PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	A protocol for prospective study of vitamin D, obesity, and leptin in relation to bladder cancer, incidence and survival.
AUTHORS	Gislefoss, Randi Elin; Stenehjem, Jo; Hektoen, Helga; Andreassen, Bettina Kulle; Langseth, Hilde; Axcrona, Karol; Weiderpass, Elisabete; Mondul, Alison; Robsahm, Trude

VERSION 1 – REVIEW

REVIEWER	Lars Holmberg Prof emeritus, Cancer epidemiology King's College London and Uppsala University
REVIEW RETURNED	15-Sep-2017

GENERAL COMMENTS	This paper is in the category “Cohort profile” or “Protocol presentation” and does not contain results. The investigators present an interesting protocol related to overweight, vitamin D exposure and bladder cancer. The questions – especially regarding overweight – are highly relevant for cancer prevention. Bladder cancer is an important public health problem. The overall study design and the setting are well suited for the inquiry and present some features that provide unique opportunities in terms of quality of exposure data, follow-up information and statistical precision. Given this opportunity to provide new insights into the problem area, this reviewer thinks that there are some more aspects that could be investigated by developing the questions, and following that also the analytical strategy further. Two aspects come to mind in regard of the current literature, and certainly more themes could be developed if the theory is better penetrated: • The current protocol indicates that overweight and vitamin D exposure will mainly be seen as probably related, but still independent risk factors. Some investigators have raised the issue if the effect of overweight is in fact mediated by influencing vitamin D levels. Studies hitherto have not disentangled the contribution of this possible pathway. This protocol could develop approaches to do so. This aspect would also have general interest beyond bladder cancer. • It is a challenge that BMI and the vitamin D levels will have been measured at many different points in time before occurrence of the cancer. Thus, the exposure may have been measured during initiation, promotion or even late progression to metastatic disease.

	It would seem that this study might have some statistical precision to provide further information on a longitudinal perspective. Once again, this would also be of general interest in cancer etiology. The study can be an important source of information regarding bladder cancer, especially if the hypotheses and analytical strategy could be developed further, preferably a priori and not as a result of data exploration later.

REVIEWER	Christel Häggström Department of Biobank Research, Umeå University, Sweden
REVIEW RETURNED	17-Nov-2017

GENERAL COMMENTS	General comments This is a study protocol describing of Vitamin D, obesity and leptin in relation to bladder cancer risk and survival, using data from a biobank linked to nation-wide registers in the design of 1) cohort study and 2) nested case-control study. I have no major comments. Minor comments: • I think one of the main limitations for hypothesis 1b and 2b are lack of data of bladder cancer treatment, which may affect survival of bladder cancer to a larger extent than pre-diagnostic BMI or Vitamin D levels. Please consider adding this limitation to the text. • The hypotheses in the aims and data analysis plan are not consistent. Do you plan to investigate only BMI/obesity or also other anthropometric measures in hypothesis 1? Also, for hypothesis 2 it is written on page 8 line 21 that only muscle-invasive bladder cancer will be selected as cases – if this is true please rewrite the hypotheses accordingly. • It is unclear how and from where the T stage information can be retrieved (assumingly from medical charts, but the data is already de-identified?). It is also unclear how data of T stage will be handled in the analysis. • Table 1 footnote is unclear. Also, if possible, consider to add a reference to expected proportion of the population exposed with vitamin D deficiency + high leptin levels. • Please declare all abbreviations (including 25(OH)D) upon first use. • This is not a comment, but something that the authors can consider in the study: In hypothesis 1b and 2b, the obesity paradox might be an issue (Mayeda and Glymour, CEBP, jan 2017).

VERSION 1 – AUTHOR RESPONSE

Reviewer comments:

Reviewer #1.

Comment 1.1: The current protocol indicates that overweight and vitamin D exposure will mainly be seen as probably related, but still independent risk factors. Some investigators have raised the issue if the effect of overweight is in fact mediated by influencing vitamin D levels. Studies hitherto have not disentangled the contribution of this possible pathway. This protocol could develop approaches to do so. This aspect would also have general interest beyond bladder cancer.

Response 1.1: We appreciate this comment and have included more details on how we will do these analyses (Sub-study II p8 and Statistical methods p9).

