Author's response to reviews

Title: Vitamin C supplement use may protect against gallstones: an observational study on a randomly selected population

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Author's response to reviews: see over
The authors thank the editors and reviewers for the valuable comments. According to their suggestions we performed major revisions and critically discuss several points raised. The appropriate changes are now included in the text and are given below. We believe that these have considerably strengthened the manuscript. The changes are addressed in a point-by-point fashion.

Point-by-point statement to the reviewers’ comments

Reviewer 1

Major issues:

1. The authors state that the identification of an influence of vitamin C on gallstone prevalence rates was the primary objective of the study. Was this pre-defined? The same population was employed earlier to study an influence of variation of the beta3 adrenergic receptor gene on gallstone formation (Klass et al., AJG 2007). This should be mentioned in the current manuscript.

The presented study objective was a pre-defined objective of the EMIL-study. Although the EMIL-study was initiated to assess the prevalence of Echinococcus multilocularis infection in an urban population sample, several other primary objectives have been part of the study plan. Among these were the influence of Vitamin C intake on gallstone prevalence as well as the influence of the beta3 adrenergic receptor gene on gallstone formation. Following the reviewer’s suggestion we deleted the word “primary”, included an explanatory sentence and the above mentioned reference into the manuscript.

2. The findings are interesting and well explained but one limitation certainly is the size of the study and the small number of participants that took vitamin C supplementation. This should be mentioned as a limitation of the study. In addition, the gallstone prevalence rate appears low compared to other studies. Do the authors agree and have an explanation for this finding?

The authors agree with the reviewer: the larger a population sample, the better. The presented study features, at least to our knowledge, the largest published randomly selected population in which the influence of vitamin C on gallstone prevalence was analysed. Sample size is (nearly) always a limitation to population-based studies. Therefore we think it is unnecessary to mention this common limitation as it would only increase the manuscripts length with an information every reader can judge for himself. If the Editor/Reviewer thinks it important to stress this limitation of the study, we are willing to include such a paragraph into the discussion. Gallstone prevalence varies between different countries and regions and is strongly influenced by the selection of the study population. The gallstone prevalence in the manuscript lies within the expected range for a randomly selected southern German population.
3. Two very important risk factors for gallstone formation are rapid weight loss and the number of pregnancies. Was this assessed in the questionnaire used? Could these factors have influenced the results?

As rapid weight loss was not assessed in the questionnaire the authors can’t rule out a possible influence of this factor. However we think it is unlikely that the group of participants taking Vitamin C supplementation differs significantly from the other participants in this respect. Although in the literature rapid weight loss was established as a major risk factor for gallstone disease, this factor is less important in randomly selected population samples than in patient populations. The number of persons undergoing excessive rapid weight loss can be assumed to be relatively small in our sample.

Number of pregnancies was assessed. These results are already published and neither pregnancy (yes/no) nor the number of pregnancies did exert any influence. The analysis of pregnancy as a risk factor for cholecystolithiasis has lead to different results in the literature which range from no effect to a prevalence that is reduced by a factor of 40 in comparison of nulliparae to women who have been pregnant.

4. A positive family history was identified as an important risk factor for gallstones. The known genetic risk for gallstones should be discussed and the important results of the Swedish twin study could be referenced as a confirmation for genetic factors influencing gallstone risk (Katsika et al., Hepatology 2005).

We agree with the reviewer. Genetic risk is an important risk factor for gallstone disease. Following the reviewer’s suggestion we added the mentioned reference as evidence for this important connection.

5. Page 12: The sentence at the end of the first section of the discussion is unclear. Why did the results for the effect of lipid levels differ in the descriptive analysis and in the multiple regression models?

Although pathological lipid levels yielded an effect in the descriptive analysis, this association couldn’t be confirmed if adjusted for age, gender, BMI, and family disposition. Multiple logistic regression showed that other variables in the model altered the association (possibly BMI or age).

Minor:

1. In the abstract, the background section states the aims of the study. Instead, a sentence stating the background/hypothesis of the study could be given. The first sentence of the methods-section is lacking a verb.
We apologize for the unclear sentences and missing information. The missing information was added to the abstract.

2. Newer data on the economic impact of gallstones in the U.S. were published in GASTROENTEROLOGY earlier this year and should be cited in the first paragraph of the introduction.

The reviewer is right: By the time of submission of the manuscript, we missed to notice the recently published new data. The information was “updated” according to the reviewers helpful suggestion and the reference \(^{12}\) was added.

3. In the introduction “liver cells” should be replaced by the more specific term “hepatocytes”.

Following the reviewer’s suggestion we replaced the term.

4. In the introduction, the description of the results of the study by Gustafsson is unclear. The term “conditions of gallstone formation” should be replaced by a more detailed description of the results (changes in the bile salt composition and biliary phospholipids levels resulting in prolonged nucleation times).

The unclear description was clarified according to the reviewer’s suggestions.

Reviewer 2

Although the study is interesting, the association between gallstones and vitamin C is known since several decades. The work is well written. The major problem is the small number of cases for the reliability of the statistical analysis. I strongly suggest that the manuscript needs to be seen by a statistician.

The reviewer has addressed no specific minor or major issues.

