



## REVIEW ARTICLE | OPEN ACCESS

# Chronic remote ischemic conditioning for cardiovascular protection

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New treatments are needed to prevent adverse left ventricular remodeling following acute myocardial infarction (AMI), in order to prevent heart failure and improve clinical outcomes following AMI. Remote ischemic conditioning (RIC) using transient limb ischemia and reperfusion has been reported to reduce myocardial infarct (MI) size in AMI patients treated by primary percutaneous coronary intervention. Whether RIC can improve clinical outcomes is currently being investigated. Interestingly, repeated daily episodes of limb RIC (termed 'chronic remote ischemic conditioning', or CRIC) have been shown in experimental and clinical studies to confer beneficial effects on post-AMI cardiac remodeling and chronic heart failure. In addition, the beneficial effects of CRIC extend to vascular function, peripheral arterial disease, and stroke. In this review article, we focus on the therapeutic potential of CRIC as a strategy for cardiovascular protection and for improving clinical outcomes in patients with cardiovascular disease.

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## Introduction

Acute myocardial infarction (AMI) and the heart failure (HF) that often follows, are among the leading causes of mortality and morbidity worldwide. As such new treatments are needed to reduce myocardial infarct (MI) size, in order to prevent resultant adverse left ventricular (LV) remodeling, and reduce the risk of HF (Hausenloy et al., 2017). In this regard, the heart can be protected against acute lethal ischemia/reperfusion injury (IRI) by subjecting the myocardium to brief non-lethal cycles of alternating ischemia and reperfusion, a phenomenon termed 'ischemic preconditioning' (IPC) (Murry et al., 1986). However, IPC requires the intervention to be applied before the onset of acute myocardial ischemia (which is not possible in AMI patients), and for it to be applied to the heart directly (making the intervention invasive). Interestingly, the 'conditioning' stimulus can be applied to an organ or tissue away from the heart, either prior to ischemia, during ischemia, or at the onset of reperfusion, thereby easing its clinical application in AMI patients - a phenomenon referred to as 'remote ischemic conditioning' (RIC) (Przyklenk et al., 1993). Crucially, the RIC stimulus can be applied both simply and non-invasively, by simply inflating and deflating a pneumatic cuff placed on the upper arm or thigh to induce 3 to 4 cycles of brief ischemia and reperfusion to the limb (Kharbanda et al., 2002; Hausenloy and Yellon, 2008). A single RIC stimulus applied to the limb has been shown to reduce peri-operative myocardial injury in patients undergoing coronary revascularization by either PCI (Hoole et al., 2009) or CABG (Hausenloy et al., 2007), and has been demonstrated to reduce MI size in ST-segment elevation myocardial infarction (STEMI) patients treated by primary percutaneous coronary intervention (PPCI) (Botker et al., 2010; White et al., 2015), although not all studies have been positive. However, in large clinical outcome studies, a single limb RIC stimulus was shown to not improve clinical outcomes in CABG patients (Hausenloy et al., 2015; Meybohm et al., 2015). Interestingly, emerging data suggests that repeated daily episodes of limb RIC, termed chronic remote ischemic conditioning (CRIC), may have beneficial effects over and above a single RIC stimulus following AMI (Wei et al., 2011) and in stroke (Meng et al., 2012).

In this review article we focus on the therapeutic potential of CRIC as a strategy for cardiovascular protection. The reader is referred to other articles investigating the use of CRIC as a strategy for neuroprotection (Hess et al., 2015; Khan et al., 2018).

## CRIC and post-infarct remodeling

The first study to introduce the concept of CRIC, was by Shimizu et al. (2010) in a small study, who demonstrated in 5 healthy volunteers that repeated daily episodes of limb RIC (3 x 5 min cycles of inflations and deflations of a pneumatic cuff placed on the upper arm) for 10 days reduced neutrophil adhesion and activation. This finding confirmed that the anti-inflammatory effects observed with a single limb RIC stimulus could be extended to CRIC. Based on these findings and the knowledge that excessive oxidative stress and inflammation contribute to post-AMI adverse LV remodeling, Redington's group (Wei et al., 2011) went on to investigate the effect of CRIC on subsequent post-AMI adverse LV remodeling in a rat model. They found that CRIC (3 x 5 min cycles of unilateral hindlimb ischemia and reperfusion) applied either daily or every 3 days for 28 days prevented post-AMI adverse LV remodeling (preserved LV function, less LV dilatation, and attenuated LV hypertrophy and myocardial fibrosis), and improved survival, in a dose-dependent manner at 12 weeks, and to a greater extent than a single limb RIC stimulus applied prior to reperfusion (Wei et al., 2011). The beneficial effects of CRIC were shown to be associated with less oxidative stress and inflammation (reduced

