ACTA PHYSIOLOGICA

Acta Physiol 2016, **216**, 395–406

**REVIEW**

# Adaptive response to hypoxia and remote ischaemia pre-conditioning: a new hypoxia-inducible factors era in clinical medicine

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Received 3 June 2015,
revision requested 1 July 2015,
revision received 2 August 2015,
accepted 2 October 2015
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## Abstract

Transient ischaemia leads to tolerance to subsequent protracted ischaemia. This 'ischaemia pre-conditioning' results from the induction of numerous protective genes, involved in cell metabolism, proliferation and survival, in antioxidant capacity, angiogenesis, vascular tone and erythropoiesis. Hypoxia-inducible factors (HIF) play a pivotal role in this transcriptional adaptive response. HIF prolyl hydroxylases (PHDs), serving as oxygen sensors, control HIF$\alpha$ degradation. HIF-mediated ischaemic pre-conditioning can be achieved with the administration of PHD inhibitors, with the attenuation of organ injury under various hypoxic and toxic insults. Clinical trials are currently under way, evaluating PHD inhibitors as inducers of erythropoietin. Once their safety is established, their potential use might be further tested in clinical trials in various forms of acute ischaemic and toxic organ damage.

Repeated transient limb ischaemia was also found to attenuate ischaemic injury in remote organs. This 'remote ischaemic pre-conditioning' phenomenon (RIP) has been extensively studied recently in small clinical trials, preceding, or in parallel with an abrupt insult, such as myocardial infarction, cardiac surgery or radiocontrast administration. Initial results are promising, suggesting organ protection. Large-scale multi-centre studies are currently under way, evaluating the protective potential of RIP in cardiac surgery, in the management of myocardial infarction and in organ transplantation. The mechanisms of organ protection provided by RIP are poorly understood, but HIF seemingly play a role as well.

Thus, Inhibition of HIF degradation with PHD inhibitors, as well as RIP (in part through HIF), might develop into novel clinical interventions in organ protection in the near future.

*Keywords* heart, hypoxia, hypoxia-inducible factors, kidney, pre-conditioning, prolyl hydroxylase.

 doi: 10.1111/apha.12613 

Protracted significant decline in tissue oxygenation leads to progressive cellular injury, as a consequence of depleted cellular energy stores and the generation of reactive oxygen species. Common clinical scenarios may lead to local or systemic hypoxia. Myocardial infarction, ischaemic stroke, organ harvesting for transplantation or surgical procedures associated with cessation of arterial blood supply are prime examples for local organ hypoxia, while intense and protracted hypotension, as in shock, cardiorespiratory arrest and resuscitation are examples of systemic hypoxia. Interestingly, susceptibility to hypoxia varies between tissues and seems to depend on baseline oxygen supply and on metabolic rates, as well as on the ability to shift energy metabolism towards anaerobic glycolysis and to use alternative energy resources. Thus, significant physiological hypoxia should not be defined as absolute value of oxygen tension, but rather as a reduction in oxygen tension below that normally experienced by an organ or specific cell types (Nikinmaa 2013). Most importantly, tissues adapt to sublethal ischaemia by the upregulation of many genes involved in cell survival. Hypoxia-inducible factors (HIFs) play a central role in this inherent capacity to acquire resistance to hypoxia. There is ample clinical and experimental data indicating that HIF induction during repeated short episodes of ischaemia provides resistance to a subsequent protracted ischaemic event. This phenomenon, termed *ischaemia pre-conditioning*, can now be reproduced by pharmaceutical stimulation of HIF response with so-called hypoxia-mimetic agents. Furthermore, clinical trials and animal studies show that transient tissue ischaemia provides hypoxia tolerance also in adjacent tissues and even in remote organs, a phenomenon called *remote ischaemia preconditioning* (RIP). Organ protection provided, for instance, by repeated short periods of limb ischaemia with the use of a sphygmomanometer cuff has recently been the focus of many clinical trials. The mechanisms responsible for RIP are speculative and may include HIF activation.

This review outlines the role of HIF in hypoxia pre-conditioning and illustrates the protective potential of pharmacologic stimulation of HIF and of remote ischaemia pre-conditioning in various medical fields.

### *Hypoxia-inducible factors (HIFs)*

HIFs are ubiquitous key regulators of the cellular response to acute hypoxic stress, triggering the expression of numerous genes involved in cell physiology and survival, including those related to cell metabolism, proliferation and programmed death. In addition, HIF-mediated genes regulate antioxidant capacity, cellular pH, vascular tone and microcirculation and enhanced oxygen carrying capacity and angiogenesis (Gunaratnam & Bonventre 2009, Nangaku *et al.* 2013).

