Journal of Clinical and Translational Research
Peer review process file 05.202005.004

Hemodilution causes glycocalyx shedding without affecting vascular endothelial barrier permeability in rats

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Review timeline

Received: January 7, 2020
Editorial decision: March 4, 2020
Revision received: March 9, 2020
Editorial decision: April 6, 2020
Revision received: April 14, 2020
Editorial decision: April 14, 2020
Published online: May 12, 2020

1st editorial decision

4-Mar-2020

Ref.: Ms. No. JCTRes-D-20-00002
Hemodilution causes glycocalyx shedding without effecting vascular endothelial barrier permeability in rats
Journal of Clinical and Translational Research

Dear PhD Ergin,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript.Also, please ensure that the track changes function is switched on when implementing the revisions. This enables the reviewers to rapidly verify all changes made.

Your revision is due by Apr 03, 2020.

To submit a revision, go to https://www.editorialmanager.com/jctres/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #2: General:
This study aims to elucidate the role of the glycocalyx as a barrier to permeability during shedding of glycans induced by normovolemic hemodilution. The study is well done with a comprehensive spectrum of physiological measurements. It is concluded that shedding of the glycocalyx, as evidenced by increased amounts of circulating syndican-1, heparan sulfate and hyaluronan, is not accompanied by a disruption of the permeability barrier. The measurements of macromolecule retention in the circulation and lack of tissue accumulation of fluid support this conclusion. However, there are some major concerns as to whether or not all parameters that govern fluid balance have been accounted for, as noted in the following.

Specific:
(1) Given that fluid exchange is proportional to the difference between hydrostatic and colloid osmotic pressures, and that MAP falls significantly in almost all cases studied here, it would not be surprising that a loss of the potential for filtration is offset by diminished resistance to transvascular flow through the blood-tissue interface. What assurances do you have that intravascular pressures at the exchange site are not adversely affected by the induced hypotension? Other studies on isovolemic hemodilution have clearly shown a decrease in arteriolar pressures, which is presumably followed by a loss in pressure at the sites of filtration.

(2) Did you measure plasma colloid osmotic pressure during the hemodilution maneuvers? Was that consistent with the fall in arterial pressures?

(3) The control experiments, performed by simply observing the status of the variables you have measured without any sham exchange may not be adequate to ensure that other factors have not been lost which regulate barrier function. It would seem logical to perform control experiments using plasma from donor animals, and separate experiments with the HES and other substitutes seeded with red cells at the initial hematocrit (i.e. no dilution). Have you considered doing so?

(4) Was there any indication that HES was sequestered in the endothelial surface layer and thus serves to replace HS and HA to maintain the barrier function of the glycocalyx?

(5) Have you attempted to pharmacologically inhibit shedding of the glycocalyx with MMP inhibitors or heparanase scavengers during the ANH?

Reviewer #3: The authors investigate the effects of normovolemic hemoddilution on the integrity of the endothelial glycocalyx in an experimental model with rats.
The design of the study is good and the results are interesting.
Two Comments: 1) The authors describe - based on their results, that a degraddation of the endotehilal glycocalyx can occur even without vascular leakage. Already since 2004 it is known, that the endothelial glycocalyx is one component of a "double-barrier" of blood vessels (Endothelial glycocalyx as an additional barrier determining extravasation of 6% hydroxyethyl starch or 5% albumin solutions in the coronary vascular bed. Rehm M, Zahler S, Lötsch M, Welsch U, Conzen P, Jacob M, Becker BF. Anesthesiology. 2004 May;100(5):1211-23. According to this model even a remarkable degraddation of the endothelial glycocalyx can be compensated by the second component of the vascular barrier, the edothelial cells and a resulting tightening of interendothelial gaps. As the results of the present investigation confirm this model, it should be mentioned/cited in the manuscript.

2) The calculation of the plasma volume and intravascular volume have to be presented in the methods section.

Reviewer #4: The authors should provide a better timeline for the experiments. The sequence of injury to the vascular barrier in shock is thought to be glycocalyx then endothelial layer, resulting in increased vascular barrier permeability... Were the experiments carried out long enough to address this ??