leucocyte accumulation, less NF- $\kappa$ B activation, and attenuated expression of pro-inflammatory cytokines, IL-1 $\beta$  and TNF- $\alpha$ ) (Wei et al., 2011). The findings from this study suggested that CRIC applied after AMI mediate distinct benefits on post-AMI LV remodeling when compared to a single limb RIC stimulus given at the time of AMI.

In a follow-up study by the same research group, Rohailla et al. (2014) went on to show in a murine AMI model that CRIC (4 x 5 min cycles of unilateral hindlimb ischemia and reperfusion) daily for 9 days reduced MI size to a level similar to that with a single RIC stimulus, and this beneficial effect was associated with downregulation of mTOR and subsequent upregulation of pro-autophagy proteins. Whether this effect of CRIC on autophagy signalling contributes to the beneficial effects of CRIC on post-AMI remodeling is not known and remains to be determined. In a subsequent study, Yamaguchi et al. (2015) investigated in a rat AMI model the effects of CRIC (5 x 5 min cycles of bilateral hindlimb ischemia and reperfusion) daily for 28 days, initiated 4 weeks following AMI, thereby testing the effects of CRIC in the chronic phase of AMI. They found less oxidative stress and improved LV remodeling (preserved LV function, less LV dilatation, and attenuated myocardial hypertrophy) in CRIC-treated hearts compared to control, suggesting beneficial effects of CRIC in the chronic phase of AMI healing (Yamaguchi et al., 2015). Interestingly, the effects of CRIC were associated with upregulation of miR-29a and miR-30a (negative regulators of fibrosis) in myocardial tissue and circulating exosomes (Yamaguchi et al., 2015).

The first clinical study to test the effects of CRIC in AMI patients as a strategy to prevent adverse LV remodeling was by Vanezis et al. in 2018 (Vanezis et al., 2018). They randomized 73 STEMI patients treated by PPCI with LV ejection fraction <45% to receive either CRIC (4 x 5 min cycles of upper arm cuff inflation and deflation) initiated 3 days post-STEMI and applied daily for 28 days, or sham. Unfortunately, they found no effects of CRIC on post-AMI remodelling at 4 months post-AMI in terms of LV chamber size or function as assessed by cardiac MRI. The reasons for failure of CRIC to have beneficial effects post-STEMI are not clear but may be due to delaying the application of CRIC to 3 days post-infarction, a critical period when inflammation and oxidative stress contribute to adverse LV remodeling. In the original experimental study reporting beneficial effects of CRIC in a rat model of AMI, a single RIC stimulus was given prior to reperfusion and CRIC was initiated the next day (Wei et al., 2011). This issue should be addressed by the ongoing Chronic Remote Ischemic Conditioning to Modify Post-MI Remodeling (CRIC-RCT) study (NCT01817114), which is testing the effect of a single limb RIC stimulus applied prior to reperfusion followed by CRIC (4 x 5 min cycles of upper arm cuff inflation and deflation) applied daily for 28 days on post-AMI LV remodeling. In addition, the ongoing Comprehensive Remote Ischemic Conditioning in Myocardial Infarction (CORIC-MI) study is investigating the effect of a comprehensive CRIC protocol comprising RIC (5 x 5 min cycles of bilateral thigh cuff inflation and deflation) applied both prior to and immediately following PPCI, and repeated daily for 28 days on post-STEMI LV remodeling assessed by cardiac MRI at 28 days (Song et al., 2018).

In summary, CRIC has been shown in experimental animal studies to prevent post-AMI adverse LV remodeling, but whether it is effective in AMI patients is currently being tested.