We consider the statistics to be on a sound basis and admit the sample size to be a limitation. However considering the difficulty to acquire a randomly selected population sample with an acceptable response rate we regard the sample size of our study as pretty good (Reviewer 3 even mentioned “the large sample size” as a “strength” of the report). As far as we know it is the largest randomly selected population study analysing all the well-established risk factors and the influence of vitamin C supplementation on
gallstone prevalence. All other large studies on gallstone prevalence and a comparable thorough assessment of multiple risk factors were performed in biased populations (patients, specific professional groups,...).

Reviewer 3

*This is a population-based study investigating the association between vitamin C supplementation and prevalence of gallstones using abdominal ultrasound examination as the tool of diagnosis. The report indicated that there was an inverse association between supplementation of vitamin C and gallstone prevalence. The strength of the report is the large sample size in a retrospective study.*

The study was designed as a prospective observational, population-based study. The association of vitamin C supplementation with gallstone prevalence was an objective of the initial study protocol and was not part of a retrospective analysis.

1. *In the Abstract: “To assess the possible influence...........” would be most appropriate as Aim or Purpose rather than Background.*

We apologize for the misleading heading. We added complementary information to clearly state the aim of our study.

2. *In the Results of Abstract: “......Female gender, hereditary predisposition......were associated with risk of gallstone formation” This is a cross-sectional retrospective analysis rather than a prospective study, so it would be more appropriate to report as association with prevalence of gallstones instead of risk of gallstone formation. For same reason, it would not be appropriate to report a “protective effect” because this is not a clinical trial or prospective study.*

Although the study objective (association of vitamin C supplementation and gallstone prevalence) was assessed prospectively, the reviewer is right that we didn’t perform a clinical trial (testing the influence of vitamin C supplementation on gallstone formation) or a longitudinal study. We checked the manuscript for misleading formulations according to the reviewer’s suggestion. We do not report a protective effect of vitamin C on gallstone formation (we only describe an association of these parameters) but like to introduce the hypothesis that there “might be a protective effect”.
3. In the Conclusions, for the same reason it would not be appropriate to conclude that “……..a protective effect on the development of gallstones”

See above.

4. In the statistical model there is a covariate of inflammatory bowel disease, presumed as personal history, but it is was not reported in the tables.

The variable “inflammatory bowel disease” couldn’t be assessed properly (too many persons in a test screening of the questionnaire didn’t know their status) and therefore was eliminated from the final questionnaire. We therefore hadn’t the chance to integrate this (certainly interesting) variable into the logistic regression models. By mistake this variable was still mentioned in the “methods section” of the manuscript. After realizing this mistake, we immediately deleted this wrong information from the manuscript text.

5. In the results the authors reported regular vitamin C use and duration, but there was no dosage information and its analysis. For example, one tablet daily for one year vs. two tablets daily for six months may differ in the associations.

An addendum to the questionnaire was a detailed assessment of the subject’s medication and dietary supplementation products. The names, mode of intake over the last two weeks and duration of intake were recorded. Only subjects with a regular daily intake of a certain period of time (see figure 1) were assigned to the respective groups. Initially we planned to calculate the exact dosage of ascorbic acid per day (and per year) but this was not possible because too many subjects didn’t know the exact name of their vitamin supplementation products. We checked all commercially available products in Germany (products containing solely ascorbic acid as well as multi-vitamin supplementation products) for their contents: Practically all of these contain at least 50 mg of ascorbic acid per unit. So, one can assume that all persons taking regular vitamin C supplementation products in our study took at least 50 mg of ascorbic acid per day. Nonetheless a proper calculation of dosage was not possible and therefore not included into the study report.

6. Because all the results were reported as odds rather than relative risk as this is a cross-sectional retrospective analysis, it would be better to use risk of gallstones in the manuscript.

We decided to use the wording “reduced prevalence of gallstones” as suggested by another reviewer to describe the direction of effects. This was recommended due to the cross-sectional design of the study.
7. What are the associations between vitamin C supplement and gallstone prevalence among participants with vs. without diabetes and others such as low vs high physical activity, obese vs. normal weight in BMI, males vs females, etc.

Stratified analyses also revealed a reduced prevalence of gallstones in subjects with vitamin C supplementation. No important interactions were found. Effects in different strata were similar (ORs about 0.5 to 0.6). Moreover, effects were similar to the crude effect (OR=0.55) estimated in the total population. However, a difference in ORs occurred when stratifying the analysis for diabetes. Results showed an increased prevalence of gallstones for vitamin C supplementation among subjects with diabetes. However, this result is not reliable and should be interpreted with caution as the sub-population of subjects with diabetes is very small (N=59). Therefore, we decided not to include detailed information on this in the manuscript.

8. There is no validation study on the use of vitamin C for the data collected in the questionnaire.

It is true that there was no validation study. We tried to validate the given information on vitamin C intake by asking similar questions at different times during the questionnaire: Firstly we asked for regular vitamin C supplementation intake (yes/no), than we asked for the duration of regular vitamin C intake and finally we asked for the name, dosage, mode of intake and duration of intake of the supplementation product.

9. It would be better to address the issue of misclassification in the assessment of exposures.

We are sorry, but we don’t understand to which part of the manuscript the reviewer refers by the above statement.

References:


4. Walcher et al. Pregnancy is not a risk factor for gallstone disease: Results of a randomly selected population sample. World J Gastroenterol. 2005;11(43):6800-6


