

July 4, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 11786-review), and an editorial certification from American Journal Experts: <http://www.aje.com> in PDF format (file name: 11786-language editorial certificate).

Title: Delayed Ethanol Elimination and Enhanced Susceptibility to Ethanol-Induced
Hepatosteatosis after Liver Resection

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Name of Journal: *World Journal of Gastroenterology*

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

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

(1) MATERIALS AND METHODS (Page 7) : “Eight-week-old male Wistar rats from Charles River Japan, Inc. (Tokyo) were randomly assigned to the PH or the Sham-operation (Sham) groups.” This section seems to be rather opaque, the author should explain this in further detail, and how the randomized groups were conducted.

We agree with that and “Eight-week-old male Wistar rats from Charles River Japan, Inc. (Tokyo) were randomly assigned to the PH or the Sham-operation (Sham) groups” is revised to “Eight-week-old male Wistar rats from Charles River Japan, Inc. (Tokyo) were randomly assigned to either the

PH or the Sham-operation (Sham) group for a better chance at detecting if the observed changes were due to chance or due to the PH itself” in the 3rd paragraph, line 3-6 of page 8.

- (2) The study sample size appears to be rather arbitrary, which is also the biggest method problem. Is there theory-guided rationale for such a sample size? The author should provide a description about sample size calculation. In the current situation, four groups were compared, and the largest sample size is only ten, which is quite far from the requirements of statistical analysis. Such a small sample size makes the whole analysis results quite unreliable.**

Referenced to the previous studies, such as “Al Asmari AK, Al Omani S, Elfaki I, Tariq M, Al Malki A, Al Asmary S. Gastric antisecretory and antiulcer activity of bovine hemoglobin. *World J Gastroenterol.* 2013;19:3291-3299” and “Molotkov A. Duester G. Genetic evidence that retinaldehyde dehydrogenase Raldh1 (Aldh1a1) functions downstream of alcohol dehydrogenase Adh1 in metabolism of retinol to retinoic acid. *J. Biol. Chem* 2003; 278: 36085–36090 (reference 12 of the present study)”, we inducted 4 or 5 PH rats and 6 Sham rats into the 1st stage of the present study; 7 Sham-control rats, 8 Sham-ethanol rats, 10 PH-control rats, and 10 PH-ethanol-rats into the 3rd stage of the present study. However, we agree with you about the comments on the sample size, and we will pay attention deeply about that in our future study.

- (3) Similar to point 2, the sample size is so small. The parametric statistical methods used in the manuscript are very inappropriate including the descriptive statistics, (means ± standard deviations). The non-parametric statistical should be conducted, and the median and quartile should be used to describe the results.**

In the present manuscript, all of the data, which be expressed as means ± standard deviations, were continuous data, so parametric statistical method was used in the present study. We re-edited “All of the values were expressed as the means ± standard deviations (SDs). Extreme value was excluded by Smirnov - Grubbs test when necessary. The statistical significance was assessed by

1-way analysis of variance (ANOVA) using Statcel2 for Windows software (OMS Publishing, Inc., Saitama, Japan)” to “Continuous data was expressed as the means ± standard deviations (SDs). Extreme value was excluded by Smirnov - Grubbs test when necessary. The statistical significances of the continuous variables were assessed by 1-way analysis of variance (ANOVA) using Statcel2 for Windows software (OMS Publishing, Inc., Saitama, Japan)” in the 3rd paragraph, line 2-5 of page 14.

- (4) MATERIALS AND METHODS (Page 8)** According to the author’s statement, there are 6 sham operation and 30 PH. “Two PH rats, died from PH operation, were discarded from the preliminary examination.” The total should be 34. But in the Abstract part, the author mentioned “Pair-feeding was performed with a controlled diet or with a 5-g/dL ethanol liquid diet for 28 days in 35 age-matched male Wistar rats with a one-week recovery after undergoing a sham operation or PH. Please explain this.

