Remote Ischaemic Preconditioning - Translating Cardiovascular Benefits to Humans

James A. Lang and Jahyun Kim
DOI: 10.1113/JP282568

Corresponding author(s): James Lang (jlang1@iastate.edu)

The referees have opted to remain anonymous.

Review Timeline:

| Event                  | Date        |
|------------------------|-------------|
| Submission Date        | 20-Dec-2021 |
| Editorial Decision     | 04-Mar-2022 |
| Revision Received      | 03-May-2022 |
| Accepted               | 17-May-2022 |

Senior Editor: Ian Forsythe

Reviewing Editor: Mike Stembridge

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)
Dear Dr Lang,

Re: JP-TR-2021-282568 "Remote Ischaemic Preconditioning - Beyond the Heart" by James A. Lang and Jahyun Kim

Thank you for submitting your Topical Review to The Journal of Physiology. It has been assessed by a Reviewing Editor and by 2 expert referees and I am pleased to tell you that it is considered to be acceptable for publication following satisfactory revision.

The reports are copied at the end of this email. Please address all of the points and incorporate all requested revisions, or explain in your Response to Referees why a change has not been made.

NEW POLICY: In order to improve the transparency of its peer review process The Journal of Physiology publishes online as supporting information the peer review history of all articles accepted for publication. Readers will have access to decision letters, including all Editors' comments and referee reports, for each version of the manuscript and any author responses to peer review comments. Referees can decide whether or not they wish to be named on the peer review history document.

I hope you will find the comments helpful and have no difficulty in revising your manuscript within 4 weeks.

Your revised manuscript should be submitted online using the links in Author Tasks Link Not Available. This link is to the Corresponding Author's own account, if this will cause any problems when submitting the revised version please contact us.

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I look forward to receiving your revised submission.

Yours sincerely,

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EDITOR COMMENTS

Reviewing Editor:

Thank you for your submission. The review is very well written and the content will be interesting to our readership. In
addition to the comments provided by our two expert referees, please also consider the following in your revision.

The abstract figure communicates the overall picture of RIPC, but not the issues highlighted in the review e.g. timing, cofounders etc. I think this requires a little more focus throughout, as numerous confounders are mentioned (comorbidities, age, sex, frequency, tissue size, physical activity) but not how each of these might affect the response. Perhaps this could be incorporated here, or in a separate figure in the main body. I also think these concepts need to be unpacked and their potential impact explained in the text.

Given the thrust of the article is about the translation from animal model to human application, please make sure that the study population is clearly stated when discussing findings e.g. line 34- important to highlight Bromage meta-analysis was in animals vs. Kharbanda et al was humans. The lack of translation from animals to humans could also be highlighted in an additional figure, where distinct benefits in animals are shown on one side, and the few that “breakthrough” to humans shown on the other.

A more direct “future directions” section could be added at the end, to communicate to the reader what needs to happen to overcome the translational issues that stand at the moment.

I agree with reviewer 2 in that, while the title is punchy, it could be tailored more to the article content.

Senior Editor:
This is an interesting review, which with appropriate revision should appeal to a wide audience. In addition to the referees comments, please consider adding a figure to explain the background to a broad audience.

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REFEREE COMMENTS
Referee #1:

The central topic of this review is relevant. Several reviews have focused on RIPC, with relatively few studies focusing on the potential factors being involved in (or understanding) the poor translation of RIPC. The focus on these novel model and the potential impact of repeated RIPC is important, and provides a good rationale. I have a number of suggestions.

In the abstract, the authors mention fitness as one of the factors to potentially play a role in the efficacy of RIPC (or translation of RIPC in humans). In the paper, this factor has not been mentioned (or perhaps once under 'physical activity'). Acknowledging and discussing the role of fitness or exercise training in efficacy of (R)IPC seems sensible in light of this review. Some studies in animals and humans have been performed to address this topic. Recent reviews (Thijssen J Physiology 2022, Thijssen JAMA Cardiol 2018) have discussed this topic. Also because the similarity between repeated exercise (i.e. training) and repeated RIPC, I think this should be added to the review.

