Peer Review File

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Review Comments

Comment 1: 48 hours appears to be a long time between harvest and research. Most articles appear to act within 24 hours at most when assessing bladder physiology. Particularly with a study examining ischemia, it would appear that this may be an excessive timeframe. Have the authors validated 48 hours as an appropriate timeframe after extraction? Also, were some tissues used sooner, ie: Within 24 hours, and others used later? If so, the times should be included in the results or this could be considered an additional confounding variable.

Response 1: Thank you for this comment. From a practical standpoint, we needed to extend the usual 24 hours of harvest to experiment time to 48. To ensure the viability of all bladders, we completed 2 pre-test fill and void cycles to ensure the bladder was contracting and contracting to the same degree as those that were used at 24 hours. Those bladders that did not seem viable were not used. This should however be considered as a possible confounding variable and so the following text has been added to page 10:

Changes in text: Bladders were used between 24-48 hours after harvest. The time variability may be a confounding factor. To account for this, all bladders underwent 2 pre-study fill-void cycles to ensure adequate baseline contractility.

Comment 2: There is not enough information regarding the statistical analysis. A Student's t-test is only appropriate in this occasion if calculated as "unpaired", but this is not stated. Also, include some information on how pressure values were normalised.

Response 2a: Thank you for pointing out this omission. All analyses were paired Student T-tests because bladder pressure data were compared to their own baselines. To address these the last two lines of the statistical analysis section has been revised as follows on page 7:

Changes in text 2a: Two-tailed paired Student's t-tests were used to compare normalized pressure values, and a p value of <0.05 denotes statistical significance.

Response 2b: In order to explain how the pressure values were normalized the following text was added to page 7:

Changes in text 2b: Pressure values were normalized by calculating the ratio of each passive, total and active to the value of the control fill

Comment 3: There is a gender disparity between the groups. This is usually best to be controlled. However, at the very least a statement should be included in the discussion regarding the limitation of gender as an uncontrolled variable.

Response 3: Thank for you for this comment. We agree that gender of the pig is best to be controlled. Due to practical circumstances we were unable to do so. Therefore, the following text has been added in the limitations section to page 10:

Changes in text: Limitations of the current study include the relatively small sample size, use of male and female pigs, and the recognition that in-vitro porcine bladders may not faithfully represent the human condition.

Comment 4: It is helpful to have the breed and weight both listed, and this was a positive addition to the paper.

Response 4: Thank you for bringing up this point, we have now added the mean weight, in addition to the range, as requested.

Changes in text: On page 7, the following text is included: All pigs were of mixed breed, with main breed percentages from Yorkshire, Hampshire, Duroc, and Guinea Hog breeds. All pigs weighed between 215-375 lbs. prior to slaughter, mean = ….

Comment 5: The pig is increasingly being used as an effective model for human research into bladder dysfunction. A statement to this extent could be included with a reference to other recent research in the field such as: https://bmcurol.biomedcentral.com/articles/10.1186/s12894-020-00619-0 (doi: https://doi.org/10.1186/s12894-020-00619-0).

Response 5: Thank you for this. We have added the following to Page 9 as well as include the suggested reference:

Changes in text: Increasingly, porcine models are being used as a model to examine human physiology. This has been seen most well known in cardiac models, but has also been used in gastrointestinal models as well as pulmonary models. Recently this model has been used in the Urologic world to model bladder physiology and to be better understand pathology.

Comment 6: What was the rationale for using more than one abattoir to source samples of tissues? Assuming this study was conducted in the united states, pigs may have been subjected to a range of additives which could impact muscle function, such as ractopamine. Using a single abattoir and similar breed of pig is a more effective way to control such activities, with a wide variability coming from different regions, farms and abattoirs. The use of a variety of breeds and a number of different abattoirs introduces additional variables.

Response 6: We chose to use 2 abattoirs in order to obtain enough bladders to achieve statistical significance. Both facilities function solely as abattoirs in order to slaughter animals provided by individual customers. These were not large-scale commercial facilities. Thus, additives used in commercial pig farming cannot be ruled-out but are extremely unlikely. In order to address this issue, the following has been added to the text in the limitations section on page 10:

Changes in text: These bladders were obtained from 2 abattoirs, so there could be some variability in the pig diet prior to slaughter. However all bladders were harvested by study authors immediately after slaughter in order to standardize handling and experimentation.

Comment 7: . Reference 16 and 23 are presented to support the findings, but these are both in rats, rather than pigs or humans. This should be mentioned.

Response 7: Thank you for this comment, we agree this should be mentioned and have added the following clarification statement to page 9-10:

Changes in text: … as has been demonstrated in rat models

Comment 8: . I am content that the porcine bladder is a suitable model, and does link very well to human tissue. However, it would be effective to cite examples of where porcine tissue has been effectively used in research related to human studies.

Response 8: We agree and have added the following to page 9 with supporting references:

Changes in text: Increasingly, porcine models are being used as a model to examine human physiology. This has been seen most well known in cardiac models, but has also been used in gastrointestinal models as well as pulmonary models. Recently this model has been used in the Urologic world to model bladder physiology and to be better understand pathology.

Comment 9: The article consistently relates the findings to detrusor function, but not the urothelium and lamina propria. The fact that in porcine tissue the urothelium and its underlying lamina propria contracts, and would contribute to any contractile responses. This should be mentioned with reference to https://onlinelibrary.wiley.com/doi/full/10.1111/iju.13172 (doi: https://doi.org/10.1111/iju.13172), and https://onlinelibrary.wiley.com/doi/abs/10.1111/aap.12000 (doi: https://doi.org/10.1111/aap.12000). However, after this mention, it is fine to suggest that an impact of ischemia on the urothelium is likely to have minimal impact on the results, as it is apparent that most of the contraction would arise from the detrusor.

Response 9: Thank you for this consideration. We have added the above mentioned references and the following on page 10:

Changes in text: In previous porcine strip studies, it has been shown that the lamina propria also contracts, in conjunction with the detrusor muscle. The urothelium and lamina propria respond to prostaglandins, like the detrusor muscle, as well as serotonin. This model measured the total contraction, without examining the exact contribution of detrusor vs lamina propria or urothelium, however the majority of the contract is sustained by the detrusor.

Comment 10: Spelling error in reference 16: Chronic

Response 10: This has been corrected

Changes in text: Chronic