The following text has been added: “In sub-study I, we will investigate the association between BMI and BC, and for sub–study II we will in addition include vitamin D levels. Overall we will focus on disentangle the relationship of vitamin D, BMI and BC. This will be done in two ways:

1. By implementing regression models including and interaction effect of vitamin D and BMI on BC.
2. By testing the hypothesis whether the effect of BMI on BC is mediated by vitamin D using mediation analysis34.”

Comment 1.2 It is a challenge that BMI and the vitamin D levels will have been measured at many different points in time before occurrence of the cancer. Thus, the exposure may have been measured during initiation, promotion or even late progression to metastatic disease. It would seem that this study might have some statistical precision to provide further information on a longitudinal perspective. Once again, this would also be of general interest in cancer etiology.

Response 1.2 We acknowledge this comment. Minimum time from blood draw to diagnosis will be 5 years. Unfortunately, this information was missing in the paper and has now been added (Sub-study II p8). Studies have shown that seasonally adjusted measurement of 25(OH)D at different time-points in life have little intra-individual variability (Berwick, M and Erdei, E.O 2013). The exact onset of carcinogenesis is impossible to monitor, it is therefore important that a potential relationship between exposure and cancer is investigated to minimize the possibility of reverse causality, i.e. that the BC tumor itself is affecting the 25(OH)D level. The use of pre-diagnostic samples of serum collected at least 5 years before diagnosis will thus be of importance.

Reviewer #2

Comment 2.1 I think one of the main limitations for hypothesis 1b and 2b are lack of data of bladder cancer treatment, which may affect survival of bladder cancer to a larger extent than pre-diagnostic BMI or Vitamin D levels. Please consider adding this limitation to the text.

Response 2.1 We agree with the reviewers comment. Lack of treatment data is a limitation of this study and will be of importance when evaluating the survival data. We have therefore added this as a limitation to the study (Textbox p3 and 2nd paragraph in study strengths p12).

Comment 2.2 The hypotheses in the aims and data analysis plan are not consistent. Do you plan to investigate only BMI/obesity or also other anthropometric measures in hypothesis 1?

Response 2.2 Thank you for making us aware of this inconsistency. The text has been corrected accordingly (Aims p5) We plan to investigate BMI, height, weight, Body Surface Area, and weight change over time.

Comment 2.3 Also, for hypothesis 2 it is written on page 8 line 21 that only muscle-invasive bladder cancer will be selected as cases – if this is true please rewrite the hypotheses accordingly.

Response 2.3 We appreciate this comment. The project group has discussed the limitation of only including muscle-invasive cases and have decided to include all T stages (Ta, Tis and T1-T4) that are high grade tumors. The text has been corrected (Sub-study II p8).

Comment 2.4 It is unclear how and from where the T stage information can be retrieved (assumingly from medical charts, but the data is already de-identified?).

Response 2.4 The T-stage information has been retrieved from histological reports. The verification phase has been done on identified data, but the analyses will be done on de-identified data.

Comment 2.5 It is also unclear how data of T stage will be handled in the analysis.

Response 2.5 In study 1 all cases will be included. In study 2 the cases will be selected randomly among the included stages. The analyses will stratify on stage.

Comment 2.6 Table 1 footnote is unclear.

Response 2.6 We agree. The footnote has been corrected (Table 1 p10).

Comment 2.7 Also, if possible, consider to add a reference to expected proportion of the population exposed with vitamin D deficiency + high leptin levels.

Response 2.7 We appreciate this comment and have added a few sentences and two references on the expected proportion of the population exposed with vitamin D deficiency + high leptin levels (Sub-study II p10 and ref #27 and #29)

Comment 2.8 Please declare all abbreviations (including 25(OH)D) upon first use.

Response 2.8 Thank you for noticing, the text has been corrected.

Comment 2.9 This is not a comment, but something that the authors can consider in the study: In hypothesis 1b and 2b, the obesity paradox might be an issue (Mayeda and Glymour, CEBP, jan 2017).

Response 2.9 Thank you for this remark. However, as we use height and weight measured many years before diagnosis, presumingly before any ongoing cancer disease, this paradox might be of minor relevance. We will consider this reference and the obesity paradox for the original research papers.

