

Author's response to reviews

Title: Plasma folate levels are associated with the lipoprotein profile: A retrospective database analysis

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Author's response to reviews: see over

Dear Editors,

thank you for the valuable comments on our manuscript and the opportunity to send a revised version. We answered the reviewers' comments and changed the manuscript accordingly. All changes in the manuscript are highlighted. We hope that the manuscript is now acceptable for publication in the journal.

Best regards,

Alexander Semmler, Michael Linnebank

Detailed answers to the reviewers' comments are as follows:

Reviewer 1:

The relations between folate and HDL cholesterol are already reported (Imamura A. et al. Metabolism 2009 available in the web; Villa P et al. J Clin Endocrinol Metab 2005;90:4622-9.)

These important references are now included in the manuscript.

Major Compulsory Revisions,

1. The authors mentioned the calculation using 1743 subjects. However, they determined only 123 individuals in terms of total cholesterol as shown in Table 1. How did they make Table 2 for multiple analysis. They calculated coefficient b from only 123 subjects? Please describe how many samples are used for multiple analysis in Table 2.

We included 1743 patients in the analysis. In all of these patients folate and vitamin B12 levels were known. However, not all plasma lipoprotein levels were available in these patients. We agree that 123 patients in the case of total cholesterol is very limited, and that

that data are of preliminary nature. The numbers for LDL cholesterol, HDL-cholesterol and triglycerides are adequate for statistical analysis. Therefore these data seem promising enough to warrant future studies. The number of subjects included in each analysis was added in all tables. Concerning statistical analyses, the lipoproteins were dependent variables (one after another/ one test per lipoprotein) with each test using the covariables folate, vitamin B12, age and gender, which were available for all subjects. As these were five separate analyses, we set the level of significance to $\alpha=0.01$. All data are now log transformed before statistical analysis.

2. Coefficient B is not suitable in Table 2. The standardized partial regression coefficient, which is correspondent to r value in a binary relationship, should be expressed in this table.

Coefficient B has been replaced with the standardized partial regression coefficient Beta.

3. The relationship of Gender (male) with HDL cholesterol, LDL cholesterol, and LDL/HDL must be negative according to the plot. (B value must be -14, not 14 as the relation of Gender with HDL)

The linear regression analysis was repeated after log transformation as suggested by the reviewer (see below first minor revision). The calculated BETA values show a significant negative relationship of male gender with HDL-C, no relationship with LDL-C, and a positive relationship with LDL-C/HDL-C.

4. What is the meaning of “ $B<0.01$ ” in Table 2? These values must be mistakes.

(these are P values?)

This was a mistake. B was exchanged against the standardized partial regression coefficient Beta.

5. HDL means high density lipoprotein. “HDL” in the text means HDL cholesterol, not high density lipoprotein. Please use correctly terminology.

This was corrected throughout the manuscript.

6. They recruited 1743 sample data, but they did not contain clinical conditions. For example, how many subjects with diabetes, hypertension, or dyslipidemia are included? In addition, how many subjects are treated with statins or antidiabetic medicine? These are very important to explain the results.

We agree with the reviewer that it would be important to include these data. However, due to anonymous data extraction, information on diagnosis, diet and body mass index were not available. The results of the actual study are promising enough though to warrant future studies where these parameters are to be included.

Minor Essential Revisions

1. Triglycerides ranged widely. They calculated these values for linear multilogistic regression. If they use logarithmically transformed values of triglycerides, the results may be different even in linear multilogistic regression. Try calculation.

The statistical analysis was repeated after log transformation as suggested by the reviewer.

2. They showed both F values and P values. Only P values are sufficient for the presentation, in particular in Abstract.

This was changed according to the reviewers suggestion (Abstract, Table 3).

Reviewer 2:

- *Abstract: The effect size of the association of vitamin B with lipids is very low, and in quartile analysis, vitamin B12 did not show significant results. Thus, data on vitamin B12 should be omitted from the abstract.*

A: This was done. Additionally, as vitamin B12 no longer showed a correlation with the lipoprotein-profile after the log transformation of the data we also omitted vitamin B12 from table 3.

- *From the results, the remark concerning defined sets of parameters can be omitted.*

A: This was done.

- *The sentence “In addition, patients with high folate levels had higher vitamin B12 levels and vice versa.” can be omitted from the results. This is redundant (see linear regression).*

A: This was done.

Reviewer 3:

Body mass index (BMI) of subjects should be included in the study. The relationship between BMI vitamin B12, folate and plasma lipid levels, should be also studied and discussed.

We agree with the reviewer that it would be important to include body mass index. However, due to anonymous data extraction, information on body mass index was not available. The results of the actual study are promising enough though to warrant future studies where this parameter should be included.

The physiological relevance of the differences in the levels of HDL and LDL observed in subjects with high or low levels of folate, should be better described in the discussion.

This was added to the discussion (second paragraph).

Minor revisions

- In table 3 subjects were divided based on levels of vitamins in different groups. The number of subjects in each group should be inserted.

This was done.