Format for ANSWERING REVIEWERS

October 13, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 13702-review.doc).

Title: Vitamin D deficiency and liver disease

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Name of Journal: *World Journal of Hepatology*

ESPS Manuscript NO: 13702-edited

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 reviewers

(1) **Reviewed 02976740*:* The manuscript "Vitamin D deficiency in chronic liver disease" presents an over of the literature on the role of vitamin D in chronic liver disease, chronic hepatitis C virus infection, and non-alcoholic fatty liver disease. (NAFLD) The authors further discuss the synthesis and regulation of vitamin D and the biological (cellular) plausibility for its role in liver diseases. Although my research does not generally focus on hepatology, I greatly enjoyed reading this manuscript, and believe it is a strong contribution to the literature on vitamin D deficiency that will be well received from a multidisciplinary group of readers. The review is comprehensive, offers a generally clear presentation of complex biological phenomena, and may advance the study of vitamin D deficiency in liver disease. Notwithstanding these many strengths, some aspects of the manuscript could likely benefit from revision. First, although the authors have seemingly surveyed a vast body of literature, they do not specify how the selected articles for inclusion. The authors should specify whether studies were selected systematically based on a set of explicitly defined search criteria (and if so, the search strategy should be mentioned in a methods section). If the authors have not conducted a systematic search, they should acknowledge this towards the end of their review. Relatedly, it is not clear how or why the authors choose to highlight certain studies but not others. For instance, they discuss a study of Targher and colleagues (page 15) but do not identify other individual studies. They also present a table summarizing the literature on vitamin D and hepatitis C, but they do not present similar tables for NAFLD or chronic liver disease. Second, the manuscript can be considerably shortened without comprising its central objective. Specifically, the sections on vitamin D synthesis and regulatory mechanisms of vitamin D synthesis seem somewhat extraneous (and the latter section is a bit confusing). They can likely be incorporated in briefer form in the section titled "Vitamin D: Functions and their implications in liver diseases." Although the overview sections on vitamin D synthesis and regulation are quite insightful and summarize a large body of literature, these sections detract from the focus of the manuscript. Further, the first paragraph in section 8 can be condensed, as I suspect that the readers of this journal are familiar with the epidemiology and public health significance of NAFLD. Third, the organization of the**

manuscript can be improved slightly. The authors should note in their introduction the objective of their manuscript. The section on vitamin D functions in liver diseases can also probably be moved after the discussion of vitamin D deficiency in each of the three types of diseases. Fourth, in place of the aforementioned sections that could be removed, the manuscript may benefit from a brief discussion of the limitations of previous studies of vitamin D and liver diseases. They note some of these limitations in their review of two meta-analyses on page 13, but their implications for the overall literature on vitamin D and liver disease (particularly issues pertaining to the methods of vitamin D assessment) should be addressed. Doing so may also help strengthen the authors' recommendations for future research at the conclusion of the manuscript. These recommendations should also likely be expanded and clarified, as this manuscript may serve a strong research agenda for future research. Some minor comments are addressed below: ? Given the numerous abbreviations that appear throughout the manuscript, it would be helpful to include a box at the beginning of the manuscript that defines these terms. ? The authors should please clarify the functional significance and 3 genotypes of the polymorphism discussed at the bottom of page 13 to top of page 14.

According to the suggestion of the reviewer, we have included a) how selected articles for inclusion; b) a table for NAFLD; c) we have condensed the first paragraph in section "VITAMIN D AND NONALCOHOLIC FATTY LIVER DISEASE (NAFLD)"; d) we have included the objective of our manuscript; e) we have included at end of manuscript (after conclusion) a box with abbreviations; f) we have modified the functional significance of polymorphism

(2) **Reviewed 02975652: This is an interesting and detailed review on the complex interplay between vitamin D status and chronic liver disease. There is remarkable discussion of data on cell-mediated and humoral immune functions related to vitamin D, in association to the mechanisms controlling the evolution of liver diseases. There are such sections where the description is unfortunately appearing somewhat generic, and would therefore benefit from further analysis of available data, more detailed description of the involved mechanisms, as well as semplification of presented Literature, so as to make the writing easily readible for a vast audience. For examples I would suggest expanding the following subjects: how vitamin D modulates SVR (page 13, middle paragraph), which proinflammatory cytokines specifically modulate liver damage (page 12, second paragraph; page 16, line 5-14). The review by Adams JS & Hewison, Nat Clin Pract Endocrinol Metab 2008, should be quoted. The description on the relationship between vitamin D and metabolic syndrome, obesity and diabetes should be more thorougly detailed. This paper is clearly directed to hepatology readers, howevere obesity and metabolic syndrome remain the strongest determinant of vitamin D deficiency in current era, and I suggest to expand this subject by also quoting some papers on the association between vitamin D and diabetes and obesity (Song Y et al, Diabetes Care 2013,36:1422; Bellan M et al, Cardiovasc Diabetol 2014,13:57; Afzal S et al, Lancet Diabetes Endocrinol 2014, 2:298). It is well known that obesity promotes the onset of NAFLD due to increased hepatic lipid synthesis secondary to excess free fatty acids; subsequent association with oxidative stress on mitochondrial and with the increase of proinflammatory cytokines can definitely trigger a progression of steatosis to nonalcoholic steatohepatitis (NASH) and cirrhosis (Tilg H et al, N Engl J Med 2000 343: 1467-1476). Studies in vivo and in vitro have clearly documented that oxidative activity controlled by cytochrome P450 is reduced in the fatty liver (MT Donat et al, Drug Metab Dispos, 2006 34: 1556-1562). As introduced by the Authors, studies in humans have observed a decrease in the levels of 25OH-Vit. D with the progression from healthy subjects to patients with hepatic steatosis**

