHFs), and prostacyclin (PGI_2). Although each of these mediators may contribute to conduit artery FMD, this measurement is largely thought to be a more specific index of NO-dependent vasodilatation. Multiple studies have shown that a single bout of RIPC increased FMD or preserved FMD following IR injury during both the first window (∼4 h) and the second window (24–72 h) of protection (Contractor et al., 2013; Kharbanda et al., 2002; Loukogeorgakis et al., 2005; Liu et al., 2015; Moro et al., 2011). These findings collectively suggest that RIPC may be a stimulus for NO release, particularly during the reperfusion periods of RIPC as shear stress along the endothelium stimulates endothelial nitric oxide synthase to produce NO. However, considering the short (∼3–5 s) half-life of NO, it seems that such a response would be limited to only a localized area as opposed to having a distant or 'remote' effect. This is consistent with findings in the skin microcirculation where NO-mediated vasodilatation, tested by intra-dermal inhibition with N^ω-nitro-ʟ-arginine methyl ester (ʟ-NAME) during local heating (39°C), was unaffected by RIPC (Lang et al., 2019). Furthermore, maximizing shear stress and NO release by cycling more quickly through ischaemia and reperfusion periods (i.e. 5 s arm ischaemia interspersed with 10 s reperfusion over a 30 min period) increased FMD by ∼50% as well as the NO contribution to cutaneous local heating-mediated

vasodilatation, but no 'remote' effect was observed in the opposite arm (Hodges et al., 2017, 2018). However, using a typical RIPC protocol that incorporated longer periods of ischaemia (i.e. 5 min periods), a remote effect of increased cutaneous vasodilatation in response to acetylcholine or local heating was observed but the specific contribution of NO was not assessed (Kim et al., 2021). One explanation is that NO bioavailability may increase during the ischaemic periods in the RIPC protocol (Totzeck et al., 2015). Circulating nitrite, a byproduct of NO oxidation with a longer half-life (30–60 min), reduces to NO by reacting with haemoglobin or myoglobin during hypoxia or ischaemic stress (Rassaf et al., 2014). Alternatively, increased endothelium-dependent vasodilatation following a single bout of RIPC may rely on other non-NO endothelial mechanisms.

Other endothelial mechanisms that may explain the effects of a single bout of RIPC include EDHFs and PGI$_2$; however, few studies have examined their respective contribution. EDHFs act through K$^+$ channels to elicit vascular smooth muscle hyperpolarization and vasodilatation. In the skin circulation, most of the EDHF response occurs through calcium-activated potassium (K$_{Ca}$) channels; however, ATP-sensitive potassium (K$_{ATP}$) channels have an important role through their interaction with NO (Brunt & Minson, 2012; Fujii et al., 2020; Leung & Vanhoutte, 2017). In conduit arteries, the RIPC-induced preservation of the FMD response following IR injury was lost after inhibition of K$_{ATP}$ channels with glibenclamide, thereby indicating that the protective effects of RIPC were mediated by K$_{ATP}$ channel activation (Loukogeorgakis et al., 2007). However, the loss of protection with glibenclamide was tested only ~30 min after RIPC (i.e. only during the first window of protection). PGI$_2$, catalyzed by rate-limiting cycloogenase (COX) enzymes, is a potent vasodilator that acts on vascular smooth muscle to elicit relaxation. A COX-2 inhibitor also abolished the protective effects of RIPC following IR injury; however, this was evident only at 24 h after but not immediately after RIPC (Liu et al., 2015). Summarily, these findings indicate that endothelial mechanisms besides NO importantly contribute to RIPC-induced vasculoprotection. Furthermore, these data suggest that the protective effects in the first window are more dependent on EDHFs whereas the second window may be more dependent on PGI$_2$. But, vascular measurements at additional time points following RIPC, particularly when the delayed effect peaks ~48 h post (Kim et al., 2021), are needed to confirm this notion.

With respect to endothelium-independent pathways, these mechanisms appear to be unaffected in both the first and second window following a single bout of RIPC (Loukogeorgakis et al., 2005, 2007). Interestingly, the underlying protective mechanisms appear to shift as repeated bouts of RIPC are implemented over several days

(Depre et al., 2010; Shen et al., 2008). Thus, repeated RIPC stimulates different adaptive mechanisms to potentially elicit additive or longer lasting effects than that seen with only a single bout of RIPC.

Repeated RIPC and vascular function. The potential for additive benefit by implementing repeated bouts of RIPC, often over several days, is important considering that improvement in vascular function is related to lower risk for future cardiovascular events (Schachinger et al., 2000). Studies that have examined the vascular effects of repeated RIPC have typically used a similar protocol (i.e. periods of 3–4 cycles of 5 min limb arterial occlusion followed by 5 min reperfusion) but have implemented RIPC sessions daily, or every other day, over a period of time ranging from 1 week to as long as 2 months. From a clinical perspective, it is important to determine the effective minimal intervention period (i.e. dose and frequency) that maximizes vascular improvements. Although this remains unclear, a similar amount of improvement in cutaneous endothelial function was observed between 1 and 2 weeks of RIPC (Kim et al., 2020). Furthermore, FMD was not further increased by 8 weeks compared to 2 weeks of RIPC sessions administered every other day (Jones et al., 2015). Since a single bout of RIPC has a biphasic response and the second window upregulates cardiovascular protective mechanisms (Bolli et al., 2007), repetitive stimulation during the peak of the second window (i.e. 48 h post-RIPC or every other day) may be an optimal frequency for inducing the endothelium-mediated effects with a repeated RIPC protocol (Kim et al., 2022). But, the optimal frequency of RIPC as well as how long benefits remain after completing RIPC requires further investigation. With respect to duration, ~1–2 weeks may be sufficient to maximize the endothelial benefits of repeated RIPC; however, a longer duration may be needed to fully realize angiogenic or endothelium-independent effects.

Compared to only a single bout, repeated RIPC appears to provide additional cardiovascular protection through endothelium-independent mechanisms. Thus, there may be further clinical benefit by administering multiple bouts of RIPC (Table 2). The pattern and underlying mechanisms of cardiovascular protection with repeated RIPC may be similar to that observed after short-term aerobic exercise (Thijssen et al., 2018; Thijssen et al., 2022). In heart failure patients, 1 week of RIPC increased coronary artery flow reserve by ~20% (Kono et al., 2014). Furthermore, 1–3 weeks of RIPC increased FMD or blunted the decline in FMD with IR injury in Type II diabetics (Maxwell et al., 2019), coronary heart disease (Liang et al., 2015) and stroke patients (Hyngstrom et al., 2020). Longer RIPC interventions (>6 months) attenuated recurrent stroke rate (7.9% *vs*. 26.7%) (Meng et al., 2012), and increased thrombolytic activity and

Table 2. Clinical studies using repeated bouts of remote ischaemic preconditioning

Study	Patients	Location	Cycles	Period	Outcomes
Kono et al. (2014)	Chronic heart failure (n = 10) Healthy control (n = 10)	Bilateral upper arm	4 × 5 min	2×/day for 1 week (14 bouts)	• ↑Coronary flow reserve (20%) – in both groups • ↓HR (10%)
Meng et al. (2015)	Older ischaemic stroke (n = 30; age 84 ± 2 years) Standard treatment (n = 28)	Bilateral upper arm	5 × 5 min	2×/day for 180 days (360 bouts)	• ↑Tissue plasminogen activator • ↓CRP, IL-6, PAI-1, platelet aggregation rate • ↓Stroke recurrence • ↓Transient ischaemic attacks
Liang et al. (2015)	Coronary heart disease prior to CABG surgery (n = 20) Standard treatment (n = 20)	Bilateral upper arm	4 × 5 min	3×/day for 20 days (60 bouts)	• ↑FMD (100%) • ↑Endothelial progenitor cells (20%) • ↑STAT-3 activation • ↓Blood [troponin] after surgery (50%)
Shaked et al. (2015)	Type 2 diabetics (n = 22) Standard treatment (n = 12)	Bilateral upper arm	3 × 5 min	Bi-weekly (6 weeks)	↑Diabetic ulcer healing (reduced ulcer area per unit time)
Mi et al. (2016)	Cerebral small vessel disease (n = 9) Standard treatment (n = 8)	Bilateral upper arm	5 × 5 min	2×/day for 360 days (720 bouts)	• ↑MCA flow velocity • ↓White matter lesions • ↓Dizziness handicap inventory
Wang et al. (2017)	Cerebral small vessel disease (n = 14) Standard treatment (n = 16)	Bilateral upper arm	5 × 5 min	2×/day for 360 days (720 bouts)	• ↑Visuospatial and executive abilities • ↓Plasma [triglyceride] • ↓Plasma [total cholesterol] • ↓Plasma [LDL cholesterol] • ↓Blood [homocysteine]
Pryds et al. (2019)	Chronic heart failure prior to PCI surgery (n = 21) Healthy control (n = 21)	Unilateral upper arm	4 × 5 min	1×/day for 28 days (28 bouts)	• ↓CRP • ↓Calprotectin (marker of intestinal inflammation)
Maxwell et al. (2019)	Type 2 diabetics (n = 11) Standard treatment (n = 10)	Unilateral upper arm	4 × 5 min	1×/day for 1 week (seven bouts)	• ↓FMD attenuation with IR injury (25% less)
Hyngstrom et al. (2020)	Chronic stroke (n = 12) Standard treatment (n = 11)	Unilateral thigh	4 × 5 min	Every other day for 2 weeks (seven bouts)	• ↑FMD (40%)

Remote ischaemic preconditioning cycles are provided in periods of occlusion and reperfusion.
Abbreviations: CABG, coronary artery bypass graft surgery; CRP, C reactive protein; FMD, flow mediated dilatation; HR, heart rate; IL-6, interleukin 6; LDL, low density lipoportein; MCA, middle cerebral artery; PAI-1, plasminogen activator inhibitor-1; PCI, percutaneous coronary intervention; STAT-3, signal transducer and activator of transcription 3.

reduced markers of inflammation and coagulation in stroke patients (Meng et al., 2015). These clinical studies suggest that repeated RIPC improved cardiovascular function in various clinical populations. Considering that peripheral limb RIPC is a safe, inexpensive and simple intervention, repeated bouts of RIPC could be beneficial in preventing subclinical CVD progression as well as improving cardiovascular function in populations with limited mobility or those at high risk of an ischaemic event.

Potential mechanisms of repeated RIPC on vascular function. Vascular adaptations to repeated RIPC may become less dependent on endothelial mechanisms in resistance vessels. One of the first studies to examine the effects of repeated bouts of RIPC in humans measured forearm blood flow before and after 4 weeks of RIPC (Kimura et al., 2007). They found that acetylcholine-mediated vasodilatation increased in the arm that was ischaemic but not the contralateral or remote arm after repeated RIPC. This is supported by work in the cutaneous microvasculature showing that the vasodilatation to local heating (39°C) as well as the specific NO contribution, verified by local perfusion of a non-specific NO inhibitor (L-NAME), was unaffected following 1 week of RIPC (Lang et al., 2019). The NO contribution to local heating at 39°C is greater than when heating at the more conventional 42°C (i.e. ∼70% compared to ∼40–50% NO contribution) (Choi et al., 2014). In a follow-up study utilizing a higher local heating temperature (42°C), 2 weeks of RIPC modestly increased the skin vasodilatation response by ∼20% (Kim et al., 2020). In this study, post-testing occurred 24 h after the last RIPC bout. Thus, the increased vasodilatation response may in part be explained by the previous single bout since testing occurred while in the second window of the most recent bout of RIPC. No effect of acetylcholine-mediated vasodilatation occurred at 2 weeks of RIPC (Kim et al., 2020). Collectively, these studies indicate that NO does not appreciably contribute to the repeated RIPC response in the microcirculation of young, healthy adults. However, similar to a single bout of RIPC, repeated bouts may have an effect on endothelial mechanisms that are non-NO mediated.

Conversely, there may be a greater endothelial effect from repeated RIPC in conduit vessels. One week of RIPC prior to vascular IR injury abolished the reduction in FMD (Luca et al., 2013). Interesting, they further demonstrated that COX did not contribute to these protective effects. However, much of this attenuation occurred after a single bout and the effect of added bouts of RIPC is difficult to ascertain considering that FMD was tested 24 h after the last RIPC bout (i.e. still within the delayed window of the last RIPC bout). A study that controlled for the second window measured FMD both 24 h after (effect of both single and repeated bouts) and 8 days after the last RIPC bout (effect of repeated RIPC alone) found that brachial FMD was augmented at both time points following a 1 week daily RIPC protocol (Jones et al., 2014). In this study, the cutaneous vasodilatation response to local heating was unaffected by repeated RIPC. Thus, the adaptive response to repeated bouts of RIPC may depend on location within the vascular tree.

There may be a significant contribution of endothelium-independent mechanisms to repeated bout RIPC in the microcirculation. Antidromic release of paracrine mediators such as CGRP from sensory nerves (Chai et al., 2006), sensitivity of vascular smooth muscle to NO, and stimulation of angiogenic pathways may encompass the endothelium-independent component as it relates to repeated RIPC. Although there are reports to the contrary with respect to NO sensitivity (Kimura et al., 2007), sodium nitroprusside-mediated vasodilatation was increased in the cutaneous microvasculature following 2 weeks of RIPC (Kim et al., 2020) but was unaffected with only a single bout (Kim et al., 2021). One week of daily RIPC augmented maximal cutaneous vasodilatation by ∼50% and this increase was maintained 1 week after the last RIPC bout (Lang et al., 2019). These studies indicate that repeated RIPC is uniquely affecting microvascular function independent of the endothelium.

Angiogenesis may also contribute to the vascular adaptations with repeated RIPC. In the early study by Kimura et al., they demonstrated that vascular endothelial growth factor (VEGF) and endothelial progenitor cells were increased following 4 weeks of repeated RIPC (Kimura et al., 2007). Increased VEGF can improve myocardial functional recovery after ischaemia (Guzman et al., 2008). The ischaemic stress of RIPC stimulates the transcriptional activator hypoxia inducible factor 1α (HIF-1α), which may be required for the manifestation of the RIPC response (Albrecht et al., 2013; Cai et al., 2013). HIF-1α subsequently increases VEGF and multiple proteins mediating vasomotor tone and angiogenesis. These proteins increase vascular permeability, for example through VEGF binding to its receptor on endothelial cells, resulting in plasma protein leakage and creation of an inflammatory response that degrades the extracellular matrix membrane of the vascular wall via matrix metallopeptidase. Membrane degradation increases endothelial cell migration into the surrounding tissues, thereby allowing endothelial cell proliferation and the assembly of a new vessel and capillary branching. Although previous studies in humans have suggested that repeated RIPC induces angiogenesis due to elevated VEGF and increased ischaemic tolerance (Kimura et al., 2007), actual structural changes in the vasculature were not measured. Furthermore, the increase in angiogenic markers appears to require more extended periods of RIPC, such as 1 month of RIPC as opposed to 1 week

or a single RIPC bout (Guo et al., 2019; Hummitzsch et al., 2021; Kimura et al., 2007). These studies reveal an important endothelium-independent contribution to the repeated RIPC response not otherwise seen with a single bout; however, further studies are needed to clarify precise mechanisms.

Future directions

It is well established that direct ischaemic preconditioning and RIPC consistently elicit cytoprotective effects in the cardiovascular system in animal models. However, translating the benefits of RIPC to humans has proven challenging due to multiple confounding factors, misplaced timing of RIPC, and lack of knowledge regarding the alignment of underlying mechanisms with RIPC timing. Most studies in humans have limited their focus to the immediate effects of a single bout RIPC. Although the precise timing of the first window still requires further investigation, clinical studies involving emergency surgery should incorporate RIPC as soon as possible (e.g. on the way to the hospital) to allow time to maximize the benefit of the 'first window' effects. For non-emergency clinical applications, a RIPC bout should also be administered 48 h prior to a hospital visit to also incorporate the 'second window' effects, and adding additional bouts when possible to provide repeated bout RIPC effects. The latter may be more powerful considering its potential for longer lasting angiogenic or structural adaptations; however, the extent of this benefit and underlying mechanisms have yet to be elucidated. Considering the impact of propofol on large clinical RIPC studies, it is imperative that future studies identify and characterize additional confounding factors as that will likely modify a RIPC 'prescription'. Outside the context of clinical studies, the role of repeated RIPC in preventative medicine to reduce CVD progression is also warranted. A recent study indicated that 8 weeks of combined exercise and RIPC did not improve vascular function more than RIPC alone (Maxwell et al., 2021); however, the additive or overlapping effects of exercise and RIPC required further characterization. Additionally, RIPC may have neuromuscular and neuroprotective effects and thus could have application to exercise performance or with maintaining cognitive function. Perhaps similar to exercise, a single bout is better than none but in order to maximize beneficial effects, multiple bouts of RIPC are needed.

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Additional information

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No competing interests declared.

Author contributions

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Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

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