PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Protocol for the combined cardiometabolic deep phenotyping and registry-based twenty-year follow-up study of the Inter99 cohort
AUTHORS	Bjørnsbo, Kirsten; Brøns, Charlotte; Aadahl, Mette; Kampmann, Freja; Friis Bryde Nielsen, Camilla; Lundbergh, Bjørn; Christensen, Rasmus; Kårhus, Line Lund; Madsen, Anja; Hansen, CS; Nørgaard, Kirsten; Jørgensen, Niklas; Suetta, Charlotte; Kjaer, Michael; Grarup, Niels; Kanters, Jørgen; Larsen, Michael; Køber, Lars; Kofoed, KF; Loos, Ruth; Hansen, Torben; linneberg, allan; Vaag, Allan

VERSION 1 – REVIEW

REVIEWER	Kehler, D. Scott Dalhousie University
REVIEW RETURNED	06-Nov-2023

GENERAL COMMENTS	Thank you for the opportunity to review the manuscript by Bjørnsbo et al., who describe a protocol to phenotype cardiometabolic variables in a follow up study of the Inter99 cohort which was collected in Denmark in 1999. The age of the cohort is now 50-80 years old (20 year follow up). The cohort will now undergo an extensive battery of cardiometabolic tests. A subsample will undergo 10-day monitoring of lifestyle behaviors (e.g., diet, physical activity) and glucose monitoring. Major comment: -The new investigation of Inter99 participants 20 years later will provide extensive data to better understand cardiometabolic health and dysfunction. One can imagine that these data will provide a wealth of hypothesis-generating investigations (including ones beyond the planned sub-studies). In this context, it would be helpful to understand how the Inter99 study is unique from other investigations that capture cardiometabolic phenotyping (e.g., Framingham Heart Study, MESA). Abstract: - Please provide a brief description of examples of the main analysis plan. The abstract in its current form does not allow the reader to understand how the cardiometabolic phenotyping will be analyzed. It is recognized that there could be many analyses, but you could provide examples like you do in the methods section. -Where it says “All eligible participants will be invited for a deep phenotyping…” what is the expected sample size, or the number of participants expected to be invited from the original cohort? There were 6,784 in the baseline cohort. How many of these participants are eligible and/or did not die before your intended investigation?

	Methods: -Please clarify how many participants are eligible for the follow up study in the main text of the document. It appears from Figure 1 that only the lifestyle intervention group will be included. The confusion arises when the questionnaire and reference groups are mentioned in the text, making it seem that those groups will also be eligible. The deep phenotyping follow-up study section would also suggest it is the alive lifestyle intervention group that is eligible. -Furthermore, please clarify the eligibility criteria, if there are other criteria other than participants who were in the intervention group and were alive. -There are also a number of sub-studies planned. Where possible, please also clarify how many participants were enrolled in each sub-study. Please also describe the eligibility criteria (if any) for the planned sub-studies, where not mentioned. Grammar/spelling/sentence structure: -Make sure to use past-tense language when writing about the original Inter99 Study (e.g., the first line of the methods section)

REVIEWER	Lee, David New York University School of Medicine, Emergency Medicine
REVIEW RETURNED	14-Nov-2023

GENERAL COMMENTS	Overall: This study protocol seeks to extend a longitudinal analysis of the Inter99 cohort to study cardiometabolic health at 20 years after cohort entry. The analysis is very well justified and demonstrates the critical importance of this work. Some noted addressable issues with the planned protocol: while there is an extensive number of variables and outcomes that will be examined through this work, the statistical plan section is lacking hypotheses to be tested. While this could be okay, then the study should be framed as a more exploratory analysis. If not, then perhaps hypotheses to be tested should be delineated, along with the a priori models to be analyzed. Machine learning is mentioned but sounds as if it's more of an adjunct analysis. Finally, there might be more attention needed regarding the ethics plans given use of cardiac CT, which may not necessarily be benign. Strengths/Limitations: Limitation 5 might need to highlight the country specific results of the study. Introduction: The introduction does a great job of detailing the need for the planned study. Lines 243-250: Regarding Cardiac CT, how does the study deal with the challenge of identifying incidental findings, which may increase risk of interventional procedures? Lines 345-355: How to the study investigators deal with the design looking at a multiplicity of outcomes, should p-values be adjusted as such? Or is this to be an exploratory analysis. Furthermore, given the numerous variables to be analyzed shouldn't variable selection methods be used? Otherwise, if models are to be developed based on an a priori conceptual model then that model should be described in the protocol, such that

