Author's response to reviews

Title: Dietary intake of fish, omega-3, omega-6 polyunsaturated fatty acids and vitamin D and risk of psychotic-like symptoms in a cohort of 33 000 women from the general population

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Author's response to reviews: see over
Sabina Alam
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Dear Editor,

We are grateful for your consideration of our manuscript named: **Dietary intake of fish, omega-3, omega-6 polyunsaturated fatty acids and vitamin D and the risk of psychotic-like symptoms in a cohort of 33 000 women from the general population**

We also thank the reviewers for providing valuable and useful comments. Our manuscript has been improved by accommodating the reviewer’ suggested revisions.

We look forward to continuing to work with you on revising this manuscript.

Sincerely yours,

Marina Hedelin
To follow the Journals “author instructions”, we have change the title “Introduction” to “Background”. The below described changes are marked with yellow in the manuscript text.

**Response to comments:**

**Reviewer #1 (Jessica Sontrop):**

1. **Introduction:**
   Evidence is presented to support a link between PUFAs and schizophrenia, but the authors need to clarify how the outcome of their study fits into the causal hypothesis. For instance, is there a temporal relationship between these three conditions (e.g., do these three phenomena represent a continuum or is schizophrenia often preceded by milder symptomatology or delusional ideation, or some combination thereof?). Do these conditions have a distinct onset and remain relatively constant thereafter, or can they be isolated events? What is known about the typical age of onset of the outcome, delusional ideation and schizophrenia?

Schizophrenia in adulthood is often preceded by milder symptoms and delusions during adolescence. The typical age of onset for schizophrenia is early adulthood (20-25 years of age). Expression of psychotic symptoms in populations is continuous and characterized by differing levels of severity and persistence (Rössler et al., 2007). Meta-analysis (van Os et al, Psychol Med 2009) and prospective follow-up studies indicates that up to 75-90% of developmental psychotic experiences are transitory. Persistence and clinical relevant impairment may be related to a family history of schizophrenia and environmental risk factors that might interact with the genetic risk. Self-reported psychotic experiences in the general population may represent the developmental expression of population genetic risk for psychosis (Lataster et al., 2009). This has now been further clarified in the Background, page 4, second paragraph.

2. **Title and Objectives:**
The authors’ stated objective is to evaluate the association between dietary intake of PUFAs (n3 and n6) and vitamin D and the risk of positive psychotic-like symptoms from diet. Use of the term risk implies that only new (incident) cases with the outcome (psychotic-like symptoms) were measured. However, dietary intake was measured on one occasion at baseline (1991-2) and the outcome was measured on one occasion in a follow-up survey approximately 10 years later (2002-3). Since psychotic symptoms were not measured in the baseline survey (and so prevalent cases were not excluded), the outcome is a measure of prevalence, not incidence, and therefore it is not possible to evaluate the risk of the outcome since by definition, risk is a measure of incidence.

I suggest the statement of objectives in the intro and abstract be re-worded to: To evaluate the association between the prevalence of positive psychotic-like symptoms and previous intake of PUFAs (n3 and n6) and vitamin D measured 10 years earlier.
We have followed the suggestion by the reviewer and change the word “risk” to “prevalence” in the abstract and the Background.

Similarly, the title could be re-worded to: Prevalence of positive psychotic-like symptoms in relation to previous intake of PUFAs and vitamin D measured 10 years earlier.

We have followed the suggestion by the reviewer and change the word “risk” to “prevalence” in the title. The suggested title by the reviewer does not follow the instruction from the Journal, but if the editor wishes we could change the title as the reviewer suggested.

3. Methods
The study design, although described, is not defined. The study design does not appear to fit the criteria for standard study designs, although it may be defined as a retrospective population-based case-control study. It would be misleading to describe this study as a cohort study with dietary exposure measured at baseline followed by the outcome a decade later since participants who have the outcome at study entry are unknown and can’t be excluded. Rather, participants with and without the outcome are identified, and then the researchers look back in time to see what proportion were exposed/unexposed.

