

## RESPONSES TO REVIEWERS' CONCERNS



December 25, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: **14675-review.doc**).

**Title:** Role of Ghrelin-GOAT system in the pathogenesis of Non-alcoholic fatty liver disease

**Author:** Shao-ren Zhang, Xiao-Ming Fan

**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO:** 14675

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 reviewer

**(1) Reviewer "02715825":**

**Comments:** The manuscript entitled: Role of Ghrelin-GOAT system in the pathogenesis of Non-alcoholic fatty liver disease addresses a right vision about this hormone and its pathological role in NAFLD. These are minor comments referred to style in order to improve the manuscript. **First**, please, be aware of grammar failures (Page 3, line 6, revalent) and abbreviations (DAG). **Second**, Although the two-hits hypothesis of NAFLD pathogenesis is currently the most recognized theory, the author should at least mention the multi-hit hypothesis, which is gaining strength. **Finally**, references

could be completed with current articles (Li Z, Xu G, Qin Y, Zhang C, Tang H, Yin Y, Xiang X, Li Y, Zhao J, Mulholland M, Zhang W. Ghrelin promotes hepatic lipogenesis by activation of mTOR-PPAR $\gamma$  signaling pathway. *Proc Natl Acad Sci U S A*. 2014 Sep 9;111(36):13163-8. doi: 10.1073/pnas.1411571111. Epub 2014 Aug 25 or Arslan N, Sayin O, Tokgoz Y. Evaluation of serum xenin and ghrelin levels and their relationship with nonalcoholic fatty liver disease and insulin resistance in obese adolescents. *J Endocrinol Invest*. 2014 Sep 9. [Epub ahead of print].

**Response:** Thank you for your constructive advice, the revision has been made as follows:

1)The typing error “revalent” has been corrected in “prevalent”(page4, Line16). “DAG”is the abbreviation of “des-acyl ghrelin” (page6, Line20).

2)As suggested, the “**multi-hits hypothesis**” information have been added in the paper(page5, Lines 6-12). The added information are as follows:

- Although the“two-hits hypothesis”of NAFLD pathogenesis is currently the most recognized theory, the “multi-hits hypothesis” that involves lipotoxicity, oxidative stress, mitochondrial dysfunction, a chronic inflammatory state and endoplasmic reticulum stress is getting more and more attention. The “multi-hits hypothesis” summarizes the complex factors and interactions between cytokines, FFAs metabolism, inflammation and IR in NAFLD<sup>[21,22]</sup>.

References:

21 **Polyzos SA**, Kountouras J, Zavos C. Nonalcoholic fatty liver disease: the pathogenetic roles of insulin resistance and adipocytokines. *Curr Mol Med* 2009; **9**: 299-314 [PMID: 19355912 DOI: 10.2174/156652409787847191]

22 **Liu J**, Xu Y, Hu Y, Wang G. The role of fibroblast growth factor 21 in the pathogenesis of non-alcoholic fatty liver disease and implications fortherapy. *Metabolism* 2014; pii: S0026-0495(14)00367-9. [Epub ahead of print] [PMID: 25516477 DOI: 10.1016/j.metabol.2014.11.009]

3)As suggested, the two references of **Li** (page12, Line23 to page13, Line2)**and Arslan** (page13, Lines 19-22)have been added in the paper. The added information are as follows:

- Li recently found that ghrelin activated hepatocytes lipogenesis via a mechanism of mTOR-PPAR $\gamma$  signaling pathway. The ghrelin-induced up-regulation of lipogenesis in hepatocytes was mediated by mTOR and the stimulatory effect was significantly attenuated by PPAR $\gamma$  antagonism in cultured hepatocytes and in PPAR $\gamma$  gene-deficient mice<sup>[93]</sup>.

References:

93 Li Z, Xu G, Qin Y, Zhang C, Tang H, Yin Y, Xiang X, Li Y, Zhao J, Mulholland M, Zhang W. Ghrelin promotes hepatic lipogenesis by activation of mTOR-PPAR $\gamma$  signaling pathway. *Proc Natl Acad Sci USA* 2014; **111**:13163-13168 [PMID: 25157160 DOI: 10.1073/pnas.1411571111]

- A recently study which evaluated ghrelin level and its relationship with nonalcoholic fatty liver disease and insulin resistance in obese adolescents found that ghrelin was negatively correlated with relative weight.

References:

103 Arslan N, Sayin O, Tokgoz Y. Evaluation of serum xenin and ghrelin levels and their relationship with nonalcoholic fatty liver disease and insulin resistance in obese adolescents. *J Endocrinol Invest* 2014; Sep 9 [Epub ahead of print] [PMID: 25200997]

## **(2) Reviewer "00058441":**

**Comments:** This review paper is focusing on the pathological role of Ghrelin-GOAT system for the development of NAFLD. This topic is interesting and also important for scientists who study energy homeostasis. I have some questions and suggestions: **1)** English editing: I have found lots of typing errors in this manuscript. For examples: Page3 relevant? prevalent Page5 synthesised?synthesized Page5 The concept that in addition to the important endocrine effect of acylated ghrelin, the paracrine effects of locally synthesised and acylated ghrelin may be important, was supported by identification of GOAT expression in various tissues. sentence is too complicated Page6 mediumchain? medium chain Page6 improved?improve Page6 pathways?pathway Page6 an critical therapeutic agent? a critical therapeutic agent ....(too many) **2)** GOAT appears to regulate post-translation of Ghrelin. acylated form can act on GHS-R but DAG is not. However, DAG is more abundant form and also play some roles in NASH patients. These facts make the Ghrelin/GOAT/NAFLD story very complicated and confusing. Not mention that leptin is regulating GOAT and also compete with acylated Ghrelin on the same receptor, GHS-R. I would like to suggest that authors may focus on acylated Ghrelin first without discussing DAG. Then, combing DAG and GHS-R-independent action of acylated Ghrelin in NAFLD together as one section.

