Author's response to reviews

Title: Obesity and statins are both independent predictors of enhanced coronary arteriolar dilation in patients undergoing heart surgery

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Author's response to reviews: see over
Dear Dr. Zamvar,

March 29, 2013.

We would like to submit a revised version (R1) of the manuscript entitled “Obesity and statins are both independent predictors of enhanced coronary arteriolar dilation in patients undergoing heart surgery” to be considered for publication in Journal of Cardiothoracic Surgery.

We appreciate the time and expertise dedicated by the Editor and the reviewers to the constructive criticism of this article. We are pleased that the reviewers found our work of clinical interest. We have taken careful consideration of all comments and suggestions, and we have revised our manuscript. Point-by-point responses are provided to the reviewers’ criticisms in the accompanying rebuttal letter.

We hope that the Editor will share our belief that comments raised by the reviewers are adequately addressed in the rebuttal letter and in our revised manuscript, which we hope will be considered for publication.

Thank you for your consideration in this matter.

Sincerely yours,

Dr. Zsolt Bagi
Authors' Comments to Reviewers:

Reviewer #1

We appreciate the time and expertise dedicated by the reviewer to the constructive criticism of our study. We are very pleased to receive valuable criticisms, which we have considered during the revision of our manuscript.

1. This group reported in 2007 (citation 11) that obese hypertensive patients have increased coronary vasodilator responses compared to lean hypertensive patients. The patient population utilized in this study largely mirrors these groups as 89% of non-obese and 86% of obese patients studied were hypertensive. Thus, the results presented in Figure 1 are confirmatory of this previous work and no additional experiments are presented to provide additional mechanistic insight into coronary arteriolar function in these patients.

RESPONSE:

We agree with the reviewer. In this study we enrolled patients with similar characteristics to our earlier study. We did so in order to critically examine whether or not the observed effects of obesity on coronary microvascular responsiveness (Fulop et al. Arterioscler Thromb Vasc Biol. 2007 Nov;27(11):2348-54.) were influenced by the patients’ medications (Please see clarified scope of our study on Page 4, Lines 8-15 and Page 12 – lines 1-8). To test this postulation we investigated 64 consecutively enrolled patients, which allowed us to apply robust statistical models; with a greater statistical power for detecting the impact of medications and the influence of other confounding factors, such as obesity on microvascular responses. For example, power analysis of the 2-way ANOVA demonstrated a power of 0.81 to detect large (f=0.40) interactions in our study. We have employed these statistical methods to our patients following recommendations by and in agreement with Dr. Lan, a statistician in the Department of Biostatistics and Epidemiology at Medical College of Georgia; also coauthor of this paper.

We also agree with the reviewer that statistical tool(s) to investigate vasomotor responses does not provide mechanistic insight. This approach however is widely used and accepted to detect the influence of confounding factors in clinical studies involving patients with different characteristics.

Taken together, the main focus of our present study is to examine the influence of various factors likely affecting vasomotor responsiveness using a statistical approach. We believe that our present findings are novel and valid. However, we share the reviewer’s concern regarding the lack of insight into the underlying mechanisms, which is due to the approach and should be evaluated in future studies.

Major Compulsory Revisions:

1. This group's previous work (citation 11) demonstrated that the increased dilation in obese hypertensives (compared to lean hypertensives) was due primarily to enhanced endothelium-independent dilation since BK responses were not different but those to SNP were enhanced. Why were SNP responses not determined in this study? The results of the
present study should be more clearly discussed in the context of the previous report by this group (citation 11) in the Discussion. Furthermore, in the absence of SNP responses, the conclusion at the beginning of the Discussion that ‘obesity and statins are independent predictors of enhanced endothelium-dependent dilation of coronary arterioles’ is not supported as these results could be explained entirely by enhanced smooth muscle endothelium-derived vasodilators as reported in citation 11.

RESPONSE:

The reviewer refers to our earlier mechanistic study in which the endothelium-dependent agonist, bradykinin and the NO donor, sodium nitroprusside were used and both found to cause enhanced coronary dilation in obese hypertensive patients. Based on our results we proposed enhanced sensitivity of vascular smooth muscle cells to exogenous NO. However we could not exclude the possibility of involvement of endothelial mechanisms.

In our present study only bradykinin was used, as it, what we believe, is a more potent vasodilator in the diseased human heart than an NO donor. Indeed, bradykinin is released during tissue ischemia in myocardial infarction and heart failure, to restore myocardial perfusion. Another and more practical reason for using bradykinin, is that it causes a rapid dilator response, which can be repeated and detected in reliable manner. This property of the bradykinin response is particularly important when using it as a variable in statistical tests.

The results of our previous study are described in the revised manuscript (Page 12, lines 2-5).

2. The text regularly refers to ‘enhanced dilator function of coronary arterioles from obese patients’. This statement is not supported by the data since ‘maximal’ dilation to BK is similar between obese and non-obese and with and without statin use. These statements must be amended to refer to the noted differences in arteriolar sensitivity (ie, EC50) among the various groups. The title must also be similarly amended to accurately reflect the data set.

RESPONSE:

As suggested by the reviewer we have modified the text and provided clarification regarding the increased coronary sensitivity to bradykinin, but not the overall magnitude of vasodilation in obese patients (please see revised Abstract). Moreover, motivated by the reviewer’s suggestion, we have constructed a new graph and analysis to show the effects of obese vs. non-obese populations segregating subgroups depending on the use of statins (New Figure 1C). This new data demonstrates a significantly enhanced magnitude of dilations to bradykinin in obese subgroups with our without statins. Discussions regarding this observation now are incorporated into the revised manuscript (Page 13, lines 15-18). Thank you for the suggestion.

3. The Discussion is currently too long. The attempt to clearly describe the cited studies is appreciated; however, these descriptions should be shortened and more time should be spent integrating the present results with the groups previous work (see comment 1).

RESPONSE:
We have made efforts to shorten the whole manuscript, while discussing our data and addressing issues suggested by the Editor and reviewers for the revision. We also include further clarification regarding the main focus of the present study in view of our earlier observation (Introduction, Page 4, Lines 8-15 and Discussion Page 12 – lines 1-8).

4. Presentation of the data as ‘trends’ is inappropriate, particularly with p values of 0.2 and above as reported. This is done in the text in reference to the difference in BK dilation between obese and non-obese patients and to the effect of ACE inhibitors on coronary dilation. These are not significant differences and should be addressed accordingly.

**RESPONSE:**

The reviewer is correct and we have modified the Abstract and the text, which avoids the phrase ‘trend’ when statistical insignificance is detected, P > 0.05.

5. It is not clear to this reviewer if the use of complex statistical analysis on such a small patient population is appropriate. Thus, I am recommending further statistical review.

**RESPONSE:**

Before employing statistical analyses, power calculations were performed in order to determine the robustness and validity of our analyses in our sample size. As indicated in the original submission the analysis had high power (0.81) do detect large differences, but exhibited less power to detect small changes. Furthermore, when performing the statistical analyses we worked closely with a statistician, Dr. Lan, who advised us and also contributed and validated the analysis. Thus, we are confident in the validity of our approach.

6. Please report the percent development of spontaneous tone for the coronary arterioles used in the study in the Results.

**RESPONSE:**

As suggested by the reviewer, the active and passive diameters as well as the magnitude of spontaneous tone are described in obese and nonobese patients (Abstract, and Page 8, lines 15 -20).

7. It is unclear why BMI was correlated with % Dilation to BK in Figure 1B when the percent dilation curves were not different between groups. It seems more appropriate that such an analysis be done between BMI and the EC50 for BK since this variable is significantly different between the groups. Figure 1B is not necessary in its current form.

**RESPONSE:**

RE: Figure 1B. In the original submission Figure 1B shows a positive ‘trend’ instead of a negative one as one may expect in obesity. We thought showing this data carries an important message. However, following the reviewer’s suggestion we have removed this analysis and data from Figure 1 and from the text. Following the reviewer’s comment we have performed analyses investigating the relationship between BMI and EC50 of bradykinin induced responses using linear regression. This analysis, also did not
reveal any significant correlations (P=0.117) and therefore we are not incorporated it into the revised manuscript, as suggested.