As shown in Figure 1, HIFs are heterodimers, consisted of cytoplasmic $\alpha$ and $\beta$ subunits, which undergo nuclear translocation, assembling and binding to hypoxia response elements (HREs) along DNA strands. The HIF$\alpha\beta$ heterodimers, either directly or through mediators such as miR-687, induce transient epigenetic changes in the DNA, with histone modification and chromosomal conformational changes, leading to transactivation of HIF-target genes (Nangaku *et al.* 2015). Over 2000 HREs were identified along the mouse genome and there are over 200 genes clearly controlled by HIF (Schödel *et al.* 2011, Khamaisi *et al.* 2015). The long list of acknowledged HIF-target genes includes hexokinase, phosphofructokinase, aldolase, enolase, glucose transporters, adrenomedullin, erythropoietin (EPO), haeme-oxygenase-1 (HO-1), carbonic anhydrase-9 (CAIX), vascular endothelial growth factors (VEGF), transforming growth factor (TGF), insulin-like growth factor (IGF) and its binding proteins, transferrin, transferrin receptor and ceruloplasmin, nitric oxide synthases, endothelin and endothelin converting enzyme-1 (ECE-1), adrenergic receptors, and many others (Schödel *et al.* 2011, Khamaisi *et al.* 2015).

HIF is mainly regulated by oxygen-dependent proteolysis of its $\alpha$-subunit (Gunaratnam & Bonventre 2009, Nangaku *et al.* 2013). As illustrated in Figure 1, under normoxic conditions HIF$\alpha$ rapidly undergoes proteasomal degradation. This process is initiated by hydroxylation of prolyl residues on HIF$\alpha$ by specific HIF prolyl hydroxylases (PHDs). Once tagged with hydroxyl groups, HIF$\alpha$ is captured by the von Hippel-Lindau tumour suppressor, poly-ubiquitinized and disassembled. PHDs are active at normal tissue oxygenation, facilitating HIF$\alpha$ removal and preventing its nuclear translocation and binding with HIF$\beta$. By contrast, under hypoxic conditions, PHDs are inhibited, leading to HIF$\alpha$ accumulation and nuclear translocation, with subsequent HIF$\alpha\beta$ formation and binding to HRE, with induction of HIF-target genes. Thus, PHDs serve as unique oxygen sensors and play a key role in the control of HIF-dependent hypoxia-mediated gene response (Myllyharju 2013). PHD inhibition and HIF stabilization under such hypoxic conditions is an instantaneous process with a short-lived signal, allowing tight regulation of HIF-dependent gene activation.

There are at least 4 PHD isoforms, with diverse expression in different cell types (Schödel *et al.* 2009, Myllyharju 2013). Their expression and activity are regulated by HIF, either directly, or by compound

  doi: 10.1111/apha.12613

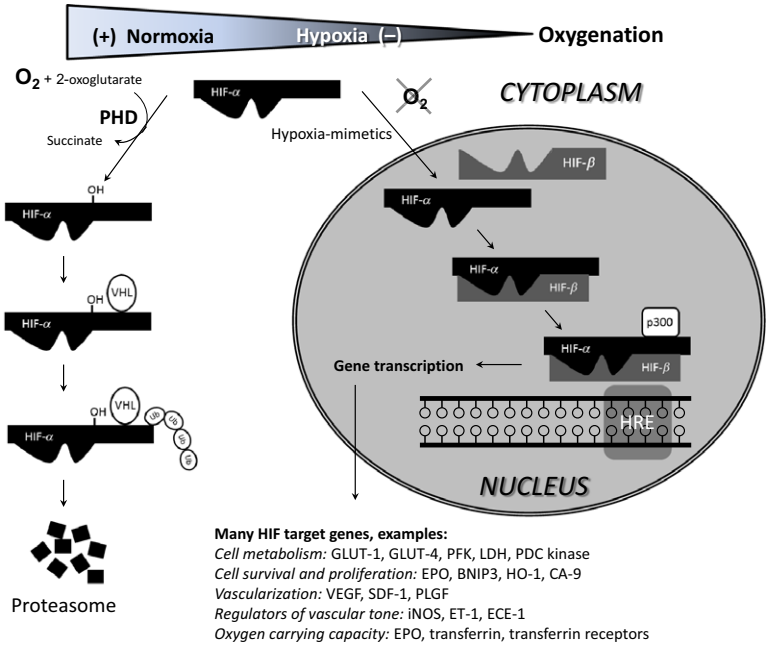


**Figure 1** HIF prolyl hydroxylases (PHDs) regulate HIF-mediated response: Tissue oxygenation (illustrated on top) governs gene expression, with HIF prolyl hydroxylases (PHDs) serving as oxygen sensors: As shown on the right, Hypoxia and hypoxia-mimetics inhibit PHDs, facilitating HIFα accumulation, nuclear translocation and binding with HIFβ and other transcription factors, such as p300, with subsequent trans-activation of HIF-target genes. By contrast, as shown on the left, PHDs are activated under normoxia and prevent trans-activation of HIF-target genes by promoting HIFα removal. HIFα proteasomal degradation is initiated by hydroxylation of prolyl residues, subsequent binding with VHL factor and poly-ubiquitination. (Abbreviations: PHD, prolyl hydroxylase; VHL, von Hippel Lindau tumour suppressor; Ub, ubiquitin; GLUT, glucose transporter; PFK, phosphofructokinase; LDH, lactate dehydrogenase; PDC, pyruvate dehydrogenase complex; HO-1, haeme-oxygenase-1; CA-9, carbonic anhydrase-9; VEGF, vascular endothelial growth factor; SDF-1, stromal cell-derived factor-1; EPO, erythropoietin; $BNIP_3$, BCL2 interacting protein-3; PLGF, placental growth factor; iNOS, inducible nitric oxide synthase; ET, endothelin; ECE-1, endothelin converting enzyme-1).

intermediate pathways, such as nitric oxide and oxygen free radicals, with complex negative and positive feedback mechanisms (Muratsu-Ikeda *et al.* 2012, Fujita *et al.* 2012).

