Author’s response to reviews

Title: Computer-controlled closed-loop drug infusion system for automated hemodynamic resuscitation in endotoxin-induced shock

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Author’s response to reviews:

Response to Reviewers’ comments:

Reviewer reports:

Joseph Rinehart (Reviewer 1): The authors are reporting on the results of a series of animal experiments studying the ability of a novel-closed-loop system to resuscitate appropriately from septic shock. The system is capable of management of both vasopressor infusion rate and crystalloid resuscitation, and in this study both ran under full closed-loop control. The concept is novel; I am not aware of any other dual-loop systems managing both vasopressors and volume resuscitation simultaneously. As I am very active in this space myself I think the topic is of interest to the anesthesiology community and the future of our specialty and that work like the authors have performed is an essential step in the validation of a controller. The current manuscript could be much improved before publication, however.

Response: We thank Dr. Rinehart for his detailed review of our manuscript and constructive comments.
Major Comments:

1. The main issue with the current manuscript is the statistical methodology and reporting. Specific comments follow, but overall there is a lot of loose language in the results (terms like "reasonable" or "close to" are used without definition or qualification), there are no statistical comparisons presented in the results section, and significant differences are stated but not supported. Even if all of these were resolved, however, the underlying statistical approach itself is very weak. I would propose two possible approaches to resolving this problem:

Response: Following the reviewer’s suggestion, we have extensively revised the statistical methodology and the way of reporting. We have deleted the loose languages such as “reasonable”. In results section, we have provided more detailed data supporting significant differences in statistical comparison.

a. The first would be to re-frame this manuscript as a case-report. With only 8 animals it is a small sample appropriate for this format and narrative descriptions of the cases themselves (as the authors have presently in the results) more acceptable. The narratives in this case should be expanded to include all of the animals in each group (as opposed to reporting on a single animal as the authors currently do in the results), and group-wise summary measures reported where possible. Comparisons between the groups should still be made. If this is the approach taken, the authors should strictly limit conclusions drawn to "feasibility" and should not draw any specific safety or performance conclusions in the manuscript.

b. The second option (which could result in a stronger publication), would be to revise the statistical methodology to a more robust reporting for this type of study. Reporting of at least Varvel's criteria would be my suggestion (PMID 1588504). For examples of practical application of this approach in published studies see PMID 28368936 or PMID 16931977. If the authors revise their analysis of the performance in this manner, they may be able to make more performance-related conclusions from this work, which would obviously be stronger. All 8 animals could be included in an overall assessment, but obviously with the two different subgroups it would be better to assess each group individually (else why bother having the subgroups).

Response (continued): We adopted the second option in the revised manuscript. The precision of control of mean arterial pressure (AP) and cardiac output (CO) by our system was assessed by analyzing performance error (PE) as reported previously (PMID 1588504) (PMID 28368936). However, the way of application was minorly changed from the previous study. In the previous study, PE calculations were applied to data from start to end of the closed-loop control of AP,
since the previous system is required to maintain AP noted at the start of the control (PMID 28368936). On the other hand, our system is required to substantially increase and restore AP and CO to their respective target values. Therefore, PE calculations were applied to data from 1 h to the end of the closed-loop control of AP and CO. In addition, we analyzed “response time” to evaluate how fast our system restored AP and CO. To explain these points, we rewrote “Data analysis and Statistics” in “Methods” section in the revised manuscript as follows,

“Data analysis and Statistics” in “Methods” section (Line 8, Page 11)

To evaluate the rapidity of the control of AP and CO by the new system, we calculated response time required for AP and CO to reach respective acceptable ranges, which were defined as AP*-5 mmHg, i.e. 65 mmHg, for AP [1] and CO* - 10 ml·min⁻¹·kg⁻¹ for CO, respectively. To evaluate the precision of the control of AP and CO by the new system, we analyzed performance error [21, 22]. In each animal, the following parameters were calculated for AP and CO: (1) percentage performance error (PE; defined as the difference between each measured value and the target value, expressed as a percentage of the target value); (2) median performance error (MDPE; defined as the median of all values of PE); (3) median absolute performance error (MDAPE; defined as the median of the absolute values of PE |PE|); (4) wobble (a measure of the variability of PE around MDPE, calculated as the median value of the differences between each value of PE and MDPE); and (5) divergence (a measure of the trend of change in |PE| with time). Derivation of these parameters has been described previously [21, 22]. Since a steady state was reached within 1 h after activation of the system, PE were determined every minute from 1 to 4 h after activation.

Response (continued): We reported data of the performance error analysis in “Results” section, where time traces of absolute performance error (|PE|) were newly shown in Fig. 2d, 3d, 4d, 5c, and 5d. We compared PE parameters between the groups, and summarized them in Table 2 in the revised manuscript.

Please review “Results” section, Fig 2, 3, 4, and 5, and Table 2 in the revised manuscript.