This reviewer is uncertain as to the duration of the experiments

the authors should not equate glycocalyx integrity with indirect measurements such as shedding of GC components...for that matter what do the authors attribute as to why these is shedding of syndecan 1 in even the control group

Reviewer #5: The experimental research paper submitted to JCT Res (No. 200002) addresses the clinically relevant question whether acute normovolemic hemodilution (ANH), conducted with different replacement fluids (balanced hydroxylethyl starch, balanced crystalloid or isotonic saline), leads to different extents of disturbance of the endothelial glycocalyx and/or the vascular barrier permeability (VBP). That is, the aim of the study is to determine if one of the chosen fluids provides greater protection against deterioration of the vascular barrier and, thus, to formation of edema in the course of ANH than the others. Groups of 6 rats each per group were subjected to stepwise ANH to hematocrit levels of 35, 25, 20 and, finally, 15%, except for a control group for which there was no blood-letting. The VBP was assessed by searching for edema formation (weighing wet weight vs dry weight of selected organs) and by determining the rate of extravasation of 3 marker macromolecular substances of different molecular size/weight.

Although only a relatively small number of animals per group has been studied here, the work is original and of potential clinical relevance for the fields of intensive care, trauma therapy and surgery. All in all, the paper is well-structured and potentially makes for informative and interesting reading. However, several major concerns need to be expressed after reviewing the work in its submitted form.

Major criticisms.

1. The authors do not provide exact details concerning the duration of the potential disturbance at each level of ANH or on the total duration of the observations. The supplemental Figure 1 (see Methods, section Protocol) does not enhold this information. This information should be added to the Abstract and the Methods and critically discussed (see below).
2. A major concern is whether the (still to be provided) duration of the perturbation incurred by ANH was, at all, principally sufficient to allow development of overt damage to the vascular permeability barrier. In other words, the study fails to bring about those changes encountered in clinical practice in any of the experimental groups. It is, obviously, impossible to detect a beneficial effect of one fluid over another if there is no overt damage. A positive damage group must be established by intensifying the extent of the perturbation ensuing from ANH in the study.
3. The data suggest that some shedding of the glycocalyx was incurred by ANH, but that this was not accompanied by detectable increases in vascular permeability (tissue edema, extravasation of tracer substances). The authors should discuss this result in the context of the "double barrier" formed by the glycocalyx and the endothelial cells. According to the "double barrier concept", expressed, e.g., by M. Rehm et al. (Anesthesiology 2004), both the endothelial cells and the glycocalyx need to be perturbed before the vascular permeability barrier is seriously breached, i.e., before edema rapidly develops (see also Discussion, p. 16, 2nd paragraph).
4. P. 12, § 3.4.1, Fig. 4: Can you explain why there was less extravasation of Texas-Red 40kD tracer in all the ANH groups than in the Controls? This result is rather confusing. Why does the barrier become tighter in ANH? The "explanations" proffered to this aspect in the Discussion on p. 16 are not really convincing or helpful. Also, please define how the "Ratio" was established (last value S6 / value at 2 min after injection ??).
5. P. 17, lines 05-09: The submitted data do not "show" that excess volume of applied crystalloids might accumulate in the intestine or in lymphatic vessels.
6. Generally, major corrections must be performed concerning the quality of the language in the text and in correcting grammatical errors. There is a complete lack of syntax in the English in some parts of the text (see, e.g., p.6 -7, §2.7, first paragraph, and again p.7, sentence on lines 29-32; p. 16-17, sentence on lines 59-05).

Minor criticisms.

1. P6. § 2.6.1, last sentence: The times of withdrawal of blood samples for measuring of tracer levels given here do not comply with the sampling protocol (S1-S6) depicted in Supplemental Fig. 1 (p. 5).
2. P6, §2.6.2: Was surface water dried from the sampled organs prior to determining wet weight? Especially in the case of the heart, was intraventricular fluid sponged away before weighing? In this light, the identical ratios given for wet/dry weight of heart and lung tissue (Supplemental Fig. 3) are totally unbelievable.