Nuclear HIFα can be detected in tissues subjected to acute hypoxia, such as at the border of tissue infarcts, in the hypoxic renal medulla in various models of acute renal failure, or in the subendothelial hypoxic myocardium (Heyman *et al.* 2011). HIF stabilization under such conditions is functional, with evident local induction of HIF-dependent genes, such as haeme-oxygenase-1 or VEGF.

Under hypoxia, different cell types express different HIFα isoforms and target genes. For instance, renal hypoxia leads to HIF-1α expression in tubular cells, while endothelial and interstitial cells produce HIF-2α. Medullary thick ascending limbs and collecting ducts express HO-1 in response to HIF-1α, while EPO is specifically regulated by HIF-2α in interstitial cells in the juxta-medullar renal cortex (Paliege *et al.* 2010). Furthermore, the extent of HIF activation is cell type-specific. For example, hypoxia leads to strong and abundant HIF in medullary collecting ducts, but to a substantially lesser extent in neighbouring thick ascending limbs (Nangaku *et al.* 2013). This difference might be attributed to varied distribution of PHD isoforms, to different rates of HIF synthesis, and to additional factors affecting HIF synthesis and degradation, including oxygen free radicals, nitric oxide, Sentrin/SUMO-specific proteinase (SENP)1, miR-687 and miR 205 and many others (Gunaratnam & Bonventre 2009, Berchner-Pfannschmidt *et al.* 2010; Nangaku *et al.* 2015), beyond the scope of this review. The complex scheme of HIF generation and degradation, with differential expression and regulation of various components, may explain cell- and organ-specific susceptibility to hypoxic insults, with diverse phenotypic outcomes and biological function. For instance, enhanced susceptibility of thick ascending limbs to severe medullary hypoxia, as compared with neighbouring hypoxic collecting ducts, may stem from their lower capacity to express HIF (Rosenberger *et al.* 2008). Interestingly, crosstalk may exist between various cell types, with HIF-mediated transactivation of

 

genes in one cell type affecting gene expression and protection from injury in different neighbouring cell types. For instance, targeted enhancement of HIF expression in medullary thick ascending limbs attenuates proximal tubular injury in nearby S3 proximal tubules (Schley *et al.* 2011), and cross-stimulation of HIF and STAT3 occurs transversely between different renal cell types, possibly via paracrine mechanisms (Nechemia-Arbeli *et al.* 2013).

### *Ischaemic pre-conditioning*

In 1986, Murry *et al.* studied dogs subjected to acute myocardial infarction induced by prolonged occlusion of the circumflex artery (Cx). They found that 4 cycles of short (5′) transient occlusion of the Cx prior to the prolonged (40′) ischaemia resulted in a 75% reduction in the final infarct size (Murry *et al.* 1986). This breakthrough study was supported by subsequent clinical observations in patients undergoing acute ST-elevation myocardial infarction (STEMI) in the pre-primary coronary intervention (PCI) era. Clinical outcome was substantially improved if the acute event had been preceded by transient episodes of chest pain, with a 2/3 reduction in the composite endpoint of death, congestive heart failure and cardiogenic shock in non-elderly patients (Abete *et al.* 1997). This phenomenon, termed ischaemic pre-conditioning, is schematically illustrated in Figure 2a.

HIF plays a pivotal role in ischaemia pre-conditioning, and the upregulation of HIF-dependent genes in this setting is considered tissue protective. This is clearly shown in transgenic animals with defective HIF production, subjected to injury. For instance, the extent of brain infarction following ligature of middle cerebral artery (Baranova *et al.* 2007), or the degree of chemical ileitis induced by C. difficile toxin (Hirota *et al.* 2010) is markedly increased in such animals, as compared with HIF-competent controls, subjected to similar insults. In addition, the cardioprotective efficacy of ischaemic pre-conditioning during subsequent ischaemia is lost in HIF1α mutated mice, as opposed to substantial myocardial salvage in naïve mice (Cai *et al.* 2008). By contrast, enhancement of HIF signal in mice with conditional knock-out of von Hippel Lindau tumour suppressor confers protection, attenuating rhabdomyolysis-induced acute kidney injury (Fähling *et al.* 2013). In the same fashion, infarct size was attenuated in hearts of transgenic mice overexpressing HIF-1α, characterized by enhanced expression of VEGF and increased capillary density (Kido *et al.* 2005). Conceivably, upregulation of protective genes offsets the noxious insults, providing tolerance and endurance, with improved local microcirculation, antioxidant capacity and other factors essential for cell survival. Indeed, HIF signal stabilization and the upregulation of HIF-dependent genes have been clearly documented in a host of hypoxic conditions. For instance, renal parenchymal HIF accumulation is noted at the border of renal segmental infarction, in hypoxic medullary regions in models of acute kidney injury, in the diabetic and chronic tubular-interstitial renal disease and in transplanted kidneys, associated with regional expression of HIF-dependent genes such as HO-1 (Nangaku *et al.* 2013). Indeed, detection of HIF stabilization might be regarded as an indicator for the presumptive role of hypoxia in various forms of acute (Persson 2013) and chronic kidney injury (Heyman *et al.* 2008). In the same fashion, HIF appears at the subendothelial myocardial ischaemic region in a model of congestive heart failure (May *et al.* 2008), and HIF-stimulated VEGF transcription is initiated at the penumbra surrounding brain infarction (Marti *et al.* 2000).

