PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Accelerated menopausal changes as human disease model 'FOCUM' for the development of osteoarthritis and other degenerative disorders: protocol for a prospective cohort study
AUTHORS	Molendijk, Eveline; Schiphof, Dieuwke; Oei, Edwin; de Vos, Robert-Jan; Bos, P. Koen; Van Meurs, J; Lubberts, Erik; Zillikens, M. Carola; van der Eerden, Bram; Kavousi, Maryam; Schouten, Boris; de Rooij-Duran, Mariëlla; Bierma-Zeinstra, Sita

VERSION 1 – REVIEW

REVIEWER	Guo, Zhe Beijing Jishuitan Hospital, Department of Radiology
REVIEW RETURNED	01-Jul-2022

GENERAL COMMENTS	Strength It is a novel human model that focuses on the influence of a simulated “sudden menopause” on the development of osteoarthritis. Weakness 1. Please clarify the menopause status of all the participants in the “Eligibility criteria“, which will influence the level of endogenous sex hormones. 2. Please specify which reproductive factors will be included in the questionnaires, e.g., age of menarche and menopause, parity, breastfeeding. 3. Lifestyle such as smoking, drinking and physical activity should be included in the questionnaires.

REVIEWER	Heuch, Ivar University of Bergen, Department of Mathematics
REVIEW RETURNED	01-Jul-2022

GENERAL COMMENTS	The basic ideas behind the work outlined in this protocol seem very reasonable. As it is difficult to obtain accurate results about associations between factors changing during menopause and development of degenerative disorders, it is natural to create something similar to an accelerated menopause by focusing on women who terminate use of oral contraceptives (OC) at the same time. This will presumably lead to a data set with larger changes in relevant hormone levels, creating better opportunities to study relationships with risk of osteoarthritis (OA) and other disorders.

	The details of the protocol for a pilot study appear in general to be well justified. My remarks below relate mostly to minor points which the authors may take into account by providing some brief additional clarifications. 1. Although the approach suggested seems attractive, I am not entirely convinced that the "acceleration" of menopausal changes attained by letting the study period overlap with termination of OC use is really equivalent to changes caused by natural menopause. To what extent can menopause also involve other changes, not determined directly by changes in levels of the hormones included in OC? I wonder if the authors could comment on these aspects, which seem to be fundamental to the proposed observational design. 2. I am a bit in doubt about the procedure suggested for selecting candidates for the study. It seems to me that authors really want to study relationships during a phase when large changes occur in women, both because of actual menopause and because of termination of OC use. Of course the exact time of menopause can be difficult to define, and conventionally it is frequently required that a year must have passed without menstruation. In the eligibility criteria described on page 6 in the methods section (considering page numbers shown in the upper righthand corner) it is emphasised that participants must have been using OC for a certain number of years (depending on current age) and that they must intend to stop the OC use. As far as I can see, not very much is said about other aspects of the menopausal status in these criteria. Maybe that is not necessary in this situation, but I somehow feel that the way the eligibility criteria are formulated here, they may not be sufficiently restrictive to ensure that only women are included who are at the stage in the menopausal transition that the authors actually have in mind. 3. This remark is related to remark no. 2 but rather deals with the technical aspects of the recruitment. It is stated on page 6 in the methods section that women will be recruited from pharmacies in the Rotterdam area on the basis of age and OC use. I wonder if the actual procedure proposed for selecting women should be specified in slightly more detail. How is it possible to make certain that the women included are going to stop OC use quite soon? An answer to that question might be that the relevant age range will give the correct selection of women in any case, in particular if nearly everybody follows the general advice described in the first paragraph of the discussion on page 14, that OC use can be stopped at the age of 52 years. In this way, perhaps the upper limit of 60 years on current age at inclusion is not going to be of much significance in the actual selection. In any case, I would appreciate a few more details in the manuscript on how women will be approached to establish a relevant group of candidates for "accelerated menopausal changes". 4. The section on statistical methods indicates on page 10 that principal component analysis will be used on the change in protein levels over time. I am not quite certain what kind of analysis the authors want to carry out at this point. Principal component analysis is often used to extract only a few variables (defined as linear combinations of the observed values) expressing most of the observed variation in a larger set of variables. Such methods may seem reasonable as a first step in the data analysis, but that