3. Some abbreviations in the legend to the Table 1 are inappropriate: FABF is not needed, whereas CVP is missing.
4. P9, Text lines 29-32: bicarbonate levels are not provided in Table 1
5. P. 11, lines 36-39: The statement "syndecan-1 levels were significantly higher than at baseline" does not hold for the Control group.
6. P.16, lines 22-24: microcirculatory disturbance was only evident in the NaCl-group.

Author's rebuttal

Response to the reviewers

Reviewer #2:

General:

This study aims to elucidate the role of the glycocalyx as a barrier to permeability during shedding of glycans induced by normovolemic hemodilution. The study is well done with a comprehensive spectrum of physiological measurements. It is concluded that shedding of the glycocalyx, as evidenced by increased amounts of circulating syndican-1, heparan sulfate and hyaluronan, is not accompanied by a disruption of the permeability barrier. The measurements of macromolecule retention in the circulation and lack of tissue accumulation of fluid support this conclusion. However, there are some major concerns as to whether or not all parameters that govern fluid balance have been accounted for, as noted in the following.

Specific:

(1) Given that fluid exchange is proportional to the difference between hydrostatic and colloid osmotic pressures, and that MAP falls significantly in almost all cases studied here, it would not be surprising that a loss of the potential for filtration is offset by diminished resistance to transvascular flow through the blood-tissue interface. What assurances do you have that intravascular pressures at the exchange site are not adversely affected by the induced hypotension? Other studies on isovolemic hemodilution have clearly shown a decrease in arteriolar pressures, which is presumably followed by a loss in pressure at the sites of filtration.

Answer: We thank to the reviewer for this interesting observation. We agree with the reviewer comment about the given fluids that have a proportional difference of osmotic and colloidal pressure leading to hypotension and reduction of vascular resistance. However, in this study, we also showed that type or composition of the given fluids did not increase the vascular barrier permeability even though presence of hypotension (low hydrostatic pressure) or differences of plasma colloidal pressure in short-time of hemodilution. On the other hand, an extended duration of hemodilution and anemia can definitely adversely affect the intravascular pressure and vascular resistance, finally vascular barrier. We also added a "hypotension" as a factor to contribute microcirculatory dysfunction in second paragraph of discussion.

(2) Did you measure plasma colloid osmotic pressure during the hemodilution maneuvers? Was that consistent with the fall in arterial pressures?

Answer: we did not measure the plasma oncotic pressure during the hemodilution process because we have targeted the alteration of vascular barrier function and glycocalyx degradation during the change of hematocrit level. Study based on the alteration of oncotic pressure should be consider in future.

(3) The control experiments, performed by simply observing the status of the variables you have measured without any sham exchange may not be adequate to ensure that other factors have not been lost which regulate barrier function. It would seem logical to perform control experiments using plasma from donor animals, and separate experiments with the HES and other substitutes seeded with red cells at the initial hematocrit (i.e. no dilution). Have you considered doing so?

Answer: We agree with the reviewer about adding of the new groups as plasma exchanged group or even maybe albumin group for concerning deep fundamental research in study but here we have chosen to perform study including a having more clinical translational capacity with the most frequently fluid being used in peri-operative settings.

(4) Was there any indication that HES was sequestered in the endothelial surface layer and thus serves to replace HS and HA to maintain the barrier function of the glycocalyx?

Answer: The most important pharmaceutical properties of HES solution is to include a colloid molecule with size of 130kDa and require lesser amount than crystalloid in order to maintain the intravascular colloidal pressure, tissue microcirculation and glycocalyx integrity (we added a new figure to show fluids amount needed for Hct 15%, see in Fig 2).

(5) Have you attempted to pharmacologically inhibit shedding of the glycocalyx with MMP inhibitors or heparanase scavengers during the ANH?

Answer: No, we did not attempt to use glycocalyx degradation inhibitors, but those might be considered in future studies.

Reviewer 3

The authors investigate the effects of normovolemic hemoddilution on the integrity of the endothelial glycocalyx in an experimental model with rats.

The design of the study is good and the results are interesting.

