Author's response to reviews

**Title:** Acute effects of remote ischemic preconditioning on cutaneous microcirculation - a controlled prospective cohort trial

**Authors:**

Robert Kraemer (robertkraemer@arcor.de)
Johan Lorenzen (j.m.lorenzen@gmail.com)
Mohammad Kabbani (m.kabbani@gmx.de)
Christian Herold (herold.christian@mh-annover.de)
Marc Busche (busche.marc@mh-hannover.de)
Peter M. Vogt (vogt.peter@mh-hannover.de)
Karsten Knobloch (kknobi@mh-hannover.de)

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**Author's response to reviews:** see over
Reply to Reviewer's report 1

Title: Acute effects of remote ischemic preconditioning on cutaneous microcirculation - a controlled cross-over trial

Version: 1 Date: 3 June 2011
Reviewer: Stewart Walsh

Reviewer's report:
The authors present the results of a prospective study of the effects of RIPC on cutaneous microcirculation in healthy volunteers. It is a potentially useful study but a few points need to be addressed.

Major Compulsory Revisions

This is not a crossover trial. It is a prospective cohort study and should be described as such.

We absolutely agree and changed that.

Please include error bars for all datapoints in the figures. This is particularly important for Figure 2, where a significant difference in only 2 data points is used to conclude that post-capillary filling pressure falls after RIPC.

Error bars included in all figures.

Did the authors consider incorporating a Bonferroni correction for multiple comparisons?

We performed a Bonferroni-Holm-procedure as standard in multiple t-tests of statistical analysis.

The discussion is very superficial. It largely revisits material already covered in the introduction. The authors should focus on the strengths and weaknesses of their work and its relation to other literature. For example, Hoole et al found no effect of RIPC on myocardial microvascular function. How does their work relate to this? If RIPC has no effect on skeletal muscle microvascular function could it alter its potential utility in flap surgery?

We agree to the Reviewer that the discussion should be more precise. Consequently, we added the recommended questions and further current scientific literature:

Although it is generally accepted that IP increases flap survival by enhancing microvascular perfusion, the molecular and microcirculatory mechanisms are still not completely understood. The noninvasive application of a tourniquet at the hindlimb to induce IP was introduced by Kuentzche et al. and demonstrated to be as effective as the invasive clamping of the flap's pedicle itself, including both adipocutaneous and muscle flaps. [1]
Recently, remote ischemic preconditioning demonstrated a modulation of hepatic microcirculation with consecutive reduction of the effects of ischemic reperfusion injury in an in-vivo animal model. RIPC in that setting significantly increased red blood cell velocity, sinusoidal flow and sinusoidal perfusion along with decreased neutrophil adhesion and cell death. For remote preconditioning the tourniquet was tightened around the limb until no flow was detected. The procedure involved 5 min of ischemia followed by 5 minutes of reperfusion. This was repeated four times. [i] Regarding myocardial perfusion, Hoele et al. could not find an effect of RIPC on coronary microvascular resistance or coronary flow in humans. [ii] Nevertheless, Thielmann et al. found that myocardial injury after coronary artery bypass grafting was reduced by RIPC [iv] Zimmermann et al. demonstrated RIPC as protective from acute kidney injury in patients following cardiac surgery. In that study remote ischemic preconditioning was applied by an automated thigh tourniquet consisting of three 5-min intervals of lower extremity ischemia separated by 5-minute intervals of reperfusion. Within 48 h after surgery there was a significant both absolute and relative risk reduction due to preconditioning. [v] Another recent trial found no evidence that remote ischemic preconditioning provided protection of kidney function in children undergoing operation for complex congenital heart disease. Four cycles of five minutes RIPC were applied in that study. [vi] The clinical and experimental data about RIPC in current literature remains confusing. An equal microcirculatory effect on different kinds of tissue obviously is not mandatory in different studies. A dose effect dependency can still not be included in RIPC because of the fact that there is still a non-equivalent effect apparent in different trials with identical dosages of RIPC. The main reason for this heterogeneous scientific data could be the indisputable heterogeneity of clinical studies concerning with RIPC, especially in terms of methodology and cohorts. [vii] Thus, basic scientific research on RIPC is mandatory and must include investigations on healthy cohorts. For this reason, we included only young, healthy subjects in our study and focused our investigation on immediate and short-term effects of RIPC on a currently scientifically non-investigated body area.

Do the authors intend to examine skeletal muscle microvascular function?

As our trial elucidated the short term results of RIPC and documented the beneficial effect of the intermittent, repetitive component of RIPC on microcirculation, further studies should now focus on intermediate and long term effects of RIPC on cutaneous microcirculation. A further field of application of RIPC should also be part of further investigations calling muscular microcirculation as ischemia of muscle does not necessarily lead to muscle necrosis, but can lead to progressive microvascular dysfunction at an early stage with consecutive microcirculatory impairment as a vicious circle up to a compartment syndrome. [viii] Therefore, an increase of cutaneous oxygen saturation and capillary blood flow as well as a decrease of venous stasis as immediate effects of RIPC in our study might also be beneficial for reoxygenation-associated inflammation due to reperfusion injury in muscular trauma or surgery.
Reply to Reviewer's report 2

Title: Acute effects of remote ischemic preconditioning on cutaneous microcirculation - a controlled cross-over trial

Version: 1 Date: 18 September 2011
Reviewer: Derek J Hausenloy

Reviewer's report:
The study is novel and interesting and aims to investigate potential mechanisms underlying RIPC. However, it needs further data to confirm preliminary findings.

• Major Compulsory Revisions
1. The authors demonstrated improvements in tissue oxygenation and capillary blood flow and resistance in direct response and at the time of the RIPC stimulus in the arm. However, the authors need to demonstrate that the microcirculatory changes are present up to 3 hours after RIPC stimulus as this would correspond to when surgery on any flap would be occurring.

We agree to the Reviewer that there is an inalienable need for further elucidation of intermediate to long term effects of RIPC. Nevertheless, there is a lack of data about microcirculatory effects in healthy humans in general, but especially on skin perfusion. Up to date, there is no data available in current scientific literature that addresses skin perfusion under RIPC. Therefore, we performed this preliminary study on immediate effects of RIPC on skin microcirculation, which has to be elucidated before raising more efforts in several ways. One effort could be to investigate the recommended 3 hours period after RIPC. Surely, intermediate to long term effects are mandatory for scientific evaluation, but as 15 minutes of follow up are an arbitrary time interval, it would be the same with 3 hours of follow up. Actually, in a surgical setting usually RIPC can be administered directly before action takes place, e.g. free flap ischemia, certain stress to soft tissue due to endoprothetics, etc. Consequently, from our point of view as surgeons we first need to know about the immediate effects for the basic knowledge. As a next step, we need long-term results not only dealing with microcirculation but also with clinical outcome, which contributes to the second concern of the Reviewer: 2. The observed changes may only be haemodynamic response to RIPC stimulus elsewhere- the authors need to provide evidence that they actually contribute to protection.

Providing evidence of protective effects of RIPC on soft tissue is definitely not the issue of the current work. Providing evidence of protection needs either clinical or laboratory end points for a study which both has already been addressed by multiple studies in current literature which come to different and confusing results. As stated above, besides these various studies, no study has tried to quantify the effects of RIPC in general, demonstrate the advantageous effects of repetitive RIPC and furthermore demonstrate that on cutaneous microcirculation, what we all did with this work as basic science about RIPC. Of course, the next steps must follow doing long-term outcome trials as RCTs on protective effects on skin and muscle tissue after RIPC.

3. It would be interesting to see if the microcirculatory response to proportional to
We absolutely agree to the Reviewer about the interesting question if RIPC to a leg with bigger body mass would have a proportional higher effect than RIPC on an arm with less body mass. The same interesting question is about dose-dependancy of RIPC in one extremity. Up to date, there still is a lack of basic scientific data about what is the optimum length of ischemia with consecutive reperfusion and which dosage has the most beneficial effects for tissue protection, which we additionally stated in our discussion. To answer this question you need a standardized experimental setting with only little environmental influence on microcirculation. Our experimental model in this study has quantified an immediate effect on cutaneous microcirculation due to RIPC for the first time in current literature. After having confirmed our standardized experimental setting as being capable of demonstrating effects of RIPC, we can now evaluate different dosages and different body areas as arms and legs for the application of RIPC. This is the content of our future studies and was not the issue of the current work.

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests


3 Hoole SP, Heck PM, White PA, Khan SN, O'Sullivan M, Clarke SC, Dutka DP: Remote ischemic preconditioning stimulus does not reduce microvascular resistance or improve myocardial blood flow.

Epub 2008 Dec 23.


preconditioning reduces myocardial injury after coronary artery bypass grafting with crystalloid cardioplegic arrest.


v Zimmerman RF, Ezeanuna PU, Kane JC, Cleland CD, Kempananjappa TJ, Lucas FL, Kramer R:

Ischemic preconditioning at a remote site prevents acute kidney injury in patients following cardiac surgery.


vi Pedersen KR, Ravn HB, Povlsen JV, Schmidt MR, Erlandsen EJ, Hjortdal VE:

Failure of remote ischemic preconditioning to reduce the risk of postoperative acute kidney injury in children undergoing operation for complex congenital heart disease: A randomized single-center study.


Zhang L, Bail H, Mittmeier T, Haas NP, Schaser K.