	reviewers can be confident that the final model selected was not chosen based on testing a variety of models. While machine learning techniques are described later, given the discussion prior to it, they sound adjunct analyses rather than the primary analytic approach which would use conceptual models to choose variables to be examined. Line 374-375: I am not certain that we can describe a Cardiac CT scan as necessarily harmless. While it may be indicated for some persons, there are a large number of incidentalomas that might be identified through the procedure, especially among a large number of participants. For instance, a patient might be found to have a mass, which might lead to a biopsy, which may in turn lead to significant complications from such. Regular CT scans are not advised (as the research has shown) for cancer screening in the general population, so then, the argument that only benefit will occur from these scans cannot be made. Some consideration and detail should be put forth about how this will be handled in a rationale way that is consistent with both clinical care standards and also patient preferences.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1 - Dr. D. Scott Kehler, Dalhousie University

Comments to the Author:
Thank you for the opportunity to review the manuscript by Bjørnsbo et al., who describe a protocol to phenotype cardiometabolic variables in a follow up study of the Inter99 cohort which was collected in Denmark in 1999. The age of the cohort is now 50-80 years old (20 year follow up). The cohort will now undergo an extensive battery of cardiometabolic tests. A subsample will undergo 10-day monitoring of lifestyle behaviors (e.g., diet, physical activity) and glucose monitoring.

1. Major comment:
   The new investigation of Inter99 participants 20 years later will provide extensive data to better understand cardiometabolic health and dysfunction. One can imagine that these data will provide a wealth of hypothesis-generating investigations (including ones beyond the planned sub-studies). In this context, it would be helpful to understand how the Inter99 study is unique from other investigations that capture cardiometabolic phenotyping (e.g., Framingham Heart Study, MESA).

   Thank you for this suggestion to clarify further the uniqueness of our 20 years follow up study of the Inter99 cohort. Due to limitations of space, it will not be possible for us to provide a complete review of all details in the current study that differentiates it from other large cohort studies. However, perhaps the most important differentiation dimension towards other prospective cohorts within the field of cardiovascular diseases including the MESA study is that the Inter99 cohort up front included a detailed assessment of diabetes (as well as prediabetes) status including standard

75-gram oral glucose tolerance tests (OGTT´s) at the baseline, 1-year, 3-year, and 5-year follow-up examinations. This, together with the combined registry based and clinical deep phenotyping examinations on diabetes status and incidence rates over a 20-year period, allow us with great certainty to understand the extent to which subtle or overt elevations of plasma glucose levels over a period of two decades may or may not be causally related to the various early subclinical and/or overt cardiometabolic outcomes as determined in the deep phenotyping and/or registry based assessments after 20 years. Likewise, we in our outcome assessments have a relatively wider coverage of diabetes related complications and co-morbidities related outcomes compared with other studies including the MESA study that are more explicitly focused on atherosclerotic and cardiovascular outcomes. This, for instance, illustrated by our detailed outcome assessments of liver fat and fibrosis, as well as retinal, kidney and nerve changes, after 20 years.

We believe that much of the uniqueness of our study set up is already well described in the section/paragraph below in the Discussion. However, to further explain how we envision the diabetes related uniqueness of our study, we have added a paragraph to the Discussion:

L. 437

Compared with other prospective cardiometabolic studies including the FHS (Framingham Heart Study) and MESA (Multi-Ethnic Study of Atherosclerosis), a unique feature of the Inter99 cohort, is its detailed assessment of glucose tolerance with standard 75-gram oral glucose tolerance tests in all participants at the baseline examinations, as well as our broader focus on diabetes related cardiometabolic outcome variables including assessments of subclinical diabetes related disease manifestations in arteries, liver, eye, kidney, and nerves at the 20-years follow-up examinations.

2. Abstract:
Please provide a brief description of examples of the main analysis plan. The abstract in its current form does not allow the reader to understand how the cardiometabolic phenotyping will be analyzed. It is recognized that there could be many analyses, but you could provide examples like you do in the methods section.

We have added the following sentence to the Abstract to serve as an example of the type of analyses that we can and will perform.

L. 57

A main purpose is to investigate whether low birthweight independent of genetics, lifestyle and glucose tolerance predicts later common T2D cardiometabolic co-morbidities.