Specific comments:

P6L58 - P7L4 - These rules appear to be "on/off" rules rather than "adjustments" to ongoing rates. The authors need to report what portion of operation time in the cases the system was infusing fluid, what the total volume infused for each animal was, what triggered infusion in the animals at what point. Overall reporting on this dimension of the system is almost non-existent.
Response: Following the reviewer’s suggestion, we newly indicated “on/off” status of the infusion of fluid (Ringer’s acetate, RiA) in Fig. 2a, Fig. 3a and Fig. 4a to report what portion of operation time in the cases the system was infusing RiA.

Please review Fig. 2, Fig. 3 and Fig. 4 in the revised manuscript.

Response (continued): Data at 4 h in each time trace of accumulated volume of RiA infused in Fig. 2a, 3a, and 4a indicate the total volume infused. We explained this, and added data of the total volume of RiA infused in each animal of Fig. 2 and 3, and the median of the total volume infused in the 8 animals in “Results” section in the revised manuscript as follows.

Please review Fig 2, 3, and 4.

Second paragraph in “Results” section (Line 1, Page 14)
Data of the cumulated volume of RiA infused at 4 h indicates total volume of RiA infused in this animal (75 ml·kg\(^{-1}\)).

Third paragraph in “Results” section (Line 10, Page 14)
Total volume of RiA infused was 80 ml·kg\(^{-1}\).

Fourth paragraph in “Results” section (Line 19, Page 14)
Total volume of RiA infused was 77 (64-89) ml·kg\(^{-1}\).

Response (continued): To make it clear what triggered infusion in the animals at what point, we briefly described the generation of the command of drug infusion by the control computer in the revised manuscript as follows.

“Hemodynamic Data acquisition, processing, and command generation” in “Methods” section (Line 23, Page 10)

If the feedback loops were closed, simultaneously with the analogue to digital signal conversion, i.e. every 5 ms, Computer 1 calculated the infusion rate of NA using Ki and Kp, determined the on/off status of the RiA infusion at 15 ml·min\(^{-1}\) based on the ”if-then” rules, and sent command signals to the pumps.
Response (continued): RiA infusion was triggered when the subject’s stressed blood volume (V) was less than the target blood volume (V*). We briefly explained this in “Results” section as follows.

Second paragraph in “Results” section (Line 22, Page 14)

Until about 30 min after activation of the system, RiA infusion was continuously activated, since V was lower than V*. Once V was controlled to V*, the status of RiA infusion was changed between “on” and “off” so that V was maintained at V*.

P7L34 - rather than "make the system clinically feasible", I believe the authors intend "test the clinical feasibility"?

Response: Thank you for your suggestion. We have rewritten the sentence following your suggestion in the revised manuscript.

Second paragraph in “Animals” in “Methods” section (Line 13, Page 7)

In 4 dogs (group A), AP and CO were measured invasively. In the other 4 dogs (group B), AP and CO were measured less invasively to test the clinical feasibility of the system [11].

Statistical methodology - using mean and SD to report and ANOVA to test in n=8 (or actually n=4 in two different groups) animals is too few data points to assume the normality of the distribution of the sample mean. The variables should be tested for normality using something like Shapiro-Wilk if mean ± SD and ANOVA are to be used. Otherwise (or if the distributions are shown to violate normality by Shapiro-Wilk), non-parametric reporting with median & quartiles is preferred and repeated measures testing would be by the Friedman test.

Response: Thank you for your suggestion. The Shapiro-Wilk test indicated non-normal distribution in some data sets of hemodynamic variables and parameters. We therefore reported all the data as median and interquartile range in the revised manuscript. We also used non-parametric tests for comparisons between different groups and also among different time points in the revised manuscript. We rewrote “Data analysis and Statistics” in “Methods” section in the revised manuscript as follows,

“Data analysis and Statistics” in “Methods” section (Line 23, Page 12)
Group data are expressed as median (interquartile range). The level of statistical significance was defined as P < 0.05. Mann-Whitney U test was used to compare parameters between different groups. Friedman test followed by the Wilcoxon signed rank test was used to compare variables or parameters among 3 time points (Baseline, Shock, and Resuscitated), where a Bonferroni correction was applied to maintain □ at 0.05 such that the significance criterion was P < 0.0167 (0.05/3) [23]. We used the non-parametric Spearman correlation coefficient to examine associations between variables, and interpreted the correlations coefficients using Cohen’s conventions [23] (.10 small, .30 moderate, .50 large). To determine 99% confidence interval (CI) of the difference between variables, we used a bootstrap technique (1000 replicates) [17, 24]. Statistical analyses were performed using commercially available software (Statistica, Statsoft, Inc., Tulsa, OK, USA).

Results

P12L11 - "decreased significantly". Here and throughout results, please provide numerical data to support any report of "significance". This should include median and 25th, 75th quartiles (assuming non-parametric reporting) for both groups being compared (in this case before & after induction of sepsis), and a specific p-value. Ideally the 95% confidence interval of the difference between the groups would be included as well for robust reporting.

