Format for ANSWERING REVIEWERS

March 2nd, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 16185-review.doc).

Title: An Increase in Liver Markers is Associated with a Higher Risk of Impaired Fasting Glucose and Type 2 Diabetes: The Korea National Health and Nutrition Examination Survey 2010-2011

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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) The paper by SH Ko et al investigates the association between liver enzymes and the risk to have IFG or type 2 diabetes (T2DM) in the population of the KNHANES survey older than 30 years. They observed that higher levels of ALT and/or GGT and lower levels of AST/ALT increased the risk to have IFG and T2DM and that the levels for the increased risk are lower than that considered pathological. Ethical requirements are fulfilled. The reading of the paper suggests some comments: In particular, minor comments:

1) Because the population is wide, it should be better to express dispersion as standard deviation instead of SE.

Response: We thank the reviewer for the thorough review. The KNHANES used a complex, stratified, multistage, probability-cluster survey design, which represents the whole Korean population. As the KNHANES looks at the precision of the estimated population, the tables in our manuscript are expressed in SE rather than SD. We have also conferred with a biostatistician, who has confirmed the use of SE. We feel that the use of SE more accurately represents the data and are sorry that we cannot agree with the thoughtful suggestion of the reviewer.

2) Why did the Author use geometrical mean to express AST, ALT and GGT? (see Table 1)

Response: The reviewer's point is well taken. We used the geometrical mean as the AST, ALT and GGT did not follow normal distribution. This had been outlined in the text as **If necessary, logarithmic transformation was performed to achieve a normal distribution.** (page 6, line 28-29)

3) Please, insert the reference of table in results to help in the reading of the paper.

Response: Thank you for your kind comment. We have inserted these into the Results section. (page 8, line 10 ; page 9, line 4, and 17)

Also, in order to help with the reader's understanding, we have inserted the sex-specific quartile levels of the Liver markers below Tables 2-4.

4) The Authors used 2 models in the multivariate analysis. However, the model 2 only included hypercholesterolemia but non hypertriglyceridemia. It is well known that higher triglycerides are connected with higher liver enzymes and NAFDL and a new model 2 or a model 3 with the correction also for this factor is mandatory.

Response: We thank the reviewer for an excellent point. Upon review of your comments, we have added Hypercholesterolemia to the previous model 2 and constructed a new model 2, which we have applied to tables 2-4.

The revised sentences were inserted into our manuscript in the "Results" section as follows;

At page 9, lines 7-16;

- When adjusted for age, BMI, smoking, alcohol intake, regular physical activity, education level, income level, hypertension, hypercholesterolemia, and **hypertriglyceridemia**, the ORs across quartiles of AST/ALT were 1, **0.67, 0.42, and 0.30** in men and 1, **0.51, 0.23, and 0.28** in women (P for trend < 0.001). Compared with the Q1 of ALT, the ORs for type 2 diabetes were **2.22** (95% CI, **1.381–3.559**; P for trend < 0.001) and **3.16 (95% CI, 1.990–5.026**; P for trend < 0.001) for Q4 of men and women, respectively. The positive association between GGT and type 2 diabetes was consistently present among both men (adjusted OR, **3.05; 95% CI, 1.913–4.877**; P for trend < 0.001) and women (adjusted OR, **2.23; 95% CI, 1.459–3.397**; P for trend < 0.001), when Q4 of GGT was compared with Q1.

At page 9, lines 28-32, page 10, lines 1-2 ;

- When the combination of ALT (Q4), GGT (Q4), and AST/ALT (Q1) was compared with the other quartiles, the ORs for prevalence of type 2 diabetes were **3.21 (95% CI, 1.829–5.622**; P < 0.001) in men and **4.60 (95% CI, 3.217–6.582**; P < 0.001) in women. In the same adjusted models, men and women who were in Q4 of ALT and GGT and Q1 of AST/ALT had **1.99 and 2.40** times increased risks of IFG, respectively. The increase in risk was generally greater for type 2 diabetes than for IFG.

(2) Although several epidemiology studies have shown the close relations between concentrations of ALT/AST/GGT and high risk of type 2 diabetes, it is still unclear whether there is a relationship between ALT/AST/GGT and impaired fasting glucose (IFG). This study compared the relationships of AST, ALT and GGT with both type 2 diabetes and impaired fasting glucose (IFG) in a nationally representative sample of Korean adults, and further determined whether AST, ALT and GGT have an incremental effect on the prevalence of type 2 diabetes and IFG. Data presented in this manuscript is impressive; however, two issues need to be addressed before considering this work for publication.

Q1: As increased serum ALT and GGT levels reflect hepatic insulin resistance and impaired insulin secretion, it is necessary for the authors to investigate whether ALT and GGT are associated with hepatic insulin resistance in the development of IFG and type 2 diabetes.

Response: Thank you for pointing out a very valid point. As the reviewer mentioned, ALT and GGT have been reported to be associated with hepatic insulin resistance. Hepatic insulin resistance is usually measured with isotope dilution methods or by an indirect method involving Oral Glucose Tolerance Tests. However, the KNHANES measured only the fasting glucose state, which precluded the measurement of hepatic insulin resistance. This has been inserted into the Discussion as a limitation as below. (page 13, lines 3-6)

Fourth, though hepatic insulin resistance may have contributed to the increase in diabetes or IFG risk, we could not measure hepatic insulin resistance as currently accepted methods such as the isotope dilution methods or oral glucose tolerance tests were not done [33].

Also, as previous reports have noted, ALT and GGT have been reported to be correlated with hepatic

insulin resistance, which may have contributed to the increase in DM and IFG risk. This has also been inserted into the Discussion section. (page 12, line 13-15)

In addition, GGT and ALT have also been reported to be correlated with hepatic insulin resistance, which may have contributed to the increase in diabetes or IFG risk [26].

Q2: Although the authors provide convincing data showing that the AST/ALT were negatively correlated with type 2 diabetes and IFG, the precise meaning of AST/ALT in evaluating the risk of type 2 diabetes is largely unclear. Thus, the authors should address the significant of AST/ALT in their result discussion.

Response: We thank the reviewer for this excellent suggestion. An increase in AST/ALT ratio has been suggested as corresponding to improvements in hepatic insulin sensitivity, possibly due to decreasing NAFLD. A higher AST/ALT ratio may be associated with lower hepatic fat accumulation and improvements in hepatic insulin sensitivity. The risk in diabetes or IFG associated with lower AST/ALT levels may have been due to this factor and this has been clarified in the Discussion section. (page 12, line 8-10) as below.

One is that increased serum ALT, GGT and **decreased AST/ALT** levels reflect hepatic steatosis or visceral obesity [21-23]

(3) I read the paper with interests, entitled "An increase in liver markers is associated with a higher risk of impaired fasting glucose and type 2 diabetes: the Korea National Health and Nutrition Examination Survey 2010-2011" by Ko SH et al. My comments are as follow. The authors investigated the association between liver markers and the risk of type 2 diabetes and impaired fasting glucose (IFG) based on nationally representative, cross-sectional survey, demonstrating higher levels of GGT and ALT and lower AST/ALT within the physiological range are independent, additive risk factors of type 2 diabetes and IFG. It is interesting paper consists of important information consists of appropriate figures and tables.

Response: We thank the reviewer for the very kind comments.

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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