ANSWERING REVIEWERS

February 15, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: Manuscript revision NO_ 16258.doc).

Title: Four Sample Lactose Hydrogen Breath Test for Diagnosis of Lactose Malabsorption

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 16258

The manuscript has been improved according to the suggestions of reviewers:

1. Format has been updated

2. References and typesetting were corrected

3. Revision has been made according to the suggestions of the reviewer

Reviewer #1

Question1. Was the order that the subjects received the different doses of lactose randomised?

Answer1: all participants underwent HBT at 10g, 20g, 40g lactose on three different days, each separated by 7-14 days in randomized order using random number table.**(see Page 9)**

Question 2.I'd like to know a little more about the symptoms that those with LI developed.

How about results from the telephone call after the test?

Answer 2: LI symptoms in 96.1% (49/51) IBS-D patients and 92.7% (38/41) HVs occurred between 90-180min after lactose intake. Two IBS-D patients developed moderate diarrhea at 215min, 245min after lactose intake. one HV developed severe diarrhea at 230min, two HVs developed severe bloating and borborygmi in 255, 300min after lactose intake. (see page 18)

Question 3. It would help many readers to explain early on in the introduction, for example, what the genotypes relate to and what 'CC' means as opposed to 'CT" and 'TT'.

Answer 3:This is excellent suggestion, genotypes about lactose deficiency have added in the introduction. **(see Page 7)**

In 2002, Enattah et al.[8] reported single-nucleotide polymorphism C/T-13 910 above the structural gene coding for the enzyme lactose on the short arm of chromosome 2q.21-22. Many recent studies showed CC-13910 was a good predictor of intestinal lactose deficiency and LI, there was an excellent agreement between genetic testing of lactose C/T-13 910 and LHBT. With LHBT as the gold standard, the CC-13 910 genotype had a high sensitivity (97%) and specificity (95%) with high kαvalue (0.9) in diagnosing lactose deficiency.

Question 4. Would any less than 4 samples be equally helpful and if so what timing? Wouldn't expanding the regime to >3 hours further increase the diagnostic yield?

Answer: As previous studies, three sample (0, 120,180) was also almost equally helpful in diagnosing LM compare with 4 sample (0, 90, 120, 180) for LHBT in our study. But considering small intestinal bacterial overgrowths (SIBO) could bring about false positive results on LHBT, the H2 value of 90min could help us estimate whether the participant exist SIBO, when this point value

was very high, even higher than that of 120min or 180min, SIBO should be considered for oro-caecal transit time in this study population was 60~90 minutes. However in a population with high prevalence of LD, SIBO would not alter the number of patients diagnosed with LM. **(see page 16-17)**

Hydrogen breath concentration at all three doses (10g, 20g, 40g) of lactose most often increased after 90min of lactose intake and peaked between 150-180 min, and 96.1% (49/51) IBS-D patients and 92.7% (38/41) HVs developed LI symptoms between 90-180min after lactose intake, therefore, expanding the regime to >3 hours little increase the diagnostic yield. (see page 18)

Question 5. It may help to speculate, based on the results, what the authors propose to be the optimal dosage of lactose to be? Clearly LI is a very common diagnosis in this cohort and rates increase with larger lactose challenges.

Answer 5: We propose 20g was the optimal dosage for LHBT. High doses (i.e. 40g, 50g**)** are appropriate for epidemiologic studies of lactase deficiency. As our another study ***(Yang J, Deng Y, Chu H, et al. Clin Gastroenterol Hepatol 2013;11:262-268)*** showed the number of patients that reported LI symptoms was significant lower after ingestion of 20g than 40g lactose (controls: 22% vs. 68%; D-IBS: 47% vs. 85%); with concordant results for LM diagnosis for 20g and 40g lactose HBT in 88% of participants. Moreover, participants that reported a high number of different symptoms (≥3) and / or a high total symptom score (TSS≥5) were very likely to have D-IBS and these criteria may identify patients that should benefit from nutritional intervention.

Question 6. Minor stuff :- 'rats' should be 'rates, page 11 'Detection rates of LM and LI'. Do you really mean 'our Swiss study' on line 4 , page 14 in discussion. Reference is from other

authors. Lactase deficiency is not totally (but almost) 'universal' line 12, page 14.

Answer 6: We have revised "rats" to rates **(see page 13)** and Lactase deficiency was abbreviated as LD.

This study was cooperated with division of Gastroenterology & Hepatology, University Hospital Zürich, Zürich, Switzerland. Reference 11 was also from division of Gastroenterology & Hepatology, University Hospital Zürich, Zürich, Switzerland conducted by Prof Michael Fried and Mark Fox.

Review #2

Question: It is good for readers. A minor English correction is required

Answer: We have made appropriate edits and correction in some clerical error. Revised portion are marked in red in the paper.

4.The manuscript have been reviewed and revised by one of corresponding authors, **Mark Fox** , a native speaker of English, who have worked in NIHR Nottingham Digestive Diseases Biomedical Research Unit, Queen's Medical Centre, Nottingham, United Kingdom. So we think it don't need for employing a professional English editing service for this manuscript.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology.*

Sincerely yours,

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