Response: Following the reviewer’s suggestion, we provided numerical data in the main text in Results section and also in Table 1 to support any significance in the revised manuscript. All group data were expressed as median (interquartile range). In Table 1, we provided specific P-values for each statistical comparison. In main text, we provided specific P-values only in case of statistically significant difference. Since significant P-value was corrected by a Bonferroni correction, we determined 99%, instead of 95%, confidence interval (CI) of the difference between variables with use of a bootstrap technique (1000 replicates).

First paragraph in “Results” section (Line 3, Page 13)

Intravenous LPS induced endotoxin shock in all the 8 animals (Baseline vs Shock in Table 1). After induction of endotoxin shock, AP decreased significantly from 95 (91-108) to 43 (39-45) mmHg (99% CI of difference, -81 to -40 mmHg; P=0.012), CO decreased significantly from 112 (104-142) to 62 (51-73) ml·min⁻¹·kg⁻¹ (99% CI of difference, -96 to -41 ml·min⁻¹·kg⁻¹; P=0.012), while heart rate increased significantly from 115 (111-140) to 161 (141-176) bpm (99% CI of difference, 17-71 bpm; P=0.012). As for the cardiovascular parameters, V decreased significantly from 22 (17-38) to 11 (10-22) ml·kg⁻¹ (99% CI of difference, -27 to -6 ml·kg⁻¹; P=0.012), S decreased significantly from 52 (38-55) to 27 (18-31) ml·min⁻¹·kg⁻¹ (99% CI of difference, -36 to -12 ml·min⁻¹·kg⁻¹; P=0.012), while R did not change significantly. Blood
lactate level increased significantly from 1.8 (1.6-1.9) to 3.0 (2.5-3.5) mmol·L⁻¹ (99% CI of difference, 0.6-2.1 mmol·L⁻¹; P=0.012), Ht increased significantly from 30 (29-31) to 43 (42-45) % (99% CI of difference, 11-16%; P=0.012), while SaO₂ decreased significantly from 99 (99-100) to 98 (95-98) % (99% CI of difference, -5.2 to -0.7%; P=0.012). In group B, SvO₂ did not change significantly.

Fourth paragraph in “Results” section (Line 4, Page 15)

At 4 h of hemodynamic resuscitation, AP and CO were significantly increased to 70 (69-71) mmHg (99% CI of difference, 22-36 mmHg; P=0.012) and 130 (125-138) ml·min⁻¹·kg⁻¹ (99% CI of difference, 54-97 ml·min⁻¹·kg⁻¹; P=0.012), respectively, but blood lactate level, Ht and SaO₂ were not significantly different from those observed before resuscitation (Shock vs Resuscitated in Table 1).

P12L24 - P13 - These two paragraphs describe individual cases (one animal from each group). This would be borderline if the authors intended to submit a "case report", but these paragraphs do not constitute statistical reporting of the results of an experiment. Please instead provide statistical summaries (medians & quartiles) of each group of four animals, and statistical comparisons where appropriate.

Response: Following the reviewer’s suggestion, we provided Table 2 summarizing performance error (PE) calculations for all the animals in the revised manuscript. PE parameters were also summarized for each group of A and B. Results of the statistical comparisons between group A and B by Mann-Whitney U test were also described in 5th paragraph in “Results” section in the revised manuscript.

Please review Table 2 in the revised manuscript

Fifth paragraph in “Results” section (Line 20, Page 15)

To highlight the precision of control of AP and CO, time courses of |PE| in AP and CO from 1 to 4 h after activation of the system are shown for each animal in group A (Fig. 5c) and B (Fig. 5d). There were no statistically significant difference in any of the PE parameters for AP and CO between group A and B (Table 2).

P13L7-41 - This paragraph is now reporting some summary measures better, but there are still many uses of vague terminology like "reasonable accuracy and stability" (P13L26) that are
undefined and unsupported by any tests or measures. What did the authors define "reasonable" accuracy?

Response: As responded to the major comments, throughout the revised manuscript, we have deleted the loose languages “reasonable” to avoid confusions.

No comparisons between group A and group B are reported. No statistical test results are reported in the results.

Response: Following the reviewer’s suggestion, we reported and compared the control performance of our closed-loop system between group A and B. In Fig. 5 in the revised manuscript, we newly focused on the control performance of our system. The parameters evaluating the performance were summarized in Table 2. Exact P-values were reported for each comparison in Table 2. Results of the statistical comparisons between group A and B using Mann-Whitney U test were also reported in 5th paragraph in “Results” section in the revised manuscript.

