Reviewer's report

Title: Fluorescence imaging in vivo visualizes delayed gastric emptying of liquid enteral nutrition containing pectin

Reviewer: Shinsuke Nakayama

Accelerated gastric emptying possibly causes diarrhea and/or the symptoms of dumping syndrome. To increase the viscosity of liquid meal with addition of dietary fiber is a possible clinical treatment.

The authors examine the effects of pectin, which increases the viscosity of liquid meal at a low pH, on the gastric emptying rate in mice anaesthetized with 2.5% isoflurane. Liquid meals with or without pectin are administrated. The gastric emptying rate is assessed by in vivo imaging of the fluorescence of GastroSenseTM750 contained in the liquid meal. The experiments are interesting, but I have several comments to improve the manuscript.

1) P4, L73: ‘To our knowledge, the transition of EN from the stomach into the intestine has not been assessed in vivo as changes in the physical properties of a gelation agent determined using real-time imaging.’ I wonder what the authors claim here? We have monitored the duodenal motility as well as the gastric emptying rate using rapid MRI in humans (Teramoto et al. 2012, 2014).

2) In relation to the comment #1, the effects of pectin on gastric emptying have already been examined. Furthermore, accelerated gastric emptying has been reported from Gunma University (Shinomiya et al. 2007).

Please more clearly and carefully explain what is the point of this study in the Introduction and Discussion sections.

Imaging analysis has already been performed in humans. Also, the effects of pectin have already been examined. Do the authors want to emphasize the animal model used in this study?

If the authors want to claim the discrepancy between the present results and previous study reporting acceleration of gastric emptying upon adding pectin, please more carefully compare experimental conditions between studies.

3) Experimental protocols: The procedures of food administration, imaging, and treatment of mice between imaging, are hard to follow in this manuscript. Please describe them more clearly. A new figure showing the protocols will help readers to understand how measurements were carried out.
4) P4, L90: ‘The Committee for the Care and Use of Laboratory Animals at Otsuka Pharmaceutical Factory, Inc. approved the surgical and experimental procedures’. Did you apply any surgical operation in mice?

5) P4, L94: ‘After a 24 h fast without water for the last 2 h’. Please describe why this treatment was necessary.

6) P4 L95: ‘the baseline value of fluorescence at the body surface of mice was monitored’. Why did you do it? How did you use ‘the baseline value’ in the following fluorescence imaging?

7) P5 L98: ‘the mice were gavaged with 10 µL/g of body weight ---’ is unclear.

8) P5 L108: ‘The total flux of regions of interest was quantified using IVIS® imaging software (Perkin Elmer).’ is unclear.

9) P3, L50: ‘A bolus injection or the rapid infusion of liquid enteral nutrition (EN) into the stomach helps nutrients and water to rapidly flow into the small intestine’. I agree that the viscosity of liquid meal possibly change the gastric emptying rate. However, as textbooks of Physiology (e.g. Hunt) describe, the gastric emptying rate is normally controlled by the calorie contained. Also, we have visualized a similar mechanism comparing ingestions of water and liquid meal. So, I wonder that the first sentence in the Introduction may not be justified, unless administration conditions and personal differences etc are stated.

10) Figure 1: The fluorescence was less at 30 min than that at 45 min. Were mice fed more meal containing fluorescent reagent? Is this due to a limitation of fluorescent imaging? How much depth can you visualize and quantify the fluorescence in your fluorescent imaging system?