We apologize if the description of our study design was confusing in the previous version of the manuscript. We have now revised the text, at page 6, and hope that it is completely clear now. However, we respectfully disagree with reviewer 1 in describe the study as a “retrospective population-based case-control study”. All women were recruited into our study in 1991 when they answered a questionnaire including dietary exposure (food frequency questionnaire), based on which levels of fish intake were evaluated. In 2002 a new questionnaire was sent to all women, and psychotic symptoms (considered as outcome in the present article) was measured. This study design is defined as a cohort study according to K. Rothman (Modern Epidemiology, third edition, chapter 7, Lippincott Williams & Wilkins 2008). However, as correctly pointed out by the reviewer and fully acknowledged by us in the discussion (page 16, paragraph 2 (lines 3-5)) and in the methods section at page 10, line 1, the study has as its main limitation the fact that the outcome status among participants was unknown at study entry.

We would like to clarify that we did not investigate diagnosis of schizophrenia but instead reported psychotic symptoms and that we do not have the information about when the symptoms started – indeed it is possible that they had started before study enrolment. Our RR (or RRR to be correct, see the comment below) is, in this case, a valid estimate of the association between exposure (dietary assessment) and outcome (reported psychotic symptoms). However, a direction of an association could never be ruled out by statistical analysis. Since, the outcome status among participants was unknown at study entry we cannot draw any conclusions about causality, only about the existence of associations (negative or positive). Based on the hypothesis under study we interpreted the RR<1 as a negative association (for simplicity we refer to it from now on “decreased risk”) and RR>1 as a positive association (for simplicity we refer to it from now on as
“increased risk”). This is now described more in detail in the methods section, at page 9-10.

We would also like to explain that the study participants were divided according to their response to the psychotic symptoms questionnaire into three groups (low, middle and high level symptoms) according to the criteria described in the method section. Therefore we used multinomial (polytomous) logistic regression for the statistical analysis, which is the adequate statistical method. The estimated associations given by a multinomial logistic regression are relative risk ratios (RRR). For simplicity of language we abbreviated ‘relative risk ratio’ to ‘relative risk’. The definition of the association parameter is stated in the statistical method section (page 9, paragraph 4), and we have now included a clarification on the abbreviation we have used throughout the paper (page 9, last paragraph 1.

3.1 Since the outcome has three categories, the cases or controls may be divided into two groups of increasing or decreasing severity.

Please see our response to comment 3. Earlier, in additional analysis, we divide the women into two groups, those with no psychotic-like symptoms and those with any psychotic-like symptoms and investigated the relationship with fish or fatty acids. However, since the effect was most apparent for those with the high level of psychotic-like symptoms, groupings into two categories dilute the effect. Therefore, we think it is more appropriate to have three categories.

3.2 This study could only be defined as a retrospective cohort study if there is strong evidence that development of the outcome rarely occurs before the age of 50 (which was the mean age of study participants at baseline).

Please see our response to comment 3. The women were between 30-49 years of age at the enrolment and the mean age was 50 years of age at the completeness at the follow-up questionnaire.

4. Discussion:
Given the j-shaped relationship observed between the outcome and dietary intake of vit D and PUFAs, the first paragraph of this discussion describing a protective effect on the outcome with “increasing” dietary intake of PUFA and vitD is highly misleading (a greater intake of fatty fish was associated with the highest level of positive psychotic-like symptoms).

We agree with the reviewer that the text was misleading and have now changed the text at page 14, first paragraph.

4.1 Moreover, given that the outcome is a measure of prevalence that could theoretically precede the dietary exposure, it is not possible to draw any conclusions regarding
temporality or causation. It is possible that the outcome itself determines preference for dietary items with higher or lower PUFA or vit D – “a protective effect” of these foods cannot be claimed based on these data.