Please review Table 2 in the revised manuscript

Fifth paragraph in “Results” section (Line 10, Page 15)

There were no significant differences between group A and B in time-averaged infusion rate of NA over the period of 4 h [group A: 0.8 (0.6-1.2) μg·kg-1·min-1 versus group B: 0.7 (0.5-1.1) μg·kg-1·min-1], and in total volume of RiA infused [group A: 71 (58-88) ml·kg-1 versus group B: 84 (74-89) ml·kg-1]. To highlight the rapidity of control of AP and CO, time courses of AP-AP*, and those of CO-CO* during 1st h of closed-loop control are shown for each animal in group A (Fig. 5a) and B (Fig. 5b), where response times were the durations for AP and CO to reach the horizontal broken lines. Response times were less than 60 min, except in two animals, in CO control in group A (blue line in Fig. 5a, 75 min) and in AP control in group B (green line in Fig. 5b, 131 min). There were no statistically significant difference in response times of AP and CO between group A and B (Table 2). To highlight the precision of control of AP and CO, time courses of |PE| in AP and CO from 1 to 4 h after activation of the system are shown for each animal in group A (Fig. 5c) and B (Fig. 5d). There were no statistically significant difference in any of the PE parameters for AP and CO between group A and B (Table 2).

Discussion
Again, the authors use vague terminology like "stably and accurately" and "showed good performance", but these terms and the criteria used to establish them are not defined in the manuscript. See comments above about evaluating performance, also.

Response: Following the reviewer’s suggestion, we did not use the vague terminology in the revised manuscript. Instead, in the paragraph, we evaluated the performance of our system based on the SSC guidelines and the previous reports on PE as follows.

First paragraph in “Discussion” section (Line 3, Page 17)

To the best of our knowledge, we are the first to succeed in automated closed-loop control of hemodynamic resuscitation in endotoxin shock. Rapidity of control of AP by this system satisfies the SSC guidelines, which recommend that AP should be recovered to more than 65 mmHg within the initial 6 h of hemodynamic resuscitation [1]. Rapidity of control of CO by this system seems acceptable in that CO was restored to baseline level within 4 h after activation of the system. Precision of control of AP and CO were evaluated by the PE parameters (Table 2). MDAPE in AP and CO were 2.5 and 2.4%, respectively, which are smaller than that reported previously in closed-loop hemodynamic control by other groups [22, 25]. Other PE parameters of AP and CO were comparable to or smaller than those reported previously [22, 25]. Furthermore, even when the system was modified to a less invasive, clinically feasible version, rapidity and precision of control of AP and CO were not worsened. In this study, although we observed performance of this system during 4 h period, the period may be extended without difficulty in clinical settings until the infection causing sepsis is resolved. This system may be a powerful clinical tool in rescuing patients with septic shock.

The authors have not discussed weaning in the present manuscript. If this work was done previously it should be cited (the cited reference appears to be from a different group).

Response: We did not systematically evaluate the weaning of drug infusion in this study, since it would require observation period far longer than 4 h. The cited reference (Critical Care 2008;12:R155) demonstrated that the weaning of vasopressor infusion took on average about 29 h. However, in principle, the negative feedback mechanisms used in our system automatically quit drug infusions once they are no longer required. Indeed, in two animals, infusion rate of NA was temporarily increased more than 0.5 μg/kg/min, but was reduced to zero by the end of the period of 4 h. We reported on the two animals in “Results” section, and discussed these points in “Clinical perspective” in “Discussion” section in the revised manuscript as follows.

Fourth paragraph in “Results” section (Line 17, Page 14)
In two animals, infusion rate of NA was temporarily increased more than 0.5 μg·kg⁻¹·min⁻¹, but was reduced to zero by the end of the period of 4 h.

First paragraph in “Clinical perspective” in “Discussion” section (Line 18, Page 21)

Our system may be used for early hemodynamic resuscitation as well as for weaning from hemodynamic support. No closed-loop control systems for early resuscitation of septic shock have been reported. Only one clinical trial reported that closed-loop control for weaning from NA infusion in septic patients has beneficial effects on clinical outcomes [8]. We did not systematically evaluate the weaning of drug infusion in this study, since it would require observation period far longer than 4 h. In the clinical trial [8], the weaning from NA infusion took more than 24 h. However, in principle, the negative feedback mechanisms used in our system automatically quit drug infusions once they are no longer required. Indeed, we observed that NA infusion was quitted by the end of 4 h period in 2 out of the 8 animals.

Table 1 - The exact p-values should be reported for each comparison. How much volume (total) was given during the resuscitation? Moreover, there are two distinct subgroups being combined in this table; what were the differences? Why are they being grouped all together?

Response: We newly reported the exact P-values for each comparison in Table 1, and also in Table 2. We have reported how much volume was given as responded above. As introduced in “Background” section, primary objective of this study was to evaluate our new system developed by extending previously reported closed-loop systems (J Appl Physiol. 2006;100:1278-86.)(IEEE Trans Biomed Eng. 2016;63:1699-708.). In this study, we made group B to confirm that performance of the closed-loop control was not worsened even if AP and CO were measured less invasively. These are why the two groups were combined in Table 1 and in Fig. 4, while the performance of the closed-loop control of AP and CO were compared between the two groups in Table 2 and Fig. 5.

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Response: We thank again Dr. Rinehart very much for his extensive review of our manuscript and highly constructive comments.