8. **Discussion of the results with regard to their specific physiological relevance is inadequate.** Specifically, what potential relevance is a modest increase in coronary arteriolar sensitivity to BK (with obesity or statin treatment) in heart disease patients? The discussion of the obesity paradox is appreciated but does not address mechanisms underlying the control of coronary blood flow. Since BK stimulates the release of endothelium-derived dilators and it can be assumed that most of the patients have ischemic heart disease (given the large percentage of CABG patients) it would be pertinent to discuss the increased role for endothelial-derived dilators like NO in the control of coronary flow in ischemia compared to non-ischemic states where NO plays little role.

**RESPONSE:**

Regarding the importance of NO in the ischemic myocardium, recently we have found that in isolated human coronary arterioles, the NO synthase inhibitor, L-NAME had no effect bradykinin-induced coronary vasodilation (Feher et al, Circulation J, accepted manuscript in press). This finding is in accordance with previous observations in isolated human coronary arterioles (Miura et al, Circulation 1999; 99(24):3132-8) and indicates only minor, if any, involvement of NO in bradykinin-induced coronary arteriolar responses in disease. Response to bradykinin, however, is still substantial, as it was shown in this and many other previous studies of the human coronary arteriole. Thus, we believe that bradykinin plays a crucial role in maintaining coronary vasodilation in the diseased human heart, via activating mechanisms other than NO.

Regarding the potency of bradykinin, it is difficult to predict the concentration at which bradykinin causes increased myocardial perfusion in the human coronary microcirculation in vivo. However, we would like to point out that even a small change in receptor sensitivity and small enhancement in the magnitude of bradykinin-induced dilation may induce substantial changes in myocardial perfusion, as the blood flow is proportional to the fourth power of the vessel radius according to Poiseuille's Law.

9. **Given the worsened clinical outcome of patients who are morbidly obese and underweight (presented in the Discussion), do the noted differences in BK sensitivity remain after the 7 patients who fall into these categories are removed from the data set? Inclusion of these patients appears somewhat confounding in the analysis of obese vs non-obese, especially in light of the obesity paradox.**

**RESPONSE:**

We agree with the reviewer’s comment. According to our original study design we enrolled consecutive patients undergoing heart surgery, without setting any enrollment and exclusion criteria. Therefore, we are not able to exclude patients retrospectively from our study cohort and data analysis; and at this point we can only speculate how this might affect the overall results. In one of our ongoing projects we are recruiting morbidly obese patients and studying their coronary vasomotor behavior, also in the context of the obesity paradox. Thank you for the excellent recommendation.
Minor Essential Revisions:
1. In the Abstract, it appears that the SD or SE reported for the BK responses in obese and non-obese patients is incorrect as it does not correspond to that shown in Figure 1A. The Abstract values are 25% and 27% while in the figure these appear to be ~5%.

RESPONSE:
The reviewer is correct. The original Abstract indicated mean +/- SD when demonstrating the BK response. This has been revised and corrected to SEM.

Reviewer #2
We are very pleased that the reviewer found our work of clinical interest.

1. Figure 1 B shows the majority of the patients are BMI Between 25-30. This is the range for overweight patients. When BMI is >35, the vasodilation respond drops dramatically. Here you show only 3 patients. Have you ever done vasodilation respond in patients, whose BMI is > 35. Also can you explain why one of the patient vasodilation was negative?

RESPONSE:
In our study design we have enrolled consecutive patients undergoing heart surgery and we have not set any enrollment and excluding criteria in this study. Therefore we can only speculate as to how morbid obesity would affect the magnitude of coronary dilations. We are currently recruiting morbidly obese patients and studying their coronary vasomotor behavior, also in the context of the obesity paradox.

Regarding the reviewers comment on negative vasodilation, the negative responses indicate arteriolar constriction (please also see our response to point #2 for further comments on this). Usually we use the label “change in diameter” in depicting dilatation and constriction in the same figure. However, the other reviewer suggested this panel to be removed from Figure 1.

