Reviewer's report

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

Version: 3 Date: 27 March 2008

Reviewer: Douglas Curran-Everett

Reviewer's report:

General
The authors answered some of my questions, but now that I understand how they did their experiment, I continue to have concerns about their statistical analysis. I expect my concerns mirror those of Dr. Selke, whose comments I did not see.

Major Compulsory Revisions

Experimental design. As I mentioned in my initial review, because the conditions were done in the same order, it is impossible to differentiate a pressure effect from an order (or time) effect. Measurements at -50 mmHg are related to the measurements at -25 mmHg simply because they came afterwards. The authors must use an analysis that takes into account the correlation among the measurements at -25 mmHg and -50 mmHg. Some form of repeated measures analysis of variance is appropriate. Because there are different options for this kind of analysis, I encourage the authors to consult with a statistician at their institution.

Comment

Methods, sample size. As I mentioned in my initial review, if all 6 responses change in the same direction, then $P = 0.03$ (2-tailed binomial test). If 5 responses change in one direction and the 6th response changes in the opposite direction, then $P = 0.21$ . It is important to be able to justify sample size. In the US, as far as I am aware, all animal use and grant applications must justify the number of animals that will be used. As I am sure the authors recognize, just the fact that they have been using 6 animals for so long is not sufficient justification. A statistician could help estimate the sample size necessary for this kind of experiment. If the number is close to 6, all the better, but at least the authors will be able to provide a specific explanation.

Minor Essential Revisions

None

Discretionary Revisions
None

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests.