Reviewer’s report

Title: Dietary Diallyl Disulfide supplementation attenuates ethanol-mediated pulmonary vitamin D speciate depletion in C57Bl/6 mice.

Version: 3 Date: 4 February 2015

Reviewer: Diane Allen-Gipson

Reviewer’s report:

Reviewer’s Comment for BMC Nutrition_ McCaskill et al 2015.
Title: Dietary Diallyl Disulfide Supplementation Attenuates Ethanol-mediated Pulmonary Vitamin D Speciate Depletion in C57BL/6NCrl Mice

In this article the author investigated a proposed mechanism of DADs in providing availability of vitamin D in lung tissue and pulmonary epithelium lining environment.

Furthermore, the authors sought to demonstrate nutritional supplement of diallyl disulfide (an organo-sulfur compound) may prove beneficial in minimizing chronic alcohol depletion of vitamin D levels which may be associated with alcohol-related respiratory infections/injuries.

Major Compulsory Revisions:

(A) Background
- Although the author described very effectively Vitamin D metabolic pathway however failed to link the role of Vitamin D, alcohol consumption and/or the respiratory infections associated with chronic consumption of alcohol. Respiratory complications associated with chronic alcohol use is an area not fully well understood and merit gaining a better understanding of the underlying mechanism associated with alcohol-related respiratory complications. This is quite critical as mentioned by the author that it has been well documented in liver maladies but less obvious health-related ramifications e.g., severe respiratory infections [lines #147-153]. Furthermore, the author did not provide a rationale for nutritional supplementation of diallyl disulfide as a nutrient capable to blunt alcohol-mediated depletion of Vitamin D in the airways particularly the mucosal and epithelial layer (epithelium lining environment; ELE) of the larger airways. Although it has been well documented that DADs gave chemopreventive properties as well as a modulator of CYP2E1 activity; It would have been advantageous for the author to demonstrate activities of CYP2E1 and CYP27B1 as being blunted or increased, respectively. Providing protein expression of these enzyme does not correlate directly to the activity of these respective isoform as indicated in Figure 5 (Result section).

(B) Methods
- In Vivo Model: This section should be more clearly written to describe all the
animal groups e.g., Grp 1 - control (No EtOH0; Grp 2 (EtOH only); Grp 3 (EtOH and DADs); Grp 4 (DADs only). As written, I was not sure how many groups were involved in the study. Furthermore, Figure 3 A & B suggested there were actually six groups based on the labeling of the graph (ethanol/D3) and ethanol/D3/DADS). This need to be clarify.

- Ethanol Exposure, Lung Tissue and Blood Sera can be combined together and methodologies be more concise.

- Quantification of 25 (OH)D3 and 1,25 (OH)2D3 methodologies can be combined since author used ELISA for detection in both tissues and BALFs.

- Phase 1 Metabolizing Enzyme Assay methodologies need to be more concised and suggest the author to include activity measurements of CYP2E1 and/or CYP27B1, respectively.

(C) Results

- Read more like a Method Section and did not explain why the observed result occurred for example, line 301 stated “The serum levels of 25 (OH)D3 in ethanol-fed C57BL/6NCRL mice, when normalized to total protein, were statistically unchanged” Why did that happened? The author failed to explain this observation however mentioned “statistically significant 51% reduction in 25(OH)D3 in lung tissue of ethanol-fed mice as compared to the non-ethanol fed controls” How was the 51% calculated and why was this observation observed in the ethanol-fed mice when the serum levels were statistically unchanged? How many animals were used, n= 8? This section would need to be revised and figure should be denoted as Figure 1 A or B to provide more clarity. A conclusion to the observed data is needed as well.

- Figure 2, 3, 5 similar comment as above. For Figure 5 as mentioned suggest that there are 6 groups involved in this study? Please clarify?

- Figure 4, what does the protein levels of VDR means? Is this a good thing? Does it mean the lung is leaky due to the injury from ethanol consumption or is it that ethanol increases VDR levels as an indication that Vitamin D levels are decreased? This was not clearly explain in this section.

(D) Discussion

- The author indicated the incongruent results may be attributed to an abundance of dietary vitamin D precursor provided in standard rodent chow (lines 351-353). What was the baseline levels of Vitamin D in the control groups (no EtOH or DADs)?

- The author mentioned that alveolar macrophages are an integral players in host infection response and are modulated by 1,25 (OH)2D3[lines 374-375] and implied TLR2/1 mediated immune response could be impacted[lines 375-379] Did BALF provided any evidence of macrophages or immune cells involvement? Direct measurement of CYP2E1 and CYP27B1 is essential to support your findings particularly since measurement of protein does not always relate to the availability or activity of the enzyme.
- Most of the discussion was speculative and warrant more experimentations to delineate the role of vitamin D and DADs in chronic alcohol-related respiratory infections/injuries

Minor Essential Revisions:
Some grammatical errors (verb-subject agreement), typos and formatting (page 8 line 186 “dially[mm1]” highlighted red; line 215 “was formulated” underlined in blue.
Figure legends are not all formatted the same example Figure legend 2 vs Figure legend 5

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**
I have no competing interests