SUNY Downstate Medical Center
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March 28, 2016

Dr. Shui Qiu
Scientific Editor
World Journal of Hepatology
8226 Regency Drive
Pleasanton, CA 94588 USA

RE: Lipogenesis in Huh7 cells is promoted by increasing the fructose: glucose molar ratio

Dear Dr. Qiu:

On behalf of my co-authors, I would like to express my thanks to you and to the editorial board of the *World Journal of Hepatology*, for carefully considering our above titled manuscript, and for offering a salient review of this work. The narrative below provides a point-by-point response to the comments of the expert reviewers. All manuscript revisions are indicated as "track changes" in the revised paper:

Reviewer 1: *This is an interesting study that reveals fructose is linked to lipogenesis in a concentration dependent way. Some minor style corrections: authors must write the aim also at the end of their introduction. The authors present comparisons between diferrent concentrations of glucose and between diferrent concentrations of glucose:fructose. It would be interesting to compared lipogenesis between glucose groups and glucose:fructose groups.*

I would like to thank the reviewer for these salient comments. The aim of our work has now been clearly stated at the conclusion of the Introduction. Our experiments, in fact, did include "glucose alone" experiments. I have clarified this point on page 7 of the revision: "*Glucose-mediated lipogenesis*. As shown in figures 1 and 2, triglyceride and cholesterol content [μg/mg cell protein] did not differ significantly among Huh7 cells incubated for 24 hours in media containing 0.65, 0.68 or 0.72 mM glucose per plate. Further increases [>0.72 mM] in glucose molar concentration did not result in any additional enhancement in cellular lipid content [data not shown]." The Discussion [page 8] also states: "For these studies, the cell culture media monosaccharide content of 0.72 mM [glucose alone or glucose plus fructose] was found to maximize hepatocellular lipogenesis. This molar amount was determined following a series of experiments, employing a step-wise increase in sugar content and based on previous human studies showing

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serum total monosaccharide concentrations of ~0.50 mM following a fructose-rich meal[24]. Higher amounts of monosaccharide [>0.72 mM] in the Huh7 incubating media did not yield statistically significant increases either in cellular TG or in cellular C content, while further increases [>400 mM] in media osmolality resulted in decreased cell viability."

***Reviewer 2**: 1- Question: Why authors did not use regular hepatocyte instead Huh7 cells for this experiment? 2- In the introduction and discussion section the author should not compare the finds in the present experiment with what happens in patients, this is an in vitro cell culture model using Huh7 cells, not a translational research.*

1. Huh7 cells [an ATCC certified cell line] were used because of the requirement, in this series of experiments, to assure a reproducible, standardized hepatocyte model, one that remains essentially unmodified through multiple passages. We employed an immortal cell line with a predictable and consistent metabolic and biochemical profile. These essential characteristics cannot be duplicated using a primary hepatocyte culture. To further emphasize the applicability of this model, the first paragraph of page 5 now states: "These experiments employ an established, immortal and metabolically active human hepatocellular carcinoma cell line, Huh7, used extensively in studies of hepatocyte metabolism[13-15]."

2. I would like to thank the reviewer for this important recommendation. Accordingly, the Introduction now states: "In light of the above clinical and experimental data, the present study seeks to establish the influence of fructose on hepatocyte lipogenesis and provide a basis for future, translational investigations of fructose-mediated lipid biosynthesis. These experiments employ an established, immortal and metabolically active human hepatocellular carcinoma cell line, Huh7, used extensively in studies of hepatocyte metabolism[13-15]. Since facilitated uptake of glucose and fructose by the transmembrane GLUT2 transporter is demonstrated in Huh7 cells [16], these cells provide an excellent model for studies of carbohydrate-induced lipogenesis. Accordingly, the studies herein were carried out to determine whether hepatocyte lipogenesis, in an in vitro cell culture model, is modulated by adjusting culture media monosaccharide content and concentration."

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Reviewer 3*: Title, aim and conclusion did not consistent and this study didn't analyze about risk factors. I think, in abstract conclusion the statement " These investigations provide evidence diets rich in fructose may be risk factors for hepatic steatosis and non-alcoholic fatty liver disease" would be move to the aim of study or to the discussion/suggestion.*

I would like to apologize to the expert reviewer, because I am not certain I understand the above suggestion. Nevertheless, I have attempted to modify the importance of this work as being directly applicable to clinical medicine. Therefore, the Conclusion now states: "In an *in vitro* hepatocyte model, glucose or fructose plus glucose support total cell mass and lipogenic activity. Increasing the fructose:glucose molar ratio [but not glucose alone] enhances triglyceride and cholesterol synthesis. These investigations demonstrate fructose promotes hepatocellular lipogenesis, and they provide evidence supporting future, *in vivo* studies of fructose's role in the development of hepatic steatosis and non-alcoholic fatty liver disease." Furthermore, as indicated in the response to Reviewer 2, we clarify the importance of these studies vis. their providing a basis for future, translational investigations.

Reviewer 4:

I would like to express my thanks to the expert reviewer, for this extensive, referenced analysis of our work. The reviewer has provided further evidence supporting the relevance of these studies and, since no specific recommendations for revision were contained in this review, no further manuscript modifications were made.

I would again like to thank you, the editorial board of *WJH* and the expert reviewers for this careful consideration of our work. I am confident the responses above adequately address the recommendations of the reviewers. Additionally, all editorial revisions have been completed and these comply with the comments provided in the manuscript (including all figures inserted as powerpoint images).

Sincerely yours,

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