VERSION 2 – REVIEW

REVIEWER	Lars Holmberg Prof emeritus Sweden/UK
REVIEW RETURNED	05-Jan-2018

GENERAL COMMENTS	Both reviewer's discussion points are addressed.

REVIEWER	Christel Häggström

	Department of Biobank Research, Umeå University, Umeå, Sweden
REVIEW RETURNED	18-Jan-2018

GENERAL COMMENTS	I have only very minor comments, and most of my previous comments have been answer accordingly. However, the authors have added "We propose a study aiming to examine anthropometric data (BMI, height, weight, body surface area and weight change over time) and serum levels of leptin, total and free 25(OH)D, in relation to BC risk and survival.. " but in the actual hypotheses includes only obesity, free and total 25(OH)D level and serum leptin levels. What about the hypotheses for the other anthropometric data? Please make sure that the aim and the hypotheses are consistent. (page 5). Moreover, these newly added anthropometric data are not included in the variable list in (page 7), and it is unclear how weight change will be measured/calculated in the cohort (as there is no information about repeated weight measurements). Also, a very minor detail is the authors have added a line about perform one part of the analysis using the R software, and in the next paragraph they have stated that all analyses will be performed using STATA software. Please change this accordingly.

VERSION 2 – AUTHOR RESPONSE

Response to the reviewers

Reviewer comments:
Reviewer #1

Comments 1:
Please state any competing interests or state 'None declared':
None declared
Response 1:
None declared is stated

Reviewer #2

Comments 2.1:
Please state any competing interests or state 'None declared':
None declared
Response: 2.1:
None declared is stated

Comments 2.2:
However, the authors have added "We propose a study aiming to examine anthropometric data (BMI, height, weight, body surface area and weight change over time) and serum levels of leptin, total and free 25(OH)D, in relation to BC risk and survival.. " but in the actual hypotheses includes only obesity, free and total 25(OH)D level and serum leptin levels. What about the hypotheses for the other anthropometric data? Please make sure that the aim and the hypotheses are consistent. (page 5).
Response: 2.2:

BSA and weight change over time have been included in the hypotheses. The aims and hypotheses are now consistent.
Comments 2.3:
Moreover, these newly added anthropometric data are not included in the variable list in (page 7), and it is unclear how weight change will be measured/calculated in the cohort (as there is no information about repeated weight measurements).
Response: 2.3:
The anthropometric data has been added to the variable list. Weight change over time will only be available for a sub-cohort that has repeated measurements.
Comments 2.4:
Also, a very minor detail is the authors have added a line about perform one part of the analysis using the R software, and in the next paragraph they have stated that all analyses will be performed using STATA software. Please change this accordingly.
Response: 2.4:
Thank you for this comments. We are sorry for the confusing text. Bothe statistical programs will be used in the study. The text has been changed accordingly.
Reviewer comments:
Reviewer #1

Comments 1:
Please state any competing interests or state 'None declared':
None declared
Response 1:
None declared is stated

Reviewer #2

Comments 2.1:
Please state any competing interests or state 'None declared':
None declared
Response: 2.1:
None declared is stated

Comments 2.2:
However, the authors have added "We propose a study aiming to examine anthropometric data (BMI, height, weight, body surface area and weight change over time) and serum levels of leptin, total and free 25(OH)D, in relation to BC risk and survival.. " but in the actual hypotheses includes only obesity, free and total 25(OH)D level and serum leptin levels. What about the hypotheses for the other anthropometric data? Please make sure that the aim and the hypotheses are consistent. (page 5).
Response: 2.2:
BSA and weight change over time have been included in the hypotheses. The aims and hypotheses are now consistent.
Comments 2.3:
Moreover, these newly added anthropometric data are not included in the variable list in (page 7), and it is unclear how weight change will be measured/calculated in the cohort (as there is no information about repeated weight measurements).
Response: 2.3:
The anthropometric data has been added to the variable list. Weight change over time will only be available for a sub-cohort that has repeated measurements.
Comments 2.4:

Also, a very minor detail is the authors have added a line about perform one part of the analysis using the R software, and in the next paragraph they have stated that all analyses will be performed using STATA software. Please change this accordingly.

Response: 2.4:

Thank you for this comments. We are sorry for the confusing text. Bothe statistical programs will be used in the study. The text has been changed accordingly.