### *Therapeutic strategies stabilizing HIF*

Since PHDs are the key regulators of HIF, initiating HIFα breakdown under normoxia, and permitting its accumulation under hypoxia, the control of PHD activity might be useful in enhancing or inhibiting cellular hypoxic stress responses. Carbon monoxide (CO) is 'hypoxia-mimetic', capable of inhibition of HIF PHDs. Cobalt compounds, and derivatives of dimethyl-oxalylglycine (DMOG), are also potent inhibitors of PHDs, and their application was found to stabilize HIF and to enhance the synthesis of HIF-dependent genes (Heyman *et al.* 2011). For example, renal erythropoietin synthesis is hastened in animals and humans given DMOG derivatives (Bernhardt *et al.* 2010, Paliege *et al.* 2010), enhancing erythropoiesis. Currently such agents are being studied in second- and third-phase clinical trials as oral substitutes for injected erythropoietin. Furthermore, HIF stabilization with comparable strategies, associated with the induction of protective HIF-dependent genes, was found to be organ-protective in renal, cardiac, neural (Corcoran & O'Connor 2013) or gastrointestinal injuries (Hart *et al.* 2011), to improve graft survival of transplanted organs and to prevent organ damage in septic shock (Hams *et al.* 2011, Heyman *et al.* 2011, Rabinowitz 2013).

The limitation of interventional HIF stabilization is the potential non-selective modification of gene expression. Some HIF-target genes may be harmful by promoting vasoconstriction (endothelin-1), thrombosis (plasminogen activator inhibitor-1) or fibrosis (fibroblast growth factor, connective tissue growth factor). Angiogenesis induced by VEGF may be either beneficial as in myocardial infarction (Bao *et al.* 2010), or