Please also see our response to comment 3. We agree that lifestyle related to diagnosis or personality may impact dietary habits. Very few participants in our population sample, even in the high-psychotic symptoms group, are likely have a disorder, which may diminish the problem of reversed causality related to psychotic or neurotic diagnosis or medication. Regarding our opinion as to the causes of the association we find in our study, we have now tried not to go beyond the evidence from our study and generally been more cautious in our interpretation at page 19, last paragraph, and page 20.

5. Limitations:
It is not clear that a single measure of dietary intake is sufficient to categorize participants into exposure categories which are then used to predict an outcome variable measured 10 years later. The references provided in the discussion to support the “stability of dietary patterns from year to year” is vague and is not specific to PUFAs or vitamin D. This is an important limitation and should be discussed further with an emphasis on how it might be corrected in future research.

We agree with the reviewer that it is an important limitation that dietary intake was only measured once. We have improved this point in the discussion at page 18, line 3 from the bottom.

5.1 For example, please discuss the difficulties and implications of measuring an exposure that may vary over time. Greater discussion of issues related to exposure intensity and duration with respect to the causal hypothesis would be informative. Is there a hypothesized induction time? If the exposure ceases is the effect of exposure thought to continue? Does it matter whether exposure is acute or chronic?

The suggestion of an induction time and the questions about an acute or chronic exposure is interesting. However, to our knowledge there is little known about these issues and thus we find it difficult to speculate about them. Thus, we have not added anything about these issues in the discussion. However, if the reviewer had any specific articles in mind we will be happy to read them and also include them in our discussion.

5.2 Assuming that it is the biological availability of PUFAs and vit D that is related to the outcome under study, the authors should provide greater detail on how well intake of PUFAs and vit D correlates with biological availability once consumed. For instance, linoleic acid and alpha-linolenic acid compete for the same desaturase and elongase enzyme systems and a high intake of LA relative to ALA can inhibit the synthesis of EPA and DHA by competitive inhibition.
As the reviewer pointed out, the balance between intake of omega-3 and omega-6 fatty acids could be of importance. It has been proposed that the ratio of omega-3/omega-6 fatty acids might be more important in inhibiting the development of several diseases, including cancer, and various inflammatory and autoimmune diseases (Simopoulos A, Cleland L. 2003). We performed additional analysis of the effect of omega-3:omega-6 fatty acids on psychotic-like symptoms. However, the results were almost similar to those of omega-3 fatty acids (For example, in women belonging to the high level symptoms group, the RRs with increasing quartiles of omega3/omega-6 ratio intake were: 0.81 (95% CI, 0.66-0.98), 0.73 (95% CI, 0.59-0.90), 1.06 (95% CI, 0.89-1.32). We have explored this further in the discussion, at page 16, line 8-17. These results could be added in the manuscript if the editor wishes.

The absorption of vitamin D is generally high at all stages of life (Prentice et al, Am J Clin Nutr 2008; 88 Suppl: 500S-6S). The bioavailability has been observed to be decreased in obese subjects secondary to deposition in adipose tissue (Wortsman J et al. Am J Clin Nutr 2000;72:690-3), but the prevalence of obesity in this population was low. A comment on the absorption of dietary vitamin D has been added at page 15, line 5.

5.3 How well does vitamin D intake correlate with serum levels? Is information available on vit D supplementation?

This is a very important comment. Vitamin D status is dependent on dietary intake as well as sun exposure, and serum levels are subject to seasonal variations. Thus, the correlation between vitamin D intake and serum levels may vary. However, Burgaz et al found that 2-3 weekly servings of fatty fish increased 25(OH)D by 45 % in a Swedish population of women (Burgaz et al. Am J Clin Nutr 2007;86:1399–404.)

A comment on how well vitamin D intake correlates to serum levels has been added at page 15, line 7.

Unfortunately, we don’t have information about vitamin D supplementation. We do have information about intake of multivitamin supplement intake, which contain vitamin D. We performed additional analysis and adjusted for multivitamin supplement intake, and the estimate did not change. This is information is now added at page 10, line 2 from the bottom (method), page 19 line 2 (discussion) and table footnotes.