Michael Kinsky (Reviewer 3):

BANE-D-17-00124
Computer-controlled closed-loop drug infusion system for automated hemodynamic resuscitation in septic shock

Uemura and authors demonstrate that acute LPS-induced shock can be successfully resuscitated with the use of autonomous closed-loop devices in an experimental animal preparation. The authors track various physiological parameters through the course of a presumed hyperdynamic and hypovolemic shock conditions and influence these parameters through the administration of vasopressors and fluid. They use two treatment groups to demonstrate that their resuscitation approach can work with less invasive input parameters. While the overall study design is quite elegant and elaborate, and the presented capabilities of the closed-loop system represent an exciting next step in the field of automated resuscitation, the authors fall short of taking full advantage of the data they gather, specifically interpretation. Given the changes in Frank Starling slope there seems to be clear evidence of acute cardiac depression, in addition to afterload and preload alterations as in a situation of endotoxin-induced shock; this finding is not appreciated enough by the author group in the discussion of their findings, let alone addressed by their experimental approach itself, as this would need to introduce inotrope drugs to the model as an additional variable. Additionally, other parameters are not discussed despite having the appropriate experimental design e.g., pulmonary artery pressure / right heart function. Substantial changes are necessary to make this interesting contribution ready for publication.

Response: We thank Dr. Kinsky for his detailed review of our manuscript and constructive comments.

Comments:

- How is CO calculated/monitored in group B? Publication 17 indicates that CO measurement needs calibration with COref, which could not have been obtained without the aortic flow probe in group B. Please comment.

Response: In group B, we measured thermo-dilution CO (COTD) with use of the pulmonary artery catheter (PAC) at baseline condition, at endotoxin shock, every 1 h during system activation (the data were not provided in our original manuscript). However, COTD was not used for initial calibration, since routine use of PAC is not recommended by SSC guideline in patients with septic shock (Crit Care Med. 2013;41:580-637.). Correlation between CO measured less invasively and COTD was significant with “large” Spearman correlation coefficient. Percentage error, an index of absolute accuracy, between CO monitored by our system and COTD was 27% (<30% is recommended, Anesthesiology 2010; 113:1220–35.). In this study, we did our best in measurements of aortic cross sectional area, and aligning the ultrasound Doppler beam along the aortic flow. Such precise presetting may be mandatory when our less invasive CO measurements
are applied to patients with difficulty in the initial calibration with reference CO. We newly added details of measurements of CO and COTD to “Methods” section, comparison between CO and COTD to “Results” section, and briefly discussed these points in the revised manuscript. Since presentation and analysis of the data was performed non-parametrically following the suggestion of Reviewer 1, we did not include the results of the percentage error calculation because it assumes normal distribution of the variables.

Second paragraph in “Echocardiography” in “Methods” section (Line 9, Page 9)

In group B, the ascending aortic cross-sectional area was measured from the parasternal short-axis image. Flow velocity of the ascending aorta was acquired using continuous wave Doppler technique (emitting frequency, 3.3 MHz) with the transducer directed from the apex and held using a mechanical arm [18]. Audio output signal encoding Doppler-shifted frequency was output continuously from the echocardiography system [18]. Analog signals of the audio output, AP, and ECG were digitized (20 kHz, 16-bit) by a laboratory computer (Computer 2) (ME-B, NEC, Tokyo, Japan), and analyzed on-line to compute CO with use of the aortic cross-sectional area as reported previously [18]. CO was continuously output from Computer 2 to Computer 1.

Second paragraph in “Preparation” in “Methods” section (Line 20, Page 8)

A pulmonary artery catheter (6F) (T173HF6, Edwards Lifesciences, Irvine, CA) was positioned in the pulmonary artery via the right jugular vein, and used to sample mixed venous blood, and to measure thermo-dilution CO (COTD).

Third paragraph in “Experimental Protocols” in “Methods” section (Line 3, Page 11)

In group B, we measured COTD by thermodilution using the pulmonary artery catheter at baseline, after the induction of endotoxin shock, and every 1 h during the activation of the system [11].

Please review Fig. 6 in the revised manuscript

Final paragraph in “Results” section (Line 1, Page 16)

In Group B, CO measured less invasively by our system and COTD were significantly correlated with large Spearman correlation efficient (Fig. 6).
In group B, CO measured less invasively showed significant correlation with COTD. Our previous study [18] indicated that an initial calibration with some reference method is desired for absolute accuracy of the less invasive CO measurement. However, routine use of the pulmonary artery catheter is not recommended in patients with sepsis [1]. Precise presetting including measurements of aortic cross-sectional area, and aligning the ultrasound Doppler beam along the aortic flow may be mandatory when our less invasive CO measurements are applied to patients with difficulty in the initial calibration with reference CO.

- Estimation of PWP from CVP is an approximation. The one-time estimation does not take into account changes in PWP during the experiment. As clearly the authors show an incredible amount of cardiovascular dynamics.

