Author's response to reviews

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

Authors:

Ippei Yamaoka (yamaokai@otsuka.jp)
Takeshi Kikuchi (Kikuchi.Takeshi@otsuka.jp)
Naoyuki Endo (Endo.Naoyuki@otsuka.jp)
Goro Ebisu (ebisug@otsuka.jp)

Version: 3 Date: 31 July 2014

Author's response to reviews:

(Reviewer 1)
This paper describes an innovative method for measuring gastric emptying in mice. It is applied to determine the gastric emptying rate of a novel pectin based enteral nutrition product. As expected, the addition of pectin to the bolus increased viscosity in simulated gastric environment. However, a viscosity measurement was only conducted at one speed on the viscometer and it is not clear how the material would respond at other speeds (e.g., is it sheer thinning or sheer thickening) (discretionary revision – may be worth mentioning in discussion). The bolus with the added pectin reduced gastric emptying rate as indicated by the florescence imaging. While this result would not be unexpected the use of the florescence imaging to measure the gastric emptying rate makes it of interest.

The paper is generally well written and easy to follow. The paper is brief and the results may be limited in scope. Moreover, I feel the authors should discuss the limitations of this approach in a little more depth (compulsory revision). However, I feel this is a worthwhile addition to the literature.

We are very grateful to learn that you are interested in our study and we appreciate your helpful comments about our manuscript. In line with your comments, we discuss the limitations of the approach in a little more depth, and compare our results with those of previous studies of the role of pectin in gastric emptying (p9 L210-L217). We thus largely revised the Discussion section both qualitatively and quantitatively. We also measured the viscosity at different shear rates in a supplemental experiment. Consequently, higher shearing speed is associated with lower viscosity of the material after adding artificial gastric juice to EN with pectin. We describe this in the revised Discussion section (Figure 1A...
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.

We are very grateful for your valuable comments that have helped us to improve our manuscript. We revised the Methods section for clarity and discuss the application of novel fluorescence imaging to the ability of pectin to delay gastric emptying rates. We also discuss the discrepancy regarding the role of pectin in gastric emptying rates between the present and previous reports. We address your questions below.

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 emptying rate using rapid MRI in humans (Teramoto et al. 2012, 2014).

We described the previous methodology of imaging gastric emptying wrongly. We intended to describe the novelty of fluorescence imaging, but indeed we should know several methods for determining gastric emptying such as real-time imaging in experimental animals and humans. We added these methods to the Introduction section (p4. L 72-82).

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.

We appreciate this comment. As you mentioned, we intended to describe an innovative method for measuring gastric emptying in mice. Furthermore, it was applied to determine the gastric emptying rate of a novel pectin based enteral nutrition product. As expected, high viscous EN delayed gastric emptying in mice. However, as far as a previous conflicting result exists, we should discuss the discrepancy. We describe studies of the additive effect of pectin on gastric emptying of a liquid meal to the revised Introduction and Discussion sections and compared differences between the present results and those obtained under various experimental conditions (p3. L67-71, p8. L184-209).

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.

We appreciate your helpful advice. We added a new figure for clarification. (Fig 1 B)

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?

We deleted the word “surgical” (p5. L106).

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

We describe why this was necessary (p.5. p109-112)

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?

We assessed the mice by imaging before EN ingestion and changed the expression accordingly.

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

We clarified this phrase in the revised manuscript (p5. LL115-120).

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

We clarified the phrase in the revised manuscript (p6. L126-132).
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.

The caloric content regulates gastric emptying rates. We describe this in the manuscript and added an example illustrating a patient with limited gastric function (p3. L50-L57).

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

The mouse shown in Figure 1 (Fig. 2 in the revised manuscript) is the same mouse at different times. Fluorescence imaging is sensitive to small differences in the depth of the stomach containing EN, supposedly up to 1-3 cm which depends on physiological condition encircling illuminant. This was discussed as a limitation (p10. L213-217).