	does not explain the change over time. The abstract states on page 3 that "Principal Component Analysis will be used to assess which changes occur due to hormonal change." I am not at all certain how this statement should be interpreted. I would rather have thought that the principal components obtained should be considered as predictor variables in the logistic regression models described on page 12 at the end of the section on statistical methods, but perhaps I have misunderstood. Anyhow, the use of logistic regression seems reasonable here, and also use of generalised estimating equations methods to take into account the time aspect of the observations. 5. The size of the sample to be considered in this study is discussed briefly in a separate section of the data collection section on page 10. I understand that the collection and handling of information may be time-consuming, so the sample size of 50 women seems quite reasonable in a pilot study of this kind. It is also difficult to carry out a more formal assessment of sample size in this case in connection with power requirements or specifications concerning lengths of confidence intervals. This is because so little is known about the distribution of the relevant predictor variables in advance. It is stipulated that about 27% of the women may develop osteoarthritis (OA) of the knee, which represents the primary outcome variable. In practice, this may correspond to approximately 14 women in the sample with OA. This may perhaps be enough for simple statistical analyses but I am not completely convinced that it will be possible to carry out any multivariable logistic regression analyses, as the authors indicate on page 12 that they want to do. I wonder if the sample size must be increased to make the project feasible if the procedures suggested by the authors should be carried out. An alternative solution may be to focus on rather simple statistical procedures in this pilot study, but in that case it will be more difficult to formulate firm conclusions. The authors already seem to be aware of this problem, as shown by the last bullet point in the strengths and limitations list on page 4. 6. Some very minor details in the second to last paragraph on page 15: I have problems understanding the statement starting with "When this pilot study shows", especially the part "by means that it is possible to include ...". Maybe the statement could be rephrased. -In this paragraph the expression "amount of participants" is used twice. To me this almost sounds disrespectful to the women involved in the study, although I am certain that the authors did not want to make that kind of impression. Perhaps the text could simply refer to "the number of participants". 7. Only an afterthought: Overall, I appreciate the attempt made by the authors to take advantage of this pilot study design to say something general about the basic procedure used, e.g. for disorders that are completely different from OA. This is clearly indicated in bullet point 2 on page 4. For such purposes the potential problems with the sample size may perhaps not matter so much. If the sample size could be made considerably larger, I would also have thought that it might be useful to include a particular control group of women currently going through menopause but not having used OC for a great many years. However, I understand that it may be difficult to recruit such women through the same procedures based on visits to

	pharmacies when the present protocol is implemented, so this idea may not be realistic in the pilot study outlined.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1: Dr. Zhe Guo, Beijing Jishuitan Hospital

Comments to the Author:
Strength
It is a novel human model that focuses on the influence of a simulated "sudden menopause" on the development of osteoarthritis.
Author response: Thank you for your constructive comments.

Weakness
1. Please clarify the menopause status of all the participants in the "Eligibility criteria", which will influence the level of endogenous sex hormones.
Author response: Thank you for this comment. In this study there are no eligibility criteria for menopausal status. We do monitor the menopausal status during the study period and we are indeed aware of the influence of the menopausal status on the level of endogenous sex hormones.

Because all the participants are currently using their oral contraceptive and started oral contraceptive use before the age of 45, we don't know if they will or will not enter menopausal state during follow-up. Due to their age, we expect that the larger proportion of the participants will enter menopausal status after stopping oral contraceptive use, but given the range of 4-5 years around the mean menopausal age of 50-51, there will be some participants who will not enter menopausal status during follow-up as well.

