ANSWERING REVIEWERS

Please find enclosed the edited manuscript in Word format (file name: 2429-review.doc).

Title: HCV NS5A promotes insulin resistance through serine phosphorylation of IRS-1 and increased gluconeogenesis in hepatocytes

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The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

Response to the reviewer's comments

We appreciate the reviewer's constructive comments. The reviewer has ably pointed out several important aspects relating to manuscript. In keeping with those concerns, additional experiments were carried out and the manuscript is thoroughly revised. All the revisions are underlined in the text.

We have revised the model (Figure 6) and changed Akt pSer^{473} to Akt Thr^{308}. This was done based on our results (Figure 3).

In addition to that, English Language Certificate duly approved by the English native speaker is attached herewith for your kind perusal.

Reviewer#2

1- As suggested by the reviewer, we have revised the text (introduction, results and discussion section) for better understanding.

2- In response to reviewer's suggestions, we have repeated the experiments in figures 1-4 and provided the better quality data.

3- We have replaced the 18s with 18 s rRNA as an internal control.

4- In response to reviewer's concern, we have changed the "gluconeogenic gene expression" with "PEPCK, G6P gene expression".

5- We have changed the diacylglycerides (ceramides) with diacylglycerides and ceramides on page 3.

6- We have corrected the errors in the text.

Reviewer#3

1- We have elaborated the methodology for better understanding.

2-We have provided the full forms of all the abbreviations mentioned in the introduction section.

3- We have replaced can't with cannot in the text.

4- We have changed FOX01 to Fox01.

5-We have revised the AIM section of the abstract.

6- We have clearly described the molecular biology technology used in the present study.

7- We completely agree with the reviewers concern, there was no specific reason to use SEM instead of SD. In our previous study, we have used SEM for data analysis (**Reference**: Hepatitis C virus nonstructural protein 5A favors upregulation of gluconeogenic and lipogenic gene expression leading towards insulin resistance: a metabolic syndrome. Fahed Parvaiz, Sobia Manzoor, Jawed Iqbal, Steven McRae, Farrakh Javed, Qazi Laeeque Ahmed and Gulam Waris. Arch Virol. Vol. 158 (11). 2013).

8- The present work was conducted in the laboratory of Dr Gulam Waris at the Rosalind Franklin University of Medicine and Science, USA, under the guidelines of institutional biosafety and ethical committee.

9- The work with infectious HCV material was performed within biosafety level 2 (BSL2) hoods with the appropriate approval from Rosalind Franklin University IRB.

Reviewer#4

As suggested by the reviewer, we have revised the text and repeated the experiments from figure 1-4 for better understanding. Previously, several studies have shown the increased serine phosphorylation of Akt (p-Akt-Ser^{473}) in human hepatoma cells infected with HCV or transfected with HCV core protein and showed that p-Akt-Ser^{473}does not play a role in insulin resistance process (Banerjee A et al., 2010; Banerjee S et al., 2008). In contrast, studies have suggested the role of phospho-Akt Thr^{308} in HCV core and HCV envelop (E2) proteins-mediated insulin resistance (Banerjee S et al., 2008; Hsieh MJ et al., 2012). Our data is consistent with these previous reports indicating that the insulin mediated p-Akt-Ser^{473} is activated by HCV infected or in HCN NS5A transfected cells and does not play a role in insulin mediated glucose metabolism or insulin resistance. Our data clearly indicate the role of HCV NS5A-mediated phospho-Akt Thr^{308} in insulin resistance process (Fig. 2).

We completely agree with the reviewer's concern regarding the status of phospho-Akt in HCV infected cells vs Akt in HCV infected liver biopsy samples. Previously, Aytug S et al., reported the decreased phosphorylation of Akt but did not clarified whether the Akt phosphorylation was at Ser^{473} or Thr^{308}. It may be possible that they have reported the decreased phosphorylation of Thr^{308} instead of Ser^{473} in HCV infected liver biopsy samples. It is well established that the phosphorylation of Akt at Thr^{308} is necessary for increased glucose uptake, whereas Ser^{473} is not (*Kondapaka S B et al., 2004; Cli Cancer Res, 10:7192-7198*). It was also not clear at what disease state the tissues were procured from the patients. Based on these reports, our results suggest the role of Akt Thr^{308} in HCV NS5A-mediated insulin resistance.