

June 15, 2015

Dear Editor,

Please find enclosed the edited manuscript in World format (file name: 18336-basic study.doc).

**Title:** The potential effect of chronic *Helicobacter pylori* infection on glucose metabolism of Mongolian gerbils.

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

**ESPS Manuscript NO:** 18336

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

1 Format has been updated

2 The responses to the reviewer's comments are as following:

Reviewer #1:

Thank you for your questions and comments.

(1) Authors studied that the *H. pylori* infection increased HbA1c and IGF-1 levels on Mongolian Gerbils as well as the expression of cytokines and apoptotic index of islet beta cells. But these parameters did not show a significant difference by *H. pylori* infection. Authors wrote a list of potential parameters to explain which *H. pylori* infection causes T2DM, but there were no significant results in this manuscript demonstrating true causalities. Authors need to add data to explain why and how *H. pylori* causes T2DM.

**Author reply:** Our study assessed the effects of chronic *H. pylori* infection on metabolic parameters of Mongolian gerbils and found that the glycated hemoglobin and glycated hemoglobin A1c (HbA1c) levels were increased significantly after 12 months infection with *H. pylori*, which is in accordance with the results published by Hsieh et al. (Eur J Clin Invest 2013;43(9):949-56) who proposed that long-term *H. pylori* infection is significantly associated with high levels of HbA1c in Chinese population. However, the major limitation of our study is that the mechanism by which *H. pylori* infection increases the levels of HbA1c remains to be elucidated. It is commonly believed that the chronic inflammation induced by *H. pylori* infection is the major link with type 2 diabetes. Nevertheless, the inflammation hypothesis was not substantiated in our analysis since the cytokines including IL-1 $\beta$ , IL-2, IL-10, IL-12, TNF- $\alpha$  and IFN- $\gamma$  were not significant different after *H. pylori* infection. The similar phenomenon is reported by Jeon et al. (Diabetes Care 2012;35(3):520-5) who also found that serological evidence of *H. pylori* infection was associated with an increased rate of incident diabetes in a Latino elderly cohort while the inflammatory cytokines did not appear to mediate the effect. An alternative hypothesis is that gastroduodenal conditions resulting from *H. pylori* infection could delay gastric emptying (Minerva Med 2010;101:115-119), which has been postulated to cause poor glucose control in insulin-dependent children with diabetes (Am J Dis Child 1992;146:718-722). In addition, the role of *H. pylori* in the production of gastric hormones such as ghrelin and leptin might explain the link between *H. pylori* infection and HbA1c levels. Regretfully, we did not test the serum levels of ghrelin and

leptin in this *H. pylori* infected Mongolian gerbil models, but we are attempting to probe these problems in the following experimentally-infected animals. Our study also showed that the  $\beta$ -cells apoptosis and insulin expression in *H. pylori* infected Mongolian gerbils were not different from the control group which indicated that the effects of *H. pylori* infection on glucose dysregulation may not through the injury of islet  $\beta$  -cells. Regrettably, the limitation of our study was that we did not detect the insulin sensitivity of *H. pylori* infected Mongolian gerbils at each time point. Therefore, we are building a new set of *H. pylori* infected animal models in order to further elucidate this issue. Besides, we are also observing the effects of *H. pylori* eradication on both human and gerbils for demonstrating the true association between *H. pylori* and diabetes.

(2) IL-8 is the most important cytokine for *H. pylori* infection. CagA positive strain of *H. pylori* is an important factor for severe inflammatory reaction and also gastric carcinogenesis through IL-8 secretion. Authors studied many cytokine expressions in this study, but they did not show the difference of IL-8 secretion between *H. pylori* infected and non-infected. I suggest that authors need to add an IL-8 study.

**Author reply:** Infection with *H. pylori* causes a cellular infiltration of both neutrophils and CD4 positive lymphocytes, as well as secretion of proinflammatory cytokines such as TNF- $\alpha$  and IFN- $\gamma$ , which is indicative of a Th1 immune response (Infect Immun 2005;73(9):5612-9). Lipopolysaccharide (LPS) is a cell wall component of gram-negative bacteria and a strong inflammatory stimulator that binds to toll-like receptor 4 (TLR-4) and causes upregulation of innate immune mediators such as TNF- $\alpha$ , IL-10 and IL-12 (Clin Exp Immunol 1998;113(3):401-6; Comp Immunol Microbiol Infect Dis 2002;25(2):85-93). Recent studies have shown that *H. pylori* LPS might play a role in immune responses, histopathology and may even interact with TLR-4 (Infect Immun 2004;72(11):6446-54). Referring to researches from Dey et al. (Acta Med Okayama 1998;52(1):41-8) and Hahm et al. (Aliment Pharmacol Ther 2003;18:24-38), we examined several serum cytokines including IL-1 $\beta$ , IL-2, IL-4, IL-10, IL-12, TNF- $\alpha$  and IFN- $\gamma$ , which might be representative for the inflammation induced by *H. pylori* infection. Regretfully, we did not have enough serum left to test IL-8 according to the reviewer.

(3) The discussion is too long, authors need to condense it.

**Author reply:** The discussion has been condensed according to the comments of the reviewer.

Reviewer #2:

Thank you for your questions and comments.

(1) In the discussion, some explanation is given regarding the possible connection between Hp-I, glucose metabolism, HbA1c and diabetes (2 last lines of page 11 and page 12). Please provide a better possible molecular explanation-theory combined by a figure linking Hp-I, HbA1c and diabetes, instead of just reporting and comparing the relevant corresponding studies with IGF. Please add an additional paragraph describing the requested molecular theory.

**Author reply:** According to the reviewer's comments, figure 6 was added to better explain the possible molecular explanation theory linking *H. pylori* infection, HbA1c and diabetes. The IGF-1 receptor (IGF-1R) and elements of the IGF-1 signal transduction pathway are expressed in pancreatic  $\beta$  -cells (J Biol Chem 1998;273:17771-17779). IGF-1, on binding to its receptor IGF-1R, activates the intrinsic tyrosine kinase activity of the IGF-1R (Int J Biochem Cell Biol 1996;28:499-510). IGF-1R then phosphorylates substrate

proteins, including members of the IRS family such as IRS1 and Shc, on selective tyrosine residues. Downstream of the IGF-1 signaling are two main pathways namely the phosphatidylinositol-3 kinase (PI3K)/Akt pathway and MAPK pathway (Science 2003,302:1710-1). IGF-1 acts via the MAPK pathway to mediate growth responses while the PI3K pathway is thought to have predominantly metabolic effects such as glucose uptake and Glut4 translocation. In addition to tyrosine phosphorylation, the IRS proteins could undergo serine phosphorylation which may attenuate signaling by decreasing the normal tyrosine phosphorylation and promote insulin resistance (J Clin Invest 2001,107:181-9). We supposed that *H. pylori* infection associated impaired glucose metabolism and upregulation of HbA1c might mediate through the IGF-1 signaling pathway. The part of content describing the requested molecular theory was added to the revised paper on page 11 line 20 to page 12 line 7 marked in red.

(2) Could *Helicobacter pylori* infection be the result and not the cause of diabetes, provided that diabetic patients harbor infectious frequently? Please add a paragraph to the discussion. Were other infections, besides Hp-I noted in the gerbils?

**Author reply:** A growing body of epidemiological evidence suggests that a higher prevalence of *H. pylori* infection in people with diabetes. Recently a prospective cohort study demonstrated that *H. pylori* infection leads to an increased rate of incident diabetes, which implicates a potential role for antibiotic and gastrointestinal treatment in preventing diabetes (Diabetes Care 2012,35:520-525). Similar results were observed by Hsieh et al showing long-term *H. pylori* infection was significantly associated with high levels of HbA1c and a higher prevalence of T2DM in Taiwanese patients (Eur J Clin Invest 2013,43:949-956). These studies suggested that *H. pylori* infection might be the cause of diabetes. Meanwhile, patients with diabetes were more prone to *H. pylori* infection than those without the disease. There are several reasons for this phenomenon. Firstly the immune system of diabetic patients was compromised and thus may lead to an increased susceptibility of *H. pylori* infection (Am J Gastroenterol 2002,97:3032-7). In addition, altered glucose metabolism may produce chemical changes in the gastric mucosa, the reduction of acid secretion and the gastrointestinal mobility that promote *H. pylori* colonization (Diabetes Res Clin Pract 1998,39:143-6). This part of content describing whether *H. pylori* infection is the cause or result of diabetes has been added to the revised paper on page 10 from line 3 to 9 marked in red. Mongolian gerbils have been frequently used to study the pathogenesis of *H. pylori* infection as they are susceptible to colonize and develop gastric diseases as a result of infection (*Helicobacter* 2011,16:389-397, Microbiology and immunology 1991,35:475-480). In addition to *H. pylori*, Mongolian gerbils could also be used as animal models of other infection, such as Baylisascaris potosis (J Parasitol 2015,101:114-5) and Babesia divergens (Biomed Res Int 2014,2014:483854).

(3) Eradication of the infection. It would be interesting to see if the eradication of the infection would alter the course of diabetes or at least of glucose metabolism (as counted in the study) in the gerbils. Even though it was not conducted, please write a paragraph in the discussion analyzing this, providing insight for future research.

**Author reply:** There are limited and conflicting data regarding the effect of *H. pylori* eradication on glucose metabolism and insulin sensitivity. As Zojaji et al. showed that *H. pylori* treatment can improve the mean HbA1c and the metabolic abnormalities in patients with T2DM, it may be beneficial for patients at risk of diabetes to be checked for the presence of *H. pylori* infection (Gastroenterol Hepatol Bed Bench 2013,6:36-40). Gen et

al. also demonstrated that successful *H. pylori* eradication significantly decreased fasting insulin and HOMA-IR levels (South Med J 2010;103:190-196). Up to date there is no study investigating the eradication of *H. pylori* on glucose metabolism in Mongolian gerbils and thus it is worth future research. The part of content describing *H. pylori* eradication on glucose metabolism was added to the revised paper on page 13 from line 7 to 13 marked in red.

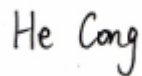
(4) Please correct some minor grammatical/syntactical errors. Just to cite a few: a. abstract...of islet b-cells were not CHANGED significantly. Please rephrase. For example...There was no modification in...etc b. abstract. The cytokines including...also showed no significant differences etc. Please rephrase...There were also no significant differences concerning the levels of cytokines including...etc c. page 3, line 2. try ranging, instead of raging (rage=anger=war) d. page 11, last line. Whereas insulin insensitivity...etc. Please replace the word whereas with although, or even though. Thank you. e. page 9...7 lines before the last line... which implicates a potential role for antibiotic and gastrointestinal treatment...please replace "implicates" with "suggests".

**Author reply:** The grammatical/syntactical errors were corrected according to the review's comments and marked in red in the revised paper.

3 References and typesetting were corrected

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

Sincerely yours,



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