Response: In our system, PWP was estimated from central venous pressure (CVP) and the ratio of tissue-Doppler tricuspid/mitral annular velocities. The Doppler velocity ratio was calibrated once, while CVP was continuously acquired for PWP estimation. PWP estimation by our method may be flawed in case of measurement error of CVP, or changes in the velocity ratio. As responded below, we did not systematically measure PWP in the present study. PAC was instrumented only in dogs in group B, where PAC was used for the sampling of mixed venous oxygen saturation and to measure COTD. As responded to later comments, further studies on this respect is required.

- The term septic shock is not equivalent to LPS-induced shock. Please clarify.

Response: As the reviewer correctly indicated, there are several concerns that the infusion of endotoxin by itself does not completely simulate sepsis/septic shock. Caution is needed in assessing the clinical efficacy of novel sepsis treatments in animal models of endotoxemia. To limit application of the present findings, we rewrote the title of the revised manuscript. We newly addressed this issue in the limitation section of the revised manuscript as follows.

Title of the revised manuscript (Line 1, Page 1)

Computer-controlled closed-loop drug infusion system for automated hemodynamic resuscitation in endotoxin-induced septic shock

“Limitation” in “Discussion” section (Line 5, Page 22)
Endotoxin administration is commonly used in animal models of sepsis, since endotoxin under some circumstances plays an important role in the pathogenesis of sepsis [36, 37]. However, there are several concerns that the infusion of endotoxin is not a suitable model with which to simulate sepsis/septic shock. Time course of canine endotoxin shock is generally different from human sepsis, with animals more often showing rapid onset of circulatory collapse [36, 37]. Although gram-positive bacteria are detected as causative organisms as frequently as gram-negative ones in patients with septic shock [2, 3, 8], endotoxin is released only by gram-negative bacteria, but not by gram-positive ones. The use of corticosteroids and anti-TNF-α has been effective in animal models of endotoxemia, but has failed in clinical trials [36, 37].

- Provide more detail regarding the increase in Hct? Should the spleen have been removed during the extensive preparation procedures as well or blood volume be calculated differently? For example, why not use indocyanine green or radio-isotopes? While on one calculation, a Hct increase from 30% to 41% could represent a vascular volume contraction of > 25%; as mentioned release of red cell mass from spleen and liver is also plausible. This reviewer has difficulty in reconciling the Hct at the end of the study [39%] along with volume repletion. Other mechanisms need to explored and/or discussed in limitations.

Response: We thank the reviewer for thoughtful suggestions. In the present animal model of endotoxin shock, hematocrit levels remained elevated (39%) at the end of hemodynamic resuscitation. As discussed in the original manuscript, low plasma expanding capacity of the Ringer solution and plasma leakage enhanced by endotoxin were two possible mechanisms for the elevated hematocrit level. Other possible mechanisms include enhanced recruitment of red blood cells into the systemic circulation from the spleen stimulated by endotoxin itself and/or by noradrenaline used for resuscitation. To unveil the mechanisms of persistent elevation of hematocrit level, additional preparations as splenectomy, and/or evaluations of plasma volume/total blood volume using the dye-dilution methods may be required. However, that is not the scope of this study. We rewrote the 4th paragraph of “Discussion” section to briefly discuss these points in the revised manuscript as follows.

Fourth paragraph of “Discussion” section (Line 19, Page 19)

Second, endothelial damage induced by endotoxin may increase plasma leakage [29], and consequently require continuous compensation from RiA infusion to maintain V at its target value. This speculation is not contradictory to the observation that Ht, an indirect marker of plasma leakage [29], increased after endotoxin injection, and did not recover to baseline level even after fluid supplementation. The persistent elevation of Ht might be attributable to other mechanisms such as enhanced recruitment of red blood cells from spleen stimulated by
endotoxin, or by infused NA [30, 31]. However, unveiling the mechanisms of the elevation of Ht is not the scope of this study.

- No control group receiving some sort of manual resuscitation is presented in this experiment, which is ok for a proof-of-concept but will be needed in the future to make inferences on how the approach compares clinically to established practice.

Response: Following the reviewer’s suggestion, we rewrote 6th paragraph in “Discussion” section in the revised manuscript as follows.

Sixth paragraph of “Discussion” section (Line 1, Page 20)

In this study, we did not compare the efficacy of closed-loop control of hemodynamic resuscitation by our system with that of manual control by care providers as was done previously [27]. The reason is that this system is not intended to replace care providers, but is intended to be used under supervision by care providers. However, in future, comparison of the closed-loop hemodynamic control by our system with the manual control by the providers will be required to make inferences on how our approach compares to clinically established practice.

- Is there a better way to produce the figures? The main focus should be on the time between shock induction and reaching steady state resuscitation, there is little information thereafter that is not discussed with the mention of the small deviations from target in the results section. Maybe focus the graphs on that first period.