The authors mention the peripheral limb vasculature as a promising tool/model. However, the peripheral vasculature importantly differs from the coronary circulation. This should be discussed. whilst some studies have examined this for the FMD and plethysmography, I am not aware of studies that have done this for laser-based skin measurements. Some discussion seems warranted around these models. In addition, some support is needed for the last statement (measurements in the peripheral limb vasculature may provide a more sensitive and accessible method).

For the repeated RIPC-section, I would recommend to discuss the studies that explore repeated RIPC in clinical populations (and clinical outcomes) in more detail. The authors can also refer to reviews that have discussed this topic previously (e.g.,
Can the authors provide a more detailed view on what the next steps must be in translating RIPC to clinical benefits? The authors discussed the timing of the RIPC, but how exactly should this be examined and applied? Same holds true for the repeated bout effect; how to deal with these effects to maximise chances that RIPC actually leads to a (clinical) benefit. Perhaps the authors can add a figure that captures these central topics and suggestions? This can help (the naive) reader and to provide guidance.

Line 9: I would prevent ‘etc’ in the abstract.

Line 84: When should the RIPC-stimulus be given then? During anaesthesia or in a repeated protocol prior to surgery (and benefit from the second window)? This latter may of interest, as the mechanisms of the first and second window differ; perhaps propofol only interferes with the first window. Can the authors please elaborate on this?

Line 134/135: can you elaborate on this? What exactly was the time difference? If this is so crucial, it is important to understand the exact timings (perhaps also related to the other studies).

Line 156: Can the authors please provide some additional insight into this study. The typical local heating protocol (that goes to 42C) indeed induces an axon-reflex, but this axon-reflex typically is 60-70% of maximum dilation (which is examined with 44C heating). The plateau phase during the 42C-protocol typically is around 80-85% of the maximum heating response. Can you please clarify this.

line 168/169: The protocol seems important in the role of NO in mediating dilation during the FMD. Green Hypert 2014. Repeating the FMD after I/R may affect the role of NO. Some caution is warranted in these conclusions.

Referee #2:

In the animal model, remote ischemic preconditioning (RIPC) has been shown to protect against reperfusion injury and significant work has been done on mechanisms of action. However, this review highlights that there are significant gaps in our knowledge translating this information to human clinical research models and clinical practice. This clearly written review summarizes what is known in the human clinical model with respect to two important ingredients of the intervention: timing and frequency of the intervention. This content area is highly significant as the intervention is safe, feasible, inexpensive and could benefit several patient populations that have disrupted endothelial cell function. The focus of this review aligns well the vision of the Journal of Physiology and would be potentially appealing to physiologists as well as clinicians. Overall enthusiasm for this manuscript is high with minor, addressable concerns detailed below. Figures and tables are clear, and all needed.

Terminology: use of the term preconditioning. It is my understanding that pre-conditioning is used within the literature to describe when the interventions of RIPC is then followed by an experimental injury. It is not clear if the term RIPC can be blanketly applied to all of the human studies which may just involve ischemic conditioning or post-conditioning.

Title: The title may be a little two broad as the review focuses on the effects on peripheral vasculature and dosage/frequency. IC has also been shown to effect other domains such as motor performance.

Intervention description: It would help readers naïve to the intervention to have a brief description of the technique closer to
the beginning of the manuscript. Because direct ischemic conditioning and remote ischemic conditioning involve tissues being stimulated it might help to illustrate key differences.

Lines 51-59: The authors may want to consider highlighting the potential neural pathway of action via stimulation of group III/IV afferents.

Line 81 "...outcomes were not improved.." please clarify which outcomes.

228-229: It would be useful to include any information known about the long term durability of IC.

Line 242-253: It might be useful to include some information on how the effects of IC compare with other therapies that improve FMD in patient populations (eg in response to exercise).

The future directions portion could be expanded on based on gaps in the literature identified by the authors. In addition, the authors could touch on other outcomes that are just being explored beyond cardiovascular domain.

Conclusions are supported by the evidence reviewed.

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END OF COMMENTS

Confidential Review 20-Dec-2021
EDITOR AND REFEREE COMMENTS

Reviewing Editor:

Thank you for your submission. The review is very well written and the content will be interesting to our readership. In addition to the comments provided by our two expert referees, please also consider the following in your revision.

We sincerely appreciate the feedback and the opportunity to provide this content. We have incorporated the reviewer comments into the paper and included our responses below.