Two Comments: 1) The authors describe - based on their results, that a degraddation of the endotehilal glycocalyx can occur even without vascular leakage. Already since 2004 it is 36 known, that the endothelial glycocalyx is one component of a "double-barrier" of blood vessels
(Endothelial glycocalyx as an additional barrier determining extravasation of 6% hydroxyethyl starch or 5% albumin solutions in the coronary vascular bed. Rehm M, Zahler S, Lötsch M, Welsch U, Conzen P, Jacob M, Becker BF. Anesthesiology. 2004 May;100(5):1211-23.

According to this model even a remarkable degraddation of the endothelial glycocalyx can be compensated by the second component of the vascular barrier, the edothelial cells and a
resulting tightening of interendothelial gaps. As the results of the present investigation confirm this model, it should be mentioned/cited in the manuscript.

Answer: We thank the reviewer for this interesting observation and agree with the suggestion to add this observation to the manuscript. We added the following passage in manuscript at page 18, paragraph 2.
"Moreover, in agreement to our findings, Rehm *et al*., found that in addition to the glycocalyx another component of the vascular endothelial barrier formed by the endothelial cells ("double barrier concept") may play a role in maintaining fluid and colloid extravasation in an isolated heart model.[16]"

2) The calculation of the plasma volume and intravascular volume have to be presented in the methods section.

Answer: As suggested by the reviewer, we added the following passage to explain the plasma volume and intravascular volume calculations in section 2.6.1.
Plasma concentrations were measured for each dye in a 96-well plate fluorometer (ClarioStar, BMG LabTech, Ortenberg, Germany) in accordance with the excitation/emission wavelengths of each dye and after obtaining a standard calibration curve: 580±20 nm/625±20 nm for FITC, 480±15 nm/530±25 nm for TexasRed and 675±10 nm/740±40 nm for Alexa 680. A retention ratio (RR) of the dye was calculated as follows: RR = final concentration at 30 min / initial concentration at 2 min. For total intravascular volume (TIV) and plasma volume (PV)

determinations, the concentration of Albumin-Alexa 680 was determined at time=2 min after bolus injection according to the following calculation:

$$\text{TIV (ml)} = \frac{- \quad \text{syringe} \times 0.1}{[\text{Albumin} - \text{Alexa plasma}]}$$

$$\text{PV (ml)} = \frac{[\text{Albumin} - \text{Alexa syringe}] \times 0.1}{[\text{Albumin} - \text{Alexa plasma}]} \times (1 - \text{Hematocrit})$$

[Albumin Alexa] Hematocrit is expressed as percentage (e.g. 15%=0.15). "0.1" corresponds to the volume (in ml) of Albumin-Alexa contained in the syringe and injected.

Reviewer 4

The authors should provide a better timeline for the experiments. The sequence of injury to 26 the vascular barrier in shock is thought to be glycocalyx then endothelial layer, resulting in 27 increased vascular barrier permeability... Were the experiments carried out long enough to address this ??

This reviewer is uncertain as to the duration of the experiments
the authors should not equate glycocalyx integrity with indirect measurements such as
shedding of GC components...for that matter what do the authors attribute as to why these is shedding of syndecan 1 in even the control group

Answers: We would like to thank the reviewer for his\her comments and suggestions for the improvement of the manuscript.

As the reviewer suggested, we added a time duration line showing the sequential Hct target in
Fig 1. Also, we added a sentence in the limitation section about the duration of experiments
possibly being a limiting factor. "Fourthly, due to technical and ethical reasons, we did not
analyze the glycocalyx and endothelial barrier function after longer time exposure of hemodilution."

As we mentioned in our previous article, an intact glycocalyx is a prerequisite for normal vascular physiology, and each component exhibits a unique function. Many of its components are essential for homeostasis (1,2) mechanosensors for flow-mediated shear stress and

autoregulation (1,3) and leukocyte-endothelial cell interactions (4). Glycocalyx shedding could
be an essential and normal response to optimal physiological condition or injury and could be appropriate or even beneficial. From this perspective, it can be stated that the glycocalyx is dynamic structure and constantly degraded and renewed not only in pathological but also during control condition.