  doi: 10.1111/apha.12613

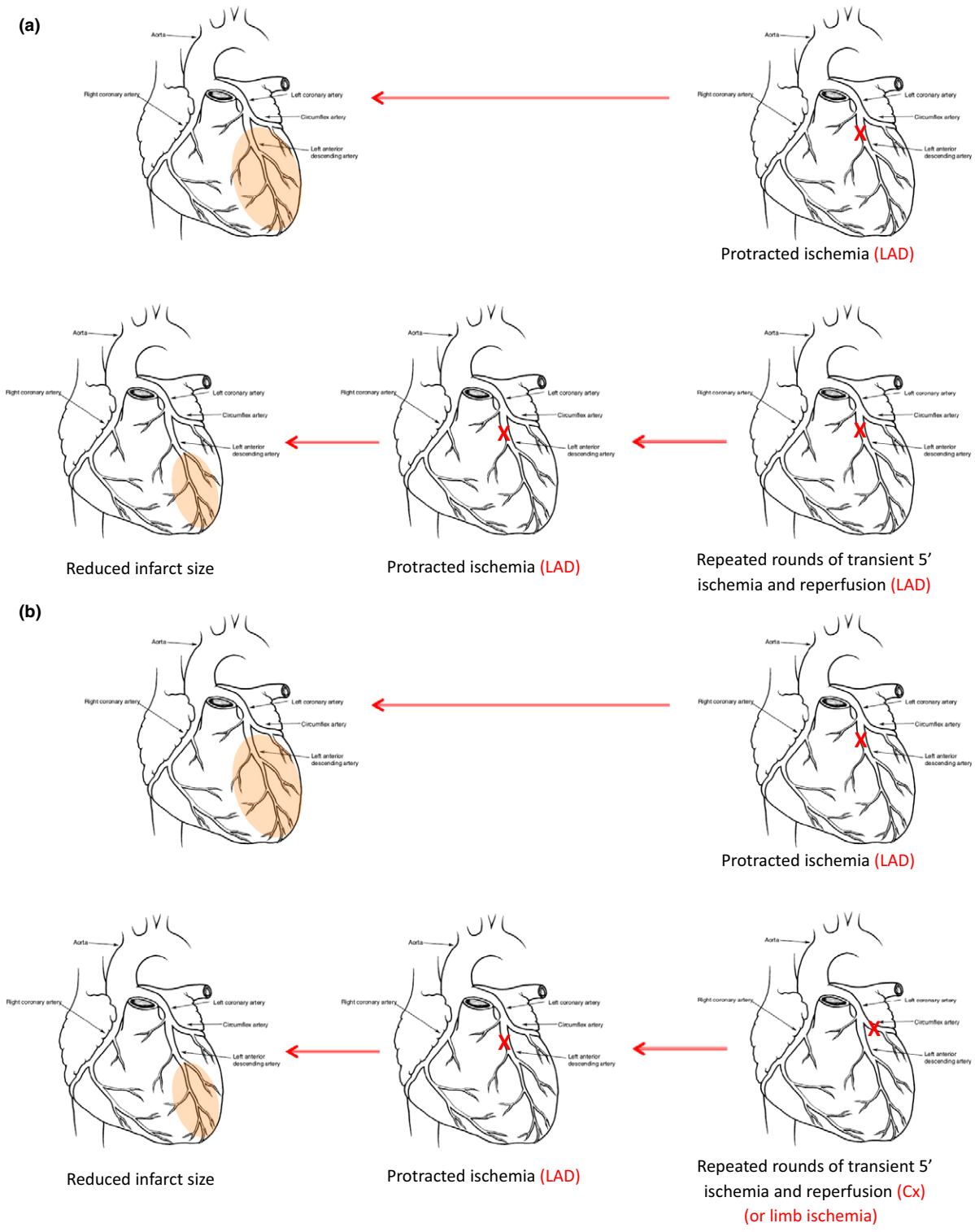


**Figure 2** Schematic illustration of ischaemic (a) and remote ischaemic pre-conditioning (b): Ischaemic injury can be mitigated by preceding transient episodes of ischaemia at the same tissue, that is ischaemic pre-conditioning (in a), at a different region in the same organ, or in an altogether different remote organs (both in b), a phenomenon termed remote ischaemic pre-conditioning. The figures represent animal studies of acute myocardial infarction conducted by Murry *et al.* 1986 and by Przyklenk *et al.* 1993, respectively. Of note, ischaemic pre-conditioning, originally carried out by Murry *et al.* at the Cx region, is presented in (a) at the LAD territory, for the purpose of convenient comparison with (b). (Abbreviations: LAD, left anterior descending artery; Cx, circumflex artery).

 

detrimental as in proliferative retinopathy and macular degeneration. Although HIF upregulation is not sufficient to induce malignancy, HIF is a survival factor in many tumours, as shown in renal clear cell carcinoma, where, inactivating mutations of both alleles of von Hippel-Lindau protein leads to persistent normoxic HIF upregulation. The aim of the pharmaceutical industry is to develop safe PHD inhibitors and strategies of administration that can be tested for organ protection in various clinical settings (Rabinowitz 2013).

### *Remote ischaemia pre-conditioning: background*

As detailed above, the concept of ischaemia pre-conditioning has been convincingly established in dogs subjected to repeated brief ischaemic episodes followed by prolonged ischaemia in the Cx territory (Murry *et al.* 1986). In 1993, Przyklenk *et al.* repeated these experiments, though with one major modification: repeated 4 cycles of 5′ transient occlusion of the Cx were followed by prolonged occlusion of the left anterior descending artery (LAD). Surprisingly, as illustrated in Figure 2b, transient ischaemia in one region of the canine myocardium successfully provided protection in a different myocardial region, with an infarct size reduction of 62% in the LAD territory, as compared with non-pre-conditioned dogs (Przyklenk *et al.* 1993). This innovative work established a new concept, namely *remote ischaemia pre-conditioning* (RIP). Later works showed that remote protection is not single-organ-specific and might be conveyed between organs, as well. For instance, intestinal ischaemic pre-conditioning ameliorated hepatic ischaemic injury (Kageyama *et al.* 2015), and transient renal ischaemia attenuated myocardial injury (Kant *et al.* 2008). Comparable cross-organ protection could be achieved by ischaemic post-conditioning as well, for instance with liver ischaemic post-conditioning preventing renal hypoxic injury (Ateş *et al.* 2002).

Remote ischaemia pre- or post-conditioning is most convenient by applying repeated short non-invasive ischaemic periods to an upper or lower limb, using a blood pressure cuff. In most animal and human studies exploring RIP, 3–5 periods of 5-min limb ischaemia are applied, with 5-min intervals of reflow. With the rapidly expanding literature on this subject, we shall narrow our review in the following paragraphs principally to clinical trials.

The mechanism(s) involved in RIP and post-conditioning are far from being elucidated. They probably involve neuronal and humoral pathways, with the potential role of various intercellular mediators and intracellular signals including inflammatory cytokines, reactive oxygen species, nitric oxide, adenosine, Akt-mTOR signalling, p38 MAPK, Erk1/2, JNK 1/2, STAT5, NFkB, miR-144, TRPAI and mitochondrial $K_{ATP}$ channels (Lim & Hausenloy 2012, Kusch *et al.* 2013, Li *et al.* 2014, Nakahashi & Mori 2014).

Recent studies indicate that HIF may play a role as well in RIP. As illustrated in Figure 3, HIF might be involved in RIP, generating protective signals arising within the transiently ischaemic limb. Furthermore, hypothetically, intrinsic HIF signal in the target organ to be protected might be activated by signals generated in the ischaemic limb.

Indeed, increased HIF-1α protein levels were detected in atrial myocardium following RIP in patients undergoing cardiac surgery, as compared with control patients, with tissues obtained just before cardiopulmonary bypass (Albrecht *et al.* 2013). Moreover, HIF-dependent genes such as haeme-oxygenase (HO)-1 were upregulated in the liver by RIP, produced by limb ischaemia (Wang *et al.* 2014).