The abstract figure communicates the overall picture of RIPC, but not the issues highlighted in the review e.g. timing, cofounders etc. I think this requires a little more focus throughout, as numerous confounders are mentioned (comorbidities, age, sex, frequency, tissue size, physical activity) but not how each of these might affect the response. Perhaps this could be incorporated here, or in a separate figure in the main body. I also think these concepts need to be unpacked and their potential impact explained in the text.

We have attached an additional table and added a paragraph that identifies potential confounders based on evidence from the literature. And, we have included these confounders into the abstract figure (now Figure 1) and how they may alter the effects of RIPC (i.e., the ‘big picture’). Lastly, we provide a summary of the effects of timing in a revised ‘future directions’ paragraph at the end of the manuscript.

Given the thrust of the article is about the translation form animal model to human application, please make sure that the study population is clearly stated when discussing findings e.g. line 34- important to highlight Bromage meta-analysis was in animals vs. Kharbanda et al was humans. The lack of translation from animals to humans could also be highlighted in an additional figure, where distinct benefits in animals are shown on one side, and the few that "breakthrough" to humans shown on the other.

We have clarified line 34 by adding “animal models”, and we have clarified ‘animal’ vs ‘human’ throughout the manuscript. This includes revising a heading that more specifically addresses animal models (i.e., “Mechanisms of RIPC and Windows of Protection in Animal Models”). Additionally, we have included text regarding the translation of RIPC from animals to humans (last paragraph under the “From Animal Model to Human Clinical Research Model” heading).

A more direct "future directions" section could be added at the end, to communicate to the reader what needs to happen to overcome the translational issues that stand at the moment.

We appreciate the comment and heavily revised the last paragraph so that it is more direct in providing guidelines and future directions.

I agree with reviewer 2 in that, while the title is punchy, it could be tailored more to the article content.

We have modified the title to be more suitable to the article content. We have revised it to, “Remote Ischaemic Preconditioning – Translating Cardiovascular Benefits to Humans”
Senior Editor:

This is an interesting review, which with appropriate revision should appeal to a wide audience. In addition to the referees comments, please consider adding a figure to explain the background to a broad audience.

We appreciate the comment and have created a new abstract figure designed to provide a broader overview of the review topic. The ‘old’ abstract figure was kept in the paper as is listed as Figure 1.

Referee #1:

The central topic of this review is relevant. Several reviews have focused on RIPC, with relatively few studies focusing on the potential factors being involved in (or understanding) the poor translation of RIPC. The focus on these novel model and the potential impact of repeated RIPC is important, and provides a good rationale. I have a number of suggestions.

In the abstract, the authors mention fitness as one of the factors to potentially play a role in the efficacy of RIPC (or translation of RIPC in humans). In the paper, this factor has not been mentioned (or perhaps once under ‘physical activity’). Acknowledging and discussing the role of fitness or exercise training in efficacy of (R)IPC seems sensible in light of this review. Some studies in animals and humans have been performed to address this topic. Recent reviews (Thijssen J Physiology 2022, Thijssen JAMA Cardiol 2018) have discussed this topic. Also because the similarity between repeated exercise (i.e. training) and repeated RIPC, I think this should be added to the review.

We appreciate this feedback and have added an additional table that show confounders that may affect the RIPC response. Also, we have added text to the paper (see below) regarding aerobic fitness and have cited the reviews indicated.

“Lastly, considering the ischemia 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; Thijssen et al., 2022).”

And, in the last paragraph we highlight a recent study as well as discuss the need for more work to elucidate the overlapping or additive effects of RIPC and exercise.

“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.”

The authors mention the peripheral limb vasculature as a promising tool/model. However, the peripheral vasculature importantly differs from the coronary circulation. This should be discussed. whilst some studies have examined this for the FMD and plethysmography, I am not aware of studies that have done this for laser-based skin measurements. Some discussion seems warranted around these models. In addition, some support is needed for the last statement (measurements in the peripheral limb vasculature may provide a more sensitive and accessible method).
We have addressed this as a limitation in the manuscript as follows; “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., 2019).”

