

RESPONSE TO REVIEWERS

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Title: Is hepatic steatosis associated with left ventricular mass index in the general population?

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We appreciate all of the reviewers' comments and suggestions. All comments have been carefully worked through, and changes made in the manuscript are listed below.

Reviewer #1

(1) Alcohol abuse should appear among exclusion criteria. Were patients with excessive alcohol intake excluded from the analysis?

Response: We did not exclude patients with excessive alcohol intake from the analysis as we hypothesize that hepatic steatosis is a multifactorial disease with alcohol consumption and obesity being the most important risk factors. According to a review on the multicausality in fatty liver disease by Völzke published in the *World Journal of Gastroenterology* in July 2012, it is common in research and clinical practice to distinguish between alcoholic and non-alcoholic fatty liver disease. But, as both entities share similar histopathological patterns and pathways, clinical presentations, prevention and treatment options, there is no rationale to distinguish between alcoholic and "non-alcoholic" fatty liver disease.

To address the reviewer's comment, we have repeated our analyses excluding participants with high risk drinking according to the recommendations of the World Health Organization (women: >40g/day, men: >60g/day). The present study population encompassed 30 individuals with high risk drinking. Analyses revealed

almost identical results (direct effect of baseline hepatic steatosis on LVMI change: $\beta=-0.13$, s.e.=0.21, $p=0.54$). We added the following part to the results section (page 12): “We further repeated our analyses after excluding 30 individuals with risk drinking according to the recommendations of the World Health Organization (consumption levels of 40g/day in women and >60g/day in men). Analyses revealed almost identical results (direct effect of baseline hepatic steatosis on LVMI change: $\beta=-0.13$, s.e.=0.21, $p=0.54$).” If requested, we will provide the data for the entire path model as online supporting information. We further added the following part to the discussion section (page 14): “With respect to alcohol consumption, analyses after excluding participants with high risk drinking did not change the results of our study. We therefore assume that alcohol consumption had no major role in the association between hepatic steatosis and LVMI. However, it needs to be considered that the number of individuals with high risk drinking was low and drinking above recommended levels is a risk factor for both hepatic steatosis and changes in cardiac structure”

(2) I don't understand the choice of defining hepatic steatosis as the presence of hyperechogenic liver pattern together with increased serum ALT levels. To the best of my knowledge, the elevation of ALT neither is required for the diagnosis of steatosis nor is sufficient for the diagnosis of NASH in the presence of ultrasonographically bright liver. The diagnosis of steatosis should be based only on ultrasonographical findings. The NAFLD fibrosis score (if all data for computing it are available) could be used for distinguishing, among patients with ultrasonographic bright liver, between those with low and those with high probability of advanced fibrosis (some of the patients will have an intermediate result). It would be interesting to verify the association of steatosis with positive NAFLD fibrosis score with LVMI.

Response: We routinely use this definition in our analyses on hepatic steatosis (e.g. Baumeister et al., Gastroenterology 2008; Völzke et al., Eur J Endocrinol 2009; Völzke et al., Int J Androl 2009; Ittermann et al., Thyroid 2012). Evidence for the importance of elevated ALT levels to detect hepatic steatosis comes from several studies. For example, Wedemeyer et al. (2010) point out that ALT is a liver-specific enzyme and predominant to AST with respect to sensitivity and specificity. Furthermore, a study

by Bellentani et al. (2000) revealed that elevated ALT levels are the most reliable marker for hepatic steatosis. Thus, it is useful to combine ALT with ultrasound findings in this context.

To address the reviewer's comment, we have repeated our analyses in the whole study population defining hepatic steatosis by liver ultrasound. Analyses revealed similar results (direct effect of baseline hepatic steatosis on LVMI change: $\beta = -0.07$, s.e. = 0.21, $p = 0.72$). If requested, we will provide the data for the entire path model as online supporting information.

(3) Table 2) misses p values for significances.

Response: We have included p-values in Table 2.

(4) Since steatosis is not associated with change in LVMI at the univariate analysis, I don't understand the sense of verifying if such an association, which doesn't exist, is mediated by blood pressure.

Response: Steatosis is associated cross-sectionally with LVMI, this was our starting point. We built our path model based on the hypothesis that blood pressure is a mediating factor involved in the pathway from hepatic steatosis to LVMI as blood pressure has been found to be a major risk factor for left ventricular remodelling. The analysis followed our initial hypothesis on potential associations but we agree with the reviewer that the lack of a relevant effect over time reduces the importance of a path model regarding hepatic steatosis. Nevertheless, the present model provides insights into the associations between the studied variables which is of interest in itself.