5.4 The authors should provide additional discussion about the hypothesized relationship between PUFAs and schizophrenia. Is the association simply due to a dietary deficiency, which can be remedied by supplementation or is it one of faulty metabolism, which may not respond to supplementation? Is it hypothesized that all people with diets deficient in PUFAs will be at greater risk for schizophrenia? Or just some? What might make some individuals susceptible and others not?
There are no current studies that can give real good answers to the question if dietary deficiency interacts with genetic vulnerability for schizophrenia. Schizophrenia is a multifactorial disorder with strong genetic vulnerability and it is very unlikely that all people with diets deficient in PUFAs will be at greater risk for schizophrenia. The vulnerability might however include metabolic aberrations. A focus on specific traits or symptoms may in future studies be more fruitful than reliance on diagnostic categories. This has been further elaborated in the discussion, at page 20.

5.5 Discuss the difficulties and implications of capturing an outcome such as positive-like psychotic symptoms which may have an ambiguous onset and variable duration. How might this be improved on in future research? Is information available on whether participants were receiving pharmacologic treatment for the outcome?

In large epidemiological studies self-reporting of symptoms is the only useful screening tool. Our study is by far the largest ever to screen such symptoms in the general population. Future research might include interview-based screening instrument of psychotic experiences to further capture and elaborate timing and severity of symptoms related to dietary intake. We had no information on whether participants were taking neuroleptics or other pharmacological treatment for psychotic symptoms.

5.6 Greater consideration of confounding is required. What is the potential for unmeasured or residual confounding?

Beside the covariates included in the multivariate analysis presented, we initially tested the effects of adjusting for BMI, level of education, country of birth, smoking, dietary intake of meat, dairy products, fruits, cereals and refined sugar, rheumatoid arthritis, gluten intolerance and diabetes. None of these covariates change the estimates substantially, and was therefore not included in the final multivariate model. This is described in the methods section and in the table footnotes.

Socioeconomic status is maybe a confounding factor. But, we adjusted for level of education, which is also a measure of SES and probably more associated with dietary habits than income. This adjustment did not change the estimates substantially and education was not included in the final model.

Other possible confounding factors may influence the results, for example drugs influencing levels of serum lipids, urbanicity or family history.

However, we do not believe that the association between these factors and fish, fatty acid or vitamin D intake is strong enough to influence our estimates substantially. Also, the ethnic homogeneity of our study population reduces the risk of confounding by population stratification. This is now described more in detail at page 18, second paragraph.

6. General comments:
Since the outcome measure in this study is a measure of prevalence not incidence, the use of the term “risk” should be removed from all parts of the manuscript including the abstract, title, objectives, and conclusions, unless it is used to refer to a “risk-factor”.

Please see our response to comment 3.

6.1 The terms odds, prevalence or proportion should be used as appropriate. Given that temporality cannot be established, all references to the “protective effect” of PUFAs or vitamin D should be removed (unless referring to the results of previous research with clear temporality). It is not possible to draw any conclusions regarding causation in this study. At most, it may be concluded that dietary intake of PUFA and vitamin D are inversely correlated in a j-shaped pattern with the outcome; however, it is not possible to draw conclusions about the direction of this relationship.

Please also see our response to comment 3 and 4.1. We totally agree that the associations found in this study are suggestive and that we cannot draw conclusions about causality. Few studies can really draw strict conclusion about causality, and certainly not ours. However, results of associations are still interesting especially in fields which are less explored. Our results are in agreement with earlier presented hypothesis of the effect of fish or fatty acids on psychiatric disorders indicating that high intake is associated with a lower risk. However, we have generally been more cautious in our interpretation and change the text at page 19, last paragraph and page 20.

7. Introduction
The introduction would be improved by including a critical appraisal of the studies discussed (e.g., should evidence from an ecological study be given the same weight as evidence from a clinical trial?)

We certainly agree with that ecological data is only hypothesis generating and have clarified this in the page 5, line 6.