Furthermore, we have added references that support the use of the peripheral circulation as a means of examining systemic or global microvascular function. Khan et al. 2008, showed a positive relationship between peripheral vascular function and coronary artery function. Also, a review paper by Minson in 2010 provides multiple references in the literature supporting that blunted skin microvascular function reflects systemic microvascular impairment and is a harbinger of cardiovascular disease. These references have been added to the manuscript.

For the repeated RIPC-section, I would recommend to discuss the studies that explore repeated RIPC in clinical populations (and clinical outcomes) in more detail. The authors can also refer to reviews that have discussed this topic previously (e.g., Landman Stroke 2019, England JAHA 2019).

We added additional information on this section, specifically with regard to the magnitude of the attenuation in recurrent stroke rate (line 269-282).

Can the authors provide a more detailed view on what the next steps must be in translating RIPC to clinical benefits? The authors discussed the timing of the RIPC, but how exactly should this be examined and applied? Same holds true for the repeated bout effect; how to deal with these effects to maximise chances that RIPC actually leads to a (clinical) benefit. Perhaps the authors can add a figure that captures these central topics and suggestions? This can help (the naive) reader and to provide guidance.

We appreciate the comment and, as a result, have heavily revised the last paragraph so that it is more direct in providing guidelines and future directions. Here we provide indications for timing as suggested.

Line 9: I would prevent 'etc' in the abstract.

This has been deleted in the abstract.

Line 84: When should the RIPC-stimulus be given then? During anaesthesia or in a repeated protocol prior to surgery (and benefit from the second window)? This latter may of interest, as the mechanisms of the first and second window differ; perhaps propofol only interferes with the first window. Can the authors please elaborate on this?

We have revised this area of the manuscript to clarify the timing of RIPC. Here, we included a reference showing that a greater latency between RIPC and surgery may explain the attenuated myocardial damage observed in their study (Botker et al 2010). This additional time before surgery may maximize protection during surgery.

We also clarified the role of propofol in the paper. Propofol has been suggested to act as a free radical scavenger, enhancing tissue antioxidant capacity, inhibiting plasma membrane calcium channels. Moreover, it can also attenuate beta adrenoceptor responsiveness (Anesth Analg. 1999)
which may explain abolished cardioprotection with RIPC. One of the protection pathways in the first window phase is the neurogenic pathway. It is possible that propofol attenuates neurogenic responsiveness and interferes with the effects of RIPC. It is interesting to consider whether the effects of propofol extend to the second window protection. However to our knowledge, no clinical study that has examined this.

Line 134/135; can you elaborate on this? What exactly was the time difference? If this is so crucial, it is important to understand the exact timings (perhaps also related to the other studies).

We have addressed detailed information here regarding the timing of RIPC. We have provided the timing in animal models and this close this section of the paper with the following: 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.

Line 156: Can the authors please provide some additional insight into this study. The typical local heating protocol (that goes to 42C) indeed induces an axon-reflex, but this axon-reflex typically is 60-70% of maximum dilation (which is examined with 44C heating). The plateau phase during the 42C-protocol typically is around 80-85% of the maximum heating response. Can you please clarify this.

Thanks for catching this. We have revised these numbers to 60-70%.

line 168/169: The protocol seems important in the role of NO in mediating dilation during the FMD. Green Hypert 2014. Repeating the FMD after I/R may affect the role of NO. Some caution is warranted in these conclusions.

Indeed, we have softened the language here regarding the role of NO.

Referee #2:

In the animal model, remote ischemic preconditioning (RIPC) has been shown to protect against reperfusion injury and significant work has been done on mechanisms of action. However, this review highlights that there are significant gaps in our knowledge translating this information to human clinical research models and clinical practice. This clearly written review summarizes what is known in the human clinical model with respect to two important ingredients of the intervention: timing and frequency of the intervention. This content area is highly significant as the intervention is safe, feasible, inexpensive and could benefit several patient populations that have disrupted endothelial cell function. The focus of this review aligns well the vision of the Journal of Physiology and would be potentially appealing to physiologists as well as clinicians. Overall enthusiasm for this manuscript is high with minor, addressable concerns detailed below. Figures and tables are clear, and all needed.