## CRIC in chronic heart failure

The first study to investigate the effect of limb RIC in chronic heart failure patients was by MacDonald et al. (2014) who found that a single limb RIC stimulus had no salutatory effects on peak VO<sub>2</sub> during peak exercise in 20 chronic heart

failure patients (LVEF < 40% with heart failure symptoms). Experimental animal studies have suggested beneficial effects with CRIC applied following AMI either in the acute period (Wei et al., 2011) or 4 weeks post-AMI (Yamaguchi et al., 2015) suggesting that CRIC may have beneficial effects in chronic heart failure. The first clinical study to test the effects of CRIC in chronic post-AMI heart failure patients was by Kono et al. (2014) who found that CRIC (4 x 5 min cycles of bilateral upper arm cuff inflation and deflation) applied twice daily for 7 days resulted in a modest increase in coronary flow reserve (CFR), assessed by transthoracic Doppler echocardiography, as a physiological index of coronary microcirculation in both healthy subjects (n = 10) and patients with chronic HF with reduced LVEF (n = 10, ischemic cardiomyopathy in six patients and idiopathic dilated cardiomyopathy in four patients). However, there were no differences in circulating inflammatory markers or cardiac size or function (assessed by echocardiography) with CRIC (Kono et al., 2014).

Pryds et al. (2017b) investigated the effect of CRIC (4 x 5 min cycles of upper arm cuff inflation and deflation) applied daily for 28 days in 22 chronic ischemic heart failure patients and 21 aged-matched controls. CRIC was shown to have no effect on either LVEF or global longitudinal strain (GLS) assessed by cardiac MRI in both heart failure and control patients, although it did improve GLS in patients with highest NT-proBNP plasma levels. CRIC also had no effect on peak cardiopulmonary exercise capacity or disease-related quality of life, although it did increase skeletal muscle power in both heart failure and control patients (Pryds et al., 2017b). This latter finding suggests that CRIC may be used to prevent the decrease in skeletal muscle function arising from cardiac cachexia. Interestingly CRIC did reduce plasma NT-proBNP levels in heart failure patients but not in control patients, and lowered systolic blood pressure in heart failure patients but not in control patients (Pryds et al., 2017b). The mechanism underlying the beneficial effects of CRIC on both NT-proBNP and GLS is not clear but may relate to less myocardial wall stress, caused by reduction in afterload (as evidenced by lowered systemic blood pressure), and this may be due to the release of known vasodilatory mediators of RIC such as adenosine and nitric oxide (Pryds et al., 2017b). A further study, using the same CRIC protocol, demonstrated a mild anti-inflammatory effect with modest reductions in C-reactive protein and calprotectin in patients with chronic ischemic heart failure when compared to control patients (Pryds et al., 2019).

Interestingly, a randomized controlled trial by Chen et al. (2018) has demonstrated beneficial effects of CRIC (4 x 5 min cycles of upper arm cuff inflation and deflation) applied twice daily for 6 weeks in patients with chronic ischemic heart failure randomized to receive either CRIC (n = 23) or standard therapy (n = 24). CRIC was reported to improve LVEF (from 39.2% to 43.4%, as assessed by echocardiography), increase exercise capacity (as assessed by 6 minute walk test), reduce NYHA class, and lower levels of plasma BNP when compared to control (Chen et al., 2018). Of note, the beneficial effects of CRIC were associated with correction of cardiac autonomic dysfunction in heart failure patients with increased parasympathetic and reduced sympathetic activity, as assessed by heart rate variability (Chen et al., 2018). Although, the beneficial effects of CRIC in this study were impressive, it must be noted that no sham control was used.

Prior experimental and clinical studies have demonstrated that a single limb RIC stimulus may reduce platelet activation (Pedersen et al., 2011; Lanza et al., 2016) and inhibit thrombus formation (Ropcke et al., 2012), suggesting that it may be useful in the setting of chronic heart failure, a condition associated with increased risk of thrombotic events (Lip et al., 2012). Pryds et al. (2017a) have investigated the effect of CRIC (4 x

5 min cycles of upper arm cuff inflation and deflation) applied daily for 28 days on platelet function in 16 chronic ischemic heart failure patients and 21 matched patients with no ischemic heart disease. CRIC was shown to have no effect on platelet aggregation in response to collagen or arachidonic acid in either heart failure or control patients. This differs from the effects of a single limb RIC stimulus that was reported to reduce platelet activation (Pedersen et al., 2011; Lanza et al., 2016). However, CRIC did increase fibrinolysis in both heart failure and control patients, suggesting it may reduce the risk of thrombosis (Pryds et al., 2017a). However, this finding differs again from a single limb RIC stimulus that had no effect on haemostasis in healthy volunteers (Kristiansen et al., 2016).