**Response:** We appreciate your advice very much, the revision has been made as follows:

**1) Typing errors** have been corrected. "revalent" has been corrected in "prevalent" (page4, Line16). "synthesised" has been corrected in "synthesized" (page7, Line22). Sentence "The concept that in

addition to the important endocrine effects of acylated ghrelin, the paracrine effects of locally synthesised and acylated ghrelin may be important, was supported by identification of GOAT expression in various tissues.” has been corrected in “In addition to the important endocrine effect of acylated ghrelin, the paracrine effects of locally synthesized and acylated ghrelin may also be important. The concept was supported by the identification of GOAT expression in various tissues(page7, Lines20-24). “mediumchain” has been corrected in “medium chain” (page8, Line22 and Line25). “improved” has been corrected in “improve” (page9, Line19). “an critical therapeutic agent” has been corrected in “a critical therapeutic agent” (page4, Line22).

2) The “**leptin**” information have been added in the paper(page8, Lines 9-17). The added information are as follows:

- Gonzalez found that exogenous leptin administration markedly increased GOAT mRNA levels in the stomach mucosa of fasted rats. It’s indicated that during fasting low-leptin levels prevent an increase in GOAT mRNA levels and therefore GOAT can be added to the list of leptin-regulated genes under this specific condition<sup>[52]</sup>. Leptin is the main signal through which the hypothalamus senses nutritional state and modulates food intake and energy balance. Leptin plays an opposite functional role of ghrelin in food intake and it also regulates ghrelin receptor GHS-R1a<sup>[53-54]</sup>.

References:

- 52 **Gonzalez CR**, Vazquez MJ, Lopez M, Dieguez C. Influence of chronic undernutrition and leptin on GOAT mRNA levels in rat stomach mucosa. *J Mol Endocrinol* 2008; **41**: 415-421 [PMID: 18835978 DOI: 10.1677/JME-08-0102]
- 53 **Nogueiras R**, Tovar S, Mitchell SE, Rayner DV, Archer ZA, Dieguez C, Williams LM. Regulation of growth hormone secretagogue receptor gene expression in the arcuate nuclei of the rat by leptin and ghrelin. *Diabetes* 2004; **53**: 2552-2558. [PMID: 15448083 DOI:10.2337/diabetes.53.10.2552]
- 54 **Lopez M1**, Tovar S, Vazquez MJ, Williams LM, Dieguez C. Peripheral tissue-brain interactions in the regulation of food intake. *Proc Nutr Soc* 2007; **66**: 131-55. [PMID: 17343779 DOI: 10.1017/S0029665107005368]

3) As suggested, we focus on **acylated ghrelin** first without discussing DAG. Then, combining **DAG and GHS-R-independent action of acylated ghrelin** in NAFLD together as one section(page 6, Line13 to page7, Line2).

The revision information are as follows:

- Ghrelin requires a posttranslational modification in which the third serine residue is covalently linked to a medium-chain fatty acid, typically octanoate<sup>[32]</sup>. The O-n-octanoylation of ghrelin is essential for binding to and activation of the GHS-R1a receptor and the procedure is unique<sup>[35]</sup>. However, only the octanoylated form of ghrelin, which represents only 10%-15% of circulating ghrelin, is able to stimulate body weight gain and food intake<sup>[36-37]</sup>.

There are two forms of ghrelin named as acylated and des-acyl ghrelin (DAG). Without food intake, both forms are reported to rise gradually in the plasma. Although some effects of DAG are still controversial and its receptor has not been identified yet, the biological activities of des-acyl ghrelin have also been reported, including gastric motility<sup>[38-39]</sup>, adiposity and glucose metabolism<sup>[40]</sup>. Further evidence for metabolic function of ghrelin has been provided by phenotypic analysis of rodents with genetic deletions of either ghrelin<sup>[41]</sup> or its receptor, GHS-R1a<sup>[42]</sup>.

### **(3) Reviewer "02079515"**

**Comments:** The paper is interesting, however it does not add relevant novelties.

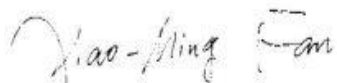
**Response:** We appreciate your suggestion very much. The underlying mechanisms leading to the development of NAFLD are not fully understood. The involvement of Ghrelin-GOAT system in NAFLD have led to the identification of pharmacological targets and the development of pharmacological compounds for the treatment of NAFLD and related diseases. we summarized research articles of this area published in recent years to illustrate the role of Ghrelin-GOAT system in the pathogenesis of Non-alcoholic fatty liver disease. We believe our review will do some help to researchers of this area to better understand this research field. Nevertheless, future studies will consider incorporating this vital component suggested by the esteemed reviewer.

The changes made following reviewer's suggestions have been highlighted in yellow in the revised manuscript.

3 References and typesetting were corrected

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

Sincerely yours,

A handwritten signature in black ink, reading "Xiao-Ming Fan". The signature is written in a cursive, flowing style.

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