and those with NASH (Targher G et al, Nutr Metab Cardiovasc Dis. 2007 17: 517-524), while levels of 25OH-Vit. D decrease with increasing degree of hepatic impairment in non-cholestatic hepatitis (Fisher L & Fisher A. Clin Gastroenterol Hepatol, 2007 5: 513-20), as well as the degree of fibrosis and to decrease hepatic expression of CYP27A1 in 'chronic hepatitis C genotype 1 (S Petta et al., Hepatology 2010 51: 1158-1167). Mortality data at page 9 should be implemented with some further discussion also on optimal VD levels (Chowdhury R et al, BMJ 2014, 348:1903; Bischoff-Ferrari, Adv Exp Med Biol 2014,810:500). When fibrosis is quoted at page 13, it would be important to quote recent data from Abramovitch S et al (Am J Physiol Gastrointest Liver Physiol. 2014 Sep 11). Data on vitamin D and AH should be adequately discussed and included in a separate chapter. Specific comments: Introduction: Please clearly and in detail mention which are the issues and how they will they be discussed in this review. Page 4 line 2: correct 25-dehydroxylation as 25-hydroxylation. Page 4, last line: correct VDR deficiency as vitamin D deficiency. Page 12, line 28: "transmembrane" vitamin D receptor is an unclear classification for a NR such as VDR, please clarify. Page 16 line 21: the abbreviation TFgB is unclear, do the Authors mean TGF-beta?

According the suggestion of the reviewer, we have included: a) more detailed description between vitamin D and metabolic syndrome, obesity and diabetes; b) we have included detailed description that obesity promotes the onset of NAFLD due to increased hepatic lipid synthesis secondary to excess free fatty acids; subsequent association with oxidative stress on mitochondrial and with the increase of proinflammatory cytokines can definitely trigger a progression of steatosis to nonalcoholic steatohepatitis (NASH) and cirrhosis; c) all references suggested by reviewer are included in the manuscript; d) discussion on optimal VD levels; e) recent data from Abramovitch S et al (Am J Physiol Gastrointest Liver Physiol. 2014 Sep 11); f) typesetting were corrected; f) we have clarified vitamin D receptor

(3) **<u>Reviewed 00351780:</u> This is a useful and generally well-written review of the role of vitamin D in liver disease. While I understand the need to include possible non-genomic activities of vitamin D, the authors need to be careful to indicate whether these non-canonical activities have been implicated in a particular process. Specific Comments: 1. Abstract: "hipovitaminosis D" should be "hypovitaminosis D" 2. Vitamin D Synthesis: The sentence "…Finally, VDR acts as a transcription factor and assists in the activation of cellular signaling pathways independent of its genomic effects[16]…." is confusing. Aren't the activities of a transcription factor inherently genomic? Very rapid, non-genomic effects of 1,25(OH)2D are known and may require plasma membrane VDR – it would be helpful to include Boland, RL. VDR activation of intracellular signaling pathways in skeletal muscle. Mol Cell Endocrinol. 2011, 347:11-16 as a reference, since the Messa reference may not be easily accessible. 3. VITAMIN D AND CHRONIC LIVER DISEASE: On p. 8, the word "casual" should probably be "causal". The word "codifies" is not correct here; it refers to arranging or systemizing, particularly a set of rules. The better word is "encode" or "encoding". 4. VITAMIN D AND CHRONIC HEPATITIS C VIRUS INFECTION: The authors state "Active vitamin D must interact with its specific transmembrane receptor (VDR) to exert its physiological functions[16,17]." While VDR can be plasma membrane associated, is there evidence it can become truly transmembrane? Is it clear that the described antiviral properties of vitamin D are non-genomic? If so, please expand. What does "b) mayority had a cross-sectional desing" mean?**

According to the suggestion of the reviewer: a) we have included the reference "Boland, RL. VDR

activation of intracellular signaling pathways in skeletal muscle. Mol Cell Endocrinol. 2011, 347:11-16"; b) typesetting were corrected; c) We have modified the sentence "… Finally, VDR acts a transcription factor……"; d) we have modified cross-sectional desing; e) we have clarified vitamin D receptor

(4) **Reviewed 02976944: I red with great interest in this review. I have some concerns as followings: 1. The discussion should be more in-depth. 2. A vitamin D signaling should be preferred.**

According to the suggestions of the reviewers we have modified the manuscript.

3 References and typesetting were corrected
4 We have included two tables and one figure in the manuscript

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

Sincerely yours,

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