3. Where it says “All eligible participants will be invited for a deep phenotyping…” what is the expected sample size, or the number of participants expected to be invited from the original

cohort? There were 6,784 in the baseline cohort. How many of these participants are eligible and/or did not die before your intended investigation?

To clarify, we have added the following sentence to the Abstract.

L. 50

In total, 6,004 eligible participants who participated in the baseline examination, will be invited to participate in the

4. Methods:
-Please clarify how many participants are eligible for the follow up study in the main text of the document. It appears from Figure 1 that only the lifestyle intervention group will be included. The confusion arises when the questionnaire and reference groups are mentioned in the text, making it seem that those groups will also be eligible. The deep phenotyping follow-up study section would also suggest it is the alive lifestyle intervention group that is eligible.

It is indeed important to make it clear how the cohort is recruited. We have described the full study population of the original Inter99 cohort for the overall understanding of the study. However, to make it clear that the current participants were recruited among those who went through a physical examination at baseline (intervention group), we have made the following adjustments to the Methods and Figure 1 including the figure text to highlight the inclusion of participants only from the intervention group who had a baseline examination performed.

L. 203

A search of the Danish civil registration register (CPR register) in December 2019 showed that 6,004 (88.5%) of the Inter99 participants, who had participated in the baseline examination, were alive and had not emigrated; and thus, eligible for inclusion (**Figure 1**). There were no other eligibility criteria for study participation.

Figure 1 – figure text.

Flow chart of participation in the Inter99 study 1999 – 2023. The dark blue column represents the Intervention group (A+B) where 52% of the invited had a baseline examination performed in 1999. The eligible participants for the 20-year follow-up study are recruited among these individuals. The light-blue columns indicate individuals followed by questionnaires (C) and the reference population (D) who are not recruited for the 20-year follow-up examinations.

5. Furthermore, please clarify the eligibility criteria, if there are other criteria other than participants who were in the intervention group and were alive.

Thank you for pointing this out, the only eligibility criteria were being alive and contactable. We have added the following to clarify.

L. 203

A search of the Danish civil registration register (CPR register) in December 2019 showed that 6,004 (88.5%) of the Inter99 participants, who were in the intervention group and had participated in the baseline examination, were alive and had not emigrated and thus eligible for inclusion (**Figure 1**). There were no other eligibility criteria for study participation.

6. There are also a number of sub-studies planned. Where possible, please also clarify how many participants were enrolled in each sub-study. Please also describe the eligibility criteria (if any) for the planned sub-studies, where not mentioned.

This is indeed important information, and we have added the following paragraphs to the Methods to clarify the number of subjects recruited for each of the sub-studies as well as the inclusion criteria.

L. 302

In total, 450 men and women who participated in the Inter99 follow-up study with detectable coronary arterial calcification (CAC≥10 Agatston units) assessed by the cardiac CT scan will be recruited to……

L. 309

The InterDAG study will recruit 1000 consecutive participants from the Inter99 follow-up study with no exclusion criteria.

7. Grammar/spelling/sentence structure:
-Make sure to use past-tense language when writing about the original Inter99 Study (e.g., the first line of the methods section)

This has been corrected.

L. 156
The Inter99 study was initiated in March 1999 as a population-based multi-factorial intervention

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Reviewer: 2 - Dr. David Lee, New York University School of Medicine, New York University School of Medicine

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1. Comments to the Author:
   Overall: This study protocol seeks to extend a longitudinal analysis of the Inter99 cohort to study cardiometabolic health at 20 years after cohort entry. The analysis is very well justified and demonstrates the critical importance of this work. Some noted addressable issues with the planned protocol: while there is an extensive number of variables and outcomes that will be examined through this work, the statistical plan section is lacking hypotheses to be tested. While this could be okay, then the study should be framed as a more exploratory analysis. If not, then perhaps hypotheses to be tested should be delineated, along with the a priori models to be analyzed. Machine learning is mentioned but sounds as if it's more of an adjunct analysis. Finally, there might be more attention needed regarding the ethics plans given use of cardiac CT, which may not necessarily be benign.

2. Strengths/Limitations: Limitation 5 might need to highlight the country specific results of the study.

   In order to address this relevant point we have added the following sentence to the Discussion as to underline this particular limitation.

   L. 493
   Finally, nearly all participants are of Danish ethnicity, as Danish literacy was a prerequisite at baseline inclusion, thus findings should be applied to other ethnic groups with caution.