Terminology: use of the term preconditioning. It is my understanding that pre-conditioning is used within the literature to describe when the interventions of RIPC is then followed by an experimental injury. It is not clear if the term RIPC can be blanketly applied to all of the human studies which may just involve ischemic conditioning or post-conditioning.
We appreciate the comment and recognize the need to provide the reader with some distinction. At the end of the introductory paragraph, we have added the following: *RIPC is typically evoked by administering 3-4 cycles of ~5 min limb ischemia, 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.*

**Title:** The title may be a little too broad as the review focuses on the effects on peripheral vasculature and dosage/frequency. IC has also been shown to effect other domains such as motor performance.

We have modified the title to be more suitable to the article content. We have revised it to, *“Remote Ischaemic Preconditioning – Translating Cardiovascular Benefits to Humans”*

**Intervention description:** It would help readers naïve to the intervention to have a brief description of the technique closer to the beginning of the manuscript. Because direct ischemic conditioning and remote ischemic conditioning involve tissues being stimulated it might help to illustrate key differences.

We agree that it would be helpful to include a description. Thus, we have clarified the ‘typical’ protocol of RIPC in the first paragraph. See our response to the first comment for the text that was added here.

Lines 51-59: The authors may want to consider highlighting the potential neural pathway of action via stimulation of group III/IV afferents.

We have highlighted this pathway as suggested. The following text and references have been added to the manuscript [under the first paragraph of the “Potential mechanisms of single bout RIPC on vascular function” heading]: *However, although there are reports to the contrary (Mulliri et al., 2016; Angius et al., 2022), a single bout of ischemic preconditioning did not alter sympathetic activity, assessed by microneurography, or reduce hemodynamic responses to static handgrip exercise or subsequent post-exercise ischemia (Incognito et al., 2017).*

Line 81 "...outcomes were not improved...." please clarify which outcomes.

We revised the manuscript to specify these markers and outcomes as follows: *“...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)”*

228-229: It would be useful to include any information known about the long term durability of IC.

We have addressed this in the manuscript by including the need to investigate how long benefits remain after completing RIPC (end of 1st paragraph under the “Repeated RIPC and vascular function” heading).

Line 242-253: It might be useful to include some information on how the effects of IC compare with other therapies that improve FMD in patient populations (eg in response to exercise).

We added two references to review articles regarding the ‘preconditioning’ effect of exercise as well as adding the following text:
“The pattern and underlying mechanisms of cardiovascular protection with repeated RIPC may be similar to that observed after short-term aerobic exercise”

The future directions portion could be expanded on based on gaps in the literature identified by the authors. In addition, the authors could touch on other outcomes that are just being explored beyond cardiovascular domain.

We appreciate the comment and heavily revised the last paragraph so that it is more direct in providing guidelines and future directions. We have also touched upon these other elements that may be affected with RIPC as follows, “Additionally, RIPC may have neuromuscular and neuroprotective effects and thus could have application to exercise performance or with maintaining cognitive function.”

Conclusions are supported by the evidence reviewed.
Dear Dr Lang,

Re: JP-TR-2022-282568R1 "Remote Ischaemic Preconditioning - Translating Cardiovascular Benefits to Humans" by James A. Lang and Jahyun Kim

I am pleased to tell you that your Topical Review article has been accepted for publication in The Journal of Physiology, subject to any modifications to the text that may be required by the Journal Office to conform to House rules.

***PLEASE NOTE***
You will need to provide an 'Additional Information' section to your manuscript at proof correction stage - see details below.

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Yours sincerely,

Ian D. Forsythe
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EDITOR COMMENTS

Reviewing Editor:

Thank you for taking the time to carefully revise your manuscript in line with the feedback received. Both reviewers are happy with the amendments made, and believe the review will an impactful addition to the literature. Thank you for your submission.

Senior Editor:

Thank you for an interesting review.

Please ensure you provide the following sections to your article at proof correction stage:

-Your MS must include a complete "Additional information section" with the following 4 headings and content:

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Acknowledgements: Acknowledgements should be the minimum consistent with courtesy. The wording of acknowledgements of scientific assistance or advice must have been seen and approved by the persons concerned. This section should not include details of funding.

REFEREE COMMENTS

Referee #1:

No further comments.

Referee #2:

The authors have substantially revised the manuscript and addressed concerns raised by reviewers and editor. Changes
have improved the quality and clarity of the manuscript. No further suggestions.

1st Confidential Review

03-May-2022