Semenza's group further established the role for HIF in RIP, using mice who underwent myocardial infarction (Cai *et al.* 2013). They found that RIP was unable to prevent myocardial injury in HIF-conditional knock-out mice and that both RIP and HIF induce IL-10 *in vivo*. Furthermore, they found that HIF transfection in these mice increases IL-10 and attenuates myocardial injury. Their findings were further complemented by *in vitro* studies using cardiomyocytes subjected to the HIFα inhibitor acriflavine or to neutralizing shRNA, showing the importance of both HIF-1α and HIF-2α-mediated IL-10 induction in RIP (Cai *et al.* 2013). Additionally, alpha-ketoglutarate, an activator of HIF PHD, was found to prevent cardiac salvage by renal RIP, again suggesting a role for HIF in RIP (Kant *et al.* 2008). Possibly, a co-stimulatory crosstalk between hypoxia/HIF and inflammatory cytokines enables upregulation of HIF in an injured organ, stimulated by inflammatory signals that are generated during RIP from other transiently ischaemic tissues (Eltzschig & Carmeliet 2011, Hams *et al.* 2011, Nechemia-Arbeli *et al.* 2013). Indeed, HIF transcription is stimulated by inflammatory response through NF-κB (Eltzschig & Carmeliet 2011). Moreover, we found in mice that IL-6-mediated STAT3 activation in tubular cells promotes HIF expression, both in tubular and in adjacent vascular endothelial cells, and vice versa, and HIF directly triggers STAT3 phosphorylation (Nechemia-Arbeli *et al.* 2013). This might be carried out through a paracrine signalling mechanism involving HIF-dependent intermediate factors, such as erythropoietin and VEGF, which are known to mediate STAT3 activation (Westenfelder *et al.* 1999, Chen *et al.* 2008). Furthermore, signalling processes may assemble with HIF and act as powerful transcription complexes, for instance, HIF/STAT3/

  doi: 10.1111/apha.12613

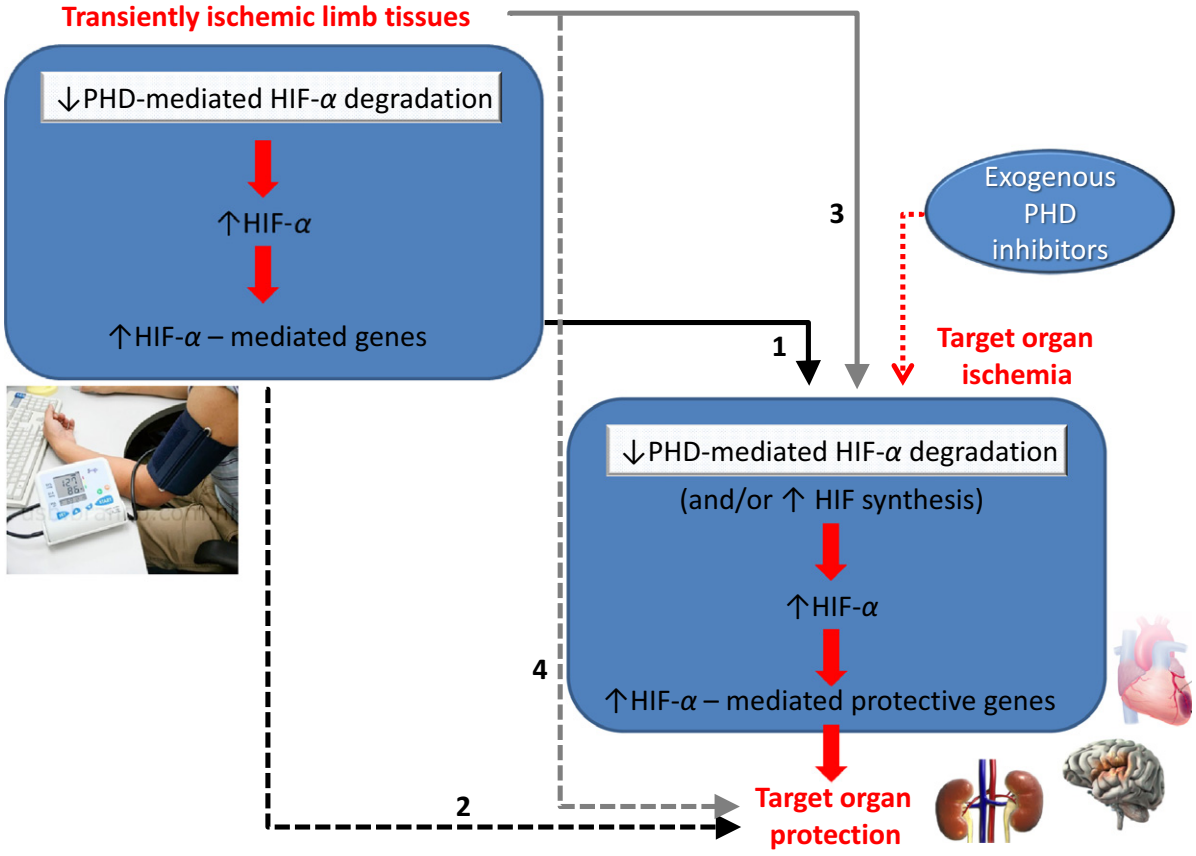


**Figure 3** HIF in ischaemic pre-conditioning and its proposed Role in RIP: HIFα degradation is blocked in response to tissue ischaemia by the inhibition of PHD, with subsequent trans-activation of hypoxia-adaptive HIF-target genes (ischaemic pre-conditioning, illustrated within the right-sided blue rectangle). HIF stabilization can also be achieved by exogenously administered PHD inhibitors. Accumulating evidence indicates that HIF might also play a central role in RIP, where ischaemia in one organ (most convenient-limb ischaemia, left-sided blue rectangle) confers protection against injury in other target organs. This could occur via several plausible pathways: HIF stabilization in transiently ischaemic limb releases HIF-mediated transmissible signals, causing HIF stabilization or transcription in the target organ (1) or provides tolerance in target organs by mechanisms unrelated to their HIF system (2). Alternatively, non-HIF-mediated neuro-humoral stimuli, originating from the ischaemic limb, may cause HIF stabilization or transcription in the target organ (3), or might induce protective systems in target organs which are unrelated to their intrinsic HIF signals (4).