1. Reitsma S, Slaaf DW, Vink H, van Zandvoort MAMJ, oude Egbrink MGA. The endothelial glycocalyx: composition, functions, and visualization. *Pflugers Arch* 2007;454:345–59.

2. Chappell D, Brettner F, Doerfler N, et al. Protection of glycocalyx decreases platelet adhesion after ischaemia/reperfusion: an animal study. *Eur J Anaesthesiol* 2014;31:474– 81.

3. Weinbaum S, Tarbell JM, Damiano ER. The structure and function of the endothelial glycocalyx layer. *Annu Rev Biomed Eng* 2007;9:121–67.

4. Constantinescu AA, Vink H, Spaan JAE. Endothelial cell glycocalyx modulates immobilization of leukocytes at the endothelial surface. *Arterioscler Thromb Vasc Biol* 2003;23:1541–7.

Reviewer 5

The experimental research paper submitted to JCT Res (No. 200002) addresses the clinically relevant question whether acute normovolemic hemodilution (ANH), conducted with differentreplacement fluids (balanced hydroxylethyl starch, balanced crystalloid or isotonic saline), leads to different extents of disturbance of the endothelial glycocalyx and/or the vascular
barrier permeability (VBP). That is, the aim of the study is to determine if one of the chosen
fluids provides greater protection against deterioration of the vascular barrier and, thus, to formation of edema in the course of ANH than the others. Groups of 6 rats each per group were subjected to stepwise ANH to hematocrit levels of 35, 25, 20 and, finally, 15%, except for a
control group for which there was no blood-letting. The VBP was assessed by searching for
edema formation (weighing wet weight vs dry weight of selected organs) and by determining
the rate of extravasation of 3 marker macromolecular substances of different molecular size/weight.

Although only a relatively small number of animals per group has been studied here, the work is original and of potential clinical relevance for the fields of intensive care,

trauma therapy and surgery. All in all, the paper is well-structured and potentially makes for informative and interesting reading. However, several major concerns need to be expressed after reviewing the work in its submitted form.

Major criticisms.

1. The authors do not provide exact details concerning the duration of the potential disturbance at each level of ANH or on the total duration of the observations. The supplemental Figure 1 (see Methods, section Protocol) does not enhold this information. This information should be added to the Abstract and the Methods and critically discussed (see below).

Answer: We would like to thank the reviewer for his\her comments and suggestions leading to improve quality of this manuscript.
As reviewer suggested that we added a time duration line showing the sequential Hct % target in Fig 1. and mentioned this addition in the method section 2.3.

2. A major concern is whether the (still to be provided) duration of the perturbation incurred by ANH was, at all, principally sufficient to allow development of overt damage to the vascular permeability barrier. In other words, the study fails to bring about those changes encountered in clinical practice in any of the experimental groups. It is, obviously, impossible to detect a beneficial effect of one fluid over another if there is no overt damage. A positive damage group must be established by intensifying the extent of the perturbation ensuing from ANH in the study.

Answer: we agree with this concern of the reviewer. However, there is a major concern about the efficiency and potential harmful effects of different types of fluids used in intensive care or perioperative setting in the clinic. That is why in this study our aim was not to demonstrate the long term effects of fluids on VBP but rather to investigate the acute effect of fluids and identify whether or not there are a benefit of using one fluid type to another in term of alteration of vascular barrier permeability in parallel to glycocalyx degradation. Furthermore, in our previous study, we already tested the relationship between the glycocalyx degradation and vascular barrier function in hemorrhagic shock model resulting no vascular barrier dysfunction occurs after aggressive fluid resuscitation despite glycocalyx shedding (Anesth Analg. 2019 Aug;129(2):598-607).

3. The data suggest that some shedding of the glycocalyx was incurred by ANH, but that this was not accompanied by detectable increases in vascular permeability (tissue edema, extravasation of tracer substances). The authors should discuss this result in the context of the "double barrier" formed by the glycocalyx and the endothelial cells. According to the "double barrier concept", expressed, e.g., by M. Rehm et al. (Anesthesiology 2004), both the endothelial cells and the glycocalyx need to be perturbed before the vascular permeability barrier is seriously breached, i.e., beforeedema rapidly develops (see also Discussion, p. 16, 2nd paragraph).

