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)	Impact of red and processed meat and fibre intake on treatment outcomes among patients with chronic inflammatory diseases: Protocol for a prospective cohort study of prognostic factors and personalised medicine
AUTHORS	Christensen, Robin; Heitmann, Berit; Andersen, Karina; Nielsen, Ole; Sørensen, Signe; Jawhara, Mohamad; Bygum, Anette; Hvid, Lone; Grauslund, Jakob; Wied, Jimmi; Glerup, Henning; Fredberg, Ulrich; Villadsen, Jan; Kjær, Søren; Fallingborg, Jan; Moghadd, Seyed; Knudsen, Torben; Brodersen, Jacob; Frøjk, Jesper; Dahlerup, Jens; Bojesen, Anders; Sorensen, G; Thiel, Steffen; Færgeman, Nils; Brandslund, Ivan; Bennike, Tue; stensball, allan; Schmidt, Erik; Franke, Andre; Ellinghaus, David; Rosenstiel, Philip; Raes, Jeroen; Boye, Mette; Werner, Lars; Lindgaard, Charlotte; Munk, Heidi; Nexøe, Anders; Ellingsen, Torkell; Holmskov, Uffe; Kjeldsen, Jens; Andersen, Vibeke

VERSION 1 – REVIEW

REVIEWER	James Galloway King's College London, UK
REVIEW RETURNED	17-Jul-2017

GENERAL COMMENTS	Well presented study plan. Justification is extensive and clear, and from a clinician's perspective, I would welcome this research. I will offer the following specific comments about the proposol: 1) The tool for capturing dietary exposure (FFQ) has important limitations, including the long term recall required (over 12 months). A number of studies have demonstrated the relationship between FFQ and objective quantification of dietary intake, and it would be important to explore these in detail - as there are several established confounders (e.g. smoking) which may introduce bias into the proposed study. I accept all food questionnaires have limitations - but I also think that one of the biggest risks to this study finding a result due to type 2 error will be due to the imprecision of the FFQ. 2) I am nervous about the sample size. The proposal contains a clear sample size - but given the non-randomised nature of the study accompanied by the heterogeneous cohort - I think they will be significantly underpowered to achieve their aims. Across the inflammatory diseases there important differences - including age - which varies substantially (e.g. Crohn's v RA).

	Given that the impact of diet may be different in the subgroups - I think some background contrasting the known differences in FFQ between subgroups would be relevant. Even perhaps some pilot data to quantify differences in FFQ in the disease groups would help? 3) Have patients been involved in the development of this protocol? It would be reassuring to know that the questionnaire burden is acceptable.

REVIEWER	Dr. David Meyre (PhD, Associate Professor) McMaster University, Canada
REVIEW RETURNED	26-Jul-2017

GENERAL COMMENTS	Study protocol by R. Christensen and colleagues: 1-The authors may provide the disease subtypes and sample sizes targeted, as well as the dates of the study in the abstract. 2-Using the “rule of the thumb” method to determine the optimal sample size is a little bit questionable. The authors may rather use power calculation tables. 3-As multiple statistical tests will be performed in the course of the study, the authors may consider using a Bonferroni correction. Using a $P < 0.05$ may considerably increase the risk of false positive association claim. 4-Please add a Strengths and Limitations of the study’ chapter. As an illustration, one limitation may be that the effects of possible interactions between biological individual differences (genetics, epigenetics, microbiome…) and dietary factors on the response to treatments will not be investigated in the study. 5-P11: please replace “Marts 2019” by “Mars 2019”

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: James Galloway
Institution and Country: King's College London, UK Competing Interests: None declared.

Well presented study plan. Justification is extensive and clear, and from a clinician's perspective, I would welcome this research.

I will offer the following specific comments about the proposol:

Comment 1) The tool for capturing dietary exposure (FFQ) has important limitations, including the long term recall required (over 12 months). A number of studies have demonstrated the relationship between FFQ and objective quantification of dietary intake, and it would be important to explore these in detail
- as there are several established confounders (e.g. smoking) which may introduce bias into the proposed study.

I accept all food questionnaires have limitations - but I also think that one of the biggest risks to this study finding a result due to type 2 error will be due to the imprecision of the FFQ.

Response: Thank you for this comment. We have added a new section:

Strengths and limitations of the study
The FFQ we use in the present study has been widely used in prospective cohort studies such as European prospective investigations in cancer and other chronic diseases1 2. It has also been extensively used and evaluated in the Danish population, and results from e.g. different methods were consistent 3 4. However, the FFQ may have some limitations, in particular in respect to missing information on portion sizes5 6. We therefore modified this FFQ to capture portion size as well5. A potential limitation is the use of the FFQ among patients who may not have the strength to fill out a comprehensive questionnaire. However, our pilot study of 10 hospital patients (50-70 years of age) showed that they were able to complete the FFQ within 40-50 minutes and no complaints were reported (personal communication). Imprecision of the FFQ will lead to large confidence intervals. The result will most likely lead to null results (rather than results due to type 2 errors). The disease groups are expected to vary in several aspects such as age, gender, and body mass index (BMI). We cannot rule out that selective diet reporting may affect responders and non-responders differentially 7. On the other hand, studies have suggested that dietary patterns are relatively stable among adults in the Danish population 8. Due to the study design and the limited number of participants, there might be differences in lifestyle between responders and non-responders not captured by this study. Similarly, this study has only limited power to detect gene-environment interactions. In order to avoid potential type 2 errors, results should be replicated in other well-characterized patients with prospectively sampled dietary information and, preferably, in cohorts from other countries in order to evaluate the robustness of the results.

Comment 2) I am nervous about the sample size. The proposal contains a clear sample size - but given the non-randomised nature of the study accompanied by the heterogeneous cohort - I think they will be significantly underpowered to achieve their aims. Across the inflammatory diseases there important differences - including age - which varies substantially (e.g. Crohn's v RA). Given that the impact of diet may be different in the subgroups - I think some background contrasting the known differences in FFQ between subgroups would be relevant. Even perhaps some pilot data to quantify differences in FFQ in the disease groups would help?

Response: Please refer to the discussion above.

Comment 3) Have patients been involved in the development of this protocol? It would be reassuring to know that the questionnaire burden is acceptable.

Response: The questionnaire and the protocol has been evaluated by the Danish Colitis-Crohn Association, represented by the director Charlotte Lindgaard Nielsen, the Danish Psoriasis Association, represented by the director Lars Werner, and three patients (with rheumatoid arthritis, axial spondyloarthritis, psoriatic arthritis, respectively) who found the project interesting and relevant and the questionnaire burden acceptable. Furthermore, our pilot study of 10 hospital patients (50-70 years of age) showed that they were able to complete the FFQ within 40-50 minutes and no complaints were reported.

Reviewer: 2

Reviewer Name: Dr. David Meyre (PhD, Associate Professor) Institution and Country: McMaster University, Canada Competing Interests: None declared.

Study protocol by R. Christensen and colleagues:

Comment 1-The authors may provide the disease subtypes and sample sizes targeted, as well as the dates of the study in the abstract.

Response: Thank you. We have added the information to the abstract.

Comment 2-Using the “rule of the thumb” method to determine the optimal sample size is a little bit questionable. The authors may rather use power calculation tables.

Response: We agree completely with the reviewer that RoT’s are made only for pragmatic reasons. We have now emphasised the more formal sample size justification in the ‘Statistical methods’ section:
As using the “rule of thumb” method to justify the sample size could be perceived as being questionable, we also estimated the statistical power to detect a difference between two dietary groups: For the contrast between groups, for a comparison of two independent binomial proportions (those with high fibre AND low meat intake vs other) using Pearson's Chi-square statistic with a Chi-square approximation with a two-sided significance level of 0.05 ($P<0.05$), a total sample size of 318 - assuming an “allocation ratio” of 1 to 2 (one third) - has an approximate power of 0.924 (i.e. >90 % statistical power) if the anticipated proportions responding are 60 % and 40 %, respectively.

Comment 3-As multiple statistical tests will be performed in the course of the study, the authors may consider using a Bonferroni correction. Using a $P < 0.05$ may considerably increase the risk of false positive association claim.

Response: This is obviously a very good point; multiplicity issues should always be considered explicitly. However, as the Bonferroni-adjusted tests are well known for their conservative nature, we would prefer not to perform these per default. In the 'Statistical methods' section we have now added: No interim analyses will be performed. All reported P values will be two-sided and will not be adjusted for multiple comparisons per default. However, due to potential issues of multiplicity - as multiple statistical tests will be performed in the study – we will interpret "statistically significant" findings in the context of whether the 95 % confidence interval (95 % CI) excludes what could be perceived as clinically important. We will use consistent language to describe the effects that might have appeared as a chance finding: "The prognostic factor appears to have little or no effect on the clinical outcome if the point estimate or the boundaries of the 95 % CI lie between 0.80 and 1.25". Thus, despite an apparently statistically significant finding (P<0.05), a relative point estimate within the range of 0.80 and 1.25 will be considered absence of a clinically meaningful effect.

Comment 4-Please add a Strengths and Limitations of the study' chapter. As an illustration, one limitation may be that the effects of possible interactions between biological individual differences (genetics, epigenetics, microbiome…) and dietary factors on the response to treatments will not be investigated in the study.

Response: Thank you for this proposal which has definitely improved the manuscript. The new section is inserted above.

Comment 5-P11: please replace "Marts 2019" by "Mars 2019"

Response: In accordance with this comment the manuscript has now been checked thoroughly by a native English speaking person.

Reference List

1. Racine A, Carbonnel F, Chan SS, et al. Dietary Patterns and Risk of Inflammatory Bowel Disease in Europe: Results from the EPIC Study. Inflammatory bowel diseases 2016;22(2):345-54. doi: 10.1097/mib.0000000000000638 [published Online First: 2015/12/31]
2. http://epic.iarc.fr/. European Prospective Investigastion into Cancer and Nutrition EPIC 2014 [
3. Togo P, Heitmann BL, Sorensen TI, et al. Consistency of food intake factors by different dietary assessment methods and population groups. The British journal of nutrition 2003;90(3):667-78. [published Online First: 2003/09/18]
4. Tjonneland A, Overvad K, Haraldsdottir J, et al. Validation of a semiquantitative food frequency questionnaire developed in Denmark. IntJEpidemiol 1991;20(4):906-12.
5. Koster-Rasmussen R, Siersma V, Halldorsson TI, et al. Missing portion sizes in FFQ--alternatives to use of standard portions. Public health nutrition 2015;18(11):1914-21. doi: 10.1017/s1368980014002389 [published Online First: 2014/11/11]
6. Tjonneland A, Haraldsdottir J, Overvad K, et al. Influence of individually estimated portion size data on the validity of a semiquantitative food frequency questionnaire. IntJEpidemiol 1992;21(4):770-77.
7. Heitmann BL, Lissner L. Dietary underreporting by obese individuals--is it specific or non-specific? BMJ (Clinical research ed) 1995;311(7011):986-9. [published Online First: 1995/10/14]
8. Osler M, Heitmann BL. The validity of a short food frequency questionnaire and its ability to measure changes in food intake: a longitudinal study. International journal of epidemiology 1996;25(5):1023-9. [published Online First: 1996/10/01]

VERSION 2 – REVIEW

REVIEWER	Galloway King's College London
REVIEW RETURNED	03-Sep-2017

GENERAL COMMENTS	The authors have reassured me, addressing my original comments. There remain minor typographical issues. I have no other comments.

REVIEWER	Dr. David Meyre (PhD, Associate Professor) McMaster University, Canada
REVIEW RETURNED	18-Sep-2017

GENERAL COMMENTS	The authors adequately addressed my comments, thank you.