p300 complexes (Gray *et al.* 2005). It is tempting to assume that organ salvage provided by physical activity (Abete *et al.* 2001) might be mediated by similar mechanisms (Zhang *et al.* 2010, Michelsen *et al.* 2012) and that remote post-conditioning might augment HIF-mediated tissue protection following ischaemia in the same manner, by the remote release of mediators that migrate to interact with HIF in ischaemic regions.

It is important to note that the mechanisms of remote ischaemic pre- and post-conditioning remain complex and poorly understood. Numerous pathways appear to be involved and HIF may not be an indispensible actor. Indeed, in one study, effective RIP cardioprotection occurred despite HIF inactivation (Kalakech *et al.* 2013).

Putative pathways of HIF-related RIP are schematically illustrated in Figure 3 and may involve the release of HIF-mediated and non-HIF-mediated signals from the ischaemic limb (black and grey arrows, respectively), which may in turn provide target organ tolerance through the activation of its HIF-mediated or non-HIF-mediated systems (solid and scattered arrows, respectively).

### *Remote ischaemic pre-conditioning for cardiac salvage*

The first clinical application of RIP was reported in children undergoing cardiac surgery for congenital heart disease. In a single-centre single-blinded study, 37 patients were randomized to 4 cycles of 5′ leg ischaemia or to sham procedure before the operation.

 

Post-operative troponin levels were lower in RIP-treated children, indicating reduced myocardial injury, and the need for post-operative inotrope support was reduced (Cheung *et al.* 2006). Comparable reduction in post-operative troponin levels following RIP was also noted in patients undergoing coronary artery bypass surgery (CABG) (Hausenloy *et al.* 2007), but most reports are small single-centre studies. A meta-analysis indicates that RIP can reduce the rise in blood troponin and diminish the incidence of myocardial infarction; however, this type of data may be affected by publication bias (Brevoord *et al.* 2012). Recently, over 1600 patients undergoing CABG were enrolled in 29 medical centres in the United Kingdom in a prospective study, randomized to RIP or sham procedure (Hausenloy *et al.* 2012). Preliminary report of this study (March 16, 2015) indeed reveals a statistically significant 15 percent reduction in myocardial injury, occurring within the first 72 h after surgery in the remote ischaemic pre-conditioning group as compared to the control patients, although this effect was not associated with improved long-term clinical outcome. RIP was also found to provide cardiac protection in a single-centre controlled study, involving 82 British patients undergoing surgery for abdominal aortic aneurism (Ali *et al.* 2007). RIP, performed by repeated cross-clamping of an iliac artery prior to the aortic surgery, prevented the rise in troponin and reduced the incidence of post-operative myocardial infarction.

The most impressive currently available study of RIP-associated cardiac protection is in patients with ST-elevation myocardial infarction (STEMI) (Bøtker *et al.* 2010). In a multi-centre Danish study, 250 patients were randomized to receive RIP (4 cycles of 5′ ischaemia of the arm with blood pressure cuff) or sham procedure during transport by ambulance and subsequently underwent primary coronary intervention (PCI). All patients underwent cardiac imaging (SPECT) immediately after PCI and a month later with assessment of systolic function, as well as the comparison of the area at risk (initial scan) and final infarction area by day 30, providing an extrapolated myocardial salvage index. RIP, performed during transfer of the patients to hospital, attenuated the decline in systolic function and substantially improved myocardial salvage. Subgroup analysis disclosed that these protective effects were most pronounced in the early post-PCI period, particularly in patients with severely compromised blood flow at the culprit lesion (TIMI flow 0–1) and in infarcts involving the LAD territory. However, mortality remained unaffected, and the differences between groups had nearly resolved by day 30, suggesting adaptive and reparative processes even in the control group (Bøtker *et al.* 2010).

These findings were recently supported by cardiac magnetic resonance technologies in an additional single-centre study of patients with STEMI, randomized to RIP and control groups. Cardiac magnetic resonance disclosed a substantial reduction in infarct size and cardiac oedema, improving myocardial salvage and reducing troponin release (White *et al.* 2015). A randomized prospective multi-centre study (ERIC-PPCI) is about to follow, looking at the effect of RIP on clinical outcomes in STEMI patients undergoing PCI (NIH ClinicalTrial.gov identifier NCT02342522).