In the 1st stage of the present study, 6 sham operation and 30 PH rats were used for the preliminary examination to determine the recovery of liver function after PH. However, 1 of 4-hour and 1 of 3-day PH group rats died from PH operation, and then 6 sham operation and 28 PH rats were conducted into the preliminary examination (Figure-1) in the present study. In the 2nd stage of the present study, the time point for the beginning of the chronic ethanol exposure (1 week after PH) was determined according to the results of the preliminary examination. In the 3rd stage of the present study, another 35 age-matched male Wistar rats with a one-week recovery after undergoing a Sham- (Sham-control, n = 7; Sham-ethanol, n = 8) or PH-operation (PH-control, n = 10; PH-ethanol, n = 10) were used to evaluate the ethanol-induced liver injury after liver resection. “The preliminary examination was performed on 6 sham operation (Sham) and 30 PH male Wistar rats (8-week-old) to evaluate the recovery of the liver weight and liver function after liver resection in rats. The time point for the beginning of the chronic ethanol exposure (1 week after PH) was determined based on the results of the preliminary examination. Pair-feeding was performed with a controlled diet or with a 5-g/dL ethanol liquid diet for 28 days in 35 age-matched male Wistar rats with a one-week recovery after undergoing a sham operation or PH” is revised to “First, the preliminary examination was performed on 6 sham operation (Sham) and

30 partial hepatectomy (PH) male Wistar rats (8-week-old) to evaluate the recovery of the liver weight and liver function after liver resection in rats. PH rats were sacrificed at the indicated time points (4, 8, and 12 hours; 1, 3, and 7 days) after PH. Second, the time point for the beginning of the chronic ethanol exposure (1 week after sham- or PH-operation) was determined based on the results of the preliminary examination. Finally, pair-feeding was performed with a controlled diet or with a 5-g/dL ethanol liquid diet for 28 days in another 35 age-matched male Wistar rats with a one-week recovery after undergoing a sham- (n = 15) or PH-operation (n = 20) to evaluate the ethanol-induced liver injury after PH” in the Abstract part, and we also revised the relevant parts in Material and method part (the 3rd paragraph, line 3 of page 8, the 2nd paragraph, line 2 of page 9, and the 1st paragraph, line 2 of page 10).

- (5) Results (P13): According to the material and methods, “The groups were divided as follows:.....ethanol liquid diet (PH-ethanol, n = 10).” Some indicators should be compared among four groups before PH or sham. How can you be sure that the baseline characteristics are consistency among four groups?**

This is a statement of fact. We compared the body weight and liver function before and 1 week after the sham- or PH-operation among the 4 groups and added the results in table-1 and the 1st paragraph of page 15.

- (6) Although the manuscript provided a number of figures to show the results, some Tables with specific value should be used, particularly in the Figure 7. The corresponding statistic results should be presented.**

We agree with that and re-edited the figure 7 to table-2 with the specific values and the corresponding statistic results.

- (7) AST (aspartate amino transferase) is also an indicators of live function, why does the author only use the ALT? Please explain this.**

This is a statement of fact. We added the AST data using the residue samples in figure 4 and

Abstract part, and revised the relevant parts in Material and method part (the 3rd paragraph, line 4 of page 13), Results part (the 2nd paragraph, line 8 of page 15).

(8) In the manuscript the author should use the hepatic steatosis instead of hepatosteatosi, and the former is more common.

We re-edited "hepatosteatosi" to "hepatic steatosis" in the manuscript exception the title due to the words limitation.

(9) The Figure legends is too long. The important results should be presented in the form of text.

We condensed the figure legends and presented the results in the form of text in the relevant parts of the manuscript (the 2nd paragraph of page 15 for figure 3 and figure 4, the 1st and 2nd paragraph of page 16 for figure 5, the 3rd paragraph of page 16 and the 1st paragraph of page 17 for figure 6).

3 References and typesetting were corrected

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

Sincerely yours,



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