On page 5, the sentence describing a “positive effect on their mental state” is vague. What was the magnitude and significance of the effect (was the effect clinically and statistically significant)? What is meant by the term “mental state”?

We agree with the reviewer that the term “mental state” is vague. We here refer to a review of several randomized trials of PUFAs treatment on schizophrenic patients. The outcome differ between studies but all of them have used validated scales, like PANSS (Positive and Negative Syndrome Scale), AIMS (Abnormal Involuntary Movement Scale), SAS (Simpson Angus Scale) or MADRS (Montgomery Åsberg Depression Rating Scale), measuring mental state, drug-related syndromes or symptoms of schizophrenia. We think this information is too detailed to fit in the Background, but we have clarified the text at page 5, line 13.

8. Methods:
The authors define the outcome as the presence of positive psychotic-like symptoms. This outcome measure was based on 20 questions on positive psychotic-like symptoms from a questionnaire called CAPE (Community Assessment of Psychic Experiences), which was derived from a modified version of the Peters et al. Delusions Inventory (PDI). A reference with psychometric statistics for the PDI is provided in the methods section. Information and references on the validity of CAPE, which are currently in the discussion section, should be moved to the methods section.

The text and references has now been moved to the methods section at page 7, first paragraph.

8.1 The psychometric properties of the outcome measure should be provided in the methods. It is not clear whether this scale has been validated in the Swedish population (e.g. in some cultural backgrounds it may be more or less acceptable to hold certain views such as belief in the telepathy or the paranormal). Based on the information currently provided, it is not clear that the outcome variable is a valid measure of positive-like psychotic symptoms. Most psychometric scales rely on validated cut-points, and it is not clear that the cut-points used for this outcome measure are valid.

There is currently no validation of CAPE-42 in the Swedish population as our group is the first to translate and use the instrument in Sweden. It is a well proven instrument by now in studies on psychosis proneness in community samples and used in seven language domains. We agree that it would have been valuable with a Swedish validation, especially for choosing cut-off points, which now has been commented in the Discussion at page 17, line 4.

8.2 How were missing data handled in the analyses? Please provide the available sample size for the multivariable analyses.

If a participant had not answered or not filled in specific dietary food items in the food frequency questionnaire (FFQ) the missing value was set to zero (0) intake, for the calculation of nutrients (e.g. fatty acids). Our experience from other studies using FFQs in Sweden, is if a respondents leave a dietary question blank it means that she/he don’t eat the specific food item asked for. But, we excluded participants with an energy intake outside the first (2261 kJ/d) and 99th (12335 kJ/d) percentiles. A person with an energy intake as low as 2261 kJ/d have probably under-reported their dietary intake or have not answered the questionnaire correctly. However, in the analysis of fish intake we excluded those with missing values. Because a potential misclassification of dietary intake could be more important for a specific food item than for a nutrient (which is a sum of several food items). We tested to exclude all with missing value on fish intake in the analysis of fatty acids and the results did not change compared to those presented in the tables.

The sample size for each multivariate analysis is presented in the tables.

9. Results:
Please report the effect measures as odds ratios, not relative risks.

We prefer to report relative risks. Please see our response to comment 3.

10. Discussion:
How do intakes of PUFAs and vit D in this cohort compare to other populations? How might this affect the generalizability of the results? Perhaps vitamin D intake is more important in a climate with longer winters and less sun exposure?

Thank you for this comment. We have added a section to the discussion where we have compared dietary intake of PUFAs and vitamin D in this cohort compared to other populations at page 17-18.

Reviewer #2 (Robert K McNamara)

This is a very interesting prospective longitudinal (10 year) study examining the role of dietary essential fatty acid and Vitamin D intake and the emergence of psychotic-like symptoms in a large cohort of Swedish women. After correcting for multiple confounding variables, it was found that there is an optimal intake level of omega-3 and omega-6 fatty acids, as well as vitamin D, required to reduce the risk of developing psychotic-like symptoms. The manuscript is well-written and the data are clearly presented, and I have only minor comments:

1) In as much as psychosis is more prevalent in males, it is not clear why males were not examined to investigate potential gender differences. Similarly, it is not clear why mood symptoms were not also evaluated. Recognition of gender differences in the prevalence of psychosis should be mentioned in the Introduction and/or Discussion.