Answer: we added a sentence according to suggestion of the reviewer “Moreover, in agreemenT
to our findings, Rehm *et al*., found that in addition to the glycocalyx another component of the vascular endothelial barrier formed by the endothelial cells (“double-barrier concept”) may play a role in maintaining fluid and colloid extravasation in an isolated heart model.[16]”in page 18 pgh 2.

4. P. 12, § 3.4.1, Fig. 4: Can you explain why there was less extravasation of Texas-Red 45 40kD tracer in all the ANH groups than in the Controls? This result is rather confusing. Why does the barrier become tighter in ANH? The "explanations" proffered to this aspect in the Discussion on p. 16 are not really convincing or helpful. Also, please define how the "Ratio" was established (last value S6 / value at 2 min after injection ??).

Answer: As suggested by the reviewer, we added the following sentences in the discussion to address the low retention rate of 40kd molecules in the control group in comparison to the diluted groups. “In comparison to a diluted group, low retention ratio of 40kDa molecules in control group indicated more leakage of small molecules from vascular space to the interstitial space in baseline condition. This may be explained by the viscosity and shear stress created by the red blood cell under baseline condition could contribute to the permeability of vascular barrier to provide osmotic balance in the intra and extra vascular spaces. However, blood viscosity and shear stress reduced by removal of red blood cell may also decrease the permeability of small molecule through the vascular barrier to maintain intravascular volume. One would expect a decrease in retention ratios for small molecules indicating an increased vascular barrier permeability for water and proteins. “

P. 17, lines 05-09: The submitted data do not "show" that excess volume of applied crystalloids might accumulate in the intestine or in lymphatic vessels.

Answer: we removed the following sentence from the text ‘This study also showed that the extra volume of crystalloids especially saline due to less urinary discharge might accumulate in intestine or lymph vessel.’

5. Generally, major corrections must be performed concerning the quality of the language in the text and in correcting grammatical errors. There is a complete lack of syntax in the English in some parts of the text (see, e.g., p.6 -7, §2.7, first paragraph, and again p.7, sentence on lines 29-32; p. 16-17, sentence on lines 59-05).

Answer: as suggested by the reviewer, the manuscript has been edited by a native English peaker to correct grammatical errors.

Minor criticisms.

1. P6. § 2.6.1, last sentence: The times of withdrawal of blood samples for measuring of tracer levels given here do not comply with the sampling protocol (S1-S6) depicted in Supplemental Fig. 1 (p. 5).

Answer: Figure 1 shows the time points between the different Hct targets and tracer 33 measurements. The tracer measurement was performed at the end of hemodiluton process (after 34 Hct %15) (see in Suppl Fig 1), not between the different Hct levels.

2. P6, §2.6.2: Was surface water dried from the sampled organs prior to determining wet weight? Especially in the case of the heart, was intraventricular fluid sponged away before weighing? In this light, the identical ratios given for wet/dry weight of heart and lung tissue (Supplemental Fig. 3) are totally unbelievable.

Answer: After harvesting, the organs were placed on gauze compress to remove extra water and blood from the organs.

3. Some abbreviations in the legend to the Table 1 are inappropriate: FABF is not needed, whereas CVP is missing.

Answer: it has been corrected.

4. P9, Text lines 29-32: bicarbonate levels are not provided in Table 1

Answer: We added the bicarbonate levels between groups in Table 1.

5. P. 11, lines 36-39: The statement "syndecan-1 levels were significantly higher than at baseline" does not hold for the Control group.

Answer: At the end of Hct 15%, level of syndecan-1 were increased in comparison to the baseline of each groups but there were no differences between the ANH groups and control at the time points.

6. P.16, lines 22-24: microcirculatory disturbance was only evident in the NaCl-group.

Answer: we corrected the Figure 5 after controlling the statistical results.

