

PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (<http://bmjopen.bmj.com/site/about/resources/checklist.pdf>) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Intake of marine n-3 polyunsaturated fatty acids and the risk of rheumatoid arthritis: protocol for a cohort study using data from the Danish Diet, Cancer and Health Cohort and Danish health registers
AUTHORS	Soussi, Bolette; Bork, Christian; Kristensen, Salome; Lundbye-Christensen, Søren; Duch, Kirsten; Cordtz, René; Christensen, Jeppe; Schmidt, Erik; Dreyer, Lene

VERSION 1 – REVIEW

REVIEWER	Fisher, Benjamin University of Birmingham, Institute of Inflammation and Ageing
REVIEW RETURNED	03-Feb-2021

GENERAL COMMENTS	<p>This is a clear and well-written protocol and addresses an area of interest. I only have a couple of comments.</p> <ol style="list-style-type: none">1. There is often a delay in the diagnosis of RA. Do the authors plan to account for this? Maybe excluding cases diagnosed within 12 months of enrolment for example.2. The authors understandably focus on long-chain n-3 PUFA commenting that n-6 PUFA tend to be pro-inflammatory. Whilst this focus seems reasonable given the extensive literature on the immunomodulatory benefits of n-3 PUFA, it could be stated that there are possible immunomodulatory actions for n-6 PUFA and their metabolites also, and not all evidence supports the pro-inflammatory role of n-6 PUFA status in RA and other diseases e.g. PMID: 29436473; PMID: 30409829. If that is the case, might the authors also want to consider investigating key n-6 PUFA?
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REVIEWER	Naumovski, Nenad University of Canberra, Faculty of Health
REVIEW RETURNED	27-Feb-2021

GENERAL COMMENTS	<p>Firstly I would like to congratulate you on an attempt to discuss such an important topic and to present the findings. I had a pleasure in reading the well formulated manuscript and some of my comments/suggestions are rather minor. I sincerely hope that this comments/suggestions assist in the improvements of the manuscript.</p> <p>Major:</p> <ol style="list-style-type: none">1. Can authors provide human ethics research approval as well as some more details on the FFQ relating to the information being collected (i.e. types of marine n-3 marine sources).
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	<p>2. In the statistical modelling, can authors also include modeling based on the place of residence (proximity to the marine food sources) if data is available (or potentially available).</p> <p>3. Prior to adjustments of modeling, it might be worth exploring the prevalence of RA in the Denmark (due to the data population set) and potentially predict the numbers within this dataset. This will reduce the error (“guessing”) in the Page 9 Line 20-21.</p> <p>4. The authors should also consider model analysis stratified by the age groups (within the population sample and within sex groups) as early onset RA is becoming more prevalent in developed world.</p> <p>5. The abstract will require adjustments following the addressing of these comments.</p> <p>Minor:</p> <p>1. The manuscript contains some typographical errors and final proof-corrections are required for grammar and academic expressions. Also, authors should consider consistent referencing.</p> <p>2. Can authors provide version of FoodCalc software, manufacturer and associated information. Also, what version of the Danish food Composition tables will be used (year of last review).</p> <p>3. Through the manuscript, authors have used abbreviations that were not defined. Please adjust.</p> <p>4. Please provide some of the units required within each parameters observed.</p>
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VERSION 1 – AUTHOR RESPONSE

REVIEWER 1:

#1 Reviewer comment:

There is often a delay in the diagnosis of RA. Do the authors plan to account for this? Maybe excluding cases diagnosed within 12 months of enrolment for example.

Author reply:

In Denmark, the national health service provides tax supported health care for the entire population and the diagnostic process with regard to RA has been systematised throughout the past decades, which most likely have limited diagnostic delay of RA in Denmark. However, we agree with the reviewer that diagnostic delay may be present. Unfortunately, no ideal solution to handle this issue exist. Excluding subjects with RA diagnosed within the first year of enrolment could exclude those individuals most sensitive to our exposures of interest and hereby introduce selection bias. The peak incidence of RA occurs in individuals 70 to 74 years of age and we consider the risk of reverse causation very limited as very few events of RA are likely to occur during the initial follow-up time.

#2 Reviewer comment:

The authors understandably focus on long-chain n-3 PUFA commenting that n-6 PUFA tend to be pro-inflammatory. Whilst this focus seems reasonable given the extensive literature on the immunomodulatory benefits of n-3 PUFA, it could be stated that there are possible immunomodulatory actions for n-6 PUFA and their metabolites also, and not all evidence supports the

pro-inflammatory role of n-6 PUFA status in RA and other diseases e.g. PMID: 29436473; PMID: 30409829. If that is the case, might the authors also want to consider investigating key n-6 PUFA?

Author reply:

The role of n-6 PUFAs in relation to development of RA is indeed an area of research that warrant further investigation. However, the effects of n-6 and marine n-3 PUFAs may be divergent and interdependent. Also, the intake of n-6 PUFA is markedly higher than n-3 PUFAs and we believe that investigation of n-6 PUFAs in relation to development of RA requires another analytical approach (substitution analyses). Therefore, we considered investigating of n-6 PUFAs in relation to risk of RA beyond the scope of this paper, but agreeable of great interest.

REVIEWER 2:

Major:

#1 Reviewer comment:

Can authors provide human ethics research approval as well as some more details on the FFQ relating to the information being collected (i.e. types of marine n-3 marine sources).

Author reply:

The study has been approved by the Data Protection Committee of Northern Jutland, Denmark (2019-87) and the North Denmark Region Committee on Health Research Ethics (N-20190031) as stressed out in the manuscript. The Diet, Cancer and Health cohort was approved by the relevant scientific Ethic Committees and the Data Protection Agency and all participants gave written informed consent at enrolment. We have added the following to the manuscript under 'Ethics and dissemination' page 9-10:

"The DCH cohort was approved by the relevant scientific Ethic Committees and the Data Protection Agency and all participants gave written informed consent at enrolment."

More details about the FFQ we have added to the manuscript under 'Data sources' page 5:

"The food frequency questionnaire covered a total of 24 questions regarding intake of fish. The food frequency questionnaire has previously been validated against two times 7-days weighted diet records and found useful for categorising according to their intake of total energy and PUFA intake.(15)"

#2 Reviewer comment:

In the statistical modelling, can authors also include modeling based on the place of residence (proximity to the marine food sources) if data is available (or potentially available).

Author reply:

All participants enrolled in the Diet, Cancer and Health cohort was recruited from in and around the two largest cities in Denmark (Copenhagen and Aarhus). Both cities are important seaports and the availability of seafood is anticipated to be similar. The median intake of marine n-3 PUFAs in the Diet,

Cancer and Health cohort is high (median 0.6 gram/day) and the major sources are seafood and in particular fatty fish (Tram et al. Eur J Clin Nutr; PMID: 33514866).

#3 Reviewer comment:

Prior to adjustments of modeling, it might be worth exploring the prevalence of RA in the Denmark (due to the data population set) and potentially predict the numbers within this dataset. This will reduce the error (“guessing”) in the Page 9 Line 20-21.

Author reply:

We have previously conducted a nationwide population-based cohort study to investigate the incidence and prevalence of RA in the adult Danish population (reference 1 in the manuscript, Soussi et al. Ann Rheum Dis; 2020;79(Suppl 1):46).

#4 Reviewer comment:

The authors should also consider model analysis stratified by the age groups (within the population sample and within sex groups) as early onset RA is becoming more prevalent in developed world.

Author reply:

We agree that RA are becoming more prevalent in the developed world. In supplemental analyses, we will conduct analyses separately among men and women. However, we are not aware of biological effects suggesting effect modification between our exposures of interest and baseline age in analyses with age at underlying time scale.

#5 Reviewer comment:

The abstract will require adjustments following the addressing of these comments.

Author reply:

We have reviewed the abstract based on the revisions made to the manuscript.

Minor:

#1 Reviewer comment:

The manuscript contains some typographical errors and final proof-corrections are required for grammar and academic expressions. Also, authors should consider consistent referencing.

Author reply:

Thank you for pointing this out. We have reviewed and corrected errors throughout the manuscript.

#2 Reviewer comment:

Can authors provide version of FoodCalc software, manufacturer and associated information. Also, what version of the Danish food Composition tables will be used (year of last review).

Author reply:

The FoodCalc program was developed for the Diet, Cancer and Health project and details are public available. We will use the latest version of the FoodCalc software (version 1.3), which we have added to the manuscript (page 6). Also, we have cited a reference to more detailed information on the FoodCalc software:

https://www.cancer.dk/dyn/resources/File/file/7/8207/1570007155/foodcalc_documentation.pdf

Further, we have specified which Danish Food Compositions tables will be used.

#3 Reviewer comment:

Through the manuscript, authors have used abbreviations that were not defined. Please adjust.

Author reply:

Thank you for pointing this out. We have specified abbreviations throughout the manuscript as suggested.

#4 Reviewer comment:

Please provide some of the units required within each parameters observed.

Author reply:

In the last paragraph in 'Exposures and outcome of interest' on page 6, we have added units as suggested:

“Established risk factors for RA were identified by review of the existing literature prior to data analysis. We identified the following covariates that needed to be adjusted for: age (years),(1) sex (women, men),(1,23–25) smoking (smoking status (never, former or current smoker), and pack years of smoking (years)),(3–5) level of education (basic school, higher education 1 to 2 year, higher education 3 to 4 year or higher education >4 years),(3) alcohol (alcohol consumption (grams per day), and alcohol abstinence (yes, no)),(26) waist circumference (cm),(27) body fat percentage (%),(27) and physical activity (hours per week);(28) and further for women: early menopause (yes, no),(29) breast-feeding (months),(29) hormone replacement therapy (<7, ≥7 years of use),(29) oral contraception (<7, ≥7 years of use),(29) and parity (number of pregnancies).(29)”

VERSION 2 – REVIEW

REVIEWER	Naumovski, Nenad University of Canberra, Faculty of Health
REVIEW RETURNED	01-Jun-2021
GENERAL COMMENTS	I would like to congratulate you on successfully addressing my comments/suggestions. Best of luck with your study and I am looking forward to the future manuscripts being generated from this work.

