

10/30/2018

Dear Dr. Wang:

Science editor of the World Journal of Gastroenterology

Thank you for your letter and for the opportunity to revise our paper "Effects of Alkaline-electrolyzed and Hydrogen-Rich Water, in a high-fat diet NAFLD Model." We have answered the reviewer's comments carefully and made all needed corrections and changes, which we hope meet your approval.

Answers to Comments of Reviewer number 1

**1-The study is observational in nature**

A – This is not observational since treatment was given and effects measured. In an observational study, individuals are observed or specific outcomes measured without any intervention or a control group. This is not the case with our study. It was randomized, controlled, and had an experimental group, etc.

**2. The authors only measured the modified gene expression by PCR. The alterations of protein expression should be analyzed.**

Although we concur that additional protein level measurements would further bolster our findings, we respectfully would like to point out that:

- a) In prior studies of (gene) expression changes, qRT-PCR analysis of changes in mRNA levels closely matched the changes at the protein level;
- b) qRT-PCR is overall a much more sensitive method of quantifying gene expression than immunoblotting;

c) As a result of a and b, many studies of NAFLD have predominantly relied on Gene expression (Kohjima et al., 2007: *Re-evaluation of fatty acid metabolism-related gene expression in NAFLD*; Greco et al., 2008 *Gene expression in human NAFLD*). A recent study proposed NAFLD signature based on gene expression ( Ryaboshapkin and Hammar 2017, *Human hepatic gene expression signature of non-alcoholic fatty liver disease progression, a meta-analysis*).

**3. Besides the preventive effect of H-HRW on NAFLD, is there any therapeutic effect of H-HRW on NAFLD.**

Based on the current study we can assume that yes, however for an answer to this question a new study should be performed starting to give hydrogen-rich water in mice who already have developed NAFLD. Additionally, the authors are aware of an ongoing clinical study where molecular hydrogen is being administered to patients with NAFLD, and the preliminary results suggest a therapeutic effect.

**4-The author should explain in more detail why average water consumption was significantly higher in H-HRW group than that in L-HRW group and control group.**

A paragraph was added to the discussion: " It is unknown why mice in the H-HRW group ingested nearly 3 times more water. Perhaps the magnesium/water reaction continued to elevate the pH after our initial measurements, which may then result in an increased thirst sensation." It is believed that acidic beverages can quench the thirst sensation better than those of higher pH.

**5. Typo error, NALFD should be NAFLD.**

All type errors were corrected.

Answers to Comments of Reviewer number 3

**1. Authors are kindly requested to express their data as mean plus/minus SD and not SEM because readers are interested in knowing the dispersion of values and not the precision of the mean due to the paucity of observations for each group.**

The data are already given as mean and SD; there was a mistranslation from the first version of the results that were written in Hebrew.

**2. Some words are lacking, i.e.,.....ACOX is one of the first in the metabolism of and is a rate-etc...**

The paragraph was corrected in the text, and missing words added.

ACOX is one of the first **enzymes** in the metabolism **of lipids** and is a rate-limiting enzyme for beta-oxidation of very-long-chain fatty acids in peroxisomes.

**3. The correct reference for the main role of metabolic syndrome determining HCC via NAFLD is the following....Could metabolic syndrome lead to hepatocarcinoma via non-alcoholic fatty liver disease? World J Gastroenterol. 2014 Jul 28;20(28):9217-28.**

Reference was corrected.

We hope the revised manuscript will now meet the criteria for the *World Journal of Gastroenterology*. We are happy to make any further revisions if needed.

Sincerely

Karen Jackson