2nd editorial decision

6-Apr-2020

Ref.: Ms. No. JCTRes-D-20-00002R1
Hemodilution causes glycocalyx shedding without effecting vascular endothelial barrier permeability in rats
Journal of Clinical and Translational Research

Dear author(s),

Reviewers have submitted their critical appraisal of your paper. The reviewers' comments are appended below. Based on their comments and evaluation by the editorial board, your work was FOUND SUITABLE FOR PUBLICATION AFTER MINOR REVISION. Please note that the reviewers rightfully comment on the use of the English language. The editorial board would like to stress the need for language polishing. The paper cannot be accepted without the authors properly addressing this recurrent issue.

If you decide to revise the work, please itemize the reviewers' comments and provide a point-by-point response to every comment. An exemplary rebuttal letter can be found on at http://www.jctres.com/en/author-guidelines/ under "Manuscript preparation." Also, please use the track changes function in the original document so that the reviewers can easily verify your responses.

Your revision is due by May 06, 2020.

To submit a revision, go to https://www.editorialmanager.com/jctres/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #5: The authors of the experimental research paper submitted to JCT Res (No. 200002) have addressed most of my previously outlined criticisms in an adequate manner. However, some issues remain to be clarified/revised.

Remaining criticisms.

1. To finally clarify the exact details concerning the time course/duration of the experimental observations may I suggest extending the text on p. 4 in line 56 as follows: ….until successive targeted levels of hematocrit were reached after approximately 45 min, each.
2. The data of figs. 2 and 3 are difficult to reconcile. Where does the extra added volume of the balanced crystalloid and the NaCl 0.9% protocols go? This is not discussed by you , e.g., on p. 19. Although I criticised your use of the term "show" concerning the fate of that excess volume of applied crystalloids in the originl submission, this volume might, hypothetically, accumulate in the intestine or in lymphatic vessels. To anesthesiologists, this would correspond to the ominous "third space".
3. Generally, corrections must still be performed concerning the quality of the language in the text and in correcting grammatical errors. Examples noted on quickly reading your paper are to be found on p. 10, line 48, p. 13, line 42, p. 18, line 31 and line 34, p. 21, line7.

Author’s rebuttal

Reviewers' comments:

Reviewer #5: The authors of the experimental research paper submitted to JCT Res (No. 200002) have addressed most of my previously outlined criticisms in an adequate manner. However, some issues remain to be clarified/revised.

Remaining criticisms.

1. To finally clarify the exact details concerning the time course/duration of the experimental observations may I suggest extending the text on p. 4 in line 56 as follows: ….until successive targeted levels of hematocrit were reached after approximately 45 min, each.

Answer 1: We would like to thank to the reviewer comments, suggestion and contribution to improve the quality of presented study.
As reviewer suggest, we added the following sentence in method part of manuscript.
'' until successive targeted levels of hematocrit were reached after approximately 45 min, each.''

2. The data of figs. 2 and 3 are difficult to reconcile. Where does the extra added volume of the balanced crystalloid and the NaCl 0.9% protocols go? This is not discussed by you , e.g., on p. 19. Although I criticised your use of the term "show" concerning the fate of that excess volume of applied crystalloids in the originl submission, this volume might, hypothetically, accumulate in the intestine or in lymphatic vessels. To anesthesiologists, this would correspond to the ominous "third space".

Answer 2: We added a new sentence in relevant paragraph of discussion as 'Due to less urinary discharge, this extra volume of crystalloids especially saline might accumulate in third space.''

3. Generally, corrections must still be performed concerning the quality of the language in the text and in correcting grammatical errors. Examples noted on quickly reading your paper are to be found on p. 10, line 48, p. 13, line 42, p. 18, line 31 and line 34, p. 21, line7.

Answer 3: We sent the manuscript in English editing service to correct the all the grammatical errors in the text as suggestion of reviewer.

3rd editorial decision

14-Apr-2020

Ref.: Ms. No. JCTRes-D-20-00002R2
Hemodilution causes glycocalyx shedding without affecting vascular endothelial barrier permeability in rats
Journal of Clinical and Translational Research

Dear authors,

I am pleased to inform you that your manuscript has been accepted for publication in the

Journal of Clinical and Translational Research.

You will receive the proofs of your article shortly, which we kindly ask you to thoroughly review for any errors.

Thank you for submitting your work to JCTR.

Kindest regards,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Comments from the editors and reviewers: