Answering reviewers

Dear Dr Wei,

Thank you very much for your letter and advice. We have revised the manuscript, and would like to re-submit it for your consideration. We have addressed the comments raised by the reviewers, and the amendments are highlighted in red in the revised manuscript. Point by point responses to the reviewers comments are listed below this letter. In addition, the revised manuscript has been edited and proofread according to the journal style.

We hope that the revised version of the manuscript is now acceptable for publication in your journal.

I look forward to hearing from you soon.

With best wishes,

Yours sincerely,

Naiqian Zhao, MD
Corresponding author

We would like to express our sincere thanks to the reviewers for the constructive and positive comments, and their patience and preciseness.

Replies to Reviewer #1

1. Why did authors use HepG2 cells?

Answer: HepG2 cells represent a relatively well-differentiated cell model of hepatocyte function and have a wide variety of liver-specific metabolic functions including the function related to triglyceride metabolism (see

reference below). Therefore, they are often used as a cellular model for researching hepatic steatosis.
Reference: Javitt NB. Hep G2 cells as resource for metabolic studies: lipoprotein, cholesterol, and bile acids. FASEB J. 19901;4:161-8.

2. In M & M section, Is HepG2 cell a hepatoma cell line or hepatoma cell?

Answer: "a hepatoma cell line" has been removed in the revised version (page 7, line 13).

3. In M & M section, Do not repeat "Company, city, state, country" twice such as Invitrogen, Carlsbad, CA, USA.

Answer: The repetitions have been removed in the revised version.

4. In M & M section, Please describe the primer sequences of G0S2.

Answer: The primer sequences of G0S2 see page 8, line 14-15 in the revised version.

5. In M & M section, Authors described "To measure the nuclear C/EBPβ protein level, nuclear protein extracts were isolated using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher Scientific, Waltham, MA, USA)." In results section, which of other proteins did authors use for Western blotting?

Answer: Some sentences have been changed in the "Western blot analysis" section (page 9, line 3-8) and some other sentences were highlighted in red (page 9, line 15-17 and page 12, line 1-3) to address this issue.

6. In abstract section, Authors described "This provide evidence linking G0S2 expression to palmitate-induced hepatic lipogenesis with important implications for the treatment of obesity-associated fatty liver disease." Authors should describe how important these evidence for the treatment of obesity-associated fatty liver disease. Please discuss more.

Answer: This sentence has been removed from the abstract section. Moreover, several sentences have been added (page 14, paragraph 2, line 2-6) in the revised version to address this issue.

7. In Figures 3 and 5, please use larger size of fonts.

Answer: Corresponding changes have been made in the revised version.

Replies to Reviewer #2

Recently, cytokeratin 8-18, or TPS/TPA, are reckoned as important for hepatocyte integrity and play a direct role in NAFLD/NASH as evident in…..Eur J Clin Invest. 2007 Jan;37(1):48-53. Serum concentrations of the tissue polypeptide specific antigen in patients suffering from non-alcoholic steatohepatitis. On the other side there is a strict link between palmitate and cytokeratins 18, as evident in……Reproduction. 2017 Apr;153(4):369-380. doi: 10.1530/REP-16-0576. Oleate attenuates palmitate-induced endoplasmic reticulum stress and apoptosis in placental trophoblasts. In fact, by immunoprecipitation, protein-protein binding assays and by immunoelectron microscopy authors identified a direct linkage between LD-binding proteins and the intermediate-sized filaments (IF) cytokeratins 8 and 18 (also designated as keratins K8 and K18). Specifically, in gradient fractions of higher density supposedly containing small LDs, they received as co-precipitations cytidylyl-, palmitoyl- and cholesterol transferases and other specific enzymes involved in lipid metabolism, as evident in….. (2013) Lipid Droplets, Perilipins and Cytokeratins – Unravelled Liaisons in Epithelium-Derived Cells. PLoS ONE 8(5): e63061. https://doi.org/10.1371/journal.pone.0063061. Authors are requested to comment these aspects in relation to their data referring to the suggested pieces of research.

Answer: NAFLD refers to a spectrum of disease ranging from steatosis to non-alcoholic steatohepatitis (NASH) with different degrees of fibrosis that can progress to cirrhosis and hepatocellular cancer. The development of NASH is frequently described by a "two-hit" mechanism, in which excessive hepatic lipid accumulation constitutes the "first hit", followed by a ''second-hit'' or ''multi-hits'' such as oxidative stress, endoplasmic reticulum stress, inflammation, apoptosis, and fibrosis. The studies mentioned above show that cytokeratins 8 and 18 are implicated in hepatocyte integrity, the composition of lipid droplet, and palmitate-induced endoplasmic reticulum stress and apoptosis. However, it is far from clear how cytokeratins 8 and 18

affect these pathological processes. Further mechanistic study will be needed to explore the relationship among palmitate, cytokeratins 18 and hepatic lipid accumulation. We are grateful for your bringing these problems to our attention.