

Author's response to reviews

Title: Stroke and plasma markers of milk fat intake - a prospective nested case-control study

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Author's response to reviews: see over

Dear Editor,

Thank you for considering our manuscript titled : 'Stroke and plasma markers of milk fat intake - a prospective nested case-control study' (MS 4259238142492618) for publication in the *Nutrition Journal*.

We greatly appreciate all the valuable comments that we have received. See comments and answers to the reviewers below. The manuscript has now been revised in accordance with comments and queries from the reviewers.

We hope that the changes we have made to the manuscript now make it suitable for publication in *Nutrition Journal*.

Sincerely,

Eva Warensjo on behalf of all authors

In addition, in table 1 the last row with data for 15:0+17:0 in cholesteryl esters has been added, since this was accidentally dropped at some point during the finalization of the manuscript.

Reviewer 1

Major compulsory revisions :

1. As there was no a priori reason for a different effect in men and in women, results (table 2) should present the ORs for men, for women and for men & women together (including gender in the model).

A: This is an important comment. The table has been updated with the data on all participants. Gender was not included in the model since gender was one of the matching criteria.

2. Table 2 should present not only crude ORs, but also ORs adjusted for variables measured in all or almost all subjects, i.e. BMI, smoking, cholesterol, systolic and diastolic blood pressure.

A: Table 2 has been updated with this information.

3. It is not clear to me whether dietary records (and record of physical activity) were made in all subjects or in a subsample (as stated in the discussion, page 10, line 6). According to the data given in table 3, the number of subjects varies with the item considered (for example, 115 for fruits and 66 for vegetables, from a total of 124 women), which would rather indicate that the questionnaire was sent to all subjects, but that only a part of them completed it correctly. Is it the case ? If yes, the adjusted ORs presented in table 3 partly rely on questionable data (incomplete questionnaires), and are thus of questionable interest. Instead, the authors had better restrict the calculation of ORs adjusted for dietary variables to the subsample of subjects having sent correctly completed questionnaires. This subsample and its size should be described in methods or in results, not incidentally

mentioned in the discussion. As they only rely on a non-random subsample of women, the results of these further adjustments are indicative and could be mentioned only in the text.

A: The participants in the present study were nested within two ongoing population-based health surveys starting in 1985 and still ongoing. Data collection is done at the participant's local primary health care centre. In the earlier parts of the surveys the FFQs were not optically readable, and not fully harmonized between the health care centres. This caused restrictions in the availability of dietary and physical activity data for statistical inference. Optically readable data were ready to use. In addition, sections where food questions used for aggregation into food groups were identical in all versions were used as the food group, i.e. fruits, in specific models. To clarify this the text in the method section now reads *“For the present study dietary data were not available in all individuals since optically readable FFQs were not available in the early phases of the basic surveys. Further, early non-optically readable FFQs were not identical between health centres. The analyses involved dietary data from the optically readable FFQ, and aggregates of questions (food groups) in selected sections where the FFQ questions were identical.”*

This is the explanation why the numbers vary in the models. We have done this to gain power for some models, but we have also run models employing only subjects with optical FFQs. The results follow the same trend (text added to the footnote in Table 3 *“For varying n-values see the Material and Methods section. Models employing the optically readable FFQs (n=66) followed the same trend as data presented in the Table”*). For the dietary data exclusion criteria are defined (Winkvist et al., Nutr J 2009), but none of the included FFQs met any of these criteria.

Further, the first sentence under the heading *Biomedical analyses* has been rewritten from *“Every participant filled out a questionnaire,”* to *“Participants were asked to fill out a questionnaire, ...”*

Similar reasons are behind the missing data on physical activity. Thus, we have now more carefully commented regarding the FFQs in the methods section, page 6, but kept Table 3 (but with addition to the second footnote) since we believe it gives valuable information to the reader.

4. The part of the discussion which deals with the associations between dairy food /milk components and stroke (page 11, last paragraph, and page 12) needs to be revised and improved. Potassium and magnesium are present in only moderate amounts in milk, which is not a main source of these elements, contrary to calcium. Some dairy products are low in sodium, but others are often high (cheeses). More importantly, the fact that, in the present study, the OR for C17:0 is not attenuated by adjustment for blood pressure does not support the hypothesis that the association found is mediated by an effect of dairy products or of dairy fat on blood pressure. The same is true for cholesterol and BMI (Reference 38). The association reported in ref. 39 (healthy dietary pattern) brings little information, if any, on the effect of dairy fat or dairy products on stroke. At variance, I suggest that this part of the discussion

should be focused on studies dealing with milk/dairy products/dairy fat and stroke. Some other references from cohort studies could be added :

Ness AR et al. Milk, coronary heart disease and mortality. *J Epidemiol Community Health* 2001 ; 55 : 379-82.