In our baseline questionnaires, we do ask the participants if they had an irregular menstruation cycle, longer periods of amenorrhea and menopausal complaints before starting oral contraceptive use, in order to determine if they were or were not already in menopausal transition. We don't use this information as an eligibility criteria. We don't know the menopausal state of the participants when they start. During the 2 years of follow-up we will determine whether or not the participants entered menopausal state, based on the date of their last menstruation.

Author action: We added this limitation in the 'Strengths and limitation of this study' section:
- The model consists of women who stop oral contraceptive use at menopausal age, whereby it is not known in advance whether the woman will then directly enter the menopausal state.

And in the 'Discussion' section:
- As mentioned before, the model consists of women who stop OC use at menopausal age, so it is expected that the majority of the participating women will enter menopausal status after stopping OC use. But given the range of four to five years around the mean menopausal age of 50 to 51, there will be some participants who will not enter menopausal status during follow-up. For this pilot study, no additional tests are used to ensure that all women enter menopausal state after stopping OC.

2. Please specify which reproductive factors will be included in the questionnaires, e.g., age of menarche and menopause, parity, breastfeeding.
Author response: Thank you for this remark. In the baseline questionnaire we included age of menarche, parity and age of first and final start of oral contraceptive use. Additionally, we included questions about whether or not the participant gave birth and/or gave breastfeeding, used hormones or had an intra-uterine device within three months before starting oral contraceptive use for the last

time.
Author action: We added the following sentence in the 'Data collection – data collection methods – questionnaires' section:
- (e.g. age at menarche, parity and first and final start of OC use)

3. Lifestyle such as smoking, drinking and physical activity should be included in the questionnaires.
Author response: Thank you for this remark. A question about smoking is included in the 'background characteristics' and physical activity will be measured using the validated SQUASH questionnaire as already mentioned in the manuscript. For the DXA-scan we use the FRAX questionnaire, which includes a question about drinking habits ("Do you use more than 3 glasses of alcohol per day on average?"). We will include the answers to the FRAX questionnaire to our dataset.
Author action: We added the following sentence in the 'Data collection – data collection methods – questionnaires' section:
- background characteristics (e.g. level of education, profession and smoking)

4. Study limitations are not involved.
Author response: Thank you for this comment. As already mentioned in the 'Editorial requests', we revised the 'Strengths and limitations of this study' section of our manuscript.
Author action: See 'Editorial requests' number one.

Reviewer 2: Dr. Ivar Heuch, University of Bergen

Comments to the Author:
The basic ideas behind the work outlined in this protocol seem very reasonable. As it is difficult to obtain accurate results about associations between factors changing during menopause and development of degenerative disorders, it is natural to create something similar to an accelerated menopause by focusing on women who terminate use of oral contraceptives (OC) at the same time. This will presumably lead to a data set with larger changes in relevant hormone levels, creating better opportunities to study relationships with risk of osteoarthritis (OA) and other disorders.

The details of the protocol for a pilot study appear in general to be well justified. My remarks below relate mostly to minor points which the authors may take into account by providing some brief additional clarifications.
Author response: Thank you for the compliment, important questions and constructive suggestions.

1. Although the approach suggested seems attractive, I am not entirely convinced that the "acceleration" of menopausal changes attained by letting the study period overlap with termination of OC use is really equivalent to changes caused by natural menopause. To what extent can menopause also involve other changes, not determined directly by changes in levels of the hormones included in OC? I wonder if the authors could comment on these aspects, which seem to be fundamental to the proposed observational design.
Author response: Thank you for this comment. This is a very important question. There is still so much unclear about how menopause has influence on the development of diseases, that we hope to get some first (even though they may be small) answers to that by doing this study and using this model. With this model we want to explore if and which changes occur due to hormone change. We don't think that this "sudden" menopause is totally equivalent to the natural menopause, but we just hope to get some pieces of the large puzzle to understand the influence of menopause a little bit more than we do now.
Author action: None.

2. I am a bit in doubt about the procedure suggested for selecting candidates for the study. It seems to me that authors really want to study relationships during a phase when large changes occur in

women, both because of actual menopause and because of termination of OC use. Of course the exact time of menopause can be difficult to define, and conventionally it is frequently required that a year must have passed without menstruation. In the eligibility criteria described on page 6 in the methods section (considering page numbers shown in the upper righthand corner) it is emphasised that participants must have been using OC for a certain number of years (depending on current age) and that they must intend to stop the OC use. As far as I can see, not very much is said about other aspects of the menopausal status in these criteria. Maybe that is not necessary in this situation, but I somehow feel that the way the eligibility criteria are formulated here, they may not be sufficiently restrictive to ensure that only women are included who are at the stage in the menopausal transition that the authors actually have in mind.
Author response: For this comment we would like to refer you to question number one of the previous reviewer. Indeed, at baseline we don't know whether or not a participant will enter menopausal state after stopping oral contraceptive use during the study. For this pilot study we decided to do it this way, but maybe there are other methods or eligibility criteria that can be used for further research.
Author action: See comment one of the first reviewer.

3. This remark is related to remark no. 2 but rather deals with the technical aspects of the recruitment. It is stated on page 6 in the methods section that women will be recruited from pharmacies in the Rotterdam area on the basis of age and OC use. I wonder if the actual procedure proposed for selecting women should be specified in slightly more detail. How is it possible to make certain that the women included are going to stop OC use quite soon? An answer to that question might be that the relevant age range will give the correct selection of women in any case, in particular if nearly everybody follows the general advice described in the first paragraph of the discussion on page 14, that OC use can be stopped at the age of 52 years. In this way, perhaps the upper limit of 60 years on current age at inclusion is not going to be of much significance in the actual selection. In any case, I would appreciate a few more details in the manuscript on how women will be approached to establish a relevant group of candidates for "accelerated menopausal changes".
Author response: Thank you for this comment. The pharmacies select women who are between 50 and 60 years of age and currently using a combined oral contraceptive (with ATC-code G03AA or G03AB). After this selection, the pharmacies send a letter with information about the study and the general advice to stop OC use at the age of 52. In the letter it is also mentioned that the women need to have the intention to stop OC use when participating in this study. The women who are interested in participating, can fill in a response form.
Author action: We added the following sentence to the 'Methods – Study design and setting' section:
- Participants are recruited through pharmacies in and around Rotterdam by sending an invitation letter to women who meet the age and OC use criteria, with information about the study and the general advice to stop OC use at the age of 52. Women who are interested in participating and have the intention to stop OC use can fill in a response form and send it to Erasmus MC.

4. The section on statistical methods indicates on page 10 that principal component analysis will be used on the change in protein levels over time. I am not quite certain what kind of analysis the authors want to carry out at this point. Principal component analysis is often used to extract only a few variables (defined as linear combinations of the observed values) expressing most of the observed variation in a larger set of variables. Such methods may seem reasonable as a first step in the data analysis, but that does not explain the change over time. The abstract states on page 3 that "Principal Component Analysis will be used to assess which changes occur due to hormonal change." I am not at all certain how this statement should be interpreted. I would rather have thought that the principal components obtained should be considered as predictor variables in the logistic regression models described on page 12 at the end of the section on statistical methods, but perhaps I have misunderstood. Anyhow, the use of logistic regression seems reasonable here, and also use of generalised estimating equations methods to take into account the time aspect of the observations.
Author response: We are sorry that this section wasn't clear enough. Indeed, we want to make use of

the principal component analyses to reduce the amount of data and to find which proteins are principal components. We use the delta of the protein levels over time in the principal component analysis to explore which proteins change the most after stopping oral contraceptive use. Thereafter we will use logistic regression models to investigate if there are any associations between these proteins and the development of diseases.
Author action: In the 'Data collection – Statistical methods' section we changed the sentence "Therefore, Principal Component Analysis will be used on the change in protein levels over time." into "Therefore, principal components analysis will be used on the delta in protein levels over time.". And in the 'Abstract' section, we deleted the part "due to hormonal change" and changed it into "after stopping OC".

5. *The size of the sample to be considered in this study is discussed briefly in a separate section of the data collection section on page 10. I understand that the collection and handling of information may be time-consuming, so the sample size of 50 women seems quite reasonable in a pilot study of this kind. It is also difficult to carry out a more formal assessment of sample size in this case in connection with power requirements or specifications concerning lengths of confidence intervals. This is because so little is known about the distribution of the relevant predictor variables in advance. It is stipulated that about 27% of the women may develop osteoarthritis (OA) of the knee, which represents the primary outcome variable. In practice, this may correspond to approximately 14 women in the sample with OA. This may perhaps be enough for simple statistical analyses but I am not completely convinced that it will be possible to carry out any multivariable logistic regression analyses, as the authors indicate on page 12 that they want to do. I wonder if the sample size must be increased to make the project feasible if the procedures suggested by the authors should be carried out. An alternative solution may be to focus on rather simple statistical procedures in this pilot study, but in that case it will be more difficult to formulate firm conclusions. The authors already seem to be aware of this problem, as shown by the last bullet point in the strengths and limitations list on page 4*.
Author response: Thank you for this notification. Indeed, with this number of participants it will be difficult to give any robust or conclusive results, but hopefully these explorative statistics will give us some direction for future studies. Again, this is a pilot and explorative study, in which we don't know what to expect exactly and in which we want to see if the study is feasible or not.
Author action: We added the following sentence to the 'Discussion' section:
- With this number of participants it will be difficult to give robust or conclusive results, but the explorative statistics will give us some direction for the future.

6. *Some very minor details in the second to last paragraph on page 15: I have problems understanding the statement starting with "When this pilot study shows", especially the part "by means that it is possible to include ...". Maybe the statement could be rephrased. -In this paragraph the expression "amount of participants" is used twice. To me this almost sounds disrespectful to the women involved in the study, although I am certain that the authors did not want to make that kind of impression. Perhaps the text could simply refer to "the number of participants".*
Author response: Indeed, it was not our intention to make that kind of impression.
Author action: The "amount of participants" is changed in the "number of participants".

7. *Only an afterthought: Overall, I appreciate the attempt made by the authors to take advantage of this pilot study design to say something general about the basic procedure used, e.g. for disorders that are completely different from OA. This is clearly indicated in bullet point 2 on page 4. For such purposes the potential problems with the sample size may perhaps not matter so much. If the sample size could be made considerably larger, I would also have thought that it might be useful to include a particular control group of women currently going through menopause but not having used OC for a great many years. However, I understand that it may be difficult to recruit such women through the same procedures based on visits to pharmacies when the present protocol is implemented, so this idea may not be realistic in the pilot study outlined.*

Author response: Thank you for this nice afterthought. Of course it would be better to have some sort of control group of women. For this pilot study we decided not to do this, also because of the statistical power when you divide the group of 50 participants into two groups of 25 participants. It would be interesting to discuss which group of women should be the best control group for this study (women who do not use OC or women who continue oral contraceptive use after the age of 52?). But first we need to investigate if this pilot study is feasible and promising.
Author action: None.

VERSION 2 – REVIEW

REVIEWER	Heuch, Ivar University of Bergen, Department of Mathematics
REVIEW RETURNED	06-Oct-2022

GENERAL COMMENTS	The authors seem to have done a good job revising their manuscript. I feel the paper now provides a very reasonable protocol for the pilot study. I hope the authors will be able to extract important information from the data set that is being collected. I only noticed one very minor detail in the revised manuscript which may perhaps need some attention. In Table 1 two lines have an asterisk attached as a footnote symbol. The last one representing "Glucose, cholesterol and hormones" seems to match the actual specification of the footnote on the next page, but for the first one, "Inspection and palpation of tendons", the footnote appears to be misplaced. Perhaps the asterisk had a different significance in an earlier manuscript version and should now be deleted.