January 30, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 8210-review.doc).

Title: Impacts of common factors of life style on serum liver enzymes

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 8210

The manuscript has been improved according to the suggestions of reviewers and we are herewith enclosing our point-by-point replies to each comment.

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

Sincerely yours,
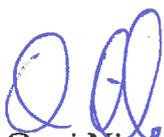
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Point-by-point replies to the reviewers

This study investigated the association of liver enzymes alteration with some potential hepatotoxic factors (fat, alcohol, smoking,...) in the general population of apparently healthy individuals. The study is well organized, the article well written, the results interesting. However, a number of points needs improvements.

Methods:

- the range of age for enrolled people should be reported (25-75 years)

> The information requested has now been given in the Methods Section (page 5).

- the measurements of lipids in blood should be mentioned

> A more detailed description on the measurements of blood lipids has been provided (page 4).

- major hepatitis virus was excluded or not investigated?

> The present material included only apparently healthy individuals with no clinical signs of liver diseases. Due to both financial and ethical considerations, analyses of hepatitis serology were therefore not performed in this population. It should also be noted that in Finland the prevalence of viral hepatitis is very low (observed rates of 1–2 cases/10000 blood donors per year) and is thus not expected to create a significant confounding factor in the present comparisons. The above aspects have now been covered in the Discussion Section of the revised manuscript.

Results:

- The effect of different factors should be separately described in this section (I.e. alcohol, age, BMI, ...) as well as the liver enzymes (i.e. Alt and separately GGT).

> We have now clarified the text on the individual effects of the different factors in the Results Section, as recommended. A summary of the statistical significances of the individual main effects and two-factor interactions between the different factors on both ALT and GGT enzyme are listed in Table 3 of the revised manuscript.

- The amount of ingested alcohol is important as well as the BMI value, however more important is the relationship between enzyme elevation and the duration of drinking and/or obesity status.

We appreciate the reviewer's excellent suggestion. However, since our study is cross-sectional we do not have follow-up data to analyze the specific relationships between enzyme elevations and duration of drinking and/or obesity status. We feel, however, that this is an important issue which should be addressed in future prospective trials. The above views have now been covered in the Discussion Section of the revised manuscript.

- It is not clear why the authors look at the results by fixing a threshold at forty years old; since the range is 25-75 it would be more appropriate the use of fifty; please motivate this decision.

We thank the reviewer for the valuable comment. The choice of using 40 years as grouping criteria was based on previous findings, which had shown an increase in the 97.5 percentile of GGT levels in both genders at the age of about 40 years (Stromme et al 2004, Scand J Clin lab Invest 2004; 64, 371-84). In addition, when testing enzyme data for establishing reference intervals, the partitioning test was also shown to be positive for age using a breakpoint of 40 years (Stromme et al 2004). In the revised manuscript, we have now provided a more detailed account on the rationale for using this cut-off and added an additional reference to support our decision.

- Figure 1 is actually reported as the last

We have now clarified the presentation and citation to Figure 1 in the Results Section.

- Table 2: what kind of statistics was performed? Please, specify in the legend

The methods used in the analyses have been clarified in the legend to Table 2 and in the Statistical Methods Section.

- Table 4 should be moved as table 1 or 2

We have now restructured the presentation according to the advice given by the reviewer and relocated Table 4 as Table 2 of the revised manuscript.

Discussion is too long and should be shortened

We have now condensed the Discussion, as recommended. However, some important new points suggested by the reviewers have also been necessary to include as additions into the Discussion Section of the revised manuscript.

The authors studied the relations of liver enzymes and anthropometric and lifestyle factors. The main findings are an association of BMI and alcohol consumption with these enzymes. Several interactions were additionally detected.

1. The study of liver enzymes in a cross sectional design cannot include outliers in order to be valid. One can assume that a small number of participants with high liver enzymes had liver disease of some sort or an acute effect of a binge. As the alcohol consumption as described is definitely skewed as well as the ALT concentration, these outliers should be removed from the analysis.

> We agree with the reviewer in that a separate consideration of outliers is of crucial importance in studies on liver enzyme normal limits. In the present study outliers were detected by the Dixon´s test and values above a mean +4SD were excluded from the population forming the basis for defining reference intervals. In the revised manuscript, we have now provided a more detailed account on the calculations of reference intervals and on the rules used for exclusion of outliers.

2. The authors performed multiple analyses in regards to age without clarifying what was the rational for them. Was there an a-priori assumption that a certain age group will differ from others? What is the age of 40 years for this matter?

> We thank the reviewer for the valuable comment. The choice of using 40 years as grouping criteria was based on previous findings, which had shown an increase in the 97.5 percentile of GGT levels in both genders at the age of about 40 years (Stromme et al 2004, Scand J Clin lab Invest 2004; 64, 371-84). In addition, when testing enzyme data for establishing reference intervals, the partitioning test was also shown to be positive for age using a breakpoint of 40 years (Stromme et al 2004). In the revised manuscript, we have now provided a more detailed account on the rationale for using this cut-off and added an additional reference to support our decision.

3. The impact of waist is important for this outcome. The authors should incorporate waist circumference into their models, along with the degree of obesity and alcohol consumption, in order to highlight this point. I believe this may change some of the interactions as presented.

> We appreciate the reviewer's excellent suggestion. In the revised manuscript, we have further emphasized waist circumference as an alternative measure of obesity. In our material BMI and waist circumference showed highly significant correlations with each other equally in men (r=0,901) and in women (0.905). BMI was chosen as the primary measure of obesity because it is a widely accepted screening tool for populations involving thousands of participants. Therefore, use of BMI data here also allows comparisons to other studies in this field in a more efficient manner. The usefulness of measuring

waist circumference has been previously underscored especially in studies of populations with known disease states, such as heart disease or diabetes. At an individual level waist circumference may also provide valuable information to the data obtained with the use of BMI only. We feel that more detailed comparisons between BMI and waist circumference in the present material together with populations with distinct disease states should be a subject of future studies and consist a separate communication.