2. Have you ever tested the vasoconstriction respond of the vessels isolated from this patient?

RESPONSE:
It is believed that the effect, dilation or constriction, of bradykinin receptor stimulation is determined by the localization of type 1 and type 2 bradykinin receptors – i.e. endothelial expression is coupled to vasodilation (Zhang X, Hypertension 1997; 30(5):1105-11) vs. smooth muscle expression is coupled to constrictor signaling (Tsukada M, Clin Exp Pharmacol Physiol 1999; 26(5-6):456-60). In the future we are planning to perform experiments to detect type 1 and type 2 bradykinin receptor expression in human coronary microvessels and to see whether the expression is altered by disease. Thank you for the suggestion.

3. Bradykinin is not the only best mediator to study endothelium dependent vasodilation. Bradykinin plays little role in coronary metabolic flow regulation. Have you ever tried the vasodilatation respond to Ach, or NO, or hydrogen peroxide. And also it will be important to study endothelium denuded vessels vasodilation respond.
RESPONSE:

In the present study we used bradykinin as it is one of the most potent coronary arteriole dilators, ex vivo. Recently we have reported that ACh elicited a diminished dilation in isolated human coronary arterioles in diabetic and non-diabetic patients with coronary artery disease. The mechanism(s) by which ACh-induced dilation are diminished are not entirely understood. In diabetic patients, we proposed that this is due to increased arginase-1 expression and consequently diminished NO synthesis in coronary microvessels (Beleznai et al, Am J Physiol Heart Circ Physiol. 2011 Mar;300(3):H777-83). We agree with the reviewer that testing the effects of bradykinin and ACh in endothelium-denuded arterioles is important, which may reveal contributions of type 1 and 2 bradykinin as well as M1 and M3 muscarinic receptors to the response. Our ongoing studies are testing these hypotheses.

4. Table 1. 55.6% of patients (BMI<30) were under the ACE inhibitor treatment. It is well known, that ACE inhibitors inhibit bradykinin degradation and increase the level of the BK in the blood. Is it possible this high level of BK decreases the sensitivity of the vessels or effects on vasodilation respond, which you observed here? Indeed, endothelium dependent effect of statins is mediated by NO. In BMI< 30 group 72% of patient were under statin treatment (BMI>30 was 53%). And also you have more patients in this group (36vs.28). All this brings to the passive diameter of the vessels you show here. It is not significant, but it can significantly affect on the calculation of vasodilation respond to BK. In this scenario, I think it will be better to induce maximal vasoconstriction and then treat with BK or other vasodilators.

RESPONSE:

The reviewer raises an important issue. In our very recent study we have investigated the direct vascular effects of ACE inhibitors in isolated coronary arterioles of obese patients and also rats on a high fat diet (Feher et al, Circulation J, accepted article in press). We have found that captopril, when administered in vitro, enhanced coronary arteriole dilation in obese subjects. In this study we found that ACE inhibitors taken prior the heart surgery had no significant effect on the magnitude of bradykinin-induced dilation in obese patents (Table 3). One of the rational explanations for this apparent discrepancy could be a decreased sensitivity to bradykinin during prolonged ACE inhibition. Indeed in the obese group less patients were on ACE inhibitors and arterioles from these patients exhibited augmented sensitivity to bradykinin. We discuss this possibility in the revised manuscript (Page 14 – last 4 lines).

RE: pre-constriction. In this study, as in our previous studies, (Szerafin at al. Circ Res. 2006 Sep 1;99(5):e12-7., Fulop et al. ATVB. 2007;27:2354, Beleznai et al. Am J Physiol Heart Circ Physiol. 2011;300(3):H777.) we used our standard approach to human coronary arterioles: no pre-constrictor agents used and normalized responses to the passive diameter. Depending on the constrictor agent(s), the contribution of dilator factors is different even in the very same vascular bed (Dora at al. Am J Physiol Heart Circ Physiol. 2002 Aug;283(2):H606-14.). Thus, we believe that our approach is feasible and provides a good estimate for the overall vasodilator effect of bradykinin.