Reviewer #2

(1) Hepatic steatosis should be defined only by the presence of fatty liver to liver ultrasound. If also high ALT are present, it implies an hepatic inflammation. Please re-evaluate the prevalence of steatosis on the basis of this definition.

Response: Please see our response to comment #2 of reviewer #1. The prevalence of hepatic steatosis defined by ultrasound was 47% (486 out of 1289 participants).

(2) Table 1: You could divide the population in 1) no criterion 2) one criterion (classifying the population for the criterion using different columns) 3) US + ALT

Response: We have calculated a table differentiating hepatic steatosis by four categories considering ALT and ultrasound which will be provided as online supplementary material.

(3) Table 2: Please analyse separately patients with no criterion and with the different criteria you used.

Response: We have calculated a table for each criterion to define hepatic steatosis which will be provided as online supplementary material.

Reviewer #3

(1) To overcome the limitation brought about by the negative results, the authors should expand the discussion about the role of blood pressure and of its pharmacological (and life style) control in LVMI where it is partially reported in the discussion at the bottom of page 13 and at the top of page 14.

Response: We rewrote the respective part of the discussion section as follows (changes underlined): “Regarding pharmacological interventions, treatment with antihypertensive drugs is indicated in the management of patients with cardiac hypertrophy, whereas the validity of data regarding the effects of antihypertensive medication on LVH regression is limited due to methodological weaknesses of existing studies. Drugs acting on the renin-angiotensin system, beta blockers, and calcium channel blockers have been shown to diminish left ventricular mass with different efficacy. In the present study population, 20.3% of the individuals with hepatic steatosis reported the intake of beta blockers, 14.6% the intake of calcium channel blockers and 20.3% the intake of drugs acting on the renin-angiotensin system. In addition to blood pressure lowering effects, these drugs may lead to LVMI regression. It might be assumed that the observed decrease in blood pressure in the present sample was attended by LVMI regression covering a potentially present association between hepatic steatosis and LVMI. Repeating our analyses after excluding individuals taking beta blockers, calcium channel blockers, and drugs

acting on the renin-angiotensin system lead to similar results. This finding indicates that the use of the respective medication did not have an influence on the association between hepatic steatosis and LVMI in the entire population as these drugs may prevent further increase of LVM or support regression of LVH.

Besides pharmacological treatment, lifestyle modification including weight loss and a reduction of alcohol and salt intake may contribute to LVH regression. The role of physical activity remains controversial. It has been demonstrated that regular physical activity is associated with lower blood pressure and reduced cardiac remodeling, while exercise can also lead to the development of LVH. In hypertensive individuals, exercise may have a positive effect on cardiac remodelling with regression or prevention of LVH."

(2) In the Results section (Sample characteristics) it is said that 29.5% of the individuals did not fulfill any criteria for hepatic steatosis which characterised the remaining 14.9%. The sum of these percentages is 44.4%. What about the others? These data are not justified by what appears in fig. 1 even if they were the results of the various exclusions indicated in the figure. About the figure, the titles of the steps (e.g. net samples, eligible subjects...) do not correspond to the steps indicated in the subsection Setting and study population, where, e.g., the term "net sample" refers to what in fig.1 is reported as "Eligible subjects".

Response: We have checked and corrected our description as follows: "At baseline, 1106 (85.1%) individuals fulfilled no or one criterion for hepatic steatosis, while 192 (14.9%) individuals had hepatic steatosis as defined by the combined presence of hyperechogenic liver pattern and increased serum ALT levels."

We further revised the flow chart and the corresponding part of the methods section.

(3) The definition of hypertension as systolic pressure = or > 140 mmHg in systole and 90 mmHg in diastole is too extensive and should consider at least one more value in a sort of scale.

Response: In the present analyses, hypertension was defined as follows (page 8): Hypertension was defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or use of antihypertensive medication.

Values of ≥ 140 mmHg for systolic and ≥ 90 mmHg for diastolic blood pressure correspond to the definition of the World Health Organization. We would like to follow this definition as we do so in most other works as well.

(4) In fig.1 the word "decreased", which appears two times, should be replaced by the word "deceased".

Response: We corrected the respective parts. The language of the paper was further checked and evaluated by a native speaker of English.

(5) In table 2, no statistical significance is reported. Even if any significance is absent, it should be clearly reported in the legend.

Response: We have included p-values in Table 2.