3. Introduction: The introduction does a great job of detailing the need for the planned study

   Thank you, we appreciate that.

4. Lines 243-250: Regarding Cardiac CT, how does the study deal with the challenge of identifying incidental findings, which may increase risk of interventional procedures?

   This is indeed important and important to clarify, and we have added a paragraph to the Methods.

   L. 400

   Performing CT-scans may result in incidental findings that need further examination and potentially treatment. As for other screening procedures this may cause both benefit (early detection and treatment) and harm (over-treatment) to the participants. The radiation dose associated with the CT-scan is relatively low and considered of minimal risk.

5. How to the study investigators deal with the design looking at a multiplicity of outcomes, should p-values be adjusted as such? Or is this to be an exploratory analysis.

Although there are several specific hypotheses, we acknowledge that many research questions will be examined. We have added the following words to the Introduction and paragraph to the Statistics:

L. 351

As described in Table 1, this study will provide a wealth of data and future analyses strategies will depend on the research question and outcome in focus. The statistical methods described below serves as an example of the methods most likely to be used, while alternative approaches will be applied when appropriate.

L.356

As the established Inter99 20-year follow-up database will form the basis for testing several research questions, the analyses should be considered explorative in nature. However, a main hypothesis of the Inter99 20-year follow-up was to investigate whether low birthweight independent of genetics, lifestyle and glucose tolerance over 20 years is related to common T2D cardiometabolic co-morbidities.

L. 370

will be employed ~~investigated~~.

6. Furthermore, given the numerous variables to be analyzed shouldn't variable selection methods be used? Otherwise, if models are to be developed based on an a priori conceptual model then that model should be described in the protocol, such that reviewers can be confident that the final model selected was not chosen based on testing a variety of models.

We agree that numerous variables will be available to investigate many research questions by different approaches. Given the explorative nature (see response above) of the project different approaches will be needed. We have added the following text to the manuscript:

L. 379

The model and variable selection will depend on the research question. One approach is the concept of causal models and causal directed acyclic graphs. For some research questions it is also possible to use genetic risk scores as unbiased instruments of exposures. When optimal prediction of disease is the main purpose, models will be compared by using C-statistics and other related approaches.

7. While machine learning techniques are described later, given the discussion prior to it, they sound adjunct analyses rather than the primary analytic approach which would use conceptual models to choose variables to be examined.

   We have decided to delete the following paragraph on machine learning in the Statistics section as this is not a primary analytic approach.

   ~~While classical statistical prediction modelling will be using risk factors identified from existing literature, machine learning techniques will be applied to develop prediction algorithms using a much wider spectrum of the available of data.~~

8. Line 374-375: I am not certain that we can describe a Cardiac CT scan as necessarily harmless. While it may be indicated for some persons, there are a large number of incidentalomas that might be identified through the procedure, especially among a large number of participants. For instance, a patient might be found to have a mass, which might lead to a biopsy, which may in turn lead to significant complications from such. Regular CT scans are not advised (as the research has shown) for cancer screening in the general population, so then, the argument that only benefit will occur from these scans cannot be made. Some consideration and detail should be put forth about how this will be handled in a rationale way that is consistent with both clinical care standards and also patient preferences.

   Thank you for raising this point as is indeed important information to consider. To clarify, we have added the following paragraph to the Ethics and dissemination section.

   L. 400
   Performing CT-scans may result in incidental findings that need further examination and potentially treatment. As for other screening procedures this may cause both benefit (early detection and treatment) and harm (over-treatment) to the participants. The radiation dose associated with the CT-scan is relatively low and considered of minimal risk.

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   COI statements:

   Reviewer: 1
   Competing interests of Reviewer: None.

   Reviewer: 2
   Competing interests of Reviewer: No competing interests.

VERSION 2 – REVIEW

REVIEWER	Kehler, D. Scott Dalhousie University
REVIEW RETURNED	02-Jan-2024

GENERAL COMMENTS	Thank you for addressing my comments as well as the other reviewers and the editor's. I have no further comments.

REVIEWER	Lee, David New York University School of Medicine, Emergency Medicine
REVIEW RETURNED	19-Dec-2023

GENERAL COMMENTS	Authors were appropriately responsive to the reviewer comments and concerns. No additional comments to suggest.

VERSION 2 – AUTHOR RESPONSE