Title: Effects of a Cholesterol-Enriched Diet on Intestinal Smooth Muscle Contraction: Inhibition of Muscarinic Receptors and their Disinhibition by the 5-HT4 Agonist - Tegaserod

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Author's response to reviews: see over
Dear Editors:

I apologize for the delay in forwarding to you the attached a revised manuscript entitled “Increased Cholinergic Contractions of Jejunal Smooth Muscle Caused by a High Cholesterol Diet is Prevented by the 5-HT₄ Agonist – Tegaserod” by Ronald Mathison and Eldon Shaffer that we are re-submitting to BMC Gastroenterology for consideration towards publication.

Taking into consideration the referee’s comments on the interpretation of the data in the manuscript the title of the manuscript has been changed from the original title “Effects of a Cholesterol-Enriched Diet on Intestinal Smooth Muscle Contraction: Inhibition of Muscarinic Receptors and their Disinhibition by the 5-HT₄ Agonist – Tegaserod”.

We would like to thank the referee for his invaluable and insightful review of our manuscript. His comments and criticisms have certainly improved the focus of the manuscript, and help clear up some ambiguities. Replies to the referee are attached at the end of this letter.

All authors have read and agreed to the content of this manuscript. The experimental research was performed with the approval of The University of Calgary Animal Care Committee, which conforms to the guidelines of the Canadian Council on Animal Care.

Sincerely,

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Reply to Referees

Re: “Increased Cholinergic Contractions of Jejunal Smooth Muscle Caused by a High Cholesterol Diet is Prevented by the 5-HT4 Agonist – Tegaserod” by Ronald Mathison and Eldon Shaffer

The replies to David Schneider (15553048225555_comment.pdf) are discussed in detail in this reply. The replies to David Q Wang (1820243612855694_comment.pdf) are included in this reply.

Significant Changes:

1) Title of manuscript has been modified to reflect the revised interpretation of the data, as recommended by the referee in point #3. The new title is: “Increased Cholinergic Contractions of Jejunal Smooth Muscle Caused by a High Cholesterol Diet is Prevented by the 5-HT4 Agonist – Tegaserod”.

2) A new figure has been added. Figure 4 now shows “for future reference and potential comparison” data on ileal contractions – referees’ point #23(d).

Major Compulsory Revisions

1. A new section was added to the results “Phasic and Tonic Contractions of Intestinal Segments” (page 9) addressing changes in baseline tone and spontaneous activity.
2. The statement “the time course for the modification of cholinergic receptor function by the cholesterol diet is not known and acute effects cannot be excluded.” This point is addressed on page 14, lines 6-8.
3. Wording that “implies that the observed mechanism necessarily results from stimulation of muscarinic receptors that are directly couples to inhibitory transduction mechanisms” has been removed from the manuscript.
4. The comparison of tegaserod’s actions, as investigated in this study, to the responses of isolated intestinal tissues in an organ to serotonin has been removed. The conclusions with regards to tegaserod’s effects have been modified to reflect an undefined mechanism of action, possibly related to modification of cholesterol metabolites in the intestine.
5. We reanalyzed some bile samples and added additional data that were not available at the time the original manuscript was prepared. As a result the N-value for the bile composition studies was increased by 6 to 7. The results remain unchanged.

Minor Essential Revisions

7. Comment on general health included on page 6, lines 5-6. An antihelmintic was used - see page 6, lines 1 & 2.
8. 64 animals were used for this study. The 32 other animals indicated in the original manuscript were part of another drug study, and the data were not used in the current
study. Clarification is provided on the use of animals, page 6, 1st paragraph, last 3 lines.

9. Acute removed and sentence reworded – page 6, last 2 lines.

10. Removed.

11. (a) Two animals were tested daily – page 6, 1st paragraph, last 3 lines. (b) Clarification on tissue is provided on page 8, lines 9-13.

12. (a) Temperature: 35°C (page 7, line 10 of section “Intestinal Contractility in vitro”). (b) Carbachol was added cumulatively (page 8, lines 3-4). (c) Pre-incubation with antagonists: 10 min (page 8, lines 9-11).

13. Use of 0.1µM TTX. Although TTX is commonly used at 1µM, there are data showing that 0.1µM TTX is effective in blocking neurogenic actions in intestinal preparations. Some citations are: Venkova K et al., Peptides. 1992; 13:193-201 (cat large intestine); Biagi B et al., Am J Physiol. 1990; 258:G223-30 (rabbit colon). A dose of 0.2µM TTX was effective in rat colon (Christofi FL et al., J Comp Neurol. 2004; 469:16-36, and 0.15µM TTX was used with mouse distal colon (Fontaine J, Lebrun P., Br J Pharmacol. 1989; 96:583-90).


15. Corrected.

16. Data analysis: Clarification on the use of statistics (page 8, last paragraph). A two-way analysis of variance was not used since the number of animals in each group was not the same.

17. Corrected.

18. (a) Corrected. (b) The sentence has been reorganized to more clearly convey the results shown in Figure 2C (page 11, 2nd paragraph, lines 1&2). (c) Clarification added on page 8, lines 11-13.


21. (a) See new discussion - page 13, lines 1-4. (b) The reviewer points out a variety of mechanisms that the high cholesterol diet could exert an inhibitory effect. We do not have data that addresses these mechanisms so they have not been discussed.

22. This sentence has been deleted. A modified discussion on this topic is presented on page 13, 1st paragraph.

23. (a) Corrected. (b) Corrected. (c) Corrected. (d) Concentration-response curves for the ileum have been included in a new Figure 4.

24. Corrected.

25. The revised manuscript only contains the terms “low cholesterol diet” and “high cholesterol diet” as defined in the methods page 6, lines 6-9.

26. Wording has been changed from “have minimal impact on gallbladder contractility” to “not adversely affect gallbladder contractility.” – page 5, lines 8-9.

27. The data remains unchanged with the normalization procedure. The principle variable in this normalization procedure is tissue weight.

28. Removed as suggested.

29. “Luminal exposure” to cholesterol and bile salts is indicated in the discussion – page 13, 1st paragraph.
30. Even though Figures 1 and 2 show the same data, but in a different combination, the authors view this duplication as important for an appreciation of the effects of two variables – cholesterol (Figure 1) and tetrodotoxin (Figure 2). For example, the data shown in Figure 1A is repeated in Figure 2A and Figure 2B, and it is not intuitive to cross compare these two Figures to arrive at Figure 1.