Response: Following the reviewer’s suggestion, we newly added Fig. 5 to the revised manuscript. Fig. 5 demonstrates time courses of AP and CO during the first hour of hemodynamic resuscitation, and how fast these variables were recovered to their respective acceptable ranges.

Please review Fig. 5 in the revised manuscript

Fifth paragraph in “Results” section (Line 13, Page 15)

To highlight the rapidity of control of AP and CO, time courses of AP-AP*, and those of CO-CO* during 1st h of closed-loop control are shown for each animal in group A (Fig. 5a) and B (Fig. 5b), where response times were the durations for AP and CO to reach the horizontal broken lines. Response times were less than 60 min, except in two animals, in CO control in group A
(blue line in Fig. 5a, 75 min) and in AP control in group B (green line in Fig. 5b, 131 min). There were no statistically significant difference in response times of AP and CO between group A and B (Table 2).

- Cardiac determinants of CO [afterload/preload/contractility] are not accounted for in the model. Please discuss.

Response: In our framework of circulatory equilibrium, the Frank-Starling slope of the left ventricle (S) comprehensively accounts for cardiac determinants of CO. We previously reported that the parameter S is analytically related with the left ventricular end-systolic elastance (Ees, an index of contractility) and arterial resistance (R, an index of afterload) as follows,

\[ S = \frac{Ees}{k/(Ees/HR+R)} \]

Where HR is heart rate and k is diastolic myocardial stiffness. In our framework of circulatory equilibrium, the stressed blood volume (V) expresses preload, which is “vascular” determinant of CO. We briefly discussed these points in association with the present findings in 5th paragraph in “Discussion” section in the revised manuscript as follows.

Fifth paragraph in “Discussion” section (Line 10, Page 19)

Although S was not selected as a control parameter, S also increased after system activation (Fig. 2b, 3b, and 4b). S is related to R, left ventricular end-systolic elastance (Ees, an index of LV contractility), heart rate (HR) and diastolic myocardial stiffness (κ) by the following formula [10, 11, 13],

\[ S = \frac{Ees/\kappa}{(Ees/HR+R)} \]

This formula suggests that increase in S observed after system activation was probably due to enhanced cardiac contractility, Ees, through beta-adrenergic stimulation by NA [16] and reduced R accompanying RiA infusion [33].

- The authors placed a pulmonary artery catheter - which can provide PAOP [PWP]; how did PWP calculated compare to PWP measured. Furthermore, LPS infusion is noted to increase thromboxane and other mediators that greatly influence pulmonary artery pressure [PAP] - what was the PAP before, during and after the LPS infusion? Did it dramatically increase? Did NA infusion make it worse? Was there evidence of right heart dysfunction? Could this
explain the cardiac dysfunction apparent in your model - evidence by increased filling pressures / lower stroke volume?

Response: In all the dogs in the present study, we estimated PWP using CVP and the Doppler indices, but did not systematically measure PAOP [actual PWP]. PAC was instrumented only in dogs in group B, where PAC was used primarily for the sampling of mixed venous oxygen saturation and COTD. However, concerns addressed by the reviewer is relevant with our technique to estimate PWP. LPS has been reported to change the mechanical properties of pulmonary artery and vein. This can adversely affect the reliability of the technique. We briefly addressed these concerns in 1st paragraph in “Limitation” section in the revised manuscript as follows.

First paragraph in “Limitation” section (Line 23, Page 22)

We estimated PWP with use of the previously developed technique [17], which uses CVP and the ratio of the tissue-Doppler tricuspid to mitral annular velocities. However, the accuracy of this technique has not yet been confirmed in subjects with endotoxemia. Endotoxin has been shown to change the mechanical properties of the pulmonary artery and vein [35]. This can adversely affect the reliability of our PWP estimation technique. Further studies on these respects are required in future.

- Please discuss, in limitations, the interaction of fluid infusion and NA - as both models use CO as primary variable. Additionally, fluid could also reduce afterload [especially crystalloid]; which could induce an erroneous feedback loop with NA.

Response: We thank the reviewer for the thoughtful suggestions. The interaction of fluid infusion and NA may induce malfunction of the system, not because CO is used to derive target parameters such as arterial resistance and stressed blood volume, but because the input of one loop (fluid infusion) can reduce feedback gain in another loop (R in response to NA). Indeed, in all the animals, we observed reduction in systemic arterial resistance (R) early after the activation of our system (Fig. 2, 3, 4), when the Ringer solution was infused at maximum speed, but infusion rate of NA was minimum and being gradually increased. This early reduction in R was most likely induced by hemodilution accompanying the infusion of the Ringer solution as suggested by the reviewer and as reported previously (Intensive Care Med. 2015;41:1247-55.). This interaction between the two feedback loops may induce control divergence, where NA infusion rate may be increased without limit. Fortunately, in this study, we did not observe such malfunction. However, this may become problem when our system is applied to subjects with extremely low sensitivity to NA. Following the reviewer’s suggestion, we discussed these points in 5th paragraph in “Discussion” section and also in “Limitation” section as follows.
Fifth paragraph in “Discussion” section (Line 6, Page 19)

Early after system activation, R initially decreased in all the animals (Fig. 2b, 3b, and 4b), when infusion rate of NA was minimum and being gradually increased (Fig. 2a, 3a, and 4a). This early reduction in R was most likely induced by hemodilution accompanying RiA infusion [33]. Thereafter, R recovered gradually and was controlled at the target value by NA infusion.

Third paragraph in “Limitation” section (Line 15, Page 22)

Infusion of RiA reduced R early after system activation. This can be an adverse interaction between the two feedback loops (Fig. 1a), where the input of one loop (RiA) can reduce feedback gain in another loop (R in response to NA). This may cause system malfunction, where NA infusion rate may be increased infinitely. Fortunately, in this study, we did not observe such malfunction. However, this may become problem when our system is applied to subjects showing R with extremely low sensitivity to NA.

- Please mention that one-time LPS-infusion is not an indisputable model of septic shock and that there is criticism regarding its accuracy in simulating actual septic shock.

Response: As responded to the earlier comments, we rewrote the title of the revised manuscript, and newly discussed the limitation of using the LPS-injected animals to completely simulate the septic shock seen in clinical practice.

- "By using this system to automatically support the macro-circulatory status, care providers may be able to spend more time on patient-specific treatments to improve micro-circulation, thereby potentially improving patient outcomes." How would caretakers specifically spend time on improving microcirculation? Please elaborate or delete this sentence. The general notion that time and resources could be saved is enough in the reviewers opinion.

Response: Following the reviewer’s suggestion, we deleted the sentence indicated by the reviewer from the revised manuscript.

- Finally, it is difficult to state that this model was effectual in resuscitation as lactate remained elevated - please discuss the value of this endpoint as a global index of resuscitation and why lactate was not restored to basal levels? Additionally, the infusion of
fluid seemed to linear - while this may have been what the model predicted; it does not seem to be clinically apparent and oversimplified. Please provide the total amount of fluid in mL/kg used for resuscitation.

Response: We have discussed the value of normalization of lactate as an endpoint in the 2nd paragraph in “Clinical perspective” in “Discussion” section. Indeed, in the dogs in this study, lactate remained elevated at 4 h post resuscitation, and was not normalized to basal levels. SSC guidelines recommend normalization of lactate levels in initial resuscitation (Crit Care Med 2013; 41:580–637). However, normalization of blood lactate level is reported to be achieved at 24-48 h post resuscitation in clinical practice (N Engl J Med 2015;372:1301-11.)(N Engl J Med 2014; 371:1496-1506.). Observation time longer than 4 h might be needed to confirm restoration of basal lactate level in the dogs in this study. We discussed these points in the 2nd paragraph in “Clinical perspective” in “Discussion” section in the revised manuscript as follows.

Second paragraph in “Clinical perspective” in “Discussion” section (Line 9, Page 21)

Optimization of macro-circulatory endpoints including AP, CO and global oxygen delivery is an initial step in the hemodynamic resuscitation of patients with septic shock [1]. Our system automates this initial step. The next step of resuscitation is to assess the adequacy of organ perfusion indicated by micro-circulatory resuscitation targets such as optimization of blood lactate concentration. However, optimization of macro-circulation does not necessary guarantee optimization of micro-circulation [34]. Indeed, in animals resuscitated by our system, blood lactate was not normalized despite the achievement of optimization of macro-circulatory endpoints. SSC guidelines recommend that blood lactate level should be normalized in initial resuscitation [1]. However, recent clinical trials in patients with septic shock noted that blood lactate level is not normalized until 24-48 h post resuscitation [2, 3]. In the dogs in this study, duration of the closed-loop control longer than 4 h might be needed to confirm restoration of basal lactate level.

Response (continued): The infusion of fluids seemed to be linear. However, as newly indicated in Fig. 2a, 3a, and 4a, on/off status of the fluid infusion was determined every 5 ms, and adjusted so that the stressed blood volume (V) was precisely restored to its target level. Hence, in our system, fluid infusion is not simplified. Data at 4 h in each trace of the accumulated volume of RIA infused in Fig. 2a, 3a, and 4a indicate the total volume infused. Following the reviewer’s suggestion, we provided the total amount of fluid in mL/kg used for resuscitation in “Results” section in the revised manuscript as follows.

Please review Fig 2, 3, and 4.
Second paragraph in “Results” section (Line 1, Page 14)

Data of the cumulated volume of RiA infused at 4 h indicates total volume of RiA infused in this animal (75 ml·kg⁻¹).

Third paragraph in “Results” section (Line 10, Page 14)

Total volume of RiA infused was 80 ml·kg⁻¹.

Fourth paragraph in “Results” section (Line 19, Page 14)

Total volume of RiA infused was 77 (64-89) ml·kg⁻¹.

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Response: We thank again Dr. Kinsky very much for his extensive review of our manuscript and highly constructive comments.