

## ANSWERING REVIEWERS

January 4, 2016

Dear Editor,



Please find enclosed the edited manuscript in Word format (file name: 151222 iMScope normal stomach for revision.docx).

**Title:** Visualization of sphingolipid and phospholipids in gastric epithelial tissue using imaging mass spectrometry.

**Author:** Nobuya Kurabe, Hisaki Igarashi, Ippei Ohnishi, Shogo Tajima, Yusuke Inoue, Yoshihiko Takahashi, Mitsutoshi Setou, and Haruhiko Sugimura

**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO:** 23688

The manuscript has been improved according to the suggestions of reviewer.

In the attached reply letter, we describe the changes made in response to the reviewer's comments point-by point. The most significant changes are:

- 1) Some mistypings of words were corrected.
- 2) The relationship between lipid distribution and disease was mentioned.
- 3) We revised the title of this paper.

We believe that our revised manuscript has been corrected properly and suitable for publication in the *World Journal of Gastroenterology*. Thank you in advance for your kind consideration.

Sincerely yours,  
Fumio TASHIRO, Ph. D./Prof.  
TEL: +81-03-5876-1717  
FAX: +81-03-5876-1639  
E-mail: ftashir@rs.noda.tus.ac.jp

We would like to appreciate the Reviewer for giving us proper comments and suggestions. We believe that our revised manuscript is sufficient to reply your comments.

**Reviewer 1's comment:**

- 1) In Abstract, the last sentence of results section, 'PC (16:0/18:2) signals uniformly distributed around the mucosae'. I guess there is a careless mistake, should 'PC (16:0/18:2)' change to 'PC (16:0/18:1)'?
- 2) In Materials and Methods section, 'The tissue blocks were then sectioned to a thickness of 8 mm at -20°C', should revise to '8µm'? And for immunohistochemistry, 4µm of specimens' thickness would be better. Have you set any positive/negative controls when conduct immunohistochemistry?
- 3) It would be better if you provide some basic information about the 5 patients who donated the gastric specimens, could their illness influence the results? Have you considered the gastric specimen from health volunteers?
- 4) All samples were from fundic gland area, so I guess the results only represent the fundic gland area and may not well reflect the whole gastric mucosae.
- 5) If any disease portions were included for consideration and analysis, there would be more information of lipids distribution and may contribute more to the gastric disease's research.
- 6) There some grammar and language mistakes need to be revised.

**Reply 1**

- 1) As reviewer requested, we changed "PC (16:0/18:2)" to "PC (16:0/18:1)" in the revised Abstract.
- 2) As reviewer suggested, we corrected "8 mm" to "8 µm". Because we used 5 µm-thick sections for immunohistochemistry, we inserted the "5 µm-thick" into the sentence "The sections were treated with a 0.3% hydrogen peroxide solution to inactivate endogenous peroxidase activity" in the Immunohistochemistry section of Materials and methods. For immunohistochemistry, we always set the positive/negative controls.
- 3) As reviewer suggested, we think that we should apply the healthy volunteers for this study. However, because we picked up the gastric portion far from the cancerous region, we think that the effect of illness do not influence the results. The subject provided the consent on research use of the residual tissue of resected stomach.
- 4) As reviewer suggested, all of our samples are from fundic gland area. Thus, we revised the previous title to "Visualization of sphingolipid and phospholipids in fundic gland mucosa of the stomach using imaging mass spectrometry" and inserted the words "of fundic gland" at the rear of the words "gastric mucosae".
- 5) As mentioned 3), we used only healthy region of fundic gland mucosa. I agree with the reviewer's insightful comments on extension of this search toward diseased portion. We will continue to do that direction and will report the results of them in near future.
- 6) As reviewer requested, we polished our grammar and language, though we are not native English speaker.