In summary, CRIC has been shown to confer beneficial effects in patients with stable ischemic heart failure, as evidenced by improvements in cardiac function, exercise tolerance, and lowering of plasma BNP levels. Whether CRIC can improve clinical outcomes such as re-hospitalization for heart failure needs to be tested in a large randomized controlled trial.

### Vascular effects of CRIC

The first clinical study to report beneficial cytoprotective effects with a single limb RIC stimulus (3 x 5 min upper arm inflation and deflation) was by Kharbanda et al. (2002) who reported less endothelial dysfunction (as assessed by flow-mediated dilatation) in the contralateral limb following a sustained episode of limb ischemia and reperfusion. Luca et al. (2013) were the first to investigate the effect of CRIC on endothelial dysfunction induced by acute ischemia and reperfusion. They demonstrated that healthy volunteers randomized to receive CRIC (3 x 5 min cycles of upper arm cuff inflation and deflation) applied daily for 7 days had improved endothelial function following acute ischemia and reperfusion, when compared to control, and to a level similar to a single limb RIC stimulus (Luca et al., 2013). These findings suggest that the protective effects of RIC on endothelial function did not display tachyphylaxis, suggesting that CRIC may confer long-term cytoprotective effects against acute ischemia and reperfusion.

Beneficial effects on vascular and endothelial function have also been reported in the brachial artery and forearm microcirculation in healthy volunteers following CRIC (4 x 5 min cycles of upper arm cuff inflation and deflation) applied daily for 7 days, suggesting vascular effects of CRIC, which extend from conduit arteries to the skin microvasculature (Jones et al., 2014). These data suggest that CRIC may be beneficial in patients with endothelial dysfunction such as occurs in diabetes and aging. Interestingly, the vascular effects induced by CRIC were shown to still be present 8 days after the end of the intervention, suggesting a 'memory' vascular effect (Jones et al., 2014) that extends beyond the usual 2-3 days observed with the 'second window of protection' (SWOP) (Marber et al., 1993). The current paradigm suggests that a single limb RIC stimulus elicits two windows of protection, the first manifesting immediately and lasting 2-3 hours, and the SWOP, appearing 12-24 hours later and lasting 2-3 days (Hausenloy & Yellon, 2010). CRIC therefore appears to extend the window of protection to 8 days - these findings need to be confirmed in other studies, and the mechanisms underlying this protective effect need to be investigated. The SWOP has been attributed to the *de novo* synthesis of cardioprotective proteins, such as inducible nitric oxide synthase and cyclo-oxygenase-2 (Hausenloy & Yellon, 2010). The mechanisms underlying this memory effect are not clear, but may be due to epigenetic changes in the vasculature, which can extend the protective effect beyond the SWOP.

A potential hemodynamic consequence of the vasodilatory effects induced by CRIC (7-28 days) have been confirmed in



several clinical studies reporting a reduction in systemic blood pressure (of about 5 mmHg) in healthy volunteers (Kono et al., 2014; Madias & Koulouridis, 2014; Madias, 2015a 2015b) and patients with heart failure (Pryds et al., 2017b), although not all studies have been positive (Kimura et al., 2007). Whether the effect of CRIC on lowering blood pressure has a 'memory' effect, i.e. persists beyond the intervention, remains to be determined. The mechanisms underlying the vascular effects of CRIC are not clear but may relate to: adaptations to shear stress, augmentation of endothelium-dependent vasodilation and production of nitric oxide (Kimura et al., 2007) circulation of vasoactive mediators (such as, nitrite, vascular endothelial growth factor or endothelial progenitor cells) (Kimura et al., 2007), and/or systemic anti-oxidant and anti-inflammatory effects.

In summary, CRIC has been shown to confer salutatory effects on vascular and endothelial function that may be beneficial to patients with endothelial dysfunction and hypertension. Further studies are needed to elucidate the mechanisms underlying this protective effect.

### CRIC in peripheral arterial disease

Peripheral arterial disease (PAD) and its complications that include wound ulcers and lower extremity amputation, exert significant morbidity and mortality, especially in diabetic patients. As such, new treatments are needed to control symptoms of PAD, prevent its progression, and reduce the risk of complications. Clinical studies have shown that a single limb RIC stimulus improved exercise tolerance in patients with symptomatic PAD (Saes et al., 2013), although not all studies have been positive (Delagarde et al., 2015). Whether patients with symptomatic PAD are already 'preconditioned' may in part explain the mixed results of RIC on PAD symptoms of intermittent claudication. An additional clinical study reported that a single limb RIC stimulus could improve the ankle-brachial-index (ABI) (Shahvazian et al., 2017), the ratio of the systolic blood pressure measured at the ankle against one measured at the brachial artery, which is used to diagnose the presence of PAD.

More recently, CRIC has been tested in experimental animal studies and clinical studies in PAD patients and in patients with diabetic foot ulcers. Using a critical limb ischemia rat model, comprising permanent ligation of the iliac artery and vein, Karakoyun et al. (2014) demonstrated that CRIC (3 x 10 min hindlimb ischemia and reperfusion) applied daily for variable periods of 1, 7, 14, and 30 days improved limb blood flow, reduced skeletal muscle injury, and these effects were associated with increased circulating endothelial progenitor cell counts and enhanced angiogenesis. This data suggests that CRIC may be a potential therapy for salvaging viable skeletal tissue in PAD patients presenting with critical limb ischemia. In a randomized controlled trial, Ahmed et al. (2019) reported that CRIC (4 x 5 min cycles of unilateral upper arm cuff inflation and deflation) applied once every 3-4 day weeks for 28 days resulted in significant improvements in pain-free walking distance and ABI at 30 days. Interestingly, a recent clinical study has shown CRIC (4 x 5 min cycles of unilateral upper arm cuff inflation and deflation) applied daily for 12 weeks to be ineffective in diabetic patients with moderate PAD (n = 18) with no effect on transcutaneous tissue oxygen tension of the instep of the feet, toe brachial index, and heart rate variability when compared to sham (n = 18) (Hansen et al., 2019). In this study 80% of patient had peripheral symmetric neuropathy that may in part explain the lack of efficacy of CRIC (Hansen et al., 2019) given the requirement of a neural pathway to mediate the salutatory effects of limb RIC (Jensen et al., 2012).

Finally, an interesting study in patients with diabetic foot ulcers has shown that CRIC (3 x 5 min cycles of bilateral upper

arm cuff inflation and deflation) applied once every 2 weeks for 6 weeks improved wound healing in diabetic patients with foot ulcers (n = 24), when compared to a sham protocol (n = 16) (Shaked et al., 2015). The mechanisms underlying this healing effect of CRIC with RIC only applied once every 2 weeks, are not clear but may relate to its beneficial vascular effects (increased skin perfusion) (Jones et al., 2014), increased tolerance to hypoxia, and anti-oxidant/anti-inflammatory effects.

In summary, clinical studies investigating the effect of CRIC in PAD patients in terms of reducing symptoms and preventing complication of PAD have been mixed, with the beneficial effects of CRIC potentially being confounded by the presence of co-existing diabetic neuropathy.

### CRIC in sepsis cardiomyopathy

Interestingly, it has been suggested that CRIC may have beneficial effects in sepsis cardiomyopathy, a condition that is associated with significant morbidity and mortality in acutely unwell patients. Using an experimental murine model of lipopolysaccharide (LPS)-induced septic cardiomyopathy, Honda et al. (2019) first demonstrated that a single RIC stimulus (4 x 5 min cycles of hindlimb ischemia and reperfusion) prevented LPS-induced cardiomyopathy as evidenced by preserved LV function, an inflammatory response, and improved survival at 7 days. They also tested CRIC (4 x 5 min cycles of hindlimb ischemia and reperfusion) applied daily for 5 days, and showed that the effects on survival at 7 days were greater than that conferred by a single limb RIC stimulus (Honda et al., 2019). Whether CRIC had greater benefits than a single limb RIC stimulus on cardiac function and the inflammatory response to LPS was not determined in this study. Whether CRIC is beneficial in the clinical setting of sepsis cardiomyopathy remains to be investigated.

### Summary and conclusions

In summary, CRIC or repeated episodes of limb RIC applied daily for 7 to 28 days have vascular and cytoprotective effects that may benefit patients with endothelial dysfunction, hypertension, chronic heart failure, and post-AMI LV remodeling. The availability of automated pneumatic cuffs for delivering the CRIC protocol should facilitate the implementation of this intervention for patient benefit, given potential issues of compliance. Future clinical studies investigating the efficacy of CRIC in other settings should use a randomized controlled trial design and include a suitable sham protocol. Further studies are needed to elucidate the mechanisms underlying the beneficial effects of CRIC, to determine whether the beneficial effects of CRIC can be recapitulated using pharmacological agents.

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**Conflict of interests:** None

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