Male were not examined as our study was focused on Women’s Health and Lifestyle. It is correct that there is gender difference in the prevalence of psychosis (Bogren M et al). However psychotic-like experiences in the general population might be more equally distributed among women and men (Varghese D et al, 2008). Recognition of gender differences in prevalence has now been acknowledged in the Discussion, at page 18, line 4-7.

2) The authors cite Edwards et al., 1998, which investigated erythrocyte lipids in patients with depression, as support for the assertion that lipid abnormalities contribute to the etiology of schizophrenia. This should be corrected.

Thank you for this comment. This is now corrected in the text at page 14.

3) Although the authors elude to the idea that the Vitamin D content of fish may be important, greater detail should be provided regarding predicted vitamin D levels in the diet to evaluate whether this is a confounding variable in the omega-3 multivariate analysis.
We agree with the reviewer and have in the discussion mentioned this on page 15, line 9-11 “Our results of the protective effect of fatty fish could in part be due to the content of vitamin D”. We can explore this further in the discussion if required.

Fish is rich sources of vitamin D and could account for some of the effect of fish on our outcome. Also, we find a high correlation between vitamin D and omega-3 fatty acids (0.77). As long as individual nutrient intake is not measured in biological samples you can never rule out what substance in the diet, in this case fish, which is responsible for the potential effect. If several substances co-exist in a food item and if you only have information about which food items the participant have eaten, an adjustment for different substances will be imaginary. When and way to adjust for dietary variables is one of the largest obstacles within dietary research.

Therefore, we decided to not include Vitamin D in the final main models. Instead we performed stratified analysis for low vs. high intake of vitamin D; however, the number of observations was too low to make any reliable conclusions. We have now described this in more detail in the methods section at page 11.

3.1 Similarly, fish oils also contain omega-6 fatty acids which may be confounding. This should be addressed.

To our knowledge fish oil supplement for sale in Sweden don’t contain any omega-6 fatty acids. We also contacted the largest distributor of dietary supplements in Sweden and they confirmed that their fish oil capsules do not include omega-6 fatty acids. Even if it would we believe this is not a big problem, because none of the women in this study reported the use of dietary supplements containing fish oil or PUFA at baseline. Unfortunately, we do not have any information about the use of such supplements during follow-up. However, according to national figures from the National Food Administration, a low number of Swedish women took fish oil supplements (1%) at the time of the study.

4) With regards to the J-shaped risk curve for omega-3 intake, it would be worth citing a case-control study finding that the diets of SZ patients contained more PUFA than healthy controls (Strassnig et al., 2005), and Peet et al. (2004) finding that medication-free SZ patients in India and Malaysia exhibited greater erythrocyte DHA levels compared with healthy controls.

Thank you for this comment. We have added the results from the study by Peet et al in the discussion section, at page 15, line 3 from the bottom.

Regarding the results by Strassnig et al., 2005 we think it is difficult to compare this with ours. In the Strassing study they have compared dietary intake among schizophrenic patient with the results from a dietary survey among the general population. In the methods section the author stated that intake of total omega-3 and omega-6 fatty acids was not available for the population survey and they were not able to make any
comparison. Although, they have information about some omega-3 fatty acids, there was no difference in the intake of these fatty acids between schizophrenic patient and the intake in the population survey. The latter information we could mention in our article. However, we question the study design by Strassing, since they compare their results with the results from another study; the source population is not the same and could therefore not be consider as a case-control study.

5) An important confounding variable not accounted for in the multivariate model is family history of psychiatric illness. This should be declared as a limitation of the study.

Unfortunately, we do not have information about family history psychiatric illness and was therefore not able to adjust for it. We have declared this as a limitation in the study, at page 18, second paragraph.