Sauvaguet C et al. Intake of animal products and stroke mortality in the Hiroshima/Nagasaki Life Span Study. *Int J Epidemiol* 2003 ; 32 : 536-43.

Larsson S et al. Dairy foods and the risk of stroke. *Epidemiology* 2009 (E-pub ahead of print). This last study in a large cohort of male smokers in Finland finds that some dairy foods may increase the risk of stroke.

A: Thank you for this comment. This section has now been rewritten and the first and last reference has been added to the reference list.

When the manuscript was prepared articles dealing with dairy intake and stroke in Asia/Japan was actively not included in the reference list since intracerebral haemorrhage is much more common there. The sentences regarding the DASH healthy food pattern has been deleted from the manuscript since the authors agree with the reviewer that this brought little information regarding the relationship between milk fat and stroke.

5. The conclusion of the article and the abstract should be revised according to the modifications brought in results. In particular, both should first give the result found in the whole sample (men and women together), before giving the results in each sex. In the abstract's conclusion, do not mention the term "dairy products", but only "dairy fat" or "dairy fat biomarker(s)".

A: Both the conclusion of the article and abstract has been revised according to the results in the entire study sample. Also we have made sure that the term dairy products have not been used.

Minor essential revisions :

A: All minor essential revisions suggested below have been included in the manuscript. Specific comments to 7, 8 and 12 have been added below.

1. page 5, line 13-14 : "Two controls...for each case and...".
line 15-16 : "In one set...was matched to the case."
2. page 6, 2nd & 3rd lines from bottom : write the complete abbreviations of unsaturated fatty acids, i.e. 16:1n-7, 18:1n-9, 18:2n-6, 20:3n-6, 20:4n-6, 20:5 n-3, 22:5n-3, 22:6n-3.
3. page 7, lines 14-15 : "15:0, 17:0 and 15:0+17:0 proportions..." : write rather : "The percentages of 15:0.....in phospholipids and cholesteryl ester fatty acids..."
4. page 8, line 12 : cancel : "(data not shown)".
5. page 8, two last lines and page 9, line 4 : do not repeat data presented in tables.
6. page 9, lin 5 : "...adjustment for some food items, alcohol or physical activity had..."
7. page 9, first sentence of the last paragraph : the finding of a higher blood

pressure in cases than in controls is consistent with the well-established fact that hypertension is a risk factor for stroke. It brings no information of your sample's representativity.

A: We agree with the reviewer and this sentence has been taken out of the manuscript.

8. page 10, lines 5-12 : see above, major point 3. I do not understand the sentence "This reflects that harmonization...for the dataset" : please rewrite clearly, or cancel.

A: The comment regarding the FFQs has been rewritten in the discussion and an explanation has been added in the methods section page 6.

9. page 10, lines 15 and 17 : avoid to repeat tabulated data.

10. page 10, line 19 : "...decreased by 60 %.."

11. page 11, end of the 2nd paragraph : what means "TIA" ?

A: TIA has now been replaced in the manuscript with proper wording for the abbreviation, transient ischemic attack.

12. page 11, first line of last paragraph : "...association with stroke, several..." (add a comma).

Reviewer 2:

Minor errors:

- page 5, line 15, randomly selected from the the MONICA and VIP cohorts for each case and rather than control ?

A: This has now been corrected.

- Reference 16: spelling error: membranes

- Reference 28, spelling error: preceding

A: Thank you for noticing this. The mistakes have now been corrected.

I suggest that the authors comment very briefly on the fact that there was an association with phospholipid 17:0 and 15:0+17:0 but not with cholesteryl ester 17:0 and 15:0+17:0. Is this because of the lower concentration of the fatty acids in this fraction and thus higher measurement errors? Or is there also a certain selectivity of incorporation of this fatty acids in the different lipid fractions? Undoubtedly phospholipids have a more functional role than CE.

A: This is an important comment. We have now added a comment regarding the lower proportion of 17:0 in cholesteryl esters in the manuscript on page 10. However, since the proportions of the milk fat fatty acids in membranes are very low a comment regarding a more functional role of the fatty acids in phospholipids have not been included. It is possible that the more functional role of phospholipids may affect the

risk of stroke, but at present we feel that this is too speculative to be included in the manuscript.