### *Remote ischaemic pre-conditioning for brain salvage*

Symptomatic occlusion of major cerebral arteries is associated with a high risk of recurrent stroke within the subsequent months. Sixty-eight patients with transient ischaemic attacks or minor strokes with documented major intracranial arterial occlusions were randomly assigned to RIP (5 cycles of 5′ arm ischaemia twice a day for 300 days) or no treatment in a study conducted in two Chinese stroke centres. The incidence of repeated stroke or TIA was markedly reduced in RIP-treated patients as compared with their controls, and their Rankin functional assessment score substantially improved over 300 days of follow-up (Meng *et al.* 2012). It is conceivable that RIP has improved survival of the penumbra region, as technetium scanning revealed improved regional perfusion.

### *Remote ischaemic pre-conditioning for renal salvage*

In the study described above in patients undergoing abdominal aortic surgery, RIP was associated with preserved renal function and was found to better predict renal outcome than suprarenal aortic cross-clamping and baseline kidney function in multi-variate regression analysis (Ali *et al.* 2007).

In an additional single-centre German study, 100 patients with renal impairment (mean plasma creatinine 1.6 mg $dL^{-1}$) undergoing elective coronary interventions were randomized to RIP and control groups (Er *et al.* 2012). Contrast nephropathy developed in 40% and 12% of control and RIP-treated patients, respectively, and risk reduction was also noted in the subgroup of patients with the more advanced stages of renal impairment. A rise in urinary neutrophil gelatinase-associated lipocalin (NGAL, a biomarker of tubular cell injury), noted in the control group, was prevented in RIP-treated patients and re-hospitalization was halved. Furthermore, among subjects requiring emergent procedures following the coronary intervention (such as coronary bypass, valvular operations or trans-catheter aortic valve

  doi: 10.1111/apha.12613

implantation), dialysis was required in 7 of 9 control patients, as compared with none of 10 individuals in the RIP group (Er *et al.* 2012).

In a most recent multi-centre study in 240 patients predisposed to acute kidney injury (AKI) and undergoing cardiac surgery, RIP (3 cycles of 5′ upper arm pressure cuff inflation following the induction of anaesthesia) significantly attenuated the incidence of post-operative AKI (38 vs. 53% in controls), reduced the release of additional urinary biomarkers of AKI (insulin-growth factor binding protein [IGFBP]-7 and tissue inhibitor of metalloproteinases [TIMP]-1) and diminished the need for post-operative dialysis (6 vs. 16%, respectively) (Zarbock *et al.* 2015). In a randomized controlled single-centre study, RIP was also found to be renoprotective in patients with STEMI undergoing primary coronary intervention (PCI), with contrast nephropathy developing in 10% and 36% in RIP and control patients, respectively (Yamanaka *et al.* 2015). Finally, in a controlled study in 48 kidney transplanted patients, earlier functional recovery and smaller increments of urine NGAL were noted in recipients assigned to RIP prior to renal grafting, as compared to paired recipients not undergoing RIP (Wu *et al.* 2014). These clinical trials suggest that RIP may be renoprotective in post-operative hypoxic AKI, in contrast nephropathy, and possibly in additional settings as well (Gassanov *et al.* 2014).

### *Additional potential clinical uses for RIP*

In a single-centre randomized controlled blinded study of 62 patients undergoing surgery for abdominal aortic aneurysm, RIP was found to attenuate pulmonary and intestinal injury (Li *et al.* 2013). A clinical trial is under way in the evaluation of RIP-related renal protection following transplantation (REPAIR website http://repair.lshtm.ac.uk/). Additional animal studies indicate that RIP might be useful in other medical fields. Animal studies showed that RIP may also be organ protective in hepatic (Tapuria *et al.* 2012) and gut ischaemia (Erling *et al.* 2013), and in sepsis-induced organ damage (Olguner *et al.* 2013) and it has been suggested that this technique might be used for additional non-ischaemic bowl disorders. The use of RIP in trauma may also improve the outcome of casualties, enhancing pre-hospital and overall survival. This is currently being studied, and preliminary results *in vivo* are promising (A. Eisenkraft, unpublished data).

## Conclusions

HIF play a central role in organ protection against various hypoxic and toxic insults. Adaptation to hypoxia can be simulated by pharmacological inhibition of PHD, a potentially useful tool in preventing organ damage. Although there is a concern regarding the safety of this treatment option, animal studies, conducted in a clinically relevant fashion (Douelsner & Bondke Persson 2013), clearly indicate the potential effectiveness in the prevention of organ damage in a host of clinical conditions. Advanced clinical trials are under way in the set-up of anaemia of chronic disease, and it is conceivable that if such agents are approved for treating anaemia, clinical trials in other scenarios requiring organ salvage will rapidly follow. Possibly, this treatment option may also be effective in chronic progressive diseases such as diabetic nephropathy (Nordquist *et al.* 2015).

RIP with repeated transient limb ischaemia is a simple, inexpensive and intriguing treatment option with favourable outcome in many preliminary clinical studies. Laboratory data suggest that HIF may be involved as well in RIP, although we are far from in-depth understanding this phenomenon. Clinical RIP trials are ongoing, even without preceding animal studies, reflecting the non-invasive and harmless nature of this intervention. If further, large-scale multi-centre randomized blinded studies establish efficacy, overcoming publication bias, this technique may be useful in additional clinical conditions such as organ harvesting for transplantation.

## Conflict of interest

CR participates in clinical studies by Astellas and AstraZeneca and has received consulting fees from Akebia. SNH conducted laboratory studies funded by FibroGen.

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