Author's response to reviews

Title: Myocardial topical negative pressure of -25 mmHg increases myocardial microvascular blood flow

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Author's response to reviews: see over
**Respond to the Reviewers**

We are very thankful to the reviewers for their constructive criticism and we have tried to change the manuscript and make comments to their concerns:

**Reviewer Douglas Curran-Everett:**

The reviewer has some concerns about the methodology and statistics:

1. How were the pressure changes applied to normal and ischemic myocardium? This can be read in the methods (page 6-9 in the paper). Mr Curran-Everett wonders why the experiments were performed in the specific order starting with normal myocardium and -25 mmHg ending with ischemic myocardium -50 mm Hg. It is not possible to apply a higher pressure level to the myocardium and thereafter a lower pressure without carry over effects in the myocardium or in the measurements. Furthermore, it is not possible to do experiments with ischemic myocardium first and then go back to normal myocardium, since the myocardium is not “normal” after being ischemic. The myocardium would then turn into “reperfused” myocardium which is not required in those experiments. I believe the question is highly adequate and of course for optimal conditions one would appeal a Latin square, but it is of reasons earlier mentioned, not possible.

2. Mr Curran-Everett question why the sample size of 6 was chosen. I believe that this is a good question. We have used 6 animals for the last 15 years and nobody has ever asked this adequate question before. I assume one reason might be that if you use six animals and one animal shows values or measurements in the opposite direction to the other animals you are still in a position to reach significance. In my perspective, I believe that if you are not able to demonstrate differences in six animals the clinical impact will decrease.

3. Mr Curran-Everett wonders how blood flow measurements were analysed and if mean blood flow was measured and over how long period of time? We analysed laser Doppler flow (ml/100g tissue/min). Analyses were made over a period of 20-30 seconds when the flow was stabilized and a mean value was taken. Mr Curran-Everett also wonders how we account for the increased variability of the flow measurements. It is a very good question and I’m afraid
that we don’t now why we have increased variability in the flow measurements. I’m afraid this is one of the concerns with measurements with the method laser Doppler flowmetry.

4. Mr Curran-Everett thinks it makes more sense to express results as differences from baseline. We totally agree. We have done so; please see figures in the manuscript.

Minor revisions:

1. Mr Curran-Everett wants us to change the p-values to exact values. We totally agree, and have now changed the p-values to exact values.

**Reviewer Frank W. Selke:**

This reviewer have some concerns about repeated multiple comparisons and I can understand his issues. However, Mr Curran-Everett however has been taken in as a statistical adviser or reviewer and therefore I believe that it is wise to change things regarding statistics according to Mr Curran-Everett opinion. Therefore, we have not changed the statistics into repeated multiple comparisons in the manuscript.

**Reviewer Ghassan S Kassab:**

Mr Kassab has nothing to add at this point